Cardiovascular Risk in Ageing Men of Different Ethnicities; Inter-Relationships between Imaging and Endocrine Markers

A thesis submitted to The University of Manchester for the degree of PhD
in the Faculty of Medical and Human Sciences

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School of Medicine
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<td>25(OH)D</td>
<td>25 hydroxy cholecalciferol or 25 hydroxy vitamin D</td>
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<tr>
<td>2D</td>
<td>2 dimensional</td>
</tr>
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<td>3 dimensional</td>
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<td>A</td>
<td>late filling by atria</td>
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<td>A2C</td>
<td>apical 2-chamber view</td>
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<td>A4C</td>
<td>apical 4-chamber view</td>
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<td>ASC</td>
<td>apical 5-chamber view</td>
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<td>ACE</td>
<td>angiotensin-converting enzyme</td>
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<td>AIx</td>
<td>augmentation index</td>
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<td>aoAIx</td>
<td>aortic (central) augmentation index</td>
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<td>aPWV</td>
<td>aortic pulse wave velocity</td>
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<td>ARR</td>
<td>aldosterone to renin ratio</td>
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<td>AV</td>
<td>aortic valve</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>brAIx</td>
<td>brachial augmentation index</td>
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<td>BSA</td>
<td>body surface area</td>
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<td>common carotid artery</td>
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<td>CMRI</td>
<td>cardiac MRI</td>
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<td>cSBP</td>
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<td>c-reactive protein</td>
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<td>cardiovascular</td>
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<td>diastolic blood pressure</td>
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<td>DT</td>
<td>deceleration time</td>
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<tr>
<td>E</td>
<td>early mitral inflow</td>
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<td>e'</td>
<td>peak early diastolic mitral annulus velocity in TDI</td>
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<td>Ea</td>
<td>peak early diastolic mitral annulus velocity in TDI</td>
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<td>EF</td>
<td>ejection fraction</td>
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<td>EMAS</td>
<td>European Male Aging Study</td>
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<td>FOV</td>
<td>field of view</td>
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<td>GTF</td>
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<td>IVRT</td>
<td>isovolumic relaxation time</td>
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<td>IVSTd</td>
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<td>LA</td>
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<td>LAV</td>
<td>left atrial (end-systolic) volume</td>
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<td>LC</td>
<td>liquid chromatography</td>
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<tr>
<td>LV</td>
<td>left ventricle (ventricular)</td>
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<td>LVEDV</td>
<td>left ventricular end-diastolic volumes</td>
</tr>
<tr>
<td>LVESV</td>
<td>left ventricular end-systolic volumes</td>
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<td>LVH</td>
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<tr>
<td>LVIDd</td>
<td>LV internal dimension at end-diastole</td>
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<tr>
<td>LVIDs</td>
<td>LV internal dimension at end-systole</td>
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<td>LVMI</td>
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<td>M/V</td>
<td>LV end-diastolic mass to volume ratio</td>
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<td>mineralocorticoid receptor</td>
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<td>velocity encoding</td>
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<td>VSMC</td>
<td>vascular smooth muscle cell</td>
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Abstract

Cardiovascular risk in ageing men of different ethnicities; inter-relationships between imaging and endocrine markers

Mohammadreza Rezailashkajani, The University of Manchester, PhD, September 2011

Cardiovascular disease varies by ethnicity in the UK. South Asians (SA) have higher coronary heart disease (CHD) and diabetes prevalence, while African-Caribbeans (AfC) have greater stroke, but intriguingly lower CHD rates despite higher blood pressures and diabetes risk than Europeans. Conventional risk factors do not fully explain such differences. This cross-sectional study tested the hypothesis that the hormones, vitamin D measured as 25(OH)D and aldosterone, would be independently associated with intermediate cardiovascular outcome markers in these ethnic groups. Community-dwelling men 40-80 years old (AfC: n=67, 55±10yr; SA: n=68, 55±10yr; European: n=63, 57±8yr) were sampled from Greater Manchester’s multi-ethnic population. The intermediate markers examined were aortic pulse wave velocity (aPWV), left ventricular (LV) mass and function, and carotid intima media thickness (CIMT), measured non-invasively by ultrasound, and hemodynamic profiling methods (the Arteriograph) in the total sample and by magnetic resonance imaging (MRI) in a subsample of 50.

Adjusted for age, systolic blood pressure and diabetes, mean(SE) aPWV by the Arteriograph, was 0.5(0.2) m/s higher in SA than AfC and Europeans (p=0.01), which paralleled known cross-ethnic CHD risk differences in the UK. By MRI, aPWV along the descending aorta in SA was 0.7(0.3) and 0.8(0.3) m/s higher than that in AfC and Europeans, but aPWV along the aortic arch was not significantly different. Unlike aldosterone, 25(OH)D was independently and inversely correlated with aPWV (unstandardised B(SE)=-0.013[0.004] m/s, p<0.001), and partly explained the ethnic variation in aPWV. Similar inverse correlations were found between 25(OH)D and LV concentricity measured by echocardiography and MRI. Compared to Europeans, SA and AfC, had 21(3) and 14(3) nmol/L lower mean(SE) 25(OH)D, respectively (p<0.01). Mean(SE) of relative wall thickness, an index of LV concentricity by echocardiography, was 0.05(0.01) higher in SA and AfC than Europeans. Lower 25(OH)D levels were also associated with higher myocardial deformation rates measured by MRI myocardial tagging (n=50), supporting previous animal experimental evidence. A one standard deviation (SD) decrease in 25(OH)D was associated with a 0.38 SD increase in absolute systolic strain rate (p=0.003) and 0.22 SD rise in diastolic strain rate (p=0.04). Right and left CIMT showed different relations with 25(OH)D and aldosterone. Left-right CIMT differences varied by ethnicity and were related to SA ethnicity and aldosterone levels.

Two related technical studies investigated the relatively new method of hemodynamic profiling, the Arteriograph, used here. The results suggested a standardisation method of aortic length estimation for purely central aPWV, which significantly improved aPWV agreement between the Arteriograph and MRI (reference method here), and was used for calibrating the Arteriograph aPWV in the above-mentioned results for the total sample.

Future well-designed trials are necessary to investigate any cause-effect relationship between vitamin D deficiency and the unfavourable cardiovascular intermediate outcomes found here in a cross-sectional design and multi-ethnic background.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.
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I also wish to thank Prof. Naveed Sattar and his team who collaborated with us on hormonal measurements, and would like to cherish the memory of his colleague, Dr Mike Wallace, who unexpectedly died while this work was in progress.

Last but not the least; I would like to warmly thank all of the participants of this study. This work was impossible without their participation.
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Dedication

To my kind wife, Delnaz
To my sweet daughter, Niki

To my dear parents
who always encouraged me to reach up high
About the Author

Mohammad-Reza Rezai (Rezailashkajani) graduated with a doctorate in medicine (MD) from Tehran University of Medical Sciences, Iran in 1999. For more than two years, he served as a general practitioner in under-privileged areas of southern Tehran province. Since then, he has spent most of his career in medical research. He developed an interest in working and learning in the interface of computer and medical sciences. He started his research career working on epidemiology and health informatics topics related to gastroenterology in Shaheed Beheshti University of Medical Sciences, Tehran, Iran where he contributed to 9 peer-reviewed publications and several informatics projects during four years. In parallel, he earned an MSc of Bioinformatics degree in the University of Manchester, UK with an Award of Distinction in 2007. He then started this PhD project after winning a Strategic Studentship followed by an ORSAS award from the University of Manchester.

At the time of closing this thesis (September, 2011), he was a post-doctoral fellow in Cardiovascular and Diagnostic Imaging Research Program, the Institute for Clinical Evaluative Sciences (ICES), Toronto, Canada where he worked on cardiovascular risk profiling of visible ethnic minority populations in Ontario.
A) Thesis Structure

This thesis is structured based on the *Alternative Format* of the University of Manchester. The reason for doing the alternative format was the appropriate quality of results for peer-reviewed publication, and my motivation to publish.

The first following chapter (Chapter B) gives a brief introduction to the context and rationale of the project. Chapter C is a literature review on the related concepts. Description of the methods and protocols used in the project is found in Chapter E. The results are formatted as six papers, two of which are already published [1, 2]. The first four papers report the core results of this PhD project. The next two are primarily technical papers focusing on comparison of a new oscillometric method of hemodynamic measurement used in this project, with the conventional methods, and suggestions for its improvement. Finally, a summary and conclusion chapter closes this thesis.
B) Context & Rationale

Atherosclerotic cardiovascular disease (CVD) including coronary heart disease (CHD) is among the leading causes of death globally accentuated by the man’s lifestyle change especially in the industrialized world in the past century. CHD alone imposed a huge total economic burden of £7.06 billion in 1999 in the UK, the highest of all similarly analysed diseases at the time [3].

CVD clinical end-points such as myocardial infarction and cardiovascular mortality vary among different ethnic groups [4-6]. Studies in the UK and worldwide have shown that South Asian population have a higher rate of CHD compared to European whites and some other ethnic groups [6-9]. The age-standardised CHD mortality rate in South Asians in the UK is 50% higher than European whites [10]. In contrast, despite higher rates of diabetes, hypertension and stroke, the African-Caribbean have a lower CHD mortality than Europeans [10, 11].

Interestingly, the conventional CHD risk factors such as diabetes, obesity and hypertension fail to fully explain these differences [12]. The contrast between ethnic groups in terms of CVD outcome should create a good background to investigate novel risk markers.

The European Male Ageing Study (EMAS), one of the largest population-based projects on male ageing in the world, is a multi-centre cohort study running in 8 European countries led by the Andrology Research Unit, University of Manchester. The participants are European men of 40-79 years selected by stratified random sampling from population registers in 8 European centres to make four equal-size samples of 10 year age bands (i.e. 40-49, 50-59, 60-69 and 70-79 years). EMAS has already compiled a comprehensive dataset containing large number of variables related to many aspects of male aging (e.g. lifestyle, biochemical, hormonal, anthropometric, and psychological). The study cohort had an initial baseline visit in 2002-2004 and follow-up visits (after 4 years) started in 2007. The study design and further methodology details have been described before [13].
In 2007, EMAS headquarters started recruiting community-dwelling men of South Asian (mainly Pakistani) and Afro-Caribbean origin from the multi-ethnic Greater Manchester population to add an ethnic dimension to the project. This ethnic study ‘linked’ to EMAS was called LINK, and the same protocol as EMAS was used for LINK participants. Compared to EMAS, recruitment of LINK men was not based on population registers and was mostly done by advertising in community centres, churches and mosques followed by volunteer recruitment.

This clinical PhD project took advantage of the appropriate setting of EMAS and LINK studies for a cross-sectional investigation of the potential involvement of the steroid hormones (vitamin D and aldosterone) in the ethnic difference in CVD beyond conventional risk factors, among ageing men of three ethnic groups: South Asian, Afro-Caribbean, and European.

Some already-known vascular and cardiac risk markers were non-invasively measured as sub-clinical CVD risk markers or intermediate outcomes. Ethnic differences in these intermediate outcomes are described here. The association of two steroid hormones, vitamin D and aldosterone, with such outcomes was also investigated in the regression models including and controlling for already-measured conventional risk factors (e.g. blood pressures, smoking and diabetes) as well as ethnicity.

As the project used a mixture of conventional and new methods of arterial stiffness measurement (e.g. as one of the intermediate CVD risk markers), opportunities rose as parallel, but necessary projects to further compare/validate the new method with the conventional ones. This created valuable opportunities for the candidate to explore some technical realms of large-artery hemodynamic measurements used as core outcome measures in the project.
B.1 PhD student’s contributions

The PhD student declares some of his major contributions to this thesis as the following:

1) Participant recruitment including contacting the participants, follow-up and reminder calls, preparing and sending medical reports to participants’ GPs when necessary (e.g. abnormal results), designing and sending back a short feedback to all participants.

2) Coordination of participant visits with clinical teams in Wellcome Trust Clinical Research Facility (WTCRF), Manchester.

3) Interviewing, consenting and providing information for participants, and accompanying them in all measurements in WTCRF.

4) Contributing to drafting and design of ethics amendments, information packages and consent forms.

5) Active contribution to design of all protocols including echocardiography and carotid ultrasound, cardiac and aortic MRI, and arterial stiffness measurement protocols.

6) Conducting blood pressure measurements and arterial stiffness tests including the Arteriograph, SphygmoCor and Omron HEM-9000.

7) Data management including developing an MS Access database for:
   a. Efficient and secure management of recruitment data including personal contacts, phone call follow-ups, and automatic letter production.
   b. Data integration from all platforms (e.g. imaging, biochemistry/hormonal, anthropometric and hemodynamic measurements).
   c. Data storage and back-up.

8) Active participation in management of a collaborative project with a world-class magnetic resonance (MR) imaging team in University of Auckland, New Zealand, and providing feedback on the imaging protocol development, exchange of large MR image datasets, etc.

9) Image analysis including:
   a. Analysis of CIMT images using Philips Qlab.
b. Analysis of all aortic images from the MRI protocol including phase-contrast flow images, aortic length measurement and aortic pulse wave transit time and velocity computations using Philips MR Workspace and MS Excel.

10) Statistical analysis including data quality checks, choosing correct statistical approach, performing the tests with SPSS and R, and interpretation of the results guided by Prof. JK Cruickshank.

11) Drafting all manuscripts in this thesis, and responding to journal reviews of the published manuscripts in collaboration with and guided by the lead supervisor, with feedback from other team members.
C) Background

This section starts with an introduction to cardiovascular risk and its assessment using traditional and newer approaches including surrogate markers of CVD mainly measured by imaging methods. This is followed by an overview of the ethnic differences in CVD and a chapter on the potential CVD roles of the two steroid hormones of interest in this project, aldosterone and vitamin D.

C.1 Cardiovascular risk & its assessment

The risk factors of CHD have been extensively studied, and can be categorized into three groups of major, emerging, and underlying (life habit) risk factors [14, 15].

Major risk factors considered to be of proven direct causality for CVD include cigarette smoking, hypertension, elevated LDL-C (low density lipoprotein cholesterol) and low HDL-C (high density lipoprotein cholesterol), diabetes mellitus, age (men ≥ 45 yr, women ≥ 55 yr), family history of premature CHD (<55 male or <65 female first-degree relative with CHD), and evidence of other clinical forms (e.g. peripheral arterial disease). Emerging risk factors are less well-defined, but some evidence supports their causal relationship with CHD. Examples of these include prothrombotic and proinflammatory factors, lipoprotein A, homocysteine, insulin resistance, small LDL particles, and evidence of subclinical atherosclerotic disease. Finally, life habit risk factors include obesity, atherogenic diet and physical inactivity [15, 16].

C.1.1 CVD risk factors tend to cluster

In many people, a constellation of multiple CV risk factors exist simultaneously. This concurrency has been noticed since decades ago, and the terms syndrome X, insulin resistance syndrome, and most recently metabolic syndrome have been used to refer to this constellation [16]. There is evidence indicating that the metabolic syndrome may increase the risk of CVD though argument continues over whether any genuine excess
Background  

Cardiovascular risk & its assessment

Risk is enforced by the “syndrome” compared with that from the sum of its components [17].

Different clinical definitions exist for metabolic syndrome. For example, the definitions used by National Cholesterol Education Program’s Adult Treatment Panel III (ATP III) identifies the components of metabolic syndrome as: 1) abdominal obesity, 2) atherogenic dyslipidemia (e.g. low HDL, and high triglycerides and/or LDL), 3) insulin resistance, 4) increased blood pressure, 5) a proinflammatory state (e.g. manifested by high C-reactive protein) and 6) a prothrombotic state (e.g. characterized by elevated plasma PAI-1 or fibrinogen). Three of the 6 components are necessary to be present in a person to be identified as having the metabolic syndrome [15]. Primarily, insulin resistance and obesity have been postulated as the root aetiologies of the metabolic syndrome though there are still many questions unanswered in its pathophysiological picture and causality relationships [16]. Further details of the metabolic syndrome are beyond the scope of this report.

C.1.2 Total cardiovascular risk assessment

Appropriate risk assessment is a necessity in CVD prevention and research. As stated above, multiple CVD risk factors tend to cluster and damage the cardiovascular system in an additive and synergistic manner. For this reason, an integrated or “total” approach to assessment of CVD risk is a must [18].

Recognition of the “cumulative interaction among cardiovascular risk factors” has driven investigators to model risk prediction, or risk stratification algorithms [18]. The Framingham score is one of the most extensively used cardiovascular risk stratification systems. The score is calculated using a prediction equation originally developed by Anderson et al using the results from 5573 cases 30-74 years old and without CVD in the Framingham Heart Study [19]. Framingham risk score uses blood pressure, total cholesterol, HDL cholesterol, smoking, glucose intolerance, and left ventricular hypertrophy (LVH) in mathematical equations to predict the probability of important clinical CVD endpoints in a certain period of time (i.e. usually a 10-year period) [19].

Despite widespread use of Framingham score, some studies have indicated that it is not accurate in some populations. For example, Brindle et al claimed that it significantly overestimated absolute cardiovascular risk for the UK population [20]. The review by
Eichler et al showed that while the Framingham scoring was “well-calibrated” for predicting first coronary events in the populations from the US, Australia, and New Zealand, it needed recalibration to address the overestimation of absolute risk in European populations [21].

The European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) have also developed a risk stratification guideline considering the total cardiovascular risk concept. Table C-1 shows the ESH guideline which stratifies CV risk in four categories of added risk [22]. Based on the number of the risk factors, and the presence of the metabolic syndrome, or target organ damage (e.g. LVH, and microalbuminuria) the individual can be stratified into low (<10%), moderate (15%-20%), high (20%-30%), or very high (>30%) total 10-year risk for developing fatal or non-fatal CVD [18, 22].

Table C-1. ESH guidelines
How hypertension management is influenced by the total CV risk concept. Adapted from [22]

<table>
<thead>
<tr>
<th>Other risk factors Subclinical organ damage or disease</th>
<th>Blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td><strong>SBP</strong></td>
<td>120-129</td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td>80-84</td>
</tr>
<tr>
<td>No other risk factors</td>
<td>Average risk</td>
</tr>
<tr>
<td>1-2 risk factors</td>
<td>Low added risk</td>
</tr>
<tr>
<td>≥ 3 risk factors, metabolic syndrome, organ damage or diabetes</td>
<td>Moderate added risk</td>
</tr>
<tr>
<td>Established cardiovascular or renal disease</td>
<td>Very high added risk</td>
</tr>
</tbody>
</table>

HT: hypertension

Total cardiovascular risk concepts have also greatly influenced the guidelines for management of CVD risk factors. The ATPIII [15] guidelines for management of high blood cholesterol, and ESH-ESC Guidelines for the management of arterial hypertension are other typical examples.
C.1.3 Continuous risk concept

Conventional categorization of continuous risk factors’ cut-points has been in use for a long time; for instance, the cut-point of 140 as the upper normal range of systolic blood pressure in clinical management of hypertension. However, risk of CVD increases even in individuals with blood pressures above optimal values, but yet below the conventional 140 mmHg cut-point for clinical definition of hypertension [18, 23]. Similar lines of evidence exist for the impaired glucose tolerance test or fasting blood glucose and the increased likelihood of developing diabetes and consequently CVD. These have led to coining of the new terms prehypertension and prediabetes [18]. While these may suggest the continuous nature of the risk factors’ hazard, many still label a continuum into categories. The problem represents the struggle between concept and practical application.
C.2 Surrogate biomarkers of CVD

Despite enhancements offered by total risk assessment models (e.g. Framingham’s score), many patients afflicted by CVD are not well stratified by such algorithms [24]. In addition, such scoring systems do not model all CVD risk factors including those yet undiscovered. Accordingly, alternative more accurate, objective and integrative measures for risk stratification of CVD have been sought.

Beyond its clinical use, total CVD risk assessment is widely used in CVD research where, for instance, there is need for measuring the change in CVD outcome by drug interventions or lifestyle modification, or simply comparing different populations’ risk. The clinical end-points of CVD, namely cardiovascular events including mortality and morbidity (e.g. myocardial infarction [MI] and stroke), are still considered as gold standards in CVD outcome measurement. However, their use in CVD research and drug discovery entails too lengthy and costly studies with larger samples in which the investigator has to wait for such events to occur to measure efficacy of a treatment or the magnitude of a risk [25].

Alternatively, some biomarkers or intermediate end-points which predict the gold-standard clinical end-points can be used as surrogates [25]. For instance, serum LDL cholesterol is extensively used as a risk marker of CHD. In addition to soluble surrogate markers, some are measured by imaging (e.g. imaging biomarkers) or other non-invasive technologies, and have shown promise in CVD research (See Figure C-1) [25]. Both vascular and cardiac measurements have been used as CVD surrogate markers.

Figure C-1. Surrogate markers of CVD, general concept

PWV: pulse wave velocity, LVM: left ventricular mass, CIMT: carotid intima media thickness.
Since arterial walls are the main target of atherosclerosis and CVD, the alterations in the arterial walls may be suitable markers quantifying “cumulative” damage of multiple cardiovascular risk factors, and aid in “total” risk stratification [26]. Two commonly measured CVD surrogate markers in the vascular tree are arterial stiffness, mainly characterizing arterial function, and carotid intima-media thickness (CIMT), a structural index. Microalbuminuria has also emerged as a biomarker roughly reflecting small vessel damage, since the delicate renal glomerular micro-vasculature is damaged in CVD [27, 28].

Among commonly measured cardiac surrogate markers of CVD are the indices of the left ventricular structure (e.g. mass and geometry/remodelling), and function (e.g. diastolic function).

The following is a primer on the vascular and cardiac surrogate biomarkers most related with this PhD project, their measurement and epidemiological importance.

It is noteworthy that there are other biological surrogate markers of CVD, and their number is on the rise with introduction of new modern imaging and bio-analytic methods. However, they fall out of the scope of this report.
C.2.1 Arterial stiffness, a large artery functional risk marker

Arterial stiffness is a general descriptive term. Generally speaking, measuring arterial stiffness is about characterizing how elastic is the artery as a tube. Arterial stiffness is indirectly measured by various indices (e.g., arterial distensibility, and pulse wave velocity) and in different scales (i.e., local, regional and systemic) described below [26, 29]. Systemic stiffness is out of the scope of this report.

C.2.1.1 Propagative model of circulation

The arterial tree in the body starts with the aorta and its major branches known as large “conduit” elastic arteries. They are capable of “cushioning” the intermittent pumping pressure of the heart, thus maintaining a steadier pressure/flow state all along the arterial tree. A “propagative model” is now widely accepted for the arterial tree and the circulatory system [29]. In this model, large conduit arteries are assumed as elastic tubes ending in high resistance (small arteries) at the periphery, and their elastic property distributed all along their length maintains a pressure wave that travels along this tree from the heart to the periphery [29].

C.2.1.2 Regional stiffness, pulse wave velocity

In a propagative model of circulation, the velocity of the travelling pulse wave (PWV) is assumed to be finite and is calculated using the Moens–Korteweg equation: \[ \text{PWV} = \sqrt{\frac{Eh}{2R \rho}} \] where \( E \) is circumferential Young’s modulus; \( h \), vessel wall thickness; \( R \), vessel radius and \( \rho \), blood density [29]. Young’s modulus is also called incremental elasticity modulus and could represent circumferential stiffness in a tube (i.e. artery) [29]. The higher is this modulus, the stiffer the artery, and thus the higher the travelling speed of the pulse wave (PWV). PWV is measured between two points along an arterial path, and therefore is a “regional” stiffness index [29].

Large conduit arteries become less elastic and more muscular as they branch toward the periphery, thus creating a “stiffness gradient”. Therefore, an increase in PWV is expected moving from proximal to peripheral end of the arterial tree [29]. For instance, PWV measured between the two points in the descending thoracic aorta is expected to
be lower than that measured between the two points at brachial and radial arteries. The aorta and its major branches are the most favourite regions for PWV measurements because of the main role they play in pressure buffering as well as the predictive value of aortic PWV for CVD events [29].

C.2.1.3 Measuring pulse wave velocity

PWV is principally measured in 2 steps: 1) determining the time interval (transit time, Δt) between the two time points at which a pulse wave crosses the two arterial points; and 2) measuring the length (L) of the path between the two arterial points. The path length is then divided by the transit time to get the velocity (\( \text{PWV} = \frac{L}{\Delta t} \)) expressed in m/s or cm/s [29].

C.2.1.3.1 Transit time measurement

The transit time of the travelling pulse wave is usually derived from the shape of the pulse waves (i.e. pulse waveform) recorded at the two points of interest along the arterial path. The rationale is detecting a similar time-point along the two waveforms as the arrival time of the pulse wave at point 1 and 2 (\( t_1 \) and \( t_2 \)) and subtracting the two time measurements to get \( \Delta t \) or transit time. Using the feet of the two waveforms is the most common method referred to as foot-to-foot method [29], and the reason is that the foot of the waveform is the least affected region by reflection waves from peripheral sites which can alter the waveform (i.e. explained later) [26]. Figure C-2 illustrates the foot-to-foot method. There are different wave foot finding algorithms; intersecting
tangents, second derivative and 10% upstroke are among commonly used ones Figure C-3 [30].

Figure C-3. Common foot finding methods
Reprinted from [30] with permission from Elsevier.

In contrast to above methods, transit time computation from a “single” pressure waveform has been recently introduced by oscillometric devices such as the Arteriograph® (TensioMed, Budapest, Hungary). The Arteriograph records the forward systolic and reflected pressure waves at the brachial artery level with a tightly wrapped arm cuff inflated above systolic pressure assuming that the major wave reflection site is almost located at the aortic bifurcation. It estimates the transit time between aortic valve and bifurcation by measuring the time interval between the (i.e. possibly the peaks of) forward and reflected waves using an undisclosed commercial algorithm [31, 32]. The Arteriograph device is described in more detail in Methods chapter (E4.1) of this thesis.

There are different methods to record the pulse waveforms needed for transit-time measurement. Such methods use the sensors detecting different arterial dynamic properties related to the pulse wave such as pressure, flow or distension [29]. This can be done non-invasively by applying a sensor over the skin, on an artery near the body surface, or by advanced imaging techniques such as Doppler ultrasound or magnetic resonance imaging (MRI). Invasive measurements (probably the gold standards) are also possible using pressure or flow sensors via intra-arterial catheterization, but are spared for validating non-invasive techniques due to considerable associated risks.
Non-invasive pressure-based methods are the most popular and use mechano-transducer sensors (e.g. Complior System®; Colson, Les Lilas, France) [33] or applanation tonometry probes (e.g. SphygmoCor®; AtCor, Sydney, Australia) [34] on the surface arteries. Recently, oscillometric techniques (Arteriograph®; TensioMed, Budapest, Hungary) [31] or a combination of volume plethysmography and oscillometry (VP-1000 Vascular Profiler; Colin & Omron Corporations, Japan) [35] have also been introduced.

Flow-based methods usually use Doppler ultrasound probes to record flow intensity/velocity at two arterial points. The examples are the proprietary method developed by Gosling and Taylor [36] and the commercial method in PulseTrace® (MicroMedical, Rochester, UK) [37], both employing continuous Doppler measurement from near-surface arteries. In contrast, 2D-guided pulse-wave Doppler as used by a typical ultrasound machine (e.g. guided by 2D ultrasound video images) has the advantage of measuring flow at precisely-defined deeper arterial sites [38, 39]. Similarly, MRI technology has been used to measure flow in cross sections of the aorta using phase-contrast MRI by velocity-encoding of the blood particles (e.g. protons) passing through an arterial cross section [40-42].

Finally, distension waveforms can be recorded with high resolution by echotracking systems [43] using radiofrequency signals [29].

The two pulse waveforms needed for transit time computation can be recorded either simultaneously (at the same time by two sensors) or sequentially (at different times with the same sensor). For instance, the Complior System® [33] uses simultaneous pressure recordings while SphygmoCor® [34] employs sequential pressure readings gated to R wave in ECG. Similarly, the Gosling-Taylor’s device records flow waves simultaneously whereas PulseTrace® [37] or most ultrasound machines [38, 39] perform sequential ECG-gated Doppler flow readings (Figure C-4). Simultaneous waveform recording methods are thought to be more accurate than the sequential (usually ECG-gated) ones which may not take into account the variation in the heart rate or in the left ventricular isovolumetric contraction time (i.e. in heart failure) [29, 32]. With MR phase-contrast imaging, ECG-gated sequential readings are usually the case though
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depending on the arterial site (e.g. arch of aorta) or the position of velocity-encoding plane, simultaneous readings may be possible.

Figure C-4. Computing transit time by Doppler flow waveforms

Two pulsed-wave Doppler traces sequentially recorded from just above aortic valve and aortic bifurcation. ECG traces below each Doppler flow waveform provide time handles. The time interval between the ECG R wave peaks and the feet of flow waveforms are measured as pulse wave arrival times, T1 and T2. The oblique lines connecting these two points are manually drawn and the software gives time interval between the two points. T1 and T2 are subtracted to yield the transit-time.

Perhaps the carotid-femoral path is the most common arterial route used for PWV measurement in clinical studies, with the common carotid artery (accessible on the neck skin) as the proximal, and the femoral artery (accessible at the groin) as the distal points to record either pressure or flow waveforms [29]. Most of the currently available longitudinal evidence from epidemiological studies also relates to carotid-femoral PWV measured by the SphygmoCor and Complior devices. However, this path also includes the partly muscular carotid and ilio-femoral paths.
More central or “purely” aortic PWV measurement is also possible by the simultaneous flow measurement at the subclavian artery and abdominal aorta by the non-commercial Gosling-Taylor’s method [36], or pulsed-wave Doppler by a typical ultrasound machine as well as by phase-contrast MRI. Such methods may be considered the most directly measured “aortic” PWV [29]. Less often, the peripheral arterial paths such as carotid-radial and femoro-tibial paths are also used for PWV though they may have less predictive value for cardiovascular risk [29].

C.2.1.3.2 Length measurement

How to measure the length of the arterial path has been a controversial arena in PWV measurement. In an invasive catheterization study, length can be directly measured by marking and measuring the length of the catheter retraction or advancement from one arterial point of interest to the other, under 2D imaging guides (e.g. X-ray) [44]. Current advanced MRI technology is also capable of offering the gold-standard for arterial length measurement even in 3D (3-dimensional) images, accounting for arterial curves and winding paths [45].

In the most widely used non-invasive methods (e.g. tonometry), the conventional approach is approximating the arterial path from the body surface measurements by a flexible tape or rigid measure. Such methods are prone to error especially in the obese subjects or those with anatomical deformities [29]. For example, controversies have hovered over the popular carotid-femoral path, and how to synthesize different surface paths to get the total length of the arterial path from carotid to femoral sites [46, 47]. Fortunately, Sugawara et al clarified this issue by comparing the lengths directly measured by 3D MRI and the surface length [45]. They showed that one of the surface methods used for carotid-femoral length estimation was more accurate and could estimate the length with 5% error of the MR measured length [45]. Such standardised approaches have been used in the recent meta-analysis to approach this controversial length issue [47].

For pure or central aortic PWV (e.g. the Arteriograph), the surface lengths from the sternal notch to symphysis pubis have been used as a surrogate estimate of total aortic length from aortic valve to bifurcation [32]. However, evidence exists, from invasive
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studies, on possible overestimation error in this method [44]. Standardized approach for central/pure aortic PWV length estimation, as reported by Sugawara et al [45] for carotid-femoral PWV, is lacking to date.

Finally, some methods as in VP-1000 Vascular Profiler (Colin & Omron Corporations, Japan) estimate the arterial path length by formulas derived from anthropometric indices such as height [35].

Different methods of surface length estimation and associated errors can be the source of inconsistencies among PWV measurement methods. Rajzer et al demonstrated that the main difference among PWV estimates by the SphygmoCor, Complior and Arteriograph is due to differences in length estimation and there is not a big difference in the transit times across the three methods [48].

C.2.1.4 Local stiffness

Compared to PWV which is a regional stiffness index, “distensibility” measures arterial stiffness at a local site (i.e. at one point in the arterial tree). It is calculated using the formula \( \frac{\Delta V}{V} \Delta P \) which is derived from the Moens–Korteweg equation. Here distensibility is expressed as change in the volume of the artery (\( \Delta V \)) normalized by the original volume (\( V \)), caused by a change in pressure (\( \Delta P \): pulse pressure) [29]. Distensibility is the inverse of stiffness and therefore the less stiff the artery, the higher is the change in its volume caused by a certain change in blood pressure (and the higher its distensibility); see Figure C-5. In many instances the volume is represented by the diameter of the artery (\( d \)), and thus: Distensibility=\( \frac{\Delta d}{d} \Delta P \). Similar formulas with cross sectional area \( \Delta A/A. \Delta P \) are used especially in MRI studies.

The numerator of the distensibility formulas above (\( \Delta A/A \), or \( \Delta d/d \)) is sometimes referred to as the arterial wall strain. The strain, unlike distensibility, is not adjusted for the pulse pressure (\( \Delta P \)).
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C.2.1.5 Measuring local stiffness

Noninvasive measurement of distensibility can be performed by echotracking systems, or ultrasound and MRI imaging. The principle is measuring the end diastolic (i.e. minimum) diameter (d) of the artery as well as its change (Δd) with pressure change during pulse strokes. The diameter change continuously plotted with time forms a distension waveform. The change in pressure (ΔP) during each stroke is usually extracted from central pressure measurements by applanation tonometry devices (e.g. SphygmoCor).

Ultrasound [49] and MRI [50] are used to capture videos of a cross-section of an artery (e.g. the aorta) to measure the change in arterial diameter during pulse strokes. With MRI and proper image analysis software, arterial volume (e.g. considering each MR image slice has a thickness) or area changes can also be used instead of the diameter changes to calculate distensibility as described before [50]. Compared to ultrasound, echotracking systems [43] like WallTrack® (Pie Medical, Maastricht, The Netherlands) use radiofrequency signals to record the distension waveforms and achieve significantly higher precision in diameter measurements. They can also provide simultaneous wall thickness data (intima-media thickness) and therefore can compute Young’s elastic modulus [29]. Echotracking systems are currently believed to be the optimal method for local arterial stiffness measurement [29].
**C.2.1.6 Reflection waves and central pressures, the concept**

In the propagative model of the arterial tree, the pressure pulse waves created by left ventricular pumping and maintained by the elastic property of the large conduit arteries travel from the aorta toward the periphery. As such pulse waves arrive at high resistance points at the periphery or at arterial branching points, wave reflections occur producing “retrograde” pulse waves travelling backward along the arterial tree and towards the central aorta and the heart. These reflection waves are believed to “augment” the blood pressure in more proximal locations of the arterial tree [29]. Figure C-6 shows the concept of reflection waves.

![Figure C-6. Pressure waveform and reflection waves concept](image)

Schematic illustration of a typical pressure pulse wave as it propagates along the arterial tree. The pulse wave form is the summation of both forward and reflected waves. Note the difference in the shape of the pulse wave and differences between systolic and diastolic pressures (i.e. pulse pressure) in a central (ascending aorta) and a more distal point (femoral artery). The reflection sites are usually the branching points and resistance arterioles in the periphery. More augmentation of systolic pressure occurs at the peripheral sites causing higher systolic and pulse pressures (compare the two pulse waves in the figure). Reprinted from [51] under reprint rules of The American Physiological Society.

If such reflections arrive in central arteries during diastole, they can help maintain diastolic blood pressure (DBP) during cardiac cycle, thus helping organ (e.g. coronary) perfusion during diastole [29]. However, changes in arterial structure and function can result in wave reflections occurring too early which could augment central systolic blood pressure (cSBP) and fail to boost DBP, hence widening central pulse pressure (cPP) [29]. Constriction in arterial and arteriolar structures, and anatomical variations (e.g. shorter height) which bring reflections points closer to the heart, are among the factors capable of producing too early or excess wave reflection, respectively. When
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PWV increases (i.e. due to stiffer conduit arteries), the faster pulse waves reach the peripheral reflections sites earlier, causing premature wave reflections [29]. Reflection waves can alter the systolic and diastolic pressures to different extents in central and peripheral arteries. Therefore, the blood pressure (BP) typically and traditionally measured in peripheral sites (e.g. the brachial artery) may differ from central pressures. The hypothesis is that the organs such as the heart and kidneys which are the typical targets of damage in hypertension are actually exposed to the pressure levels in the central arteries (i.e. aorta) rather than that in the peripheral sites [29].

Pulse pressure may be *amplified* in peripheral arteries where the reflection sites are closer and PWV is higher than central arteries. Therefore, the pulse pressure measured at the periphery (e.g. brachial artery) could be higher than that measured centrally. This is especially true in younger subjects, whose central arteries are less stiff than the peripheral ones, creating higher contrast in vascular resistance at the interface of central-peripheral arteries. This phenomenon is called *amplification* [29, 52].

**C.2.1.7 Measuring reflection waves and central pressures**

Pulse wave analysis (PWA) is used to measure reflection waves and estimate central pressures from a *pressure* waveform [29]. While pressure waveforms can be invasively recorded with intra-arterial catheters, non-invasive measurements on superficial arteries are the routine in research/clinical settings. Applanation tonometry is the most popular non-invasive PWA method usually recorded on the carotid or radial artery. SphygmoCor® (AtCor Medical, Sydney, Australia) [34], PulsePen [53] and Omron HEM9000 (Omron, Japan) [54] are examples of tonometric commercial devices.

More recently, oscillometric methods implemented in the Arteriograph (TensioMed, Budapest, Hungry) [31, 32] and PulseCor R6.5B Vascular Monitor devices (Pulsecor Limited, Auckland, New Zealand) [55] also offer similar indices of measurement, more conveniently. These use a blood pressure arm cuff at supra-systolic levels to detect the pressure waveforms at the brachial artery.

Two important indices for quantifying reflection waves are *augmentation index* (AIx) and *augmentation pressure*, derived from pressure waveform. The difference between
the first and second shoulders on the pressure waveform ($P_1$ and $P_2$) is the *augmentation pressure* (Figure C-7).

AIx can be calculated by two different formulas, usually causing confusion when comparing AIx from different devices. The typical formula is dividing the augmentation pressure by pulse pressure as $(P_2 - P_1) / PP$ [29]. This is typically used by the Arteriograph device for brachial and aortic AIx, and by the SphygmoCor for aortic or central AIx. The second formula to calculate AIx is $(P_2 - DBP)/(P_1 - DBP)$. For instance, the SphygmoCor and Omron HEM-9000 both report their radial AIx using this formula. Using the former formula usually results in negative values for peripheral AIx, and mostly positive values for central AIx although the latter is not the rule in the younger people.

Figure C-7. Pressure waveforms by applanation tonometry
Schematic waveforms recorded at a) a peripheral site (e.g. radial artery) and b) a central site (e.g. aorta). $P_1$: the first pressure peak, $P_2$: the second pressure peak (i.e. the reflected wave), AP: augmentation pressure.

To get the absolute pressure values on a pressure waveform, the waveforms need to be calibrated usually using maximum (SBP) and minimum (DBP) pressures measured by a standard method such as the conventional non-invasive manometric or oscillometric BP measurement or, as in invasive studies, by intra-arterial manometers. If such BP measurement methods are prone to error, then the BP values on the pressure waveform may also be wrongly estimated [56, 57]. The now ubiquitous semi-automatic oscillometric BP monitors have been shown to under-estimate SBP and over-estimate DBP [58, 59].

For estimation of central pressures, the pressure waveforms recorded in the carotid artery [60] are believed to agree closely with those in the aorta [29]. Nevertheless, for
radial or other peripheral waveforms, some mathematical processing or algorithms are used to estimate central pressures. The popular SphygmoCor tonometry uses a mathematical inverse generalized transfer function (GTF) to “reconstruct” (i.e. guess) the central pressure waveforms from the radial one [29, 56]. The GTF has been validated in several invasive studies calibrating the radial waveform by invasively-measured aortic MAP (mean arterial pressure) and DBP assuming that MAP remains constant in central and peripheral arteries [56, 61-64]. However, there is still controversy over accuracy of the typical “outpatient” SphygmoCor measurements where the radial waveform is calibrated by non-invasively measured brachial pressures (i.e. themselves prone to error) [65, 66]. Another source of error in radial tonometry is the use of brachial BP (as a surrogate for the radial BP) for calibrating the radial waveforms. However, radial artery tonometry is very popular due to its convenience, and being safer compared with the carotid tonometry which might be difficult to do in the obese or considered unsafe in the elderly with carotid plaques [29].

In contrast with the SphygmoCor, other devices such as the Arteriograph [31] and Omron HEM9000 [54, 67] use regression equations derived from invasive studies correlating central SBP and the late systolic shoulder, or SBP\textsuperscript{2} (e.g. the same as P\textsubscript{2} in Figure C-7) on the peripheral pressures mixed with some undisclosed computations within their commercial algorithms. In addition, the Arteriograph measures BPs at the same point of waveform recording (e.g. brachial artery), which is said to allow the calibration to be integrated with the recorded waveform.

**C.2.1.8 Systematic error/covariates in arterial stiffness measurements**

In measuring arterial stiffness, some practical precautions should be considered to reduce systematic variation and the influence of confounders [68]. Avoiding smoking, heavy meals, and caffeine-containing beverages 3 hours before, and alcohol 10 hours before the measurement have been recommended. Conducting the measurements in a temperature controlled room after enough rest and in supine position, with the subject still avoiding speaking or sleeping would also help reduce confounding effects [29, 68].
When arterial stiffness, in particular PWV, is used as an outcome of interest (e.g. intermediate risk marker) in a research study, controlling for important covariates is indispensible. Age and blood pressure are the two most important covariates needing adjustment, as confirmed by a large meta-analysis pooling individual-level PWV data from the European population [47], and a systematic review of cross-sectional studies [69]. Gender and other traditional CVD risk factors are known to relate to PWV. However, the association of gender, smoking and dyslipidemia with PWV has been found inconsistent [69]. The large European meta-analysis also showed that after controlling for age and blood pressure such associations are substantially reduced [47]. Diabetes may also be an important covariate though a systematic review by Cecelja and Chowienczyk showed inconsistency in such association in many cross-sectional studies [69].

There is controversy and inconsistent data on the effect of heart rate on PWV [70-73]. It has been shown that the effect of heart rate as a covariate is 1/10th of age and 1/4th of mean arterial pressure [47]. Similarly, the heart rate-PWV relationship may be confounded by the influence of heart rate on mean arterial pressure [70].

**C.2.1.9 Pathophysiology of arterial stiffness**

*What causes aortic and large artery stiffening by age and CVD risk factors?*

Several pathophysiological mechanisms altering the arterial walls have been implicated including elastin fibre breakdown, inflammation, fibrosis and collagen accumulation, calcification, and necrosis of smooth muscle cells in the media [29]. Among these, more importance has been attached to the changes in quantity as well as spatial array of collagen and elastin as the two important structural proteins in the vascular wall “scaffolding” [29]. Here, the role of endocrine factors such as aldosterone and angiotensin II known to augment the inflammation and fibrosis in cardiovascular system could be important [74-76], as recently highlighted in a cross-sectional study of Framingham’s population [77].
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*How can arterial stiffness contribute to CVD?*

There is a “mechanistic” consensus (Figure C-8) as declared by Laurent et al [29]. When large arteries become stiffer with *wear and tear* of age (presumably related to continuous stretch and relaxation cycles with each cardiac cycle), or under influence of other CVD risk factors, the pulse wave travels faster and there is an earlier return of the reflected waves from peripheral sites. Such early reflections arrive in late systole resulting in a higher central SBP, a lower central DBP (i.e. failure of diastolic augmentation), and thus a wider pulse pressure (PP) [29, 78, 79]. The higher SBP is an extra load on left ventricle which may lead to LVH, a well known CHD risk factor. A wide central PP along with a low DBP could also cause subendocardial ischemia [29, 79]. Exposure of the low-pressure cerebral circulation to this wide PP and the excessive pressure fluctuations between systole and diastole may increase the risk of cerebrovascular accidents [29, 78]. Finally, it has been suggested that the functional alterations in large arterial walls represented by arterial stiffness could “parallel” similar changes in coronary and cerebral circulations [29].

*Figure C-8. Arterial stiffness role in cardiovascular disease*

**C.2.1.10 Epidemiological importance & risk prediction**

Apart from a number of cross-sectional studies alluding to arterial stiffness being a “risk marker” of CVD, there is compelling evidence from longitudinal studies that arterial stiffness, in particular PWV, is an “intermediate end-point” of CVD [29, 80].

Among different non-invasive aortic stiffness measures, aortic PWV (i.e. measured *along the aortic path*) and in particular carotid-femoral PWV, have been shown to have the most reliable and independent predictive value [29] for CVD mortality among the general (mainly normal) population [81-83], hypertensive [84] and end stage renal disease (ESRD) patients [85], as well as with all-cause mortality in people with diabetes and across the spectrum of glucose tolerance [86]. This predictive value persists after adjusting for conventional CVD risk factors. For example, a recent meta-analysis of longitudinal studies by Vlachopoulos et al showed that one standard deviation (SD) increase in aortic PWV predicts a 47% rise in the risk of cardiovascular events and mortality, and a 42% increase of all-cause mortality risk adjusted for age- sex and conventional CVD risk factors [80]. This suggests arterial stiffness as an end-point/outcome “translating” multiple CV risk factors, possibly including those yet unmeasured or undiscovered, in an individualised manner for each patient [29].

While *carotid-femoral PWV* is viewed as the “gold standard” measure of arterial stiffness because of the current bulk of epidemiological evidence, data from PWV measured in more peripheral arterial regions such as brachial or tibio-femoral paths have not shown consistent predictive value [29].

Compared to regional arterial stiffness indices which show high predictive values for CVD, *local* stiffness indices (e.g. carotid distensibility) are believed to be more appropriate for “mechanistic analyses” in pharmacological and pathophysiological investigations rather than epidemiologic studies [29]. However, there is already some evidence from longitudinal studies alluding to their predictive value for CV events in ESRD patients [87]. There is also evidence showing that carotid stiffness does not parallel aortic stiffness in high risk patients (e.g. hypertension and diabetes) where aortic stiffness exceeded carotid stiffness with age [29].
Central pressures and reflection wave indices (e.g. AIx) have also been shown to have predictive value for CV events [29, 88] among hypertensive subjects [89] and all-cause mortality in ESRD patients [90].

While PWV, AIx and central pressures are all indices of arterial stiffness, they should not be used interchangeably [29]. PWV is a direct measure of arterial stiffness reflecting wall stiffness. However, AIx and central pressures are indirect measures of arterial stiffness which are themselves partly dependent on PWV, heart rate, peripheral resistance, and ventricular contractility [29]. In fact, some pharmacological agents can change AIx and central pressures without changing arterial wall stiffness and PWV [29]. In addition, age shows a better correlation with PWV in those older than 50 years and with AIx in younger subjects [91].

Defining normal and reference values of PWV is complicated by diversity in the devices and algorithms used for transit time as well as different lengths and regions used. Recently, “Reference Values for Arterial Stiffness’ Collaboration” produced a large meta-analysis of pooled European population data (Figure C-9) to define such values for carotid-femoral PWV after standardising the methods used for transit time.
and length measurement across different devices [47]. This is of utmost importance for practical use of arterial stiffness as a tool aiding in clinical risk stratification and decision making.

Another major study by Khoshdel et al included 25 studies measuring carotid-femoral PWV using only the Complior® device in non-pregnant Caucasian adults of low CVD risk, and extracted age-specific reference PWV values. It is noteworthy that the PWVs measured by Complior® are known to be rather higher than that measured by SphygmoCor® device [32, 92] though PWV from these two commonly used devices correlate very well [32]. Similar efforts for defining reference or normal values for reflection wave indices and central pressures have been lacking.
C.2.2 IMT, a large artery structural risk marker

Compared to arterial stiffness indices which more or less quantify a functional property of the large arteries, there are those markers which mainly describe structural changes. Some of such structural changes such as intima-media thickness (IMT) or plaque characteristics have been demonstrated to relate to future CVD risk.

C.2.2.1 Definition

Intima-media thickness is the total thickness of the media and intima layers of the wall of the artery measured by B-mode ultrasound. Ultrasound cannot yet discriminate between the media and intima, and an increased IMT could be due to the thickening of the intima, media or both [93].

C.2.2.2 Measurement

IMT may be measured in several accessible surface arteries. Current consensus recommends IMT measurements in the far wall (i.e. better visualized) of a plaque-free region of the distal region of the common carotid arteries (CCA) [94, 95]. Such measurements are more reproducible and show better correlation with CVD events [93-97] (Figure C-10).

Figure C-10. Sample ultrasound images of the common carotid artery
Left: Important image landmarks, and intima/media layers; blood appears black. Right: a typical plaque area, note media thickening and how thicker and bulged it looks from surrounding IMT; the image is more speckled due to specific ultrasound mode of imaging used for plaque visualization.
Properly calibrated edge-detection software can significantly improve analysis speed obviating the time consuming manual or semi-automatic analyses and the related meticulous quality control [95]. It is also recommended that IMT along the entire measurement length be averaged to get a mean IMT which is not as vulnerable as the maximal IMT (i.e. maximum IMT along a measured segment) to outliers [95]. Averaging the IMT from the left and right CCA is also an accepted approach [95].

Carotid IMT (CIMT) values vary by arterial diameter change during cardiac cycle and therefore, averaging measurements from different cardiac cycles as well as from maximal and minimal arterial diameters can increase reproducibility [98]. Off-line analysis of digitally stored video images preferably by a single observer is recommended [98, 99].

C.2.2.3 Epidemiological importance

It has been shown that CIMT is not only correlated with previous exposure to CVD risk factors and current CHD, but it can also predict future CHD-related clinical end-points in healthy individuals [25, 93]. CIMT is an index of atherosclerosis progression in large arteries as well as an independent predictor of stroke [100]. Age, male gender and black race have been associated with higher CIMT [93]. CIMT regression has been observed with lipid modifying drugs (e.g. statins) [93].

Mean IMT of CCA may underestimate the burden of atherosclerosis as atherosclerosis develops in a heterogeneous manner progressing faster in ICA and the bulb compared with CCA, the typical recommended measurement site [97]. It is now recommended that extra-cranial carotid arteries should be explored for the presence of atherosclerotic plaques [94, 97]. In fact, the presence of carotid plaques is associated with increased CVD risk disregarding the extent of CIMT [97].

Evidence suggests that IMT and plaque may be related with different CVD outcomes. Carotid plaque shows a closer relationship with hyperlipidemia and coronary atherosclerosis than CIMT which shows more consistent and slightly closer relation with stroke than CHD [101, 102]. CIMT is measured as a continuous marker whereas
plaque indices (e.g. presence, morphology, echo texture) are mostly qualitative [97, 101].

As CIMT depends on age, gender, blood pressures and ethnicity, such covariates should be considered when defining “normal” values for a population. The American Society of Echocardiography consensus suggested CIMT > 75th percentile of a reference population or over 1 mm is associated with increased CVD risk, while CIMT below 25th percentile is associated with lower risk [94]. Such cut-off values may be useful for clinical practice, but are not error-proof. CIMT association with CVD risk is known to be continuous [97, 98].
C.2.3 Cardiac structural and functional risk markers

Similar to the vascular surrogate markers reviewed above, some “cardiac” surrogate markers for CVD have also been investigated. Most are related to the structure/function of left ventricle. Structural indices of LV mass (LVM) and remodelling (i.e. geometry) are among the most studied.

Functional indices, in particular those relating LV diastolic function, also have independent predictive value for CVD outcomes (e.g. heart failure). Studying cardiac (ventricular) stiffness along with arterial stiffness markers was of interest in this project. Therefore, the markers relating myocardial relaxation and diastolic function (i.e. compared with systolic function) received more focus.

C.2.3.1 LV structure indices: mass & geometry

C.2.3.1.1 Concept and pathophysiology

An increase in LV mass, LV hypertrophy (LVH), is a common adaptive response of the myocardium to excessive overload such as hypertension. Apart from blood pressure per se (especially 24-hr monitored BP), other important determinants of LVM include age, gender, ethnicity, genetics, myocardial inotropy, volume overload, anaemia, obesity (i.e. via volume overload), diabetes (possibly via obesity [103]), arterial stiffness, and neurohormonal factors [104, 105]. Valvular heart disease such as aortic stenosis and mitral or aortic regurgitation can also cause LVH by mechanical or volume overload [106].

Physiologic LVH occurs in response to “intermittent” overload of physical training in athletes and differs from the pathologic LVH where the myocardium is exposed to “continuous” overload (e.g. typically due to hypertension) [104]. Pathologic LVH involves not only myocyte hypertrophy (as in physiologic LVH), but also some degrees of interstitial/perivascular fibrosis [104, 107]. Interstitial fibrosis itself is the result of excessive production of extracellular matrix especially type I and III collagen [107, 108]. This is both a “primary” fibrosis and a “secondary reparative” fibrotic reaction to myocyte necrosis [107].
Though excessive "mechanical load" due to hypertension could induce LV fibrosis [109], neurohormonal factors, especially the components of RAAS: angiotensin II and aldosterone are thought to have a central role in this phenomenon [105]. Evidence shows that angiotensin II might not only stimulate myocyte and smooth muscle cell hypertrophy/hyperplasia but also induce perivascular/interstitial fibrosis via TGF-β1 (transforming growth factor beta one) and interfere with collagen degradation by altering metalloproteinase activity [105]. Aldosterone is also believed to contribute to a fibrotic process via oxidative and inflammatory stress [110]. Interestingly, in addition to systemic RAAS, the local RAAS within cardiovascular tissue is currently implicated as a contributor to this fibro-inflammatory process. It is suggested that aldosterone enhances local angiotensin-converting enzyme (ACE) in cardiovascular tissue, thus augmenting fibro-inflammatory actions of angiotensin II [110]. Leptin deficiency is another neurohormonal factor implicated in LVH in experimental models [105, 111].

It is suggested that myocardial stiffening due to fibrosis and remodelling could occur parallel to vascular stiffening [112]. This is at least partly explained by neurohormonal effects such as systemic or local RAAS hyperactivity. Analogous to arterial stiffness, the interstitial fibrosis and remodelling may cause ventricular stiffness with abnormal diastolic filling and relaxation (i.e. diastolic dysfunction) [105, 113]. In addition, myocardial tissue remodelling and perivascular fibrosis in LVH may cause conduction disturbances, arrhythmias, and systolic dysfunction, and predispose to myocardial ischemia by increasing myocardial oxygen demand and interfering with coronary artery vasodilation [105, 114].
Four types of LV geometry have been described with different associated predictive values for CVD events. This is based on the relative thickness of the LV posterior and/or septal walls to its interior dimensions (e.g. chamber size), and whether LVH exists or not. Ganau et al found that 52% of hypertensive patients had normal LV, 27% eccentric hypertrophy (LVH and normal wall thickness), 13% concentric remodelling (relatively thickened LV walls, but no LVH) and 8% concentric hypertrophy (relatively thickened LV walls along with LVH) [115] (see Figure C-11).

These geometric patterns have been associated with different hemodynamic situations. Hypertensive patients with eccentric hypertrophy tend to have a more spherical shape ventricle with high stroke volume, high cardiac output and low peripheral resistance paralleled with low plasma rennin activity. In contrast, those with concentric geometry (remodelling and/or hypertrophy) have a more elliptical shape ventricle, normal stroke volumes, and high peripheral resistance associated with high plasma rennin and RAAS activity [116].

Though the above geometry classification was first developed from the perspective of hypertension [115], cardiac remodelling may well occur unrelated to hypertension, and due to a wide variety of conditions including valvular heart disease (e.g. aortic stenosis) imposing pressure/volume overloads, high cardiac flow states (i.e. anemia), cardiomyopathies (i.e. some due to genetic etiology), coronary heart disease (e.g. myocardial infarction), metabolic and storage diseases, obesity and so on which fall out of the scope of this report.
C.2.3.1.2 Measurement of LV mass & geometry

Echocardiography and magnetic resonance imaging (MRI) are among popular methods for quantifying LVM. Most imaging methods actually measure the “shell volume” of the left ventricle and compute mass by multiplying this volume by myocardial density [117]. So the principles of LVM measurement are mostly based on volumetry.

Cardiac MRI is the most accurate, but also the most expensive method with relatively low availability [118]. Detecting LVH “qualitatively” is possible via electrocardiography (ECG) which is the least costly, most available and rather specific method for LVH detection, but its main drawback is low sensitivity [118, 119]. Echocardiography (i.e. M-mode and 2D) falls in between ECG and MRI in terms of cost, sensitivity, and availability, but still misses the mildest cases of LV hypertrophy. Considering cost, simplicity, quickness, sensitivity and availability altogether, echocardiography is currently the most widely used method for measurements of LVM [118].

Other imaging modalities for LVM measurement exist which are not the focus of this report. Cardiovascular computed tomography (CCT) has emerged as an accurate method for LVM measurement. Its accuracy is comparable to MRI, and has the advantage of showing detailed coronary artery anatomies simultaneously and has been especially helpful in heart failure patients [120]. However, as with any X-ray technology, there is some extent of radiation dose unlike MRI. Single photon emission computed tomography (SPECT) is another LVM measurement method using radio-isotopes which is less popular due to radiation, high costs and shortcomings on serial studies [120].

C.2.3.1.2.1 Echocardiography

M-mode echocardiography assumes that the LV has a “prolate ellipsoid shape” with uniform wall thickness (as in Figure C-11) and a ratio of 2:1 for long to short axis lengths [117, 118]. One-dimensional measurements of LVIDd (left ventricle internal dimension in end-diastole), PWTd (posterior wall thickness in end-diastole) and IVSTd (interventricular septum thickness in end-diastole) are made (Figure C-12) and LVM is
calculated using Devereux’s formula [121, 122] guided by American Society of Echocardiography conventions [123]:

\[
LVM = 0.8(1.04[(LVIDd + PWTd + IVSTd)^3 - (LVIDd)^3]) + 0.6 \text{ g}
\]

Figure C-12. Sample M-Mode and 2D echocardiograms
A sample of M-Mode (left) and 2D (right) echocardiogram from para-sternal long axis view of a sample patient in this project. Linear measurements marked on the image are used to compute LV mass and define its geometry. IVST: interventricular septum thickness, LVID: LV internal dimension, PWT: LV posterior wall thickness LA: left atrium.

Apart from M-Mode, 2D images can also be used for the same one-dimensional LV measurements (Figure C-12) for input into Devereux’s formula. M-Mode images are of higher temporal resolution compared to 2D. However, overestimation of LV dimensions may well occur with non-perpendicular (to LV long axis) ultrasound beam applied to the septum and posterior wall. Such variability depends on the patient’s anatomy and observer’s image acquisition skills. In comparison, 2D images may be less prone to such over-estimations [117].

The accuracy and reproducibility of M-mode echocardiography is subject to errors imposed by oversimplified geometrical assumption of LV shape including uniformity of LV wall thickness and observer variability in both image acquisition and image analysis phases [117, 118].
Recently, advances in 3D echocardiography which is not based on geometric shape assumptions have opened a new horizon in echocardiographic LVM measurements. Accuracy and reproducibility levels of 3D echo are approaching that of cardiac MRI [118]. However, the technology is not widely available yet and also fails to get acceptable images in the patients with “poor acoustic windows” [118].

As stated above, four types of LV geometry or remodelling, as defined by Ganau et al, are based on increased/normal values of 1) LVM and 2) relative LV wall thickness to chamber size (Figure C-11). Echocardiography can be used to classify such structural changes. LVM can be quantified by echocardiography as described above, and LVH may be defined using normal cut-off values. To quantify concentricity, relative wall thickness (RWT) can be measured by the same end-diastolic M-mode or 2D linear dimensions needed for LVM measurement (Figure C-12). RWT is then calculated using either following formulae:

\[
RWT = \frac{IVSTd + PWTd}{LVIDd} \quad \text{or} \quad RWT = \frac{2 \times PWTd}{LVIDd}
\]

The concept is whether LV wall thickness (quantified by sum of interventricular septum and posterior wall thicknesses) is relatively high for LV internal dimension as illustrated in Figure C-11. The four aforementioned LV geometries are defined combining the practical cut-off values for an index of concentricity (RWT here) and LVMI [115, 117]. For instance, RWT>0.42 by echocardiography is defined as high, representing concentric geometry. Combined with a normal range LVMI (e.g. ≤115 g/m² for adult males) this is defined as concentric remodelling, and if accompanied by a high LVMI (e.g. LVH), concentric hypertrophy.

LV volume measurement is also among routine measurements in a typical echocardiogram. Simpson’s method of summation of discs is among the most popular ones to quantify LV volumes. The LV endocardial borders are defined by the observer sometimes assisted by the edge detection algorithms depending on the image analysis software. The AP4 (apical 4 chamber) view is usually used, and the result is a closed polygon delineating LV endocardial limits. The software then segments this polygon and uses each segment to construct a disc, and sums up the disc volumes to get the total
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volume (Figure C-13). AP2 (apical 2 chamber) view can also be used for this purpose, but is usually more difficult to obtain. If both AP4 and AP2 views are used, then the method is called 2-plane Simpson’s method. If a single plane is used then it is single plane Simpson’s method. Two-plane method gives a better estimation of each disc shape and volume. Usually both end-diastolic and end-systolic volumes (LVEDV and LVESV) are computed which can then be used to obtain ejection fraction by the following formula: EF = (LVEDV – LVESV) / LVEDV. Ejection fraction is an index of systolic function. Simpson’s method can also be used for atrial volumes.

Figure C-13. Simpson’s method, LV volumetry
C.2.3.1.2.2 MRI

Compared to echocardiography, cardiac MRI provides a “3D spatial dataset” of the heart, is not based on geometric assumptions, and offers much higher resolution and contrast of the cardiac anatomy [118]. Cardiac MRI offers the advantage of measuring the LV mass with least variability, and much lower standard deviations compared to M-mode and 2D echocardiography. Therefore it dramatically shrinks the sample size in clinical research compared to echocardiography, and is the recommended method for research projects with small sample sizes [118].

Figure C-14. LV mass measurement by MRI
A series of short-axis heart images in end-diastole acquired by SSFP (steady-state free precession) MRI sequence from a participant of this study. The series is ordered from LV base to the apex from top-left to bottom-right. The endocardial and epicardial contours have been automatically drawn using Segment software (http://segment.heiberg.se) [124]. The contours are for general illustration purpose only and may not be precise. Papillary muscles have not been segmented here.

A series of short-axis cine images of the heart (Figure C-14) are acquired typically with breath holds and ECG-gating. Breath-holds compensate for movement of the heart and mediastinum with breathing. ECG-gating makes up for intra-cardiac movements during each cardiac cycle [118]. End diastolic frames of each slice series are selected and the contours of endocardium and epicardium are drawn either manually [125] or semi-automatically. Papillary muscles are usually excluded in this process to be appended to the final mass later [125]. LV epicardial and endocardial volumes are first computed and then LVM is calculated using myocardial density and the “shell volume” which is here the difference between epicardial and endocardial volumes [125].
Two types of MR pulse sequences are used for LV mass measurement: steady-state free precession (SSFP; white blood) and segmented k-space turbo gradient echo (TGE; dark blood) [118]. LVM reference values for these two techniques are slightly different and should be considered in practice and research [118] (see Table C-2).

Table C-2. LVM upper limits by MRI and echo
Upper limit normal values for left ventricular mass indexed to body surface area (LVMI) with echocardiography and the two popular MRI sequences: SSFP (steady-state free precession) and TGE (turbo gradient echo); adapted from [118].

<table>
<thead>
<tr>
<th>Method</th>
<th>Upper limit LVMI (g/m²)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
</tr>
<tr>
<td>M-mode</td>
<td>125</td>
</tr>
<tr>
<td>2D echo</td>
<td>102</td>
</tr>
<tr>
<td>Cardiac MRI</td>
<td></td>
</tr>
<tr>
<td>TGE</td>
<td>96</td>
</tr>
<tr>
<td>SSFP</td>
<td>83</td>
</tr>
</tbody>
</table>

Compared with the traditional echocardiographic classification of LV geometry, using MRI to quantify/classify LV geometry is an area where development has recently gained momentum. This may be due to the recent rise in the availability of this expensive and complicated method, the larger population datasets just beginning to emerge [126], and better computer platforms to tackle MR image analysis.

In contrast to M-mode echocardiography where “linear” measurements of LV wall thickness and dimensions are used to “indirectly” estimate LV volume in an oversimplified shape assumption, MRI gives a more direct and realistic approach to 3D rendering of LV volume models, enabling far more accurate mass and volume estimations. Therefore, LV end-diastolic mass to volume ratio (M/V ratio) has been used as a more sophisticated index of concentricity in MRI [126, 127] compared with its echocardiographic counterpart: RWT.

Recently, Khouri et al have suggested a new “4-tier classification” of LVH based on geometry from the MR perspective [127]; see Table C-3. They showed that a modified version of the M/V ratio expressed as $M/V^{0.67}$ or $concentricity^{0.67}$ may reflect LV
Background

Concentricity more realistically than M/V ratio. They defined four classes geometry for a hypertrophied ventricle based on increased/normal values of 3 MRI indices of mass (LVM indexed to height$^{2.7}$), concentricity (concentricity$^{0.67}$) and volume (LVEDVI: end-diastolic volume indexed to body surface area). Increased values are defined as $>97.5^{th}$ sex-specific percentile extracted from normal population data.

In the classical echocardiographic categories suggested by Ganau et al [115], a concentricity index (RWT) is combined with LVMI. However, in this new classification LV chamber size plays an independent role along with the former two indices. The new system is focusing on LVH cases; and there is actually a fifth category of those with normal LVMI but high concentricity, analogous to “concentric remodelling” in the traditional classification [128]. The new classification implies that the LV remodelling may occur due to independent modelling of LV wall thickness and chamber size, rather than their interaction (as reflected in the composite index RWT) [128].

Table C-3. New LV geometry classification by MRI indices
Adapted from Khoury et al [127] and [128].

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Concentric remodelling</th>
<th>Eccentric LVH</th>
<th>Concentric LVH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Indeterminate</td>
<td>Dilated</td>
</tr>
<tr>
<td>↑ LVMI$^*$</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>↑ Concentricity$^{0.67}$</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>↑ LV EDVI$^+$</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

$^*$ indexed to height$^{2.7}$

$^+$ indexed to body surface area
C.2.3.1.3 Epidemiological importance

LV mass is positively associated with future CHD, congestive heart failure and stroke [119]. Echocardiographic LV mass (indexed to height) was shown to predict CVD fatal and non-fatal end-points in a 4 year follow-up period independent of hypertension and other conventional CVD risk factors among 3220 subjects older than 40 years and “free of apparent clinical CVD” in Framingham Heart Study [129]. Each 50 g/m increase in men’s LV mass (indexed for height) was associated with a relative CVD risk of 1.49, already adjusted for other CV risk factors [129]. A longitudinal study following-up uncomplicated hypertensives for 3 years revealed an independent 40% increase in adverse CVD events, for a 39 g/m² rise in echocardiographic LVM (indexed to body surface area) [130].

LVH may regress with good control of blood pressure in hypertensive adults and this regression has been associated with a significant decrease in future CVD clinical endpoints independent of the extent of drop in blood pressure [119, 131].

Schillaci et al showed that the nature of LV mass relation with CVD risk and its predictive power is continuous with no particular definable cut-off point; the relation persists even with LVM values below the so called “upper limits” [132].

Apart from LV mass, its geometrical variations (e.g. remodelling) has prognostic value for CVD. Longitudinal echocardiography studies have revealed a higher risk associated with concentric LV geometry (i.e. concentric hypertrophy and/or remodelling) than eccentric hypertrophy or normal geometry [114, 133-136]. However, other echocardiography studies have questioned the independent prognostic value of concentric geometry beyond LV mass in predicting CV adverse events [137, 138].

Recently, the first large population-based cohort study (MESA: Multi-Ethnic Study of Atherosclerosis) using cardiac MR to predict incident CVD events by Bluemke et al [126] showed that mass-to-volume ratio, an index of concentric geometry, more closely predicted CHD (hazard ratio (HR): 2.1) and stroke (HR: 4.2) than LV mass (HR: 1.0 for CHD and 1.2 for stroke). In contrast, LV mass more closely predicted heart failure
Background

Surrogate biomarkers of CVD

events [126]. Bluemke et al suggested that inconsistencies previously seen with the value of concentric geometry predicting CV events beyond LV mass may be due to the collective approach by previous studies combining different types of the CV events as outcome [126].

LVH is not only considered as a “marker” of future CVD events, but is also believed to “mediate” the events by compromising diastolic coronary blood supply and inducing cardiac arrhythmias attributable to myocardial structural disarray in LVH [114].

Apart from blood pressure; other factors such as age, gender, ethnicity and obesity have also been shown as determinants of LVH [119]. Echocardiographic LVH was found to be more common among older subjects and in men [119]. Comparing whites and African-American blacks has revealed a higher odds ratio of LVH among blacks even after adjusting for age, weight and systolic blood pressure [119]. A population study by Drazner et al showed that LV mass measured by MRI was higher among black men and women compared to whites even after adjusting for fat-free body mass [139]. Finally, arterial stiffness measured by PWV has been shown to be associated with LVH independent of blood pressure [114, 140].

Considering body size variation, LVM itself can not be directly compared between individuals. LVM should be indexed to a body size index (e.g. body surface area, or height) [118]. The type of LVM indexing method may influence cut-off values for LVH definition and therefore LVH prevalence among patient sub-populations varying in demographic (e.g. ethnicity and sex) or anthropometric characteristics (e.g. obesity) [119, 141]. Indexation of LVM to body surface area (BSA) is widely used in literature. Some authors suggest that indexing to height$^2$ may provide more accurate estimation for LV hypertrophy and cardiovascular risk [118, 142].
**C.2.3.2 LV (diastolic) function indices**

While conventional indices of LV systolic dysfunction such as a low ejection fraction (EF<50%) are usually associated with “symptomatic” clinical syndromes (e.g. heart failure), the indices of diastolic dysfunction may be considered as earlier harbingers of myocardial disease at subclinical stages [112, 143]. Interestingly, current evidence suggests that ventricular and vascular stiffness are parallel processes occurring with age [112, 144, 145]. Studies on patients with diastolic heart failure suggests higher myocardial collagen content and turnover [146, 147], and a decrease in phosphorylation of sarcomeric proteins [146].

Four phases have been described for LV diastolic relaxation: 1) **isovolumic relaxation** time (IVRT) is the time between closure of the aortic valve and opening of the mitral valve, 2) **early rapid filling phase** (RFP), when the mitral valve is open and blood is sucked into the LV by the pressure gradient across mitral valve, 3) **diastasis**, when the pressures in LV and LA (left atrium) equalize, and 4) **late ventricular filling or atrial systole**, when LA contraction forces the remaining LA blood into the LV [112, 148].

Echocardiographic Doppler imaging is the most popular method in studying diastolic function. More accurate methods by magnetic resonance are also gaining popularity in research settings though they are more expensive and need advanced MR image analysis techniques. An overview of some common methods quantifying diastolic function relevant to this project follows.

**C.2.3.2.1 Mitral valve inflow velocity**

The indices of “**diastolic filling dynamics**” have been used as quantitative indirect markers of diastolic function [107, 112, 148]. Echocardiographic study of the blood flow velocity through the mitral valve (**mitral inflow velocity**) using pulsed-wave Doppler is commonly used to study diastolic filling dynamics and function [112]. Guided by 2D images, the cursor of a Doppler probe can be located at a specified point, for instance the tip of mitral valve leaflets, to measure the blood flow through that point. The flow velocity with time is then traced on the screen (Figure C-15). **E velocity** reflects velocity of mitral inflow in early diastole during the rapid filling phase while **A**
velocity reflects late mitral filling due to atrial contraction [112] (Figure C-15). Main determinants of E include LV compliance and relaxation rate and the pressures in LA and LV. “A velocity” mainly depends on LV compliance and LA contractility [112]. Another related index is the deceleration time of the E velocity (DT), the time between the peak of E velocity to its intersection with baseline (normal range: 160-240ms). DT is related to how early the pressure equalisation between LA and LV occur [112].

Figure C-15. Mitral inflow velocity profile

Top: Sample echocardiogram by pulsed-wave Doppler from this study. Blood flow toward the ultrasound probe is positive (above the baseline).

Bottom: Schematic flow velocity profiles in normal state and its progress as diastolic dysfunction deteriorates.
E: early flow velocity, A: late flow velocity (during atrial systole). Note grade 2 where E/A profile looks similar to normal (pseudo-normalization). Adapted from [112].

Normally, E/A ratio is greater than one (range: 1-1.5) showing the dominant contribution of early filling [148]. As early LV relaxation delays, LV pressures rise interfering with early mitral inflow (E) so that late filling by atria (A) predominates (to compensate for poor early filling) resulting in a decrease in E/A ratio (<1) and an increase in DT (>240 ms). These characterize grade 1 diastolic dysfunction (Figure C-15). However, as diastolic dysfunction proceeds, LA pressure goes up too and E/A ratio and DT will restore to normal, a phenomenon called pseudo-normalization (i.e. grade 2 diastolic dysfunction) (Figure C-15). E/A ratio will rise higher (>2) and DT will fall below 150 ms as diastolic dysfunction proceeds to grades 3 and 4 [112, 148].
The weakness of mitral inflow velocity profiles in assessment of diastolic dysfunction is that it is “preload-dependent”. Therefore, to distinguish pseudo-normalization from normal diastolic function, mitral inflow velocity profiles are used in combination with tissue Doppler imaging (TDI) of mitral annulus longitudinal motion [112, 148].

C.2.3.2.2 Tissue Doppler imaging

TDI quantifies myocardial tissue motion which is of lower velocity than blood flow [148]. Like pulse-wave Doppler, the Doppler probe cursor is located at specified points under 2D guide. However, here a point on the myocardial tissue rather than blood is chosen, for instance lateral or septal mitral annulus. The result is the tissue velocity trace with time as in Figure C-16.

The velocity pattern of normal mitral annulus in TDI is similar to mitral inflow velocity profile. However, the early phase in TDI (i.e. Ea or e’) is thought to be less preload dependent than E and DT. With a rise in LV diastolic filling pressures, e’ velocity decreases (<10 cm/s). Therefore, the ratio of E/e’ has been used as a more accurate estimation for mean LV diastolic pressure [112, 148].

Figure C-16. Tissue Doppler imaging, mitral annulus motion

The tissue Doppler trace from lateral mitral annulus showing s’ velocity representing the systolic, and e’ and a’ velocities denoting early and late diastolic longitudinal excursion of the mitral annulus. Tissue movement toward the ultrasound probe is positive (above baseline: s’), and away from, negative (e’ and a’).
C.2.3.2.3 Myocardial strain and deformation

Myocardial strain analysis is another method for measurement of LV contractility/relaxation, and indirectly quantifying LV stiffness. The main concept is how much or how fast the intra-myocardial tissue deforms during the cardiac cycle. Strain can be defined by the percentage of deformation (i.e. change in length due to a certain amount of force) from the original length [148]. Compared to strain, strain rate is defined as the change in strain between two time points (St1, and St2) during the cardiac cycle divided by the time interval: \( SR=(St_1-St_2)/(t_1-t_2) \) [149]. Alternatively and by mixing length and time components in the formula, strain rate may be defined as the change in myocardial velocity for a given change in length (Figure C-17) [150]. While strain is expressed in percents, strain rate is measured by \( s^{-1} \).

Figure C-17. Myocardial strain concept

Schematic illustration of strain and strain rate measurement; in real world examples the intra-myocardial movement of the whole myocardial tissue and not only myocytes is quantified. S: strain, SR: strain rate, \( \Delta L=L_2-L_1 \); \( \Delta V=V_2-V_1 \)

Myocardial strain can be measured in three directions: radial, longitudinal and circumferential (Figure C-18) [149]. Circumferential strain is believed to be more reflective of myocardial twisting/untwisting [149]. Tissue Doppler echocardiography is limited to measuring longitudinal and radial motion of the myocardium whereas most of the myocardial regional motion occurs in circumferential direction.
Background

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Figure C-18. Myocardial strain directions

Myocardial regional motion and strain can be measured in three directions: radial (R), longitudinal (L) and circumferential (C).

Non-invasive analysis of myocardial strain is possible by MRI tagging [149] and ultrasound speckle tracking [150].

The MRI technique for myocardial tagging marks myocardial tissue with areas (tags) which persist for some time in each cardiac cycle and regionally move and twist as myocardium contracts and relaxes [149]. Tags can be created in an ECG-gated manner using a special type of MR pulse sequence SPAMM (spatial modulation of magnetization). Such tags can be applied in stripes or in a grid pattern (Figure C-19) by applying stripes in perpendicular directions twice [149].

Figure C-19. Myocardial tagging

Tagged mid-level short axis views in early systole (A), mid-systole (B) and late diastole (C). In mid-systole (B) myocardial systolic contraction has distorted the tagging grid existing earlier in systole (i.e. compare B and A). In contrast, in late diastole (C), myocardial relaxation has caused a reverse change (i.e. compare C with B). Images produced from a development MRI series in this project. The image output form Segment software [124] is shown.

In fact, the magnetic characteristics (i.e. saturation) of myocardial tissue is modified by selective radiofrequency excitation in the tagged areas so that those areas emit weaker radiofrequency signals over the cardiac cycle and remain marked (i.e. darker) while myocardial tissue shape changes [149, 151] (Figure C-19). The tags generated by
Background

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SPAMM method fade with time (e.g. late in diastole, as in Figure C-19) [151]. Complementary SPAMM (CSPAMM) is an optimised method in which the tags do not fade with time at the expense of longer image acquisition times [151].

The images are analysed offline which can be a complex multi-step process including image preparation (e.g. normalization of image and background intensities), endocardial and epicardial delineation, tag tracking, 2D/3D motion reconstruction, and statistical modelling for between-subject comparison [151]. A simple analysis method is marking certain points on the tagging grid (e.g. the intersection points on the grid lines), and then quantify their cardiac cycle displacements. The extent and direction of such displacements is mathematically computed and provide an estimation of myocardial regional strain [149]. The derived myocardial (circumferential) strain has been used to quantify both systolic and diastolic function [149, 152]. The sign of strain values can change depending on myocardial shortening (negative) or lengthening (positive) in different directions (Figure C-20).

![Figure C-20. Myocardial strain (rate) trace sample](image)

Traces of circumferential strain rate (top) and strain (bottom) in a patient with left ventricular hypertrophy. An E and A wave can be seen in the upper panel. Compare this with Figure C-15, and tissue Doppler s’, e’ and a’ waves in Figure C-16. Reprinted from [152], with permission from Elsevier.

In contrast to LV filling parameters which are volume-load dependent, such MR-measured strain rates are thought to be a direct index of “myocardial relaxation” [152].
However, there are still controversies about load-independence of strain and strain rate raised by a few speckle tracking studies [153-155].

Compared to MR tagging, speckle tracking on echocardiogram videos is a less expensive and more available method for myocardial strain measurement [150]. Usually, some “speckle artefacts” can be seen on normal gray-scale myocardial images acquired during routine echocardiography. Such speckles are randomly generated because of the ultrasound beam reflections and scatter. Some of these speckles remain visible during part of the cardiac cycle and the appropriate software and algorithms can track and quantify such movements within the myocardial tissue [150], similar to tracking the myocardial tags by MRI. Such image analysis is possible offline on the recorded video echocardiograms. The results of the speckle tracking image analysis are more or less similar to the multi-directional strains and strain rates in MRI. Further details of speckle tracking method are beyond the scope of this report.

C.2.3.2.4 Left atrial volume

Poor diastolic relaxation may put an extra burden on the left atrium using more force to pump the blood into a less compliant stiff left ventricle. With time this may lead to increase in the size of the left atrium. Therefore, left atrial volume (LAV) has been shown as a valuable marker for chronic diastolic dysfunction. A higher than expected LAV can be used to help distinguishing pseudo-normalization phase of diastolic dysfunction from normal [112]. LAV can be measured by both echocardiography and MRI. Single or bi-plane Simpson’s method like that used in LV volume measurement can also be employed for LAV measurement; see Figure C-13 for LV volumetry. Method details are described in Lester et al [112].

C.2.3.2.5 Epidemiological importance

Diastolic dysfunction is now recognized as a relatively common subclinical finding without overt symptoms in community dwelling individuals. A population study by Redfield et al showed that 21%, and 7% of the adults >45 yr had mild, or moderate diastolic dysfunction respectively (defined by Doppler Echocardiography) while the
prevalence of systolic dysfunction was 6% (EF<50%) [143]. The same study revealed that mild diastolic function was predictive of all-cause mortality independent of age, sex and EF [143].

Wang et al showed that TDI-measured peak early diastolic mitral annulus velocity (e’) was among the best independent predictors of cardiac death [156]. In another longitudinal study among elderly cardiac catheterization patients, Liang et al found that after adjusting for gender, age, ejection fraction, and left ventricular end diastolic pressure (LVEDP), only E/e’ ratio and LA volume (indexed to BSA) could predict a heart failure event in a mean of 10 month follow-up [157].

A large-scale epidemiological MR tagging study (Multi-Ethnic Study of Atherosclerosis, MESA) showed a relationship between regional myocardial function (i.e. lower strain rate in diastole) and carotid artery distensibility [158] and subclinical atherosclerosis (measured by CIMT) in asymptomatic individuals [159]. LV hypertrophy and concentricity (M/V ratio) and hypertension were also found to be negatively correlated with myocardial circumferential diastolic strain rate in MRI studies [152, 160].
C.3 Ethnic differences in CVD outcomes and risk

Cardiovascular morbidity and mortality vary among different ethnic groups. People of Indian sub-continental, or South Asian, origin and those of Afro-Caribbean origin are among the most visible and fastest growing immigrant populations in the UK and North America. South Asians have higher coronary heart disease (CHD) prevalence and mortality rates, experience earlier CHD events, with slower declines in premature CHD mortality than other populations (Figure C-21) [4, 5, 7, 161, 162]. The age-standardised CHD mortality rate in South Asians in the UK is 50% higher than in European whites [10]. In contrast, African-Caribbeans have lower CHD mortality than Europeans despite higher rates of hypertension, diabetes and stroke [5, 11] (Figure C-22).

Conventional risk factors such as diabetes, obesity and hypertension fail to explain these ethnic differences.
differences fully [12]. Overall, there is still a global paucity of prospective studies on CVD difference and (conventional and novel) cardiovascular risks in ethnic groups, and most of the current evidence comes from cross-sectional studies [6].

**C.3.1 Traditional risk factors**

Two large population studies in the UK, the Southall Study among South Asian, and Brent Study in African-Caribbean communities in London revealed important facts on the differences in CV risk profiles of these UK minorities [163]. The authors speculated that significantly higher insulin resistance and central obesity in South Asians were the main culprits. South Asians have higher body fat per body mass unit, and are more centrally obese compared to European whites [164, 165]. The Southall study among 1711 South Asians and 1761 Europeans revealed uniformly higher prevalence of diabetes, and insulin resistance and higher waist/hip ratios among South Asians after BMI adjustment [7]. Some studies also showed that South Asians are more insulin resistant for the same level of body fat [166, 167].

South Asians also generally have less favourable blood lipid profiles with higher triglyceride, and lower HDL cholesterol (i.e. the protective cholesterol) [7]. Accordingly, the metabolic syndrome was found to be almost 2.5 times more prevalent among South Asians compared to Europeans [168]. In addition, recent evidence also shows that not only type 2 diabetes develops in South Asians around 11 years earlier and at a lower BMI than in Europeans [169], but glycemic and hypertension control in South Asian diabetic patients is also poorer than their European counterparts [169]. Finally, lifestyle factors such as diet and lower physical activity among South Asians may also contribute to their central obesity [170, 171]. South Asians seem to consume less protein and more fat, carbohydrate and eggs and have higher total fat content in their weekly food purchase than Europeans [172-174].

A systematic review [175] of the studies comparing blood pressure levels in South Asians and Europeans revealed rather inconsistent findings. Overall, hypertension does not seem to be higher among South Asians than Europeans. However, within South Asians, there is heterogeneity regarding levels of blood pressure with Indians having relatively higher, and Pakistanis, and Bangladeshis lower blood pressures [175].
Another review showed South Asians especially Pakistani and Bangladeshi have lower pulse pressure than Europeans [176].

Similar to South Asians, African-Caribbeans are also known to suffer from higher risks of diabetes and insulin resistance when compared to Europeans. Despite similar levels of dysglycemia, the severity of insulin resistance seems to be lower in the African-Caribbean compared with South Asians [163]. Tilllin et al suggest that the “toxic effect” of diabetes manifests itself predominantly as stroke among African-Caribbeans, but as both CHD and stroke among South Asians [163]. African-Caribbean people also have a less atherogenic lipid profile with higher HDL-C and lower triglyceride levels compared to Europeans and South Asians [7, 177]. Anderson et al also showed different plasma fatty acid profiles with higher proportions of EPA and DHA and favourable hemostatic variables among African-Caribbeans [178]. This may partly explain their lower CHD rates.

African-Caribbeans in the UK, like their African-American counterparts, have higher blood pressures than people of European origin [179-181]. The Brent population-based study found that higher “resting” blood pressures may explain higher risk of stroke in Caribbean women but not in men [180]. Some of such excess risk may further be justified by failure to drop nocturnal blood pressures in African-Caribbeans [180]. A systematic review found that African-descent people had higher pulse pressures than Europeans [176]. Blood pressure control is more difficult among people of African-origin, and they may experience more severe organ damage from hypertension compared to Europeans.

Interestingly, CVD mortality differs within people of African origin. A US study showed that people of Caribbean origin (African-Caribbeans) are known to have lower CHD and CVD mortality rates compared to African-Americans, especially those born in Southern states of the US [182]. Such differences may partly be explained by more favourable Caribbean diet (i.e. lower fat and moderate alcohol consumption), and less frequent obesity and better blood lipid profiles of the Caribbean people [182, 183].
C.3.2 Novel risk factors

Despite partially explaining the scenario, these conventional risk factors still fail to fully explain the higher relative risk of CHD among South Asians compared to European whites even taking into account the insulin resistance variables [12]. Consequently, there has been an increasing interest in novel risk factors of CVD [12].

Some of the hypotheses associated with the novel factors include the increased levels of prothrombotic factors (e.g. PAI-1) [184], and biomarkers of inflammation (e.g. high sensitivity C-reactive protein [hsCRP]) [185, 186] as well as lower levels of adiponectin [187] in South Asians’ plasma. Bansal et al found lower serum adiponectin in South Asian than European infants as early as 3-6 months of age despite similar levels of insulin and lipids [188]. Adiponectin is an anti-inflammatory protein released by adipocytes assumed to be protective against vascular and metabolic dysfunction [189], and is known to be reduced in obesity, type 2 diabetes, and CHD [6]. Elevated serum levels of homocysteine [166, 190] (assumed to cause vascular endothelial dysfunction [6]), and lipoprotein A [191] (“a genetically determined factor unrelated to insulin resistance” and known as an independent risk factor for premature CHD [192]) are among other novel risk factors [6]. From a pathophysiological point of view, some factors such as insulin resistance and alterations in novel risk factors may be downstream to, rather than the causes of, the main pathological process in CVD. Prospective studies and trials are able to elucidate this.

There is evidence suggesting that the differences in CVD risk factors actually develop early in life [6, 167, 188, 193, 194]. Table C-4 from the review by Forouhi et al [6] summarizes some evidence in this regard.

Novel risk factors have also been investigated in an attempt to explain CVD risk difference between people of African and European descents. A US population study showed that the increased risk for peripheral arterial disease among African-Americans was only “modestly” weakened by either traditional or novel risk factors [195]. Among the 9 novel risk factors studied including homocysteine, CRP and prothrombin-1, the primary effect was seen with fibrinogen and lipoprotein A [195]. Kalra et al compared healthy African-Caribbean living in the UK and Jamaica and showed that poorer
vascular endothelial function and atherosclerotic markers (CIMT) in UK African-Caribbeans compared to their Jamaican counterparts was independently related to higher homocysteine and lower folate levels, and suggested that such differences may be due to dietary changes after migration [196].

Table C-4. Lifetime CVD risk comparison South Asians and Europeans
An overview of CVD risk comparison between South Asians and matched European whites from birth to adulthood. Reproduced from [6], with permission from Elsevier.

<table>
<thead>
<tr>
<th>At birth</th>
<th>Children 8–11 yr</th>
<th>Students 18–25 yr</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑Insulin and leptin in cord blood despite lower birth weight</td>
<td>↑Insulin</td>
<td>↑PWV</td>
<td>↑Insulin/metabolic syndrome</td>
</tr>
<tr>
<td>Central adiposity despite similar ponderal index</td>
<td>Stiffer blood vessels</td>
<td>↑CRP; PAI-1; Lp(a) homocysteine</td>
<td>↓Adiponectin</td>
</tr>
<tr>
<td>↓Adiponectin</td>
<td>↑Diabetes by 3–4-fold</td>
<td>↑CHD by 50–80%</td>
<td></td>
</tr>
</tbody>
</table>

Attempts have been made to find relationships between the renin-angiotensin-aldosterone system (RAAS) and high blood pressures in people of African-descent. Some studies have shown that hypertension in African-descent people accompanies low plasma renin activity and high aldosterone levels [197, 198]. A Canadian study showed that compared to French Canadians whose blood pressure was correlated with plasma renin, hypertension in African-descent Canadians was more consistently related to plasma aldosterone or aldosterone to renin ratio (ARR) [199].
C.4 Aldosterone and CVD risk

C.4.1 Physiology

Aldosterone, a component of the well known renin-angiotensin-aldosterone system (RAAS), is secreted by the adrenal gland’s zona glomerulosa in response to serum angiotensin II. This occurs when the low perfusion (e.g. due to hypovolemia) in the renal tubules, low blood pressure in the kidney, sympathetic nervous stimulation, or low serum chloride levels stimulate secretion of renin. Renin facilitates conversion of the liver-produced angiotensinogen into angiotensin I in the serum which is then converted to angiotensin II by angiotensin-converting enzyme (ACE) in the lungs. Angiotensin II is a vasoconstrictor and also promotes aldosterone secretion from the adrenal glands. Aldosterone in turn causes retention of water and sodium and excretion of potassium via the mineralocorticoid receptor (mcR) in the epithelial tissues in kidney tubules, colon, and sweat/salivary glands [200].

C.4.2 Epidemiological & clinical evidence

Clinical studies have shown that the incidence of organ damage such as LVH, cerebral haemorrhage, and proteinuria is higher in secondary hypertension induced by hyperaldosteronemia than in untreated essential hypertension [201-203]. As interesting are the associations between the plasma aldosterone concentration (PAC) and CVD. PAC has been positively correlated with LVH in untreated essential hypertension [204], and mortality in congestive heart failure patients [205]. Even within its physiologic range and among a normotensive population of Framingham Offspring Study, PAC was positively associated with incidence of developing hypertension or an increase in blood pressure [206]. Another Framingham Offspring Study among those “free of myocardial infarction and heart failure” revealed a positive association between PAC and concentric remodelling of left ventricle in women, but not men [207].

PAC has also been positively linked with increased arterial stiffness as aortic pulse wave velocity in hypertensive patients [74, 76]. However, reports of such a relationship
are inconsistent, absent in some studies [74, 208, 209], but present in others [76, 77, 209]. Such inconsistencies have been attributed to PAC variability due to diurnal variations and anti-hypertensive medications, and that PAC may not reflect local aldosterone inside the cardiovascular tissues [210]. Further evidence also points to activation of mcR within cardiovascular and adipose tissues by other hormones such as glucocorticoids [211]; these may also explain part of the damage unrelated to PAC. Finally, recent evidence suggest that salt (Na+) loading may contribute to secretion of cardiotonic steroids such as ouabain from adrenal zona glomerulosa. Such steroids in turn may contribute to hypertension and cardiovascular damage unrelated to PAC [212].

Clinical trials involving mcR antagonists have added more compelling evidence for the association of aldosterone and CVD clinical endpoints, indicating possible protective effect of aldosterone antagonism in CVD even within seemingly normal PAC ranges [110]. mcR antagonism administered to heart failure patients by spironolactone, a non-selective mcR antagonist (added to other drugs for heart failure), lowered CVD mortality and morbidity [213]. Eplerenone, a selective mcR antagonist, administered to post-myocardial infarction heart failure patients in the EPHESUS trials also decreased CVD mortality [214]. Eplerenone has also been as successful as ACE-inhibitors in LVH reduction and hypertension control in essential hypertension patients [215].

C.4.3 Mechanisms

Excess aldosterone secretion from the adrenals (hyperaldosteronism) causes secondary hypertension, indirectly leading to cardiovascular damage (e.g. LVH, proteinuria, and stroke) via high blood pressure. However, there is an emerging bulk of evidence indicating that aldosterone per se (and not via hypertension induction) is directly associated with cardiovascular damage via mcR within the heart and vascular tissues [110].
Aldosterone’s association with LV concentric remodelling and arterial stiffness are believed to be due to its proinflammatory and fibrotic effects in the myocardium and arterial walls [74, 216, 217]. Experimental studies have suggested that aldosterone’s augmentation of angiotensin II-induced oxidative stress in vascular tissue is one of the proinflammatory mechanisms leading to cardiovascular damage [218, 219]. While sodium surplus is an important and necessary accomplice of aldosterone in causing organ damage, the mechanisms are mostly unknown [212].

A review by Whaley-Connel et al [211] depicts new roles of aldosterone in the cardiometabolic syndrome and suggests that obesity could increase aldosterone levels possibly via an unidentified fatty acid stimulating aldosterone synthesis [211]. There is also increasing recognition of non-genomic effects of aldosterone which leads to activation of NADPH oxidase and reactive oxygen species generation [211]. Direct contribution of aldosterone to insulin resistance has also been suggested via such non-genomic cellular perturbations and other mechanisms (Figure C-23) [211].
C.5 Vitamin D and Cardiovascular Disease

C.5.1 Physiology & metabolism

The major source of active vitamin D in the human is that produced from ultraviolet-B (UVB) radiation of the sunlight in the skin which promotes conversion of 7-dehydrocholesterol to provitamin D3 followed by spontaneous isomerisation to vitamin D3 or cholecalciferol. Other minor sources of active vitamin D are vitamin D3 taken from animal food (e.g. fatty fish) or vitamin D2 (ergocalciferol) from plants or fungi. Vitamin D2 is produced during a similar process in plants where UVB causes conversion of ergosterol to ergocalciferol or vitamin D2 [220, 221].

Figure C-24. Vitamin D sources and metabolism in human
The main source of vitamin D is sunlight-induced skin synthesis. The most abundant circulating metabolite is 25(OH)D, whose level reflects overall vitamin D status. The most bioactive metabolite is 1,25(OH)2D.
The sunlight produced vitamin D3 in the body, and relatively minor amounts of vitamin D3 or D2 in the diet are then hydroxylated by the liver into 25-hydroxy vitamin D or 25(OH)D, which is then further hydroxylated into 1,25(OH)2D. 1,25(OH)2D is the most active vitamin D metabolite and works as a steroid hormone binding to vitamin D receptors (VDR) available on most cells, and is capable of regulating 3% of the human genome [221]. Circulating levels of 1,25(OH)2D are mainly determined by renal alpha-1 hydroxylase enzyme which is regulated by serum levels of parathyroid hormone (PTH), calcium and phosphorus [220, 221].

Despite 1,25(OH)2D being the most active metabolite, 25(OH)D is actually the most abundant form of vitamin D found in the circulation. Circulating 25(OH)D levels are measured as the best index of the total body status of vitamin D, and are known as a major determinant of tissue levels of 1,25(OH)2D [220, 221]. Current definitions of insufficiency and deficiency are primarily based on the studies investigating bone health outcomes of vitamin D deficiency [220]. Currently, serum levels of 50-75 nmol/L (20–29 ng/mL) is commonly defined as insufficiency, and <50 nmol/L (20 ng/mL) as deficiency [220, 221]. As a rule of thumb ng/mL values are multiplied by 2.5 to convert into nmol/L [221]. Finally, since the major normal source of vitamin D in human is the D3 form (from sunlight and animal food), the large proportion of circulating 25(OH)D is expected to be 25(OH)D3.

The best known action of vitamin D is its role in bone metabolism such as promoting intestinal absorption of calcium and phosphorus, and bone calcification. However, there is mounting evidence on its role in other chronic diseases including cardiovascular disease. A summary of such evidence follow.

C.5.2 Epidemiological & clinical evidence

The association between vitamin D and cardiovascular disease (CVD) was suggested at least two decades ago based on seasonality of CVD mortality and the possible protective effect of ultraviolet radiation which itself is related to human’s vitamin D status [222, 223]. The relationship between CVD and geographic latitude, altitude, and seasons has already been well established. Now it is suggested that the variation in sunlight caused by these factors and the consequent variations in vitamin D levels could
be the common explanation for these associations [223]. A prospective British study supports the evidence of “a causal link between a latitude-associated risk factor” and ischemic heart disease (IHD) [224]. Higher altitude where people are exposed to higher UVB radiation [225] is also known to correlate with lower rates of IHD [226], and hypertension [222]. Seasonal variation of IHD mortality with winter peaks and summer nadirs in both sexes [227] as well as its negative correlation with sunshine per year in males [228] fit very well with vitamin D status of the residents of northern latitudes [223]. Moreover, recent studies have revealed low vitamin D and high PTH (parathyroid hormone) levels during winters in Central Europe [229] with the winter PTH peak blunted on vitamin D supplementation [230]. Finally, the potential epidemics of vitamin D deficiency in the world might be accentuated considering ever-increasing urbanization and indoor lifestyle which restrict UVB exposure, as well as the impact of air pollution in urban areas absorbing UVB photons [223].

Current data from several cross-sectional studies have revealed that patients with acute myocardial infarction [231], heart failure [232], and stroke [233] as well as diabetic patients with cardiovascular disease [234] had lower levels of 25(OH)D. Serum calcitriol levels have been shown to inversely correlate with the extent of vascular calcification in individuals at moderate to high risk of coronary heart disease [235]; vascular calcification is a known risk factor for IHD and associated with poor 5-year survival [236]. CIMT (carotid intima-media thickness), a marker of preclinical atherosclerosis and ageing, was found to be independently associated with low 25(OH)D levels in the serum of diabetic patients [237]. Hypovitaminosis D has been shown to independently predict low fasting apolipoprotein A-I (low availability of which may increase vascular damage) in healthy British Bangladeshi adults [238]. In the same population, the status of vitamin D turned out to be a determinant of serum matrix metalloproteinase-9 (MMP-9; a determinant of vascular and myocardial remodelling) and CRP levels [239]. In contrast, an Indian study found an association between high 25(OH)D (>222 nmol/l) and IHD [240]. Considering that 25(OH)D is the main predictor of PTH in physiological conditions [241], the association of higher PTH levels and CVD might also be a tell-tale of the vitamin D-CVD association. A population-based cross-sectional study in Norway demonstrated high PTH levels in the adults with “reference-range” calcium levels were independently associated with IHD in both sexes [242].
Several cohort studies have also confirmed the link between vitamin D deficiency and CVD events and death. Interestingly, the association between vitamin D and CVD in epidemiological studies seem to happen at a particular threshold of circulating vitamin D levels below 15-20 ng/mL [243]. Wang et al followed-up 1739 Framingham Offspring Study cases and showed that low 25(OH)D (<15 ng/mL) independently and in a graded manner increased the risk of incident CVD in hypertensive subjects, but not in normotensive ones [244]. A prospective population-based study revealed that low vitamin D consumption in diet, and low serum 25(OH)D were significant predictors of stroke [245]. Melamed et al studied a general population cohort (NHANES III study) and reported 26% higher rate of all-cause mortality in those in lowest 25(OH)D quartile (<17.8 ng/mL) [246]. A cohort population study in the elderly (>65), showed that those in lowest 25(OH)D quartile (<10.5 ng/mL) had higher all-cause and cardiovascular mortality (hazard ratio: 2.1) than those in highest quartile (>26.5 ng/mL) [247]. In another cohort of the elderly (60±10 yrs) coronary angiography candidates, low 25(OH)D and 1,25(OH)2D both predicted all-cause and cardiovascular mortality independent of CHD, comorbidity and physical activity covariates [248]. Among a cohort of end-stage renal disease patients undergoing haemodialysis in Japan, CVD mortality among 1α(OH)D3 users was 70% lower than in non-users [249]. A nested case-control study revealed the relationship of low vitamin D levels and incident (early) mortality in haemodialysis patients in whom CVD is the commonest cause of death [250]. A recent prospective study found an inverse relationship between serum 25(OH)D levels and all-cause mortality among 399 elderly subjects in Scotland [251].

Despite the evidence relating CV and all-cause mortality, the relation between low vitamin D levels and important CV risk factors such as diabetes, and hypertension is inconsistent [252].

Several small clinical trials have shown some benefits of vitamin D supplementation in CVD risk factors though well-designed trial data is still limited. A 9% drop in systolic BP, and 17% drop in PTH levels was observed in the group supplied with vitamin D plus calcium compared with the calcium-only group in a clinical trial with 148 old women with pre-treatment 25(OH)D <50 nmol/L [253]. Reduction of diastolic pressure after vitamin D supplementation for four months was shown in hypertensive individuals
Background

Vitamin D and Cardiovascular Disease

with pre-treatment low ionized serum calcium, and high PTH [254]. Correction of secondary hyperparathyroidism with calcitriol for 15 weeks caused regression of left ventricular hypertrophy in 15 haemodialysis patients compared to 10 controls not using calcitriol [255]. A recent trial on 123 heart failure patients randomly receiving vitamin D plus calcium vs. placebo plus calcium showed an increase in the anti-inflammatory cytokine IL-10 and prevention of a rise in TNF-α in vitamin D group, compared to rising TNF-α in controls [256]. Finally, vitamin D supplementation for one year decreased the high MMP-9 and CRP levels in a vitamin D insufficient British Bangladeshi population [239].

There is a shortage of well-designed trials of vitamin D supplementation measuring CVD events. A systematic review of the trials of vitamin D and calcium supplementation revealed that medium to high-dose (1000 IU/day) supplementation with vitamin D, but not calcium may have a minimal protective effect on CVD events [257]. A UK randomized trial with >3000 participants found slightly lower relative risks of total CVD, nonfatal CHD events and CVD mortality, but the results did not achieve statistical significance [258]. An Australian population-based randomized trial in 302 women 70-90 yrs of age reported a lower CHD (1.3% vs. 2%) but the same stroke rates among those receiving vitamin D2 and calcium than the participants on the placebo plus calcium after a year [259]. A randomized trial among 36,282 postmenopausal women comparing vitamin D and calcium versus placebo for 7 years failed to show any benefit for vitamin D supplementation in decreasing incident CVD events [260]. However, the study has been criticized for too insufficient dose of vitamin D (400 IU/d) used in the treatment group, lack of 25(OH)D baseline measurement, the placebo group having permission for using vitamin D supplements, and that it had been primarily designed as a fracture prevention trial and not to asses CVD risk [223, 261].

C.5.3 Mechanisms

There are multiple mechanisms connecting vitamin D deficiency and CVD (Figure C-25) [223]. Experimental studies have demonstrated that vitamin D can directly suppress renin gene expression, and therefore down-regulate renin-angiotensin-aldosterone system (RAAS). VDR-knock out mice lacking vitamin D receptor have excess RAAS activation leading to hypertension, and cardiac hypertrophy [262]. In line
with these experimental studies, Tomaschitz et al recently reported independent correlation of low 25(OH)D and 1,25(OH)2D with upregulated RAAS components such as plasma renin activity and angiotensin, but not aldosterone in elderly Europeans [263].

Figure C-25. Vitamin D and CVD, possible mechanisms


Moreover, secondary hyperparathyroidism caused by vitamin D deficiency is among the main current hypotheses behind the potential CVD-inducing effect of vitamin D deficiency [223, 244]. PTH is known to induce hypertrophy of ventricular myocytes [264], fibrosclerotic changes in vascular smooth muscle cells (VSMC) [265], and “intramayocardial arteriole thickening” [266] in experimental models and in-vitro studies. PTH is also known to increase pro-inflammatory cytokines such as IL-6 (interleukin 6) [267].

Interestingly, a similar cytokine-inducing effect for IL-6 and TNF-α is attributed to low concentrations of calcitriol [268] though a prospective Framingham study recently questioned the association between vitamin D status and circulatory inflammatory
markers [269]. VDR is found in VSMCs and endothelial cells and is believed to influence VSMC proliferation [270], and play an antithrombotic role [271].

Last but not the least, vitamin D is known to influence vascular calcification probably via promoting the synthesis of matrix Gla protein, an inhibitor of vascular calcification [272]. Paradoxically, pharmacological doses of some vitamin D receptor activators (e.g. calcitriol) may cause arterial calcification and stiffness in experimental models while others (e.g. paricalcitol) do not [273].
D) Aims & Hypotheses

D.1 Hypotheses

In this multi-ethnic sample of community-dwelling men from EMAS/LINK studies, and adjusted for conventional risk factors we aimed to test the following hypotheses:

1) Surrogate CVD risk markers such as aortic PWV, CIMT, LVMI, and LV geometry are different across the three ethnic groups.

2) There is an inverse relationship between circulating vitamin D levels as 25(OH)D and the surrogate CVD risk markers such as aortic PWV, CIMT, LVMI, LV geometry and diastolic function.

3) There is positive relationship between circulating aldosterone levels and the above-mentioned risk markers.

4) Vitamin D or aldosterone can explain part of the cross ethnic differences in the above-mentioned risk markers.

The following hypotheses were also tested relating the new hemodynamic method (the Arteriograph) used for arterial stiffness indices:

1) Transit times by the Arteriograph device are not significantly different from that measured by MR phase-contrast imaging between the aortic valve and bifurcation.

2) The length of the aortic path from aortic valve to bifurcation estimated by regressions including convenient non-invasive measures (e.g. age, height) is close to that measured by MRI.

3) There is agreement between central SBP by the newer Arteriograph and Omron HEM-9000 devices and that of SphygmoCor.
D.2 Aims

A. To recruit 70-100 men of each ethnic group of South Asian, African-Caribbean and European already seen within EMAS/LINK studies, and conduct the following tests for all of them:

1) Blood pressure measurement
2) Arterial stiffness tests especially aortic pulse wave velocity, central pressures and indices of reflection waves (e.g. AIX) using a convenient non-invasive outpatient method (the Arteriograph).
3) Echocardiographic measurement of cardiac risk markers including LV volumes, mass and geometry, and indices of diastolic and systolic function.
4) Carotid ultrasound for measuring CIMT.
5) Hormonal measurements including 25(OH)D and aldosterone on their frozen samples from EMAS/LINK.

B. To re-invite a sub-sample of the men seen above with fewer confounders for a cardiovascular MRI study. The excluding criteria were known cardiovascular disease, diabetes, or use of any cardiovascular medications. Only Pakistani (not Indian, or Bangladeshis), Caribbean and European participants were invited.

The aims of MR study were:

1) to provide a more accurate estimate of cardiac risk markers such as LV mass and geometry, myocardial stiffness and diastolic function (i.e. by myocardial tagging and strain measurement), and
2) to create an opportunity to compare the aortic pulse wave velocity measurement by the non-invasive Arteriograph device, and more accurate aortic length and transit time by MRI.
D.3 Sample size and power

The following sample size calculations were performed before recruitment assuming two-sided tests, significance level (type I error) of 5% and power of 80% using simplified sample-size formula: $N=16\times(\text{SD}/E)^2$, where SD and E are standard deviation, and effect size of the outcome variable, respectively.

To detect a 10 g/m$^2$ difference in LVMI (left ventricular mass index) by echocardiography between any two groups of participants, 100 participants in each group may be necessary (i.e. SD=21 g/m$^2$ [274]), and to find 10 g/m$^2$ difference in LVMI by MRI (SSFP method) only 13 in each group (i.e. SD=9.3 g/m$^2$ [125]) could be enough. To detect 0.5 m/s difference in aortic PWV between two ethnic groups, up to 150 in each group may be needed (i.e. SD=1.55 m/s [32, 275]), and to identify 0.04 mm difference in CIMT values between two ethnic groups 88 people in each group was suggested (i.e. SD=0.094 mm [276]). Up to 62-110 participants might be necessary to find a statistically significant correlation coefficient as small as 0.3-0.4 between any two continuous variables (e.g. serum aldosterone and LVMI) in any group.
E) Methods

E.1 Participants & recruitment

The participants comprised men of South Asian, African-Caribbean and European origin 40-80 years of age living in Greater Manchester and already recruited in LINK and EMAS studies.

Original recruitment of African-Caribbean and South Asian men (LINK study) was through volunteers by advertising in community centres and media and by general practice register sampling for Europeans (EMAS) in Manchester. Ethnicity was defined by participants’ self-reporting, and 3 out of 4 grand-parents being of the same ethnic origin. Ethics permissions for all of the tests and examinations were reviewed and endorsed by both regional Greater Manchester, and the University of Manchester ethics authorities. EMAS/LINK participants from different age groups were invited by mail and followed up by phone to attend a visit including non-invasive cardiovascular tests in Wellcome Trust Clinical Research Facility (WTCRF), Manchester. The participants had to be free of severe chronic or acute disease of active malignant, renal or liver origin. Attempts were made to avoid inviting such participants using available medical history records and biochemistry tests results available from EMAS/LINK studies.

Recruitments and visits finished by the end of March 2010. Over 67 letters were sent to general practitioners for referral of abnormal results, and simplified summary feedback reports were sent to the participants.

In final analyses, 11 African origin (non-Caribbean) and 4 with mixed or other ethnicities mistakenly recruited were excluded to keep relatively homogenous ethnic groups. The following tables show an overview of the recruitment and its temporal progress.
Table E-1. Recruited participants by age group

<table>
<thead>
<tr>
<th>Age Group (yr)</th>
<th>African-Caribbean*</th>
<th>South Asian</th>
<th>White European</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-49</td>
<td>34</td>
<td>26</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>50-59</td>
<td>25</td>
<td>27</td>
<td>27</td>
<td>1</td>
</tr>
<tr>
<td>60-69</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>68</td>
<td>63</td>
<td>4</td>
</tr>
</tbody>
</table>

* Includes 11 participants with African origin excluded in the final analysis.

Table E-2. First visit recruitment progress

<table>
<thead>
<tr>
<th>Visit Year-Month</th>
<th>No seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008-June</td>
<td>1</td>
</tr>
<tr>
<td>2008-July</td>
<td>10</td>
</tr>
<tr>
<td>2008-November</td>
<td>6</td>
</tr>
<tr>
<td>2008-December</td>
<td>10</td>
</tr>
<tr>
<td>2009-January</td>
<td>13</td>
</tr>
<tr>
<td>2009-February</td>
<td>9</td>
</tr>
<tr>
<td>2009-March</td>
<td>12</td>
</tr>
<tr>
<td>2009-April</td>
<td>6</td>
</tr>
<tr>
<td>2009-May</td>
<td>21</td>
</tr>
<tr>
<td>2009-June</td>
<td>20</td>
</tr>
<tr>
<td>2009-July</td>
<td>23</td>
</tr>
<tr>
<td>2009-August</td>
<td>20</td>
</tr>
<tr>
<td>2009-September</td>
<td>5</td>
</tr>
<tr>
<td>2009-October</td>
<td>2</td>
</tr>
<tr>
<td>2009-November</td>
<td>15</td>
</tr>
<tr>
<td>2009-December</td>
<td>9</td>
</tr>
<tr>
<td>2010-January</td>
<td>10</td>
</tr>
<tr>
<td>2010-February</td>
<td>19</td>
</tr>
<tr>
<td>2010-March</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>214</td>
</tr>
</tbody>
</table>

Four participants did not have ultrasound scans.

Table E-3. MRI recruitment progress

<table>
<thead>
<tr>
<th>Visit Year-Month</th>
<th>No seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009-April</td>
<td>1</td>
</tr>
<tr>
<td>2009-July</td>
<td>4</td>
</tr>
<tr>
<td>2009-August</td>
<td>4</td>
</tr>
<tr>
<td>2009-September</td>
<td>2</td>
</tr>
<tr>
<td>2009-October</td>
<td>6</td>
</tr>
<tr>
<td>2009-November</td>
<td>6</td>
</tr>
<tr>
<td>2010-January</td>
<td>7</td>
</tr>
<tr>
<td>2010-February</td>
<td>4</td>
</tr>
<tr>
<td>2010-March</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
</tr>
</tbody>
</table>

Table E-4. Participants with complete arterial stiffness, ultrasound measurements

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>No</th>
<th>Age mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-Caribbean*</td>
<td>75</td>
<td>53.9 (41-81)</td>
</tr>
<tr>
<td>South Asian</td>
<td>68</td>
<td>54.8 (40-81)</td>
</tr>
<tr>
<td>White European</td>
<td>63</td>
<td>56.1 (45-79)</td>
</tr>
<tr>
<td>Other/Mixed</td>
<td>4</td>
<td>50.5 (42-60)</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td></td>
</tr>
</tbody>
</table>

*Includes 11 participants with African origin excluded in the final analysis.
E.2 Protocol overview

The study protocol overview is illustrated in Figure E-1. In the first visit, the participants were walked through a formal consent process and were allowed enough time for counselling about any concerns and questions about the tests. A short interview followed, asking about medical history of chronic diseases (e.g. malignancy, renal/liver disease, diabetes, asthma, and cardiovascular disease) and a drug history focusing on vasoactive medications and supplements (e.g. vitamin D).

The following were performed for each participant during the first visit:

1) Standard sitting blood pressure measurement
2) Arterial stiffness tests (pulse wave velocity and pulse wave analysis)
3) Echocardiography

All measurements happened in the morning and typically 1-2 participants were seen and finished 9.00 – 12.00 am. A typical visit started with consenting and short interview...
followed by blood pressures/arterial stiffness tests, and then echocardiography or the reverse order. Details of the protocols are described in the following sections.

Fifty eligible participants were offered cardiac MRI during a second visit at a later date. Eligibility for MR study included Pakistani (excluding other South Asians), Caribbean (excluding Africans) or European ethnicity, no history of active malignancy or chronic disease, treatment for known cardiovascular disease, and diabetes, and being free of any cardiovascular medications, and having no contraindication of MRI (e.g. metallic parts and shrapnel in the body).

### E.3 Blood pressure measurement protocol

BP measurement was performed based on published recommendations [277] after 5 minutes rest in sitting position using a validated automatic oscillometric Omron 705CP device [278]. The participant had to be sitting comfortably in a chair with back supported and legs uncrossed. Three BP measurements (in one minute intervals) were made on the left arm rested on a desk at the level of the heart. The last two measurements were averaged if not more than 5 mmHg different. If so, further measurements were made [277].

### E.4 Arterial stiffness and central blood pressures

Conventional PWV measurement devices (e.g. SphygmoCor or Complior) were not available in our clinical facility. SphygmoCor PWA module was available, but PWV module had not been purchased. A validated non-commercial continuous Doppler PWV device (Gosling Taylor device) was also available though it was very old with no industry support and the possibility of irreversible and imminent failure loomed large. A newly introduced device, the Arteriograph® (TensioMed, Budapest, Hungary) became available to our team early in the project. Because of its measuring central aortic PWV (i.e. excluding carotid and femoral paths), simultaneous reporting of PWV, reflection wave indices and central pressures, convenient use, and acceptable
availability of manufacturer service, we decided to measure the main arterial stiffness indices in this project by the Arteriograph.

The Arteriograph device had been invasively validated by the inventor [31], and non-invasively compared with the conventional devices measuring carotid-femoral PWV (SphygmoCor and Complior) showing significant correlations though not perfect agreement [32]. However, as a newly introduced device claiming to measure central (pure) aortic PWV, we decided to perform comparison studies with other methods available in our facility. These method comparison studies were run as parallel projects to the core hypotheses, integrated within the main protocol, on a subgroup of our participants during their main visits needless of re-invitations. The study results are presented in two technical manuscripts in this thesis. These comparison studies included:

1) Comparing aortic PWV and transit times by Arteriograph and phase-contrast MRI (N=47).

2) Using MRI for improving aortic path estimations for Arteriograph measurements (N=49).

3) Comparing PWA indices (cSBP and AIx) by Arteriograph and two tonometry-based devices: SphygmoCor (Atcor Medical, Australia), and Omron HEM-9000 (Omron, Japan) (N=35).

The protocols for the three devices used for arterial stiffness measurements in this project follows. The PWV measurement by MRI is presented in MRI protocol.

The preparation for all arterial stiffness measurements included avoiding smoking, heavy meals and caffeine-containing drinks for 3 hours before the visit, and alcohol the night before. The measurements were done after 10 minutes rest, supine in a quiet room with dim light and controlled temperature (21-23 ºC).
E.4.1 Arteriograph (PWV and PWA)

E.4.1.1 Device

The Arteriograph device is based on oscillometry principles. The device comprises a blood pressure cuff (available in 3 sizes), and a pump/sensor which the cuff is connected to via a rubber tube. This complex resembles a typical automatic blood pressure monitoring device, and in fact does the same job of recording the pressure waveforms by oscillometry. The sensor communicates via an infra-red port with a laptop PC where the dedicated software records and analyses the data (Figure E-2).

The device first measures blood pressures and then inflates the cuff around 35 mmHg above SBP when the pressure waveforms needed for main analyses are recorded. The software estimates the transit time by measuring the time interval between (possibly the peaks of) forward and reflected waves using an undisclosed commercial algorithm [31, 32]. Therefore a big difference from many other methods is deriving the transit time from waveforms recorded at a “single” site.

The Arteriograph assumes that the major wave reflection site is almost located at the aortic bifurcation. Therefore the time lag between the arrivals of forward and reflection waves at the brachial artery is the time needed for the wave to travel along the aorta to bifurcation and backward. The resulting transit time is halved to get approximate travelling in one direction. In the invasive validation of the device, the transit times were very close to that measured by intra-aortic catheters just above aortic valve and in the aortic bifurcation [31].

The device uses the surface length between sternal notch and symphysis pubis as an estimation of the aortic path between aortic valve and bifurcation. This is entered into the software which finally gives PWV.

The Arteriograph calculates brachial (brAIx) and central (aoAIx) augmentation indices using $P_2-P_1/PP$ formula taking $P_1$ and $P_2$ as the first and second inflection points on the brachial pulse waveform. Central SBP (cSBP) is estimated by an undisclosed commercial algorithm based on the correlation between the “late systolic shoulder” of
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**Arterial stiffness and central blood pressures**

brachial waveform $pSBP_2$ on the brachial waveforms and aortic systolic pressures derived from the inventors’ invasive study [31]. The device also reports brachial SBP, DBP, PP, and heart rate.

**Figure E-2. The Arteriograph principles of PWV measurement**

Adapted from [279].

**E.4.1.2 Protocol**

Arteriograph measurements were performed in supine position according to manufacturer instruction with the cuff wrapped around the left arm as tight as possible. While resting, the participants were given a description of what the test involved and how it felt, and were instructed not to move, or speak during the test.

The sternal notch to symphysis pubis length was measured using a flexible tape measure supplied by the manufacturer. The results were entered into the software on the laptop.

The cuff was tightly wrapped around the left arm. The device then performed a 3 minute automatic measurement. The results were checked and such measurements were
Methods

Arterial stiffness and central blood pressures

repeated until at least 2-3 acceptable readings (i.e. low standard deviation of aortic PWV) were recorded; the software warns of low quality measurements.

TensioMed Software 1.10.1.1 was used to derive the indices. The results were exported into an Excel sheet and indices averaged for the repeated measurements.

E.4.1.3 Repeatability

The Arteriograph measurements were also performed by the same operator on the second visit after cardiac MRI to compare with MRI-measured aortic PWV. This created the opportunity to test the different–session repeatability, as this had not been well reported for the device before. We selected the measurements in 20 participants whose MRI visit were less than 10 weeks (mean±SD interval: 4.9±2.8 weeks; range: 0-9 weeks) apart from the first visit, assuming that baseline PWV would remain constant during such interval.

The coefficient of variation (intra-class correlation) of the Arteriograph different–session repeatability was 5.1%(0.87) for aortic PWV, 4.5%(0.88) for transit time, 5.3% for central (aortic) SBP (0.90), 14%(0.85) for aortic AIx, 4.6%(0.89) for brachial SBP, and 4.8 (0.88) for DBP. Intra-observer variability of the sternal notch-symphysis pubis measurement was 2.4%(0.90).

E.4.2 SphygmoCor (PWA)

E.4.2.1 Device

The SphygmoCor device is based on applanation tonometry principles. It uses a metallic pen-like tonometry probe connected to a PWA electronic module communicating with a laptop with SphygmoCor software (SCOR) installed (Figure E-3). Pressure wave forms are recorded on a peripheral surface-accessible artery such as radial artery. Such waveforms are calibrated by separately measured blood pressures fed into the software. Two calibration modes exist using SBP and DBP or MAP and DBP. Such values have to be entered into the software before the measurement. However, it is possible to change them later and recalculate (i.e. recalibrate) the results.
The software uses a mathematical transfer function to guess and reconstruct the pressure waveforms in aorta based on previous invasive validations. It reports many indices including peripheral (e.g. radial) and central (aortic) blood pressures, and AIx (Figure E-4).

Radial AIx is calculated as \( \frac{P_2 - DBP}{P_1 - DBP} \), taking \( P_1 \) and \( P_2 \) as the first and second inflection points on the radial pressure waveform. Calculation of aoAIx is performed using \( \frac{P_2 - P_1}{PP} \) similar to the Arteriograph. AIx values are reported with and without adjusting for a heart rate of 75.

**E.4.2.2 Protocol**

In this project, SphygmoCor was used to get central pressures and reflection wave indices, and not for PWV. All measurements were performed by the same trained observer (i.e. the PhD student).
Methods

Arterial stiffness and central blood pressures

After enough rest (> 5 min) in supine position, blood pressures were measured using a standard automatic Omron blood pressure monitoring device, and repeated until stable (<5 mmHg different). SBP and DBP of two last measurements were averaged and entered into SCOR software. The tonometry probe was perpendicularly applied on the left wrist over the radial artery. Strong signals with uniform waveforms were recorded. The results were checked based on manufacturer recommendation guided by the “operator index” that software reports. Attempts were made to make up to 3 quality measurements with operator index > 80. The data were analysed by SCOR-2000 software, version 7.1 and exported as spreadsheet where the quality measurements (operator index >80) were filtered and averaged.

E.4.3 Omron HEM-9000 (PWA)

E.4.3.1 Device

The Omron HEM-9000 (Figure E-5) has an automatic tonometry probe that is wrapped around the wrist. This is integrated into an arm support to facilitate the positioning of the wrist. A blood pressure cuff is also provided to be wrapped around the contra-lateral arm for blood pressure measurement and waveform calibration. Both of the former components are connected to the main unit which acts as a pump, analysis and display unit. The unit stores the database of the measurements on a memory card.

After wrapping the tonometry probe around the wrist guided by some landmarks, the probe attempts to find the strongest radial pulse signal, and record radial waveforms. If...
the quality of the signal is not good, it will warn and guides on how to adjust the wrists probe. When a good signal is detected, the waveforms are recorded and calibrated by automatic contra-lateral brachial blood pressure measured immediately afterwards. It then applies an algorithm based on a linear regression model to estimate cSBP from the “late systolic shoulder” (pSBP₂) of the radial pulse waveform which has been shown to agree closely with cSBP [54, 67]. The device uses the maxima of the “multidimensional derivatives” on the recorded pressure waveforms to detect first and second inflection points corresponding to early and late systolic (pSBP₂) pressures [54, 280].

Like SphygmoCor, Omron HEM-9000 calculates radial AIx as (P₂ - DBP)/(P₁ - DBP), taking P₁ and P₂ as the first and second inflection points on the radial pulse waveform. It also reports AIx adjusted for a heart rate of 75.

**E.4.3.2 Protocol**

The participant rested supine and were given instructions to keep calm and quiet during the test and what they would expect during the measurement. The measurements were performed on the left wrists with the arm extended and supported on the arm rest with the blood pressure cuff wrapped on the right arm.

Data were exported from the memory card inside the main unit using Omron exporting software.
E.5 Echocardiography

E.5.1 Image acquisition

Trans-thoracic echocardiography was performed by the same cardiologist for all participants using a 2-4 MHz phased-array probe on a Philips/ATL HDI-5000 ultrasound machine (Philips Medical Systems, Netherlands & ATL, Bothel, WA, USA) based on general guidelines of American Society and European Association of Echocardiography [117]. The participants were asked to lie in supine or left lateral decubitus position depending on the ultrasound view.

Standard M-mode, B-Mode 2D with tissue harmonic imaging, and pulsed-wave and tissue Doppler still images and cines (i.e. videos) were acquired and digitally stored in DICOM format on a hard drive for offline analysis later. All echocardiograms were anonymous tagged by patients’ study ID and initials.

The views taken included:

1) LV para-ternal long axis (PLX) M-Mode
2) LV PLX 2D plus:
   a. Colour Doppler to check mitral valve flow
3) LV para-ternal short axis (PSX) 2D
4) LV PSX M-Mode (as a back-up for PLX M-Mode)
5) Apical 4-chamber view (A4C) plus:
   a. Tissue Doppler imaging of septal and lateral mitral annulus longitudinal excursion velocities (e’)
   b. Pulse wave Doppler of trans-mitral velocities (e.g. E and A velocities)
6) Apical 5-chamber view (A5C) plus:
   a. Colour Doppler for abnormal aortic and mitral flows
   b. Pulse wave Doppler for aortic outflow velocities
7) Apical 2-chamber view (A2C) if possible
Methods

Echocardiography

Sample images from some of the above mentioned views are shown below.

Figure E-6. Sample echocardiograms

*Top:* 2D PLX (para-sternal long axis) view.
*Bottom left:* 2D PSX (para-sternal short axis).
*Bottom right:* M-mode PLX image, guided by 2D PLX or PSX views, and used for LV mass measurement.


Figure E-7. Sample echocardiograms

*Top:* A4C (apical 4 chamber) view which guides acquisition of pulsed-wave Doppler of mitral valve inflow velocities (*Bottom left*) and tissue Doppler imaging of mitral annulus longitudinal motion (*Bottom right*).

Colour Doppler was performed in PLX, A4C and A5C views to check for any significant (i.e. moderate to severe) valvular dysfunctions (e.g. aortic/mitral stenosis and regurgitation) potentially capable of altering LV structure (e.g. hypertrophy).
E.5.2 Image analysis

Image analysis was performed by the same observer offline and blinded to patient identity using AccessPoint 2004 software (Freeland Systems, USA). LV internal dimensions in end-systole (LVIDs) and end-diastole (LVIDd), and end-diastolic posterior wall (PWTd) and septal (IVSTd) thickness were measured in M-mode PLX view. If PLX images were not available, short axis M-mode values were used. LV mass was calculated using Devereux’s formula. Relative wall thickness (RWT) was calculated as 2*PWTd/LVIDd.

LV end diastolic and systolic volumes were measured using single-plane Simpson’s method (see C.2.3.1.2.1) in A4C, and were used to calculate stroke volume (SV) and ejection fraction (EF). Left atrial end-systolic volume (LAV) was measured using Simpson’s single-plane method in apical 4-chamber view. Cardiac mass and volumes were divided by body surface area (BSA) to get body-size adjusted values. LV hypertrophy (LVH) was defined as LVMI>115 g/m$^2$ and concentric geometry as RWT>0.42 [117]. Four LV geometry categories were defined as 1) concentric hypertrophy: LVH plus concentric geometry, 2) concentric remodelling: normal LVMI plus concentric geometry, 3) eccentric hypertrophy: LVH plus normal RWT, and 4) both normal LVMI and geometry.

Peak velocity of trans-mitral blood flow (E) was measured by pulsed-wave Doppler in A4C view with the sample volume at the mitral leaflets’ tips. Tissue Doppler mitral annulus excursion velocities (e’) at septal and lateral sides in A4C view were measured and averaged.

E.5.2.1 Repeatability

Intra-observer variability for image analysis was measured on 6 randomly selected series. Coefficient of variation (intra-class correlation) of intra-observer variability for some important echocardiography measurements were LVM: 9%(0.91), RWT: 9%(0.77), EF: 5%(0.84), LAV: 11%(0.96), and e’: 5%(0.97).
E.6 Carotid ultrasound & IMT

E.6.1 Image acquisition

Carotid ultrasound protocol immediately followed echocardiography using the same Philips/ATL HDI-5000 ultrasound scanner without moving the participant. The protocol was performed in line with Mannheim Carotid Intima-Media Thickness Consensus (2004–2006) [95].

Images were acquired with a 7.5 MHz L12-5 linear wide-band probe. The participant was supine, and the neck extended and rotated 45° to the opposite side. A quick short axis carotid artery survey was followed by longitudinal views of common carotid artery. Attempts were made to get the images with the carotid bulb visible (i.e. as a landmark for image analysis) and the intima media layers as sharp as possible. Images were taken from both sides of the neck, and stored anonymously on a PC hard drive for later blinded offline analysis. Images from at least 2 cardiac cycles were recorded.

E.6.2 Image analysis

Image analysis was performed based on Mannheim consensus. IMT was measured on the far wall of the distal 10 mm of both right and left common carotid arteries [93, 95]. State-of-the-art semi-automatic dedicated edge-detection software (Philips QLab software, Philips, Netherlands) was used to get average IMT along a standard range (10 mm) over CCA of each side (Figure E-8). IMT measurements were avoided in a plaque region. A plaque is defined by Mannheim Consensus as “a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value, or demonstrating a thickness of 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface” [95]. As arterial wall thickness and IMT varies with vessel distension during each pulse (cardiac cycle), the IMT measurements were repeated at maximal and minimal vessel diameter, and the results were averaged. This practice has been shown to increase reproducibility [98].
Methods

Carotid ultrasound & IMT

Figure E-8. Semi-automatic CIMT measurement (QLab software)
A screen shot of Philips QLab software showing automatic IMT measurement over a 10 mm range in common carotid artery in an image from this study. The software automatically detects intima-lumen and media-adventitial edges on the far wall and averages IMT over the measured range. The operator may change the calliper location.

E.6.2.1 Repeatability

Though QLab does a semi-automatic analysis, it matters where the observer places the calliper on the carotid wall (i.e. how far from the bulb), or how a plaque-free area is judged. Therefore intra-observer variability for CIMT image analysis was measured. Repeat measurements were performed by the same observer (PhD candidate) on 26 randomly selected series blinded to previous results and participant identity. Coefficient of variation (intra-class correlation) of intra-observer variability were 3.8%(0.98) for right,: 1.4%(1.0) for left, and 2.3%(0.99) for average CIMT.
E.7 Cardiovascular MRI

MR scans were performed on a 1.5T Philips Intera (Philips Medical Systems, Best, The Netherlands). The protocol included both cardiac and aortic acquisitions taking about 45 minutes.

In summary, the CMRI protocol aimed to quantify LV mass, stiffness and remodelling as well as aortic stiffness. The following items were measured:

1. LV mass index and geometry
2. LV myocardial contractility and strain (i.e. surrogate of ventricular stiffness)
3. LA volume (i.e. a marker of LV diastolic dysfunction)
4. Aortic PWV and distensibility (i.e. regional and local indices of aortic stiffness, respectively)

E.7.1 Cardiac protocol

E.7.1.1 Image acquisition

E.7.1.1.1 LV mass and volumetry

All images and cines were taken using steady-state free precession (SSFP) MR sequences. All cines included 30 heart phases.

From an appropriate axial scout, a (single-oblique) vertical long axis slice was taken closely passing through apex and mitral leaflet tips (Figure E-9, top left). From this slice, a second oblique slice was planned again passing through apex and mitral landmarks to give a “true” horizontal long axis slice halving the LV (Figure E-9, top right). Image acquisition parameters were: flip angle: 60°, slice thickness: 8 mm, matrix: 192x256, field of view (FOV): 320mm, repetition/echo time (TR/TE): 3/1.5ms.

Six equally spaced short axis slices (perpendicular to LV long axis) were planned from the latter horizontal long axis slice with the most basal slice passing through the most
basal part of the myocardium and the most apical slices touching the apical endocardium (matrix: 160x256, thickness: 6 mm) (Figure E-9, top right). The slice separation was calculated for each individual based on ventricular length (variable) and slice thickness (constant).

Figure E-9. Cardiac short and long axis anatomical series by MRI
A series of short axis images (bottom) were planned using two long axis views (top)

From a basal short axis slice, three radial long axis LV slices were planned 60 degrees apart to longitudinally cover round the LV (Figure E-10). These along with short axis series were used to reconstruct LV 3D model.
Figure E-10. Cardiac anatomical series, radial long axis planning

E.7.1.1.2 Tagging

To measure intra-myocardial circumferential strain, a respiratory-gated free-breathing SPAMM (spatial modulation of magnetization) protocol was used to grid-tag the myocardium in a mid-LV short axis slice as described before [281] (flip angle 13°, thickness: 8mm, matrix: 192x256, FOV: 320mm, TR/TE: 5/2ms).

Figure E-11. Myocardial tagging
Grid tagged mid-LV short axis images of the heart. Note that in the systolic phase (right) the tags (i.e. grids) are distorted in the myocardium compared to early diastole (left). Compare this to unchanged grids in the surrounding static organs. Such contortions are quantified to get myocardial tissue deformation.

E.7.1.2 Image analysis

LVM and volume was calculated throughout the cardiac cycle using three-dimensional guide-point modelling. Briefly, a three-dimensional finite element model of the left ventricle was adaptively optimized to fit each subject’s image dataset using custom
Methods

Cardiovascular MRI

software (CIM version 7.3, Auckland MRI Research Group). This method has previously been validated in animals against autopsy LVM, in patients with regional wall motion abnormalities, against manually drawn contours, and in healthy volunteers against flow-derived measures of cardiac output [282]. The finite-element model was interactively fitted to ‘guide-points’ provided by the analyst, as well as image derived data points contributed by an image processing algorithm. LV volume was calculated at all frames of the cardiac cycle by numerical integration of the curved surfaces represented by the finite-element model. Papillary muscles and ventricular trabeculations were included with the blood pool. LVM was measured at all frames in the cine acquisition by subtracting the epicardial volume from the endocardial volume, and multiplying by an assumed myocardial density of 1.04 g/ml. At the completion of the analysis, the end-systolic frame was chosen as the frame with the minimum volume.

LV concentricity (geometry) was quantified as M/V ratio by dividing LV end-diastolic mass by its end-diastolic volume:

$$MV \text{ ratio} = \frac{LVEDV}{LVM}$$

Systolic function as ejection fraction was calculated using end-diastolic and end-systolic endocardial volumes by:

$$EF = \frac{LVEDV - LVESV}{LVEDV}$$

The left atrial volume was determined by identifying endocardial points on the three long axes (in 3 radial long axis slices described above), fitting a 3D ellipsoid to these points and calculating its volume.

The tagged slice was analysed in the custom software (CIM version 7.3, Auckland MRI Research Group) [282] to measure circumferential strain ($E_{cc}$) in 6 regions of the LV short axis: anterior, inferior, anterior lateral, posterior lateral, inferior septum, and anterior septum. Strain was calculated in percents using the formula with $L_2$ and $L_1$ being the length of the contracting/relaxing myocardium [152]:

$$E_{cc} = \frac{L_2 - L_1}{L_1}$$
E.7.2 Aortic protocol

E.7.2.1 Image acquisition

Standard planning scouts were followed by an ECG-gated breath-held para-sagittal SSFP cine of the aorta from the aortic root to the proximal thoracic aorta (Figure E-12.a). The MR image acquisition parameters were: flip angle 60º, thickness 8 mm, matrix 176x256, FOV: 320mm, TR/TE: 2.8/1.4ms, reconstructed voxel size 1.25x1.25 mm.

The exact location of the aortic bifurcation was visualized by a coronal non-breath-hold PC imaging cine (flip angle 15º, thickness: 60 mm, matrix: 128x256, FOV: 450 mm, velocity encoding (VENC): 90cm/s in three separate directions: right-left, anterior-posterior and head-foot, TR/TE: 7.5/4.4ms) providing 16 heart phases per cardiac cycle; see Figure E-12.b.

A further 10-20 contiguous sagittal slices were acquired from the aortic arch to the bifurcation to define aortic geometry and allow aortic length to be measured (flip angle: 50º, thickness: 5 mm, matrix: 179x256, FOV: 500mm, TR/TE: 2.8/1.2ms, reconstructed voxel size: 1.95x1.95mm); see Figure E-12.c.

Two free-breathing through-plane PC acquisitions were planned orthogonal to the aorta at 1) the aortic arch at the level of the pulmonary artery bifurcation, and 2) approximately 2 cm above the aortic bifurcation (Figure E-12 and Figure E-13) (flip angle: 10º, thickness: 8 mm, matrix: 160x256, FOV: 320 mm, reconstructed voxel size: 1.25x1.25mm, TR/TE: 5.4/3.1 ms, and VENC: 200cm/s) to give 75 heart phases per cardiac cycle. These locations were chosen to measure PWV over most of the arch and descending aorta in the minimum total scan time. The scans were acquired sequentially at the conclusion of the MR protocol to minimise the effect of any heart rate or blood pressure changes and to minimise the interval to the post-scan comparative Arteriograph measurements (see below).
Figure E-12. Position of MRI phase-contrast aortic slices
a) This sagittal proximal aorta cine was used to help appropriate positioning of the upper phase-contrast (PC) slice. b) This angiographic coronal image helped precise mapping of the aortic bifurcation, guiding positioning of the lower PC slice above aortic bifurcation. c) A sample mid-slice of a series of sagittal aortic still images taken for aortic length measurement; these series could be analyzed in a 3D mode too (see Figure E-15).
To compare MR-measured aortic PWV with that by the Arteriograph device, Arteriograph measurements were performed immediately after the MR scans. The device could not be taken into the scan room due to the magnetic field and safety measures. To minimize changes in blood pressure and heart rate between the MR and Arteriograph measurements, participants rolled gently from the scanner bed onto a non-magnetic trolley of the same height and were moved outside the scan room where 2-3 consecutive left arm Arteriograph measurements were made as described in E.4.1.2.

E.7.2.2 Image analysis

Aortic length and flow image analysis was performed offline by a single analyst (PhD candidate) blinded to participant identity and Arteriograph results using Philips Extended MR Workspace Software which works integrated with the Philips Intera scanner. Aortic flow and cross-sectional area for all phases of the cardiac cycle were measured from the PC images in three regions of interest: ascending aorta, proximal descending aorta and aortic bifurcation (see P₁-P₃ in Figure E-13). Cross section contours of the aorta were automatically traced by the software with minimal supervision by the analyst, and the flow and area data for the cardiac cycle (Figure E-13) exported to an Excel spreadsheet (Figure E-14). Residual eddy currents were corrected in the image acquisition phase by the “local phase correction” filter of the scanner.

(i) Transit time: The arrival time of the velocity wave was determined using “10% of the upstroke” (i.e. wave height) foot detection algorithm [30] on each of the interpolated flow/time curves. This was performed in MS Excel sheet using XatY function of a mathematical Excel package [283]. The function returns the X axis value at a specified Y axis of an interpolated curve on a XY plot. On a flow-time plot (Y: flow, X: time), this was used to get the time (on X axis) at which the flow wave height (on Y axis) reached 10% of its maximum, thereby representing the foot of the wave or arrival time (Figure E-14). By subtraction, three aortic transit times (TT) could be calculated: arch to proximal descending aorta (TT_{Arch}; from P₁ to P₂), proximal descending aorta to bifurcation (TT_{Desc}; P₂ to P₃), and arch to bifurcation (TT_{Arch+Desc}; P₁ to P₃).
Figure E-13. Aortic PWV measurement by MRI

Left: Anatomic position of the two phase-contrast slices recording aortic flow at P₁ (ascending aorta), P₂ (proximal descending aorta) at the level of pulmonary artery bifurcation, and P₃ approximately 2 cm above aortic bifurcation (Bif). The 3 aortic regions and relative computable PWVs are marked.

Middle: the resulting two short axis slices with arterial contours marked.

Right: The flow waveforms resulting from P₁-P₃. AV: aortic valve.
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Figure E-14. Foot of wave detection algorithm

a) 10% of upstroke algorithm finds the time when the flow reaches 10% of its maximum and assigns this as a fixed comparable time handle (arrival time) on each flow wave. Such arrival times are computed from each flow waveforms recorded at arterial sites P1-P3 (see Figure E-13). Transit times are then calculated from subtractions.

b) Excel screenshot showing the data imported from MR flow analysis software, and the 3 waveforms and the arrival times for P1-P3 (dotted circle at top centre) computed by the above algorithm.
(ii) Path length: Aortic path length was measured using the sagittal images and the 3D volumes rendered from the sagittal aortic slices. The points were identified near the aortic valve (AV) and at the ascending (P₁), proximal descending (P₂) and bifurcation (P₃), with the latter three coincident with the PC imaging planes above. Path lengths were then calculated as AV-P₁, LEN_{Arch}=P₁-P₂, LEN_{Desc}=P₂-P₃, and LEN_{Arch+Desc}=P₁-P₃. Total aortic length (LEN_{Total}) was calculated by summing all segmental lengths from AV to Bif including AV-P₁ and P₃-Bif.

(iii) PWV: The three MR PWV values were calculated by dividing the regional path lengths by their corresponding TT (Figure E-13). For instance PWV along the aortic arch was calculated by:

$$PWV_{Arch} = \frac{LEN_{Arch}}{TT_{Arch}}$$

(iv) Distensibility: Local aortic distensibility was calculated with the formula:

$$D = \left( \frac{A_{max} - A_{min}}{A_{min}} \right) \times \frac{1}{PP}$$

where $A_{max}$ and $A_{min}$ (mm$^2$) are the maximum and minimum cross-sectional areas, and PP (mmHg) is central pulse pressure provided by the immediate post-MR Arteriograph measurement. Three aortic distensibility values could be calculated at P₁, P₂ and P₃.
E.7.2.2.1 Repeatability

Intra-observer variability of the MR image analysis was measured with the same observer (i.e. PhD candidate) repeating the analysis of 10 randomly selected subjects, blinded to participants’ identity and previous measurements, two months after the initial analysis. Repeatability results are listed in Table E-5.

Table E-5. Intra-observer repeatability results for MR image analysis

<table>
<thead>
<tr>
<th>Measurement</th>
<th>CV (ICC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV&lt;sub&gt;Arch&lt;/sub&gt;</td>
<td>7.8% (0.91)</td>
</tr>
<tr>
<td>TT&lt;sub&gt;Arch&lt;/sub&gt;</td>
<td>7.9% (0.83)</td>
</tr>
<tr>
<td>PWV&lt;sub&gt;Desc&lt;/sub&gt;</td>
<td>3.4% (0.96)</td>
</tr>
<tr>
<td>TT&lt;sub&gt;Desc&lt;/sub&gt;</td>
<td>3.2% (0.96)</td>
</tr>
<tr>
<td>PWV&lt;sub&gt;Arch+Desc&lt;/sub&gt;</td>
<td>2.5% (0.98)</td>
</tr>
<tr>
<td>TT&lt;sub&gt;Arch+Desc&lt;/sub&gt;</td>
<td>2.4% (0.97)</td>
</tr>
<tr>
<td>Dist&lt;sub&gt;P1&lt;/sub&gt;</td>
<td>9.4% (0.97)</td>
</tr>
<tr>
<td>Dist&lt;sub&gt;P2&lt;/sub&gt;</td>
<td>8.1% (0.94)</td>
</tr>
<tr>
<td>Dist&lt;sub&gt;P3&lt;/sub&gt;</td>
<td>10.5% (0.95)</td>
</tr>
<tr>
<td>LEN&lt;sub&gt;Total&lt;/sub&gt;</td>
<td>0.7% (0.99)</td>
</tr>
</tbody>
</table>

CV: coefficient of variation, ICC: intra-class correlation, PWV, TT and Dist: transit time, pulse wave velocity and aortic distensibility measured by MR for aortic regions (levels) defined in Figure E-13.
E.8 Hormonal Measurements

E.8.1 Vitamin D

Vitamin D was measured in serum frozen samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS), one of the most precise methods available in the UK. The details of the protocol are described in Knox et al [284], and the measurements were performed by the lab where the method was initially developed. In brief, following protein precipitation of the sample with methanol and 5 minute centrifugation, the sample was poured into a sample tray of an automated solid-phase extraction (SPE) platform (CTC PAL [Presearch] for ITSP™ SPE [MicroLiter Analytical Supplies, Inc], Suwanne, Georgia, USA). This robotic platform conducted a six-step SPE process. The samples were purified and injected into an LC-MS/MS system (Waters® UPLC® with the ACQUITY® TQD [Waters Corporation, Milford, MA, USA]). 25(OH)D3 and 25(OH)D2 were quantified by electro-spray ionization MS/MS in the multiple-reaction monitoring mode [284].

The minimum quantitation limit was 4.0 nmol/L for 25(OH)D3, and 7.5 nmol/L for 25(OH)D2. Intra- and inter-assay coefficients of variation were <10% in concentration ranges of 22.5–120 nmol/L for 25(OH)D3 and 17.5–70 nmol/L for 25(OH)D2 [284].

E.8.2 Aldosterone

Serum aldosterone was measured by radio-immunoassay (Coat-A-Count aldosterone; Siemens Medical Solutions Diagnostics, Los Angeles, CA), on the same frozen samples and by the same lab as vitamin D. The procedure was performed as recommended by the manufacturer. The Coat-A-Count is a solid-phase radioimmunoassay which uses aldosterone-specific antibody. Intra- and inter-assay coefficients of variation were 2.5–5.4%, and 3.8–15.7%, respectively. According to the manufacturer, the normal expected ranges of serum/plasma aldosterone on normal sodium intake are 111-860 pmol/L in standing, and 28-444 pmol/L in recumbent position. However, the references range may vary by each lab.
F) Results

The results are formatted as six papers in two general themes. The first theme includes papers 1 to 4 reporting the core results of this project on the association of the imaging and hormonal biomarkers in a multi-ethnic background.

The second theme, papers 5 and 6, relates to the technical hemodynamic aspects of arterial stiffness measurement by the relatively new Arteriograph device as the main device providing arterial stiffness in this project, and comparisons with some conventional methods.

Each paper has its independent pagination and bibliography. Different referencing and pagination styles used for the papers distinguish them from the rest of the thesis.
Ethnic Differences in Aortic Pulse Wave Velocity Occur in the Descending Aorta and May Be Related to Vitamin D
Ethnic differences in aortic pulse wave velocity occur in the descending aorta and may be related to vitamin D

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Abstract

We studied aortic pulse wave velocity (aPWV), a predictor of cardiovascular events independent of blood pressure (BP), in a multi-ethnic sample of British men, to investigate the roles for blood levels of vitamin D and aldosterone in total and regional aortic stiffness.

Total aPWV was estimated non-invasively by the Arteriograph device (aPWVag) in 198 men with its length measure calibrated by magnetic resonance (MR). PWV over the aortic arch (archPWVmr) and descending aorta (desPWVmr) were measured by MR in a subsample (n=47).

Mean(SE) aPWVag in South Asians (SA) (n=68, age 55±10yr), at known higher coronary disease (CHD) risk than other groups, was 0.5(0.2)m/s higher than in African-Caribbeans (AfC, n=67, 55±10yr), at lowest CHD risk here, and Europeans (n=63, 57±8yr), adjusted for age, systolic BP and diabetes (p=0.01). By MR, desPWVmr in SA was 0.7(0.3) and 0.8(0.3)m/s higher than in AfC and Europeans, respectively; archPWVmr was not different. SA and AfC had 21(3) and 14(3)nmol/L lower mean(SE) 25(OH)D than Europeans (p<0.001). Unlike aldosterone, 25(OH)D was negatively correlated with aPWVag adjusted for age and systolic BP, and weakened or removed ethnic differences in aPWVag in regression models.

These data suggest that aortic stiffness as aPWV parallels CHD risk in ethnic groups, descending aortic but not arch PWV has this feature, serum 25(OH)D is an independent negative correlate of aPWV and may partly account for ethnicity-related differences in aPWV and CHD risk.

**Keywords:** Vitamin D, aorta, pulse wave velocity, ethnic groups, aldosterone
Introduction

Cardiovascular morbidity and mortality vary among different ethnic groups. People of Indian sub-continental, or South Asian, origin (SA) in Britain and North America have higher coronary heart disease (CHD) prevalence and mortality, experiencing events at a younger age and slower declines in premature CHD mortality than other populations 1-4 (Web supplement Figure S-a; see http://hyper.ahajournals.org). Conventional risk factors such as diabetes, obesity and hypertension fail to explain these ethnic differences fully 5. In contrast, African-Caribbeans (AfC) have lower CHD mortality than Europeans despite higher rates of hypertension, diabetes and stroke 2, 6, 7 (Web supplement Figure S-b; see http://hyper.ahajournals.org). Despite sharing excess hypertension and diabetes, a similar heritage and genetic background with AfC, African-Americans (AfAm) have a different vascular profile further along the epidemiologic transition to atheromatous disease than AfC 7, 8.

There has been little comparative data on arterial function of these populations. Aortic pulse wave velocity (aPWV), an index of arterial stiffness, is a powerful independent predictor of cardiovascular events and death 9-12. We studied aPWV and its determinants in a sample of men from three ethnic groups to investigate the following questions: 1) Do differences in aPWV across ethnic groups parallel their known coronary risk profiles? If so, do proximal and distal aPWV measured by more precise magnetic resonance (MR) imaging differ similarly? 2) Are serum vitamin D or aldosterone concentrations related to aPWV as a risk marker, and do they influence cross-ethnic aPWV differences?

Methods

Participants were 198 men aged 40-80 years of AfC, SA and European origin in Manchester, UK who had already been recruited to European Male Ageing Study (EMAS) 13. The participants had to be free of severe chronic or acute disease of active malignant, renal or liver origin. Ethnicity was defined by participants’ self-reporting, and 3 out of 4 grand-parents being of the same ethnic origin. Original recruitment was through volunteers by advertising in community centres and media in Manchester for SA and AfC groups and by general practice register sampling for Europeans.
**Results**

**Main study**

All participants had non-invasive oscillometric arterial stiffness and blood pressure (BP) measurement in the morning after avoiding caffeine, tobacco and heavy meals 3 hours before their visit, and alcohol from the night before. Using a standard protocol, BP was measured using a validated Omron semi-automatic device on the left upper arm, sitting, 3 times after 5 minutes rest in a temperature controlled room. The values from last two measurements were averaged and recorded.

The Arteriograph® (TensioMed, Budapest, Hungary) device was used to measure arterial stiffness indices including aPWV, estimated central (aortic) AIx (aoAIx) and systolic blood pressure (cSBP). The device records pressure waves in the brachial artery with an arm cuff, and estimates the aortic pulse wave transit time between the aortic root and bifurcation from the time interval between the peaks of forward and reflection pressure waves in each cardiac cycle using a commercial algorithm. It assumes that the corresponding aortic path is approximated by sternal-notch to pubis distance on body surface.

The Arteriograph measurements were performed up to 3 times on the left arm after at least 5 minutes of rest supine after blood pressure measurement. Central augmentation pressure (cAP) was derived from aoAIx and central PP (cPP). The coefficient of variation (intra-class correlation) for Arteriograph aPWV, aoAIx and cSBP for separate sessions in our lab were 5% (0.87), 14% (0.83) and 5% (0.90).

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) was used to measure 25(OH)D3 on frozen serum samples as described before. Serum aldosterone was measured using radio-immunoassay (Coat-A-Count aldosterone; Siemens Medical Solutions Diagnostics, Los Angeles, CA).

**Magnetic resonance imaging (MRI) sub-study**

A subsample of the men in the main study free from diabetes and cardiovascular medications and without previous histories of cardiovascular events were invited for the MR study and seen under the same conditions. The MR protocol for PWV measurement used a 1.5 T Philips Intera scanner (Philips Medical Systems, Best, Netherlands) to acquire two consecutive non-breath hold, through-plane velocity-encoded, phase-
contrast transverse aortic cine images: one from the aortic arch at the level of pulmonary artery, the other 2 cm above the aortic bifurcation (Figure 1). Image analysis was performed offline with the same analyst blinded to patients’ identities. Arrival times of the aortic pulse waves were computed from the three flow-time curves recorded at the three points: \( P_1, P_2 \) and \( P_3 \) (Figure 1), from which three transit-times could be derived for \( P_1P_2, P_2P_3 \) and \( P_1P_3 \). The lengths of corresponding aortic paths were measured on MR images. This enabled measurement of three PWV values: over the aortic arch (archPWV\(_{MR}\)), descending aorta (desPWV\(_{MR}\)) and the overall segment (aoPWV\(_{MR}\)) (Figure 1). Intra-observer coefficient of variation was 7.8% (0.91) for archPWV\(_{MR}\), 3.4% (0.96) for desPWV\(_{MR}\), and 2.5% (0.98) for aoPWV\(_{MR}\), when MR image analysis was repeated for 10 participants.

Improving \( aPWV_{AG} \) estimation with MRI aortic lengths

Comparison of the MR-measured total aortic lengths (i.e. aortic root to bifurcation) to the surface sternal notch-pubis length used by Arteriograph (as externally estimated arterial pathway) showed that the latter would overestimate the real aortic path, and that it was possible to predict MR-measured total aortic lengths from a regression model derived from age and height. Consequently, the Arteriograph \( aPWV_{AG} \) was recalculated using the transit time measured by the device, and the length of aortic path estimated by the aforementioned regression model.

Statistical analysis

Data was analysed by R statistical package version 2.11.1 \(^{16}\). Analysis of variance (ANOVA) was used to compare means of parametric (and transformed skewed) variables across ethnic groups. PWV and hormonal correlates were investigated with robust regression models \(^{17,18}\) with dummy variables (for ethnicity) adjusting for confounders with results expressed as unstandardized coefficients (B) and standard errors (SE). Multiple-testing errors were corrected for 3 pair-wise comparisons by a lower p-value, <0.02 (i.e. 0.05÷3). Spearman \( \rho \) was used for correlation between non-parametric variables.
**Results**

**Main study**

In total 67 AfC, 68 SA and 63 Europeans were measured. Most SA were of Pakistani origin (84%) with 7% Indian and Bangladeshi. All AfC were of Caribbean origin and African descent. Age and BMI were not statistically different between groups (Table 1). SA had more diabetes than AfC with none among Europeans who smoked less. Similarly, SA had higher fasting plasma glucose (FPG) while AfC had lower TC/HDL ratio and higher serum creatinine (Table 1).

**Arterial stiffness and BPs**

In univariate analysis, SBP and MAP were only borderline significantly different across groups, but SA had higher $aPWV_{AG}$ than AfC and Europeans (Table 1, Figure 2-a). Adjusting for age, BMI and heart rate, AfC’s mean(SE) brachial SBP was 6.3(2.3), DBP 3.2(1.5) and mean arterial pressure (MAP), 4.3(1.7) mmHg higher than SA. AfC’s brachial PP 3.1(1.4) mmHg was higher than both SA and Europeans. cSBP and cPP were not different.

Adjusted mean(SE) $aPWV_{AG}$ in SA was almost 0.5(0.2) m/s higher than in AfC and Europeans (Table 2) whereas AfC’s adjusted aoAIX and cAP were 7(2)% and 3.6(1) mmHg lower than SA and 7(2)% and 2.5(1) mmHg less than Europeans. Thus, SA had higher $aPWV_{AG}$ for given levels of age and distending pressures. Excluding diabetes, or replacing SBP with MAP did not change the ethnic effects for $aPWV_{AG}$ and aoAIX; entering heart rate in the $aPWV$ model (Model A, Table 2) slightly weakened but did not remove them (data not shown).

**Hormones**

Vitamin D and aldosterone were significantly different across the groups (Table 1 and Figure 3-a-b). Adjusted for age, weight, season of blood sampling, and vitamin D supplement use, mean(SE) 25(OH)D in SA was 21(3) and AfC 14(3) nmol/L lower than Europeans, and in SA lower by 7(2) nmol/L than AfC (p<0.001 for all).
Compared with AfC, aldosterone was 85(20) pmol/L higher in SA (p<0.001), and 51(21) higher in Europeans (p=0.015) after adjusting for anti-hypertensive use and BMI. SA had 34(22) pmol/L higher aldosterone than Europeans, but this was not significant (p=0.13) after adjustments. There was borderline inverse correlation between aldosterone and 25(OH)D (ρ =-0.14, p=0.06).

Arterial stiffness-hormonal relations

Neither hormone was correlated with BPs, PWV and aoAIX in univariate analysis. Adjusted for age, SBP and diabetes, 25(OH)D was inversely related to aPWV\textsubscript{AG} (B(SE)=-0.013(0.004) m/s, p<0.001), but not aoAIX (data not shown). With similar adjustments, the participants in tertiles 2 and 3 of 25(OH)D had 0.3(0.2) (p=0.1) and 0.5(0.2)m/s (p=0.015) lower aPWV\textsubscript{AG}, respectively compared to tertile 1. The correlations between aldosterone and aPWV\textsubscript{AG} were insignificant in similar models. Entering 25(OH)D in the model removed statistical ethnic differences in aPWV\textsubscript{AG}; such results were not observed for aldosterone (Table 2). An interaction-term between ethnicity and 25(OH)D in these models was not significant (not shown). Age adjustment was the main factor making 25(OH)D-aPWV\textsubscript{AG} relationship significant, notably in those >50 yr (Figure 3-c).

MRI sub-study

MRI study participants consisted of 16 Caribbean, 13 Pakistani and 18 European men whose age, BMI, brachial nor central BPs differed significantly with this relatively small sample size (Table 1). After adjusting for age and SBP, mean(SE) desPWV\textsubscript{MR} in SA was 0.7(0.3) and 0.8(0.3)m/s higher than in AfC and Europeans respectively; archPWV\textsubscript{MR} was not statistically different (Table 3). This pattern of ethnic difference in desPWV\textsubscript{MR} in the MR-study replicates the result for the aPWV\textsubscript{AG} in total sample (Figure 2-a-b), but the pattern for archPWV\textsubscript{MR} is different (Figure 2-c). Adjusted for age and SBP, higher aldosterone tertiles were associated with greater archPWV\textsubscript{MR}, but not desPWV\textsubscript{MR} (Table 3); this remained significant after adjusting for ethnicity.
Discussion

The results suggest firstly that SA men had higher aPWV than AfC and Europeans for the same levels of age and brachial/central distending pressures, so reflecting the known CHD population risk differences across ethnic groups. Despite slightly higher peripheral BPs, AfC had lower aPWV paralleling currently lower CHD risk in Britain and the Caribbean region. Secondly, the MR sub-study showed that pathology in the more elastic descending aortic segment, but not that of the aortic arch may be related to CHD risk. Thirdly, serum 25(OH)D was inversely related to aPWV independent of age and SBP; hence poor vitamin D status may account for some of the ethnic differences in aPWV, and potentially therefore some vascular disease in the population as a whole. Randomised trials to test that hypothesis are scarce.

Studies comparing arterial stiffness in SA, AfC and Europeans, particularly in community samples, are few. Our previous population-based work focused on comparing risks across glucose tolerance with relatively small numbers of controls found no difference in Doppler-measured aPWV down the descending aorta in AfC (10.1 m/s), Gujarati SA (10.5 m/s) and European (9.7 m/s) groups after adjusting for age and gender. However, those data were not adjusted for BPs, which were higher in AfC. Two studies reported higher arterial stiffness estimated by reflection wave indices among healthy and post-stroke SA subjects compared to their European counterparts. Neither measured aPWV.

We found no significant aPWV differences between AfC and Europeans. Similar results were found in different populations of AfAm and EurAm adults, using Doppler-measured aortic-femoral PWV, central aPWV, or arterial compliance by the Windkessel method. However, two other studies using the Complior device reported higher carotid-femoral PWV (cfPWV) in younger Brazilians of African descent and for British AfC compared with Europeans. The above inconsistencies may be due to different techniques, study populations, statistical approaches and arterial paths or length estimation methods used for PWV measurement, as we found in a recent European pooling project. Here we measured aPWV over a central aortic path.

Data on the relationship between vitamin D and PWV is scarce and limited to chronic kidney disease (CKD) patients, with inverse relationships found. Our study seems
the first to report such an association in a community-dwelling, multi-ethnic sample. A recent randomized trial (n=49) found a fall in cfPWV among AfAm teenagers given 2000 IU/day of vitamin D3 compared to those on 400 IU/day. These pilot results suggest promise for vitamin D as an intervention in black populations. Other data from disease-free or asymptomatic subjects primarily relates to endothelial function measured by brachial flow-mediated dilation which was positively correlated with serum 25(OH)D and improved on supplementation in deficient cases.

In our data, vitamin D was also negatively related with TC/HDL ratio, FPG and aldosterone. Including these variables in the regressions did not remove the independent correlation of vitamin D and aPWV (data not shown).

Effects of vitamin D on arterial stiffness may be via a variety of mechanisms, including endothelial function, RAS inhibition, regulation of parathyroid hormone, vascular calcification and matrix metalloproteinase activity. Paradoxically, pharmacological doses of some vitamin D receptor activators (e.g. calcitriol) may cause arterial calcification and stiffness in experimental models while others (e.g. paricalcitol) do not.

The “high-aldosterone” SA group in the total sample had higher aPWV than the “low-aldosterone” AfC, but without correlation between aldosterone and aPWV. Reports of aldosterone and PWV relationship are inconsistent. Circulating levels of aldosterone are variable, may not reflect local aldosterone inside the vascular wall and are affected by diurnal variations and anti-hypertensive medications.

Almost all SA and AfC and half of Europeans were vitamin D “deficient” (<50 nmol/L) here, as is described in Britain. Lower vitamin D among migrants of SA and African descent resident in temperate climates compared to Europeans is also well established.

Study limitations: Although the participants were community dwelling men, they were recruited as volunteers. The study sample is therefore not fully representative, although their BP and metabolic profiles are similar to those reported previously. Similarly, our MR sub-study results should be interpreted with caution as the numbers in each group are small. However, the MR sub-sample were free of known vascular disease, so these novel data generate hypotheses on the relative roles of regional differences in aortic PWV in relation to vascular events. In cross-sectional data, cause and effect between
low vitamin D or higher aldosterone levels and arterial stiffness cannot be assessed, and is in part confounded by the ethnic data. Participants were also not on standardized sodium diets.

**Perspectives**
Arterial stiffness here was greater among SA men, with lower aPWV values from both non-invasive and MR measures in AfC despite slightly higher BPs. As the data parallel CHD risk in these ethnic groups, aPWV may therefore add precision to risk estimation from BP alone. Vitamin D insufficiency may explain part of the ethnic differences in aPWV and cardiovascular risk.

**Acknowledgement**
The paper is dedicated to the memory of Professor Mike Wallace who died unexpectedly when this work was in progress. The authors wish to acknowledge the support of the Wellcome Trust Clinical Research Facility (WTCRF), Manchester where the field-work and MRI scans took place.

**Sources of Funding**
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**Disclosures**
The authors declare no conflicts of interest.
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### Table 1. General, hemodynamic and metabolic characteristics of the participants with univariate analysis (n=198) in mean±SD or median(IQR).

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (N=198)</th>
<th>MR sub –sample (N=47)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFC (N=67)</td>
<td>SA (N=68)</td>
<td>Eu (N=63)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55±10</td>
<td>55±10</td>
<td>57±8</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8 (12%)</td>
<td>24 (35%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hypertension Tx</td>
<td>8 (12%)</td>
<td>10 (15%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Dyslipidemia Tx</td>
<td>6 (9%)</td>
<td>13 (19%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>12 (19%)</td>
<td>11 (17%)</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84±11.0</td>
<td>79±11.0</td>
<td>85±14</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174±6</td>
<td>170±6</td>
<td>176±7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28±3</td>
<td>27±3</td>
<td>27±4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>130±16</td>
<td>124±14</td>
<td>126±13</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82±11</td>
<td>78±9</td>
<td>81±8</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>48±10</td>
<td>46±9</td>
<td>45±7</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>98±12</td>
<td>94±11</td>
<td>96±10</td>
</tr>
<tr>
<td>cSBP (mmHg)</td>
<td>127±20</td>
<td>125±19</td>
<td>124±12</td>
</tr>
<tr>
<td>cPP (mmHg)</td>
<td>46±11</td>
<td>45±11</td>
<td>45±8</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>65±8</td>
<td>68±11</td>
<td>61±8</td>
</tr>
<tr>
<td>archPWV&lt;sub&gt;MR&lt;/sub&gt; (m/s)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>desPWV&lt;sub&gt;MR&lt;/sub&gt; (m/s)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>aPWV&lt;sub&gt;AG or MR&lt;/sub&gt; (m/s)</td>
<td>6.8(1.7)</td>
<td>7.6(2.4)</td>
<td>6.9(1.6)</td>
</tr>
<tr>
<td>Variable</td>
<td>All (N=198)</td>
<td>MR sub−sample (N=47)</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------</td>
<td>----------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AfC (N=67)</td>
<td>SA (N=68)</td>
<td>Eu (N=63)</td>
</tr>
<tr>
<td>aoAIx (%)</td>
<td>27±13</td>
<td>33±13</td>
<td>33±10</td>
</tr>
<tr>
<td>cAP (mmHg)</td>
<td>13±9</td>
<td>16±9</td>
<td>15±7</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.3(0.9)</td>
<td>5.6(1.5)</td>
<td>5.0(0.6)</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>95±14</td>
<td>85±14</td>
<td>86±10</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.0±1.1</td>
<td>4.7±0.8</td>
<td>5.6±1.0</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.4(0.5)</td>
<td>1.1(0.3)</td>
<td>1.5(0.5)</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>3.6±0.9</td>
<td>4.4±1.1</td>
<td>4.0±1.1</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>30(16)</td>
<td>18(15)</td>
<td>43(22)</td>
</tr>
<tr>
<td>Aldosterone (pmol/L)</td>
<td>180(137)</td>
<td>295(160)</td>
<td>227(120)</td>
</tr>
</tbody>
</table>

* Significant p-values. † Chi-square p. Rest of p-values are from ANOVA test. ‡ P-values from ANOVA on reverse-transformed PWV values and log-transforms of other marked variables. AfC: African-Caribbean, SA: South Asian, Eu: European, Tx: treatment, FPG: fasting plasma glucose, TC and HDL: total and HDL cholesterol, IQR: inter-quartile range; other abbreviations defined in text.
**Table 2.** Regression models comparing $aPWV_{ AG}$ (m/s) between ethnic groups in total sample (n= 198). Note the change in regression coefficients regarding ethnic difference in $aPWV_{ AG}$ (*italic rows*) before (Model A) and after entering vitamin D (B) or aldosterone (C).

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Model A Ethnicity</th>
<th></th>
<th>Model B Ethnicity + 25(OH)D</th>
<th></th>
<th>Model C Ethnicity + Aldosterone</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>0.10(0.009)</td>
<td>$&lt;0.001^*$</td>
<td>0.11(0.009)</td>
<td>$&lt;0.001^*$</td>
<td>0.10(0.009)</td>
<td>$&lt;0.001^*$</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.02(0.005)</td>
<td>$&lt;0.001^*$</td>
<td>0.02(0.005)</td>
<td>$&lt;0.001^*$</td>
<td>0.02(0.005)</td>
<td>$&lt;0.001^*$</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.43(0.30)</td>
<td>0.15</td>
<td>0.42(0.29)</td>
<td>0.15</td>
<td>0.43(0.29)</td>
<td>0.14</td>
</tr>
<tr>
<td>AfC vs SA</td>
<td>-0.46(0.17)</td>
<td>0.008*</td>
<td>-0.37(0.18)</td>
<td>0.04</td>
<td>-0.46(0.18)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Eu vs SA</td>
<td>-0.55(0.18)</td>
<td>0.002*</td>
<td>-0.33(0.22)</td>
<td>0.13</td>
<td>-0.55(0.19)</td>
<td>0.004*</td>
</tr>
<tr>
<td>AfC vs Eu †</td>
<td>0.10 (0.15)</td>
<td>0.52</td>
<td>-0.03(0.17)</td>
<td>0.85</td>
<td>0.09(0.18)</td>
<td>0.59</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>-</td>
<td>-</td>
<td>-0.01(0.004)</td>
<td>0.03*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aldosterone (pmol/L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.00(0.00)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

* Significant p-values. Significance level for ethnic group differences (*italic rows*) is $<0.02$ after multiple-testing adjustment. †When the same model was run again with Eu as reference group.
Table 3. Three models adjusted for age and SBP comparing MR-measured regional aortic PWV (n=47) among 3 ethnic groups (A) or between the participants in higher and lowest tertiles of vitamin D (B) or aldosterone (C).

<table>
<thead>
<tr>
<th>Model</th>
<th>archPWV_{MR} (m/s)</th>
<th>desPWV_{MR} (m/s)</th>
<th>aoPWV_{MR} (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (SE)</td>
<td>p</td>
<td>B (SE)</td>
</tr>
<tr>
<td>A: Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AfC vs SA</td>
<td>0.54 (0.73)</td>
<td>0.46</td>
<td>-0.74 (0.31)</td>
</tr>
<tr>
<td>Eu vs SA</td>
<td>-0.24 (0.51)</td>
<td>0.64</td>
<td>-0.83 (0.28)</td>
</tr>
<tr>
<td>Eu vs AfC †</td>
<td>-0.78 (0.64)</td>
<td>0.23</td>
<td>-0.09 (0.22)</td>
</tr>
<tr>
<td>B: 25(OH)D (nmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 2 (23-34)</td>
<td>-0.11 (0.7)</td>
<td>0.87</td>
<td>-0.24 (0.35)</td>
</tr>
<tr>
<td>Tertile 3 (35-62)</td>
<td>-0.33 (0.57)</td>
<td>0.57</td>
<td>-0.41 (0.33)</td>
</tr>
<tr>
<td>C: Aldosterone (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 2 (178-279)</td>
<td>0.9 (0.4)</td>
<td>0.02*</td>
<td>0.02 (0.2)</td>
</tr>
<tr>
<td>Tertile 3 (280-684)</td>
<td>1.4 (0.6)</td>
<td>0.03*</td>
<td>0.25 (0.37)</td>
</tr>
</tbody>
</table>

* Significant p-values. Significance level for ethnic group differences (Model A) is <0.02 after multiple-testing adjustment. †When same model run with AfC as reference group.
Figures

**Figure 1.** Magnetic resonance aortic sagittal view shows 3 aortic paths (P₁P₂, P₂P₃, and P₁P₃), for which the 3 pulse wave velocities: archPWV<sub>MR</sub>, desPWV<sub>MR</sub> and aoPWV<sub>MR</sub>, were measured in a subsample of 47 men. AV: aortic valve, Bif: aortic bifurcation.
Results

Figure 2. PWV profiles across ethnic groups; bar charts show mean ±95%CI. Note the contrast between a-b and c, and compare to CHD risk profile in Web supplement: Figure S-a (please see http://hyper.ahajournals.org). AfC: African-Caribbean; SA: South Asian; WE: European
Figure 3. a and b) Box-plots for circulating 25(OH)D and aldosterone among ethnic groups. Thick horizontal lines are medians, lower and upper box borders are 25th and 75th percentiles, and whiskers, data range.
Figure 3. c) aPWV<sub>AG</sub> by 25(OH)D tertiles in 4 age groups among total sample. Qrt: quartile.

c) aPWV by 25(OH)D tertile for each age quartile
Web Supplement

Figure S. a) Men’s CHD prevalence by age and three ethnic groups in England, 2008, (adapted from data in 1) and b) Men’s stroke mortality trends, 1979-2003 by country of birth in England and Wales (adapted from data in 1, 2)
F.2  Paper 2: Cardiac remodelling, vitamin D and ethnicity

Low Vitamin D Levels Are Related to Left Ventricular Concentric Remodelling in Men of Different Ethnic Groups with Varying Cardiovascular Risk
Low vitamin D levels are related to left ventricular concentric remodelling in men of different ethnic groups with varying cardiovascular risk

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Abstract

Objectives: To investigate if circulating vitamin D is related to concentric remodelling among dark-skinned ethnic groups, given the difference in vitamin D status compared with European Caucasians.

Background: Despite mounting evidence on the relationship of vitamin D deficiency and cardiovascular disease, data on how vitamin D relates to cardiac remodelling is sparse.

Methods: In a community sample of 194 men: African-Caribbean (AfC) (n=64, age: 54±10yr), South Asian (SA) (n=68, 55±10yr) and European (n=62, 57±9yr), standard echocardiographic left ventricular (LV) mass (LVM) and geometry measurements were performed, with similar cardiac MR measurements in a sub-sample of 50.

Results: Concentric geometry, as relative wall thickness (RWT) >0.42, was more common among AfC (59%) and SA (49%) than Europeans (24%) (p<0.001). Mean(SE) RWT was 0.05(0.01) higher in AfC and SA, and mean(SE) 25(OH)D, 14(3) and 21(3) nmol/L lower than Europeans. In regression models, after adjusting for age, systolic blood pressure, diabetes and BMI, circulating 25(OH)D negatively correlated with indices of LV concentric geometry by echocardiography. A one SD (18 nmol/L) rise in 25(OH)D predicted a 0.21 SD drop in RWT (p=0.002).

More precise MR-measured concentric geometry as end-diastolic mass to volume ratio in the smaller sub-sample showed similar results, with a steeper fall in LV volume than mass as 25(OH)D levels declined.

Conclusions: Lower vitamin D status in men of AfC and SA origin compared to Europeans was inversely associated with LV concentricity. Trials are needed to test a cause-effect link.
Selected Abbreviations

BSA: body surface area
LVM: left ventricular mass
LVMI: left ventricular mass indexed to BSA
RWT: relative wall thickness
M/V: LV end-diastolic mass/volume ratio
LV: left ventricular
EDVI: end diastolic volume indexed to BSA
ESVI: end-systolic volume indexed to BSA
SVI: stroke volume indexed to BSA
EF: ejection fraction
LAVI: left atrial volume indexed to BSA
e’: tissue Doppler mitral annulus velocities
Introduction

South Asians and African-Caribbean people are prominent minorities in Britain and North America with different cardiovascular risk profiles from European Caucasians and African-Americans. South Asians have a higher incidence and earlier onset of coronary heart disease (CHD) while African-Caribbeans have lower rates of CHD despite higher blood pressures, stroke incidence and probably heart failure. Traditional risk factors only partially account for these differences.\(^{1-6}\)

Vitamin D deficiency is more common among African-Caribbeans and South Asians compared to Europeans in Europe, due to melanised skin and limited sunlight exposure.\(^{7-9}\) Mounting evidence on the relationship between vitamin D deficiency and adverse cardiovascular outcomes\(^ {10-12}\) raises the question whether ethnic differences in outcomes may be associated with vitamin D status. There is also a general paucity of data on the relationship of vitamin D status and cardiac geometry, especially in community samples free of overt disease.

We recruited a community sample of men from the three ethnic groups to investigate cardiac structure and function and their relation to circulating levels of vitamin D and aldosterone as a cardioactive hormone thought to be related to vitamin D deficiency.\(^ {13, 14}\) Our hypothesis was that left ventricular remodelling would be inversely related to circulating vitamin D but directly to aldosterone, independent of blood pressure and other confounders.

Methods

Participants were 40-80 year-old men of African-Caribbean, South Asian and European origin in Manchester, UK already enrolled in the European Male Ageing Study (EMAS).\(^ {15}\) Original recruitment was through advertising for volunteers in community centres and media in Manchester for South Asian and African-Caribbean groups and by general practice register sampling for Europeans. The participants did not have active malignancy or severe renal or liver disease. Ethnicity was defined by participants’ self-reporting, and 3 out of 4 grand-parents being of the same ethnic origin. The study
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protocol was approved by both regional and internal ethics committees, and informed consent was obtained from each participant.

**Blood Pressure**

Sitting blood pressure measurement was performed three times on the left arm within 40 minutes of the echocardiogram using a standard Omron semi-automatic device following at least five minutes of rest; the last two measurements were averaged.

**Hormonal measurements**

Serum frozen samples were used to measure 25(OH)D by liquid chromatography-tandem mass spectrometry (LC/MS/MS) as previously described, and aldosterone by radio-immunoassay (Coat-A-Count aldosterone; Siemens Medical Solutions Diagnostics, Los Angeles, CA).

**Echocardiography**

Trans-thoracic echocardiography was performed by a trained cardiologist (SB) using a 2-4 MHz phased-array probe on a Philips/ATL HDI-5000 ultrasound machine (Philips, the Netherlands) according to the American Society and European Association of Echocardiography guidelines. Standard M-mode, 2D and Doppler images were acquired and stored digitally for offline analysis.

Image analysis was performed by the same observer (SB) blinded to patient identity using AccessPoint 2004 software (Freeland Systems, USA). Left ventricular (LV) internal dimensions at end-systole (LVIDs) and end-diastole (LVIDd), end-diastolic posterior wall (PWTd) and septal (IVSTd) thickness were measured from the M-mode para-sternal long axis (PLX) view. If PLX images were not available, short axis M-mode values were used. LV mass (LVM) was calculated using Devereux’s formula at end-diastole. Relative wall thickness (RWT) was calculated as 2*PWTd/LVIDd. LV end diastolic and systolic volumes were measured using Simpson’s single-plane method from the apical four-chamber view, and used to calculate stroke volume (SV), ejection
fraction (EF) and left atrial end-systolic volume (LAV). Cardiac mass and volumes were divided by body surface area (BSA) to obtain body-size adjusted values.

LV hypertrophy (LVH) was defined as LV mass index (LVMI) >115 g/m² and concentric geometry as RWT >0.42. Four LV geometries were defined as (1) normal (RWT ≤0.42 without LVH), (2) concentric remodelling (RWT >0.42 without LVH), (3) eccentric hypertrophy (RWT ≤0.42 with LVH), and (4) concentric hypertrophy (RWT >0.42 with LVH).

Peak velocity of trans-mitral blood flow (E) was measured by pulsed-wave Doppler in the four-chamber view with the sample volume at the tips of the mitral leaflets, as were tissue septal and lateral Doppler mitral annulus velocities (e’) and averaged.

Intra-observer variability for image analysis was measured on six randomly selected participants. Coefficient of variation (intra-class correlation) of intra-observer variability for were LV mass (LVM): 9%(0.91), RWT: 9%(0.77), EF: 5%(0.84), LAV: 11%(0.96), and e’: 5%(0.97).

Cardiac MRI

A sub-sample of the men with echocardiograms in the main study free from cardiovascular medications, without diabetes or previous histories of cardiovascular events, were seen again for cardiac MR.

MR scans were performed on a 1.5 Tesla Philips Intera (Philips Medical Systems, Best, Netherlands). From an axial scout, a single-oblique long axis slice was planned to pass through the LV apex and centre of the mitral valve. From this slice, a second double oblique slice was planned through apex and mitral valve to provide a long axis from which short axis slices were planned. Six equally spaced ECG-gated breath-held steady-state free procession (SSFP) cines were acquired perpendicular to LV long axis with the most basal slice passing through the most basal part of the myocardium and the most apical slices touching the apical endocardium (flip angle: 60°, thickness: 6mm, matrix: 192x256, field of view (FOV): 320mm, repetition/echo time (TR/TE): 3/1.5ms, phases:
From a basal short axis slice, three radial SSFP long axis cines were planned at 60 degree increments using similar parameters.

LV mass and volume were calculated throughout the cardiac cycle using three-dimensional guide-point modelling. Briefly, a three-dimensional finite element model of the left ventricle was adaptively optimized to fit each subject’s image dataset using custom software (CIM version 7.3, Auckland MRI Research Group). This method has previously been validated in animals against autopsy LVM, in patients with regional wall motion abnormalities, against manually drawn contours, and in healthy volunteers against flow-derived measures of cardiac output. The finite-element model was interactively fitted to ‘guide-points’ provided by the analyst, as well as image derived data points contributed by an image processing algorithm. LV volume was calculated at all frames of the cardiac cycle by numerical integration of the curved surfaces represented by the finite-element model. Papillary muscles and ventricular trabeculations were included with the blood pool. LVM was measured at all frames in the cine acquisition by subtracting the epicardial volume from the endocardial volume, and multiplying by an assumed myocardial density of 1.04 g/ml. At the completion of the analysis, the end-systolic frame was chosen as the frame with the minimum volume. The left atrial volume was determined by identifying endocardial points on the three long axes, fitting a 3D ellipsoid to these points and calculating its volume.

Statistical analysis

The R statistical package (version 2.11.1) was used for data analysis. ANOVA and chi-square tests were used to compare continuous and categorical variables by ethnicity, respectively. ANCOVA with Tukey-HSD adjustments for multiple testing and/or MM-estimate regression models (robust to outliers) were used to test the association of LV concentric geometry as RWT or M/V ratio with vitamin D or ethnicity controlling for covariates. LVM rather than BSA-indexed LVMI was used as the dependent variable, controlled for BSA along with other covariates such as age and systolic blood pressure (SBP). Bivariate correlations were measured by Pearson r for parametric, and Spearman ρ for non-parametric variables. P<0.05 was taken as significant.
Echocardiography series were available from 194 men: 64 African-Caribbeans, 68 South Asians and 62 European Caucasians, with cardiac MR in 50 of these men: 17 African-Caribbean, 15 South Asian (all Pakistani) and 18 Europeans.

The reader is referred to our recent publication and Web supplement Table A for the demographics, hemodynamic and metabolic profiles of the same participants. Compared to that paper, the MR subsample here contains another 3 participants (who lacked vascular MR data). The overall demographics, hemodynamic and metabolic profiles are not significantly different here from those and age and body mass index (BMI) were not significantly different between ethnic groups.

**Cardiac indices**

By echocardiography, absolute and indexed LVM was higher in African-Caribbeans than in either South Asian or Europeans, but Europeans had the highest LV volume indices (Table 1). Mean(SE) LVM was higher in African-Caribbeans by 14(7) and 17(8) g than in Europeans and South Asians, respectively after adjusting for age, SBP and BSA. In the MR sub-sample with similar adjustments, LVM in African-Caribbeans and Europeans was 22(5) and 19(6) g higher than in South Asians. South Asians also had lower MR-measured LV volumes than the other groups (Table 1).

Concentric geometry with RWT>0.42 was more common among African-Caribbeans (59%) and South Asians (49%) than Europeans (24%) (Figure 1-a) (p<0.001) whether including or excluding anti-hypertensive users. Adjusting for age, SBP, diabetes and BMI, mean(SE) RWT by echocardiography was 0.05(0.01) higher in South Asians and African-Caribbeans than Europeans, confirming the univariate analysis in Figure 1-b-c, and Table 1. The univariate results did not reach statistical significance for ethnic differences in M/V ratio in the small MR sub-sample (Table 1, and Figure 1-d). However, when adjusted for age, pulse pressure and BMI, mean(SE) of M/V ratio was 0.10(0.05) and 0.08(0.06) g/mL higher in African-Carribbeans (p=0.03) and South Asians (p=0.15) compared to Europeans. M/V ratio distribution was skewed in South Asians; its inverse, V/M was normally distributed. Repeating the regression model, V/M was 0.12(0.06) and 0.11(0.05) mL/g lower in African-Carribbeans and South
Asians compared to Europeans (p=0.04) implying higher M/V ratios (i.e. more concentric geometry) in African-Caribbeans and South Asians, similar to the echocardiography data in the total sample.

Systolic function as measured by EF by echocardiography and MR was not different across the groups (Table 1). Diastolic function measured by mitral annular velocity (e’) was higher by 1.0(0.35) cm/s in African-Caribbeans and South Asians than in Europeans (p<0.01) after adjusting for age, SBP, heart rate, diabetes, and LVMI or RWT. There were no LAVI ethnic differences by either echocardiography or MR.

**Hormonal differences**

Details of hormonal differences from our participants were published before (also see Web supplement, Table A). In brief, South Asians (geometric mean±SD: 19±2 nmol/L) and African-Caribbean (28±2 nmol/L) had significantly lower circulating levels of 25(OH)D than Europeans (42±2 nmol/L), including when adjusted for age, weight, season and supplement use. Aldosterone levels were significantly lower in African-Caribbeans (178±2 pmol/L) than in South Asians (258±2 pmol/L) and Europeans (219±2 pmol/L), including when adjusted for BMI and anti-hypertensive use.

**Hormones and LV geometry**

RWT by echocardiography and M/V ratio by MR were both correlated with circulating 25(OH)D levels (Figure 2 a-b). Adjusting for age, SBP, diabetes and BMI, RWT was clearly negatively correlated with 25(OH)D (nmol/L); a one standard deviation (18 nmol/L) rise in 25(OH)D predicted a 0.21 SD fall in RWT (p=0.002). Using a similar model, mean(SE) RWT among participants in the 3rd and 4th quartiles of 25(OH)D were 0.04(0.015) and 0.05(0.016) lower than those in the first quartile (p=0.01 and 0.003, respectively). In similar regression models RWT and aldosterone were not significantly related (not shown). Aldosterone and 25(OH)D were weakly negatively correlated (ρ=-0.15, p=0.04).

Independent relationships of 25(OH)D with LVM and volumes were investigated separately. Overall, 25(OH)D was more closely and consistently related to LV volumes
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in all men, independent of age, SBP, diabetes and BSA; a one standard deviation increase in 25(OH)D was associated with 0.26 SD rise in LVEDV (standardized β=0.26, p<0.001); the relationship for LVM was not significant (β=0.07, p=0.3) (Web supplement, Figure B). The positive relationship between 25(OH)D and LV volume was independent of LVM in a model controlling for LVM on top of previous covariates, which showed that LVEDVI was 6(1.5) ml/m$^2$ higher in the highest 25(OH)D tertile than the lowest (p<0.001) (also see Supplement: Figure A-a). Aldosterone showed no significant relationship with LV volumes or mass in similar models (data not shown).

The closer relationship of 25(OH)D with volumes than mass was also replicated in the MR study (Figure 2-c-d); one SD increase in 25(OH)D levels was associated with 0.53 SD rise in LVEDV by MR, and 0.35 SD (p<0.01) increase in LVM adjusted for age, SBP and BSA. Vitamin D was positively associated with LVEDVI independent of LVMI in models also controlling for LVM. Participants in the highest 25(OH)D tertile had 9(4) ml/m$^2$ higher LVEDVI than those in the lowest tertile (p=0.02) (Supplement: Figure A-b).

To test if 25(OH)D or aldosterone could explain concentric geometry (RWT) differences among the ethnic groups, the ethnicity effect was tested in ANCOVA models including age, SBP, BMI, and diabetes along with 25(OH)D or aldosterone. 25(OH)D but not aldosterone significantly weakened the main ethnicity effect, decreasing the F-value from 9 to 4, and diminishing the p-value for ethnicity from highly significant <0.001 to 0.02, nearer borderline (Supplement: Table B).

Discussion

This study showed an independent inverse relationship between circulating 25(OH)D levels and LV concentric geometry in a multi-ethnic sample of community-dwelling men with significantly different vitamin D status. Compared to Europeans, South Asians and African-Caribbean groups with significantly lower 25(OH)D levels had higher echocardiographic RWT as well as a higher frequency of concentric geometry (RWT>0.42). More precise MR imaging in a sub-sample of these men confirmed the echocardiography results, especially for the inverse relationship of 25(OH)D and
concentric geometry as M/V ratio. The results may be important as concentric remodelling is associated with poorer cardiovascular outcome compared to other types of cardiac geometry\(^{23,24}\).

To our knowledge data on the vitamin D relationship with cardiac geometry is rare in community-dwelling, and especially relatively disease-free populations. A recent study among haemodialysis patients with “low” parathyroid hormone (PTH) levels also found higher RWT among patients with lower (<30ng/mL) vitamin D levels\(^{25}\). Pilz et al in a population study of 614 elderly Europeans did not find a significant association between cardiac geometry and circulating vitamin D\(^ {26}\). Their different results may be due to significantly higher average age, high prevalence of hypertension and cardiovascular disease history among their participants, as well as differences in statistical approach and vitamin D measurement methods. A study in Turkey reported higher IVST and PWT among children with severe rickets\(^ {27}\) and some sporadic paediatric cases of dilated cardiomyopathy have also been reported. The few available trials to date have demonstrated LVH regression with vitamin D (analogue) therapy in chronic renal failure patients\(^ {28,29}\).

Pathophysiological evidence links vitamin D and myocardial remodelling. Vitamin D deficiency triggers a rise in PTH levels which itself may mediate LVH\(^ {30}\). In animal experiments, vitamin D deficiency induced myocardial hypertrophy and fibrosis\(^ {31-33}\), and vitamin D treatment regressed hypertrophy\(^ {34}\). Though vitamin D deficiency can activate the renin-angiotensin-aldosterone system directly, a renin-independent relationship between vitamin D and cardiac structure was also demonstrated in VDR-knock-out mice\(^ {32}\). Another possible mechanism could be vitamin D influence on hemodynamic and arterial stiffness indices. Al Mheid et al reported the correlation between carotid-femoral pulse wave velocity (PWV) and sub-endocardial viability ratio (SEVR) with vitamin D deficiency and SEVR improvement on vitamin D normalization\(^ {35}\). SEVR has been shown to be correlated with coronary flow reserve\(^ {36}\). Our previous paper also reports an inverse relation between aortic PWV and vitamin D. Interestingly, PWV is known to correlate with concentric remodelling\(^ {37,38}\).

Ethnic differences in cardiac structure such as higher RWT in South Asian\(^ {39}\) and African-Caribbeans or Americans\(^ {40-43}\) compared to Europeans have been demonstrated
before, even in healthy subjects, and are not well explained by traditional risk factors.\textsuperscript{39, 42} Our results suggest vitamin D as a possible pathophysiological factor in accounting for such differences. However, firm conclusions cannot be made from this cross-sectional data alone.

Insignificant differences between ethnic groups in global systolic function measures such as ejection fraction have been found in other studies where alternative systolic function indices by tissue Doppler (not measured here) revealed cross-ethnic differences.\textsuperscript{39, 43, 44} As to diastolic function, our results are different from two previous reports which showed lower diastolic function as mitral annulus velocity ($e'$) in South Asians and African-Caribbeans compared to Europeans.\textsuperscript{39, 41}

In conclusion, these data suggest that circulating vitamin D status may have relevance in cardiac remodelling. Its effects need to be tested, across susceptible ethnic groups, in formal randomised trials, of which very few have been done and none in adequate dosage to effect replacement and maintenance adequately.\textsuperscript{45} Only such trials can show whether there are direct causal links or otherwise.

\textit{Study limitations}

The participants were recruited as community-dwelling volunteers, and hence may not be fully representative, although their blood pressures and metabolic profiles are similar to those reported previously. Care should be taken interpreting MR sub-study results due to small numbers in each group, although again their risk factor profiles are typical of these ethnic groups. Moreover, the choice of the adjusted covariates may affect ethnic differences in cardiac indices observed here in regression models. How reliable the cut-off points (e.g. for LVH) or reference values may be from predominantly European Caucasian populations for studies with other ethnic groups continues as a topic of debate. However, stepping beyond such categorical analysis and using cardiac indices as continuous variables (e.g. RWT and M/V ratio in linear models) partly alleviates such issues here.
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Sources of Funding

The work was a part of the first author’s PhD project supported by a BBSRC-related Faculty Strategic Studentship and ORSAS awards from the University of Manchester, The MRI scans were funded by Magnetic Resonance Imaging Facility (MRIF) and the NIHR Manchester Biomedical Research Centre.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgement

The authors wish to acknowledge the support of the Wellcome Trust Clinical Research Facility (WTCRF), Manchester where the field-work and MRI scans took place. We also wish to cherish the memory of Professor Alan Mike Wallace who died unexpectedly while this work was in progress.
References


Table 1. Cardiac geometry and function. The values are mean±SD.

<table>
<thead>
<tr>
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<th>All (echocardiography) (n=194)</th>
<th>MR sub-sample (n=50)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>AfC N=64</td>
<td>SA N=68</td>
</tr>
<tr>
<td><strong>Mass/Geometry</strong></td>
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<tr>
<td>LVM (g)</td>
<td>201±46</td>
<td>176±41</td>
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<tr>
<td>LVMI (g/m$^2$)</td>
<td>100±22</td>
<td>91±20</td>
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<tr>
<td>RWT</td>
<td>0.43±0.07</td>
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<td>M/V ratio (g/ml)</td>
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<td>2.2±0.65</td>
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<tr>
<td>LV EDVI (ml/m$^2$)</td>
<td>44±10</td>
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<td>LV ESVI (ml/m$^2$)</td>
<td>16±5</td>
<td>16±4</td>
</tr>
<tr>
<td>SVI (ml/m$^2$)</td>
<td>27±7</td>
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<td><strong>Systolic Function</strong></td>
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<tr>
<td>EF (%)</td>
<td>62.7±6.8</td>
<td>61.6±6.0</td>
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<tr>
<td><strong>Diastolic function</strong></td>
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<tr>
<td>E/e’</td>
<td>6.9±1.8</td>
<td>7.5±2.1</td>
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<tr>
<td>e’ (cm/sec)</td>
<td>9.4±2.4</td>
<td>9.5±2.3</td>
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<tr>
<td>LAVI (ml/m$^2$)</td>
<td>17±6</td>
<td>17±4</td>
</tr>
</tbody>
</table>

* Significant p values. LVM: left ventricular mass, LVMI: left ventricular mass indexed to body surface area (BSA), RWT: relative wall thickness, M/V: mass/volume, LV: left ventricular, EDVI: end diastolic volume indexed to BSA, ESVI: end-systolic volume indexed to BSA, SVI: stroke volume indexed to BSA, EF: ejection fraction, LAVI: left atrial volume indexed to BSA.
Figure 1. a) Distribution of LV geometry types in ethnic groups, b and c) RWT and M/V (end-diastolic mass/volume) ratio as indices of LV concentric geometry measured by echocardiography in the total sample (n=194), and d) M/V ratio in MR study (n=50). AfC: African-Caribbean, SA: South Asian, WE: European. Bars show mean and 95% confidence intervals.
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**Figure 2.** Correlation between circulating 25(OH)D and left ventricular (LV) concentric geometry as RWT by echocardiography in the total sample (a) and MR M/V ratio (b). Correlation between 25(OH)D and MR LV mass index (LVMI) (c) is less than that with LV end-diastolic volume index (LVEDVI) (d). r: Spearman correlation.
Web Supplement

Table A. General characteristics of participants

<table>
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<th>All (n=194)</th>
<th>MR sub-sample (n=50)</th>
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<td>AfC</td>
<td>SA</td>
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<tr>
<td>N=64</td>
<td>N=68</td>
<td>N=62</td>
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<tr>
<td>Age (yr)</td>
<td>54±10</td>
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<tr>
<td>Diabetes</td>
<td>7 (11%)</td>
<td>24 (35%)</td>
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<td>Hypertension Tx</td>
<td>8 (12%)</td>
<td>10 (15%)</td>
</tr>
<tr>
<td>Dyslipidemia Tx</td>
<td>6 (9%)</td>
<td>13 (19%)</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>12 (20%)</td>
<td>11 (17%)</td>
</tr>
<tr>
<td>Weight (kg)</td>
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<tr>
<td>Height (cm)</td>
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<td>Creatinine (mmol/L)</td>
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<td>TC/HDL ratio</td>
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<tr>
<td>25(OH)D (nmol/L)</td>
<td>28±2</td>
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<tr>
<td>Aldosterone (pmol/L)</td>
<td>178±2</td>
<td>258±2</td>
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Table B. Testing the main effect of ethnicity in ANCOVA models predicting RTW$_{\text{log}}$ in total sample. Entering 25(OH)D in the model significantly weakens main ethnicity effect though not totally removing it (last row).

<table>
<thead>
<tr>
<th>Model predictors (along with Ethnicity)</th>
<th>Ethnicity (main effect)</th>
<th>Post-hoc comparisons (RTW$_{\text{log}}$)</th>
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<tr>
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<tr>
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<tr>
<td>age</td>
<td>0.0002*</td>
<td>0.11</td>
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<tr>
<td>age, SBP</td>
<td>0.0002*</td>
<td>0.12</td>
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<td>age, SBP, DM</td>
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<td>0.10</td>
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<td>0.10</td>
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<td>0.11</td>
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<tr>
<td>age, SBP, DM, BMI, 25(OH)D$_{\text{log}}$</td>
<td>0.02*</td>
<td>0.07</td>
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*$^*$p-values were adjusted for multiple testing. Diff.: estimated mean difference.
Figure A. Compared to participants in the lowest 25(OH)D tertile (darkest gray box), those in highest tertile (white boxes) showed higher LV end-diastolic volume index (LVEDVI) at the same level (tertile) of LV mass index (LVMI). Thick horizontal lines are medians, upper and lower box borders are 25th and 75th percentile and the whiskers show data range.
**Figure B.** Relations between vitamin D or aldosterone and left ventricle (LV) mass and volume in total sample (n=194).
F.3 Paper 3: Myocardial contractility and vitamin D

Lower Circulating Vitamin D Associated with Increased Myocardial Contractility in Humans by Magnetic Resonance Imaging, Supporting Previous Animal Experimental Evidence
Lower circulating vitamin D associated with increased myocardial contractility in humans by magnetic resonance imaging, supporting previous animal experimental evidence

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Abstract

**Background:** A few animal studies have shown relationship between vitamin D deficiency and increased myocardial contractility. Similar data from the human is rare.

**Aims:** To investigate association between circulating vitamin D and regional myocardial function in disease-free participants.

**Methods:** In 50 men free of cardiovascular disease or medications, myocardial regional deformation as circumferential peak systolic strain, and systolic and diastolic strain rates were measured using Magnetic Resonance at a mid-LV level short-axis slice by myocardial tagging. Vitamin D was measured by mass spectrometry in frozen serum samples.

**Results:** Adjusting for heart rate, central systolic blood pressure (cSBP), left ventricular (LV) mass/volume ratio and body mass index, circulating 25(OH)D was inversely and independently correlated with absolute systolic strain rate. A one standard deviation (SD) decrease in 25(OH)D was associated with a 0.38 SD increase in absolute systolic strain rate and 0.22 SD rise in diastolic strain rate. The inverse correlation with absolute peak systolic strain in a similar model did not reach statistical significance (-0.04(0.02), β=-0.27; p=0.09). Testing such models including LV end-diastolic and end-systolic volumes (EDVI and ESVI) as loading indices on top of cSBP was difficult due to multi-collinearity and significant correlation of 25(OH)D with EDVI (ρ=0.60, p<0.001) and ESVI (ρ=0.57, p<0.001).

**Conclusions:** Higher myocardial deformation rates especially in systole may reflect increased myocardial contractility associated with vitamin D deficiency similar to the evidence from animal experiments.
Introduction

Despite the mounting evidence on the association of vitamin D and cardiovascular disease, the potential pathophysiological mechanisms behind the scene remain mostly unknown. Data on the relationship between cardiac structure and function and vitamin D deficiency in humans, particularly in asymptomatic subjects, is rare. The few animal studies have shown an intriguing relationship between vitamin D deficiency and increased myocardial contractility \(^1\text{-}^3\). To our knowledge, such hypotheses have not been tested in human subjects yet.

Myocardial tagging technique via magnetic resonance imaging (MRI) is among the most precise methods of measuring intra-myocardial deformation as strain and strain rate which may be used to study early changes in myocardial function and contractility at preclinical stages \(^4\text{-}^5\). In this study, we used these sensitive imaging methods to investigate the relationship between circulating vitamin D levels and myocardial function in a community sample of men.

Methods

The participants were generally healthy, community dwelling men living in Manchester, North England already recruited in European Male Ageing Study (EMAS). Exclusion criteria were severe chronic disease, active malignancy or diabetes, and being on any cardiovascular medications (e.g. statins, antihypertensives, etc).

MR imaging

MR scans were performed on a 1.5 Tesla Philips Intera (Philips Medical Systems, Best, Netherlands). In brief, six equally spaced ECG-gated breath-held steady-state free procession (SSFP) cines were acquired perpendicular to left ventricle (LV) long axis with the most basal slice passing through the most basal part of the myocardium and the most apical slice touching the apical endocardium (flip angle: 60°, thickness: 6mm, matrix: 192x256, field of view (FOV): 320mm, repetition/echo time (TR/TE): 3/1.5ms, phases: 30). From a basal short axis slice, three radial SSFP long axis cines were
planned at 60 degree increments using similar parameters. LV mass and volume were calculated throughout the cardiac cycle by three-dimensional guide-point modelling method validated before, using custom software (CIM version 7.3, Auckland MRI Research Group)\(^6\). Papillary muscles and ventricular trabeculations were included with the blood pool. The left atrial volume was determined by identifying endocardial points on the three long axes, fitting a 3D ellipsoid to these points and calculating its volume. Cardiac volumes and mass were indexed by body surface area (BSA), to adjust for body size variations.

To measure intra-myocardial circumferential strain, a respiratory-gated free-breathing SPAMM (spatial modulation of magnetization) protocol was used to grid-tag the myocardium in a mid-LV short axis slice taken mid-way between LV base and apex as described before\(^7\) (flip angle 13º, thickness: 8mm, matrix: 192x256, FOV: 320mm, TR/TE: 5/2ms).

The tagged slice was analysed in the custom software (CIM version 7.3, Auckland MRI Research Group)\(^6\) to measure circumferential strain (E\(_{cc}\)) in 6 regions of the LV short axis: anterior, inferior, anterior lateral, posterior lateral, inferior septum, and anterior septum. Strain was calculated in percents using the formula with L\(_2\) and L\(_1\) being the length of the contracting/relaxing myocardium\(^4,8\):

\[
E_{cc} = \frac{L_2 - L_1}{L_1}
\]

Circumferential strain values are negative during systolic contraction (myocardial shortening; L\(_2\)<L\(_1\)) and shift positive during diastolic relaxation (lengthening back to original length; L\(_2\)>L_1\). From the circumferential strain trace, peak strain (Ecc in %) was measured\(^8\).

The circumferential strain rates were calculated by the following formula; E\(_{cc2}\) and E\(_{cc1}\) being the circumferential strain values at time points t\(_2\) and t\(_1\) in the cardiac cycle:

\[
SR = \frac{E_{cc2} - E_{cc1}}{t_2 - t_1}
\]

Strain rate traces were computed as time derivative from strain trace, from which the minimal systolic peak was measured as systolic strain rate (SR\(_S\)), and maximal early diastolic peak as early-diastolic strain rate (SR\(_{ED}\)) in s\(^{-1}\) as described before\(^8\).
absolute values of $E_{cc}$ and $SR_S$ were used in analyses to make the results more readable as using negative systolic and positive diastolic strain rate values would be confusing. Therefore, higher values of absolute $E_{cc}$ and $SR_S$ here mean higher systolic peak shortening and maximal contraction acceleration, respectively, and a higher $SR_{ED}$ means higher maximal diastolic relaxation acceleration.

Other measurements

Standard arm blood pressures and central systolic blood pressures (cSBP) were measured using the Arteriograph device (TensioMed, Budapest, Hungary) immediately after cardiac MRI, supine on the left upper arm. Average values of 2-3 measurements were used. Central blood pressures may provide a better estimate of the pressure load on the left ventricle compared to brachial pressures.

Serum frozen samples were used to measure 25(OH)D by liquid chromatography-tandem mass spectrometry (LC/MS/MS) as previously described.

Statistical analysis

The R statistical package (version 2.11.1) was used for data analysis. Robust regression models were used to test the association of myocardial strains with vitamin D controlling for covariates. The myocardial strain covariates in the regression models were chosen based on previous literature as well as guided by univariate analysis, and included heart rate, cSBP and LV volumes as LV pressure/volume loading indices, LV geometry as end-diastolic mass to volume ratio (M/V), and body mass index (BMI). Bivariate correlations were measured by Pearson r for parametric variables or their logged transforms where appropriate, and Spearman ρ for non-parametric variables. P<0.05 was taken as significant. The regression results are expressed as unstandardized coefficients B and standard error (SE).

Results

Cardiac MR was possible in 50 participants. Participants’ general characteristics and cardiac indices are listed in Tables 1 and 2. The unadjusted regional myocardial strains
and strain rate levels by 25(OH)D quartiles are visualized as box-plots in Figure 1. Lower systolic deformation rates as $SR_s$ is seen in the highest quartile of 25(OH)D (white boxes) compared to lower quartiles (darker boxes). This trend was more visible in anterior, lateral and inferior regions (Figure 1, top row). A similar but less obvious difference between the first and last quartiles of 25(OH)D is also seen in lateral posterior and lateral anterior regions for peak systolic strain as $E_{cc}$ (Figure 1, middle row), and in lateral posterior, inferior and septal regions for diastolic strain rate as $SR_{ED}$. (Figure 1, bottom row).

In univariate analysis, 25(OH)D was inversely and significantly correlated with absolute systolic strain rate; that is lower 25(OH)D predicted higher systolic deformation rates. Correlations with global peak systolic strain and diastolic strain rate were negative but not statistically significant (Figure 2). In multiple regression models and after adjusting for heart rate, cSBP, M/V ratio (i.e. geometry) and BMI, circulating 25(OH)D levels were inversely and independently correlated with absolute systolic strain rate ($B(SE)$: -0.43(0.14), $\beta$=-0.38; $p=0.003$), and diastolic strain rate (-0.25(0.12), $\beta$=-0.22; $p=0.04$). This means that a one standard deviation (SD) decrease in 25(OH)D was associated with a 0.38 SD increase in absolute systolic strain rate and 0.22 SD rise in diastolic strain rate. The inverse correlation with absolute peak systolic strain in a similar model did not reach statistical significance (-0.04(0.02), $\beta$=-0.27; $p=0.09$). Further attempts were made testing such models including LV end-diastolic and end-systolic volumes (EDVI and ESVI) as loading indices on top of cSBP. However, 25(OH)D was significantly correlated with both EDVI ($\rho=0.60$, $p<0.001$) and ESVI ($\rho=0.57$, $p<0.001$) so that 25(OH)D and LV volumes weakened each other’s effect in the regression models due to multi-collinearity. This made investigating the independent effect of these variables on myocardial strains difficult.

**Discussion**

We found increased myocardial deformation rates especially as systolic strain rate associated with lower levels of circulating vitamin D. Higher systolic strain rates here may reflect increased myocardial contractility inversely correlated with vitamin D.
Clinical data on relationship between vitamin D and myocardial function is scarce, and to our knowledge is limited to studies on the association between more global indices of LV contraction with vitamin D. A study by Pilz et al failed to find a relationship between systolic and diastolic function measured by conventional echocardiography methods quantifying global LV contraction as ejection fraction or mitral flow velocity profiles. Our method of myocardial strain imaging by MRI provides a more sensitive assessment of myocardial function at regional level and in earlier stages of disease compared to the indices of global chamber function (e.g. ejection fraction).

Our results are consistent with the few previous animal studies suggesting that vitamin D deficiency is associated with increased myocardial contractile activity. Tishkof et al demonstrated accelerated contraction and relaxation in the isolated cardiomyocytes of the VDR (vitamin D receptor) knock-out mouse (which lack vitamin D receptor) compared to the wild type. Weishaar and Simpson also showed higher inotropy and contractility in the isolated perfused hearts of the rats with chronic vitamin D deficiency, independent of calcium levels. Zhao et al recently studied the adult rat’s isolated cardiac myocytes contracting under electrical stimulation, and observed a decrease in peak sarcomere shortening within minutes of 1,25(OH)D3 treatment.

The mechanisms behind such associations have also been investigated in animal experiments. Zhao et al demonstrated a physical association of VDR and caveolin-3 in t-tubules and sarcolemma of rat’s isolated cardiomyocytes by confocal microscopy. Another study also showed that vitamin D influence on myocardial contractility was mediated by non-genomic protein kinase C signalling via the VDRs located close to the t-tubules where potential regulation of calcium influx is possible.

The correlation of 25(OH)D and LV volumes as loading variables in our data may also suggest the hypothesis that vitamin D association with myocardial strain rates is mediated via its effect on the cardiac loading indices. However, the nature of data here did not allow testing such hypothesis.

Accelerated contractile activity of cardiac myocytes may lead to changes in cardiac function and structure with time. Therefore, our findings along with previous animal evidence may be important in understanding possible links between vitamin D.
deficiency, cardiac remodelling and heart failure pathophysiology. This may open new research avenues to explaining the mechanisms linking vitamin D and cardiovascular disease.

*Study limitations:* Our study is cross-sectional and therefore it is impossible to draw cause-effect conclusions which will demand well designed trials. We measured strain and strain rate in circumferential 2D direction only, and at mid-LV level. More extensive quantification of myocardial deformation and torsion is possible by MRI and may be more illuminating especially in larger samples.

**Acknowledgements**

The authors wish to warmly thank Neal Sheratt and other radiographers in Wellcome Trust Clinical Research Facility, Manchester for their wonderful collaboration on the MR scans. Our special thanks goes to Dr. Cristina Santa Marta from Faculty of Science, UNED, Madrid for kindly sending us her myocardial tagging protocol.
References


Tables

**Table 1. General and hemodynamic characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>53±6</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27±3</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>129±15</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>48±7</td>
</tr>
<tr>
<td>cSBP (mmHg)</td>
<td>127±18</td>
</tr>
<tr>
<td>cPP (mmHg)</td>
<td>46±10</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>60±8</td>
</tr>
<tr>
<td>Fasting plasma sugar (mmol/L)</td>
<td>5.2±0.7</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.2±1.1</td>
</tr>
<tr>
<td>25(OH)D log (nmol/L)</td>
<td>3.2±0.5</td>
</tr>
</tbody>
</table>

**Table 2. Cardiac indices measured by MRI**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass (g)</td>
<td>140±24</td>
</tr>
<tr>
<td>LV mass index (g/m²)</td>
<td>70±9</td>
</tr>
<tr>
<td>LV end diastolic volume index (ml/m²)</td>
<td>77±13</td>
</tr>
<tr>
<td>LV end systolic volume index (ml/m²)</td>
<td>31±8</td>
</tr>
<tr>
<td>LV Mass/Volume ratio (g/ml)</td>
<td>0.93±0.16</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>61±4</td>
</tr>
<tr>
<td>Systolic strain rate* (s⁻¹)</td>
<td>86±15</td>
</tr>
<tr>
<td>Peak systolic strain* (%)</td>
<td>20±2</td>
</tr>
<tr>
<td>Diastolic strain rate (s⁻¹)</td>
<td>66±15</td>
</tr>
</tbody>
</table>

* Absolute values (regardless of the sign) are shown.
Results

Paper 3

Myocardial contractility and vitamin D

Figure 1.
Boxplots showing regional myocardial strain and strain rates by quartiles of circulating vitamin D levels. Darker box colours are lower vitamin D quartiles. Absolute values of systolic strain and strain rate were used in the plots.
Results

Myocardial contractility and vitamin D

Figure 2. Correlation between circulating 25(OH)D and unadjusted myocardial strain and strain rates. Absolute values of systolic strain and strain rate were used in the plots.
Different Left and Right CIMT Correlations with Vitamin D and Aldosterone in Men of Three Ethnic Groups
Different left and right CIMT correlations with vitamin D and aldosterone in men of three ethnic groups

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Abstract

Background and Aims: To study the relationship between vitamin D and carotid intima media thickness (CIMT), a subclinical structural marker of atherosclerosis, in a multi-ethnic sample of men with known differences in cardiovascular risk and vitamin D status.

Methods: Mean CIMT was measured according to Mannheim Consensus in left and right common carotid arteries of community-dwelling men from three ethnic groups: African-Caribbean (n=64, mean±SD age: 54±10 yr), South Asian (n=68, 55±10 yr), and European (n=63, 57±9 yr). 25(OH)D was measured by mass spectrometry (LC/MS/MS). Results are in B(SE): unstandardized coefficients (standard error).

Results: Adjusting for age, systolic blood pressure (SBP) and body mass index (BMI), 25(OH)D was inversely related with left CIMT [B(SE): -0.9(0.4) microns, p=0.03]. In a similar model, aldosterone showed borderline direct correlation with right CIMT. Entering ethnicity in the model weakened 25(OH)D relation with left CIMT, but made aldosterone relation with right CIMT more significant [B(SE): 0.2(0.1) microns, p=0.02]. Hormonal associations with, or ethnic differences in average (of left and right) CIMT were marginal and did not reach statistical significance.

Median(IQR) of left/right CIMT was 0.64(0.14)/0.64(0.15) mm in African-Caribbean, 0.66(0.16)/0.58(0.13) mm in South Asian and 0.61(0.14)/0.61(0.17) mm in Europeans, respectively. Adjusting for age, SBP and BMI; right CIMT was higher in African-Caribbeans than South Asians [B(SE): 40(19) microns, p=0.03]; entering aldosterone in the model made this difference more significant [B(SE): 52(18) microns, p=0.01]. Left CIMT was not significantly different across the three ethnic groups. Left CIMT was 0.07 mm higher than right, in South Asians (p=0.03), but not in two other groups. Serum aldosterone [B(SE): -0.16(0.06) microns, p=0.006] and South Asian ethnicity [41(19) microns, p=0.03] were independent predictors of left-right CIMT differences.

Conclusions: Right and left CIMT may have different relations with 25(OH)D and aldosterone. Left-right CIMT differences also vary by ethnicity and may be related to serum aldosterone levels.
Introduction

The differences in atherosclerotic cardiovascular disease among South Asians, African-Caribbeans and Caucasian Europeans are known issues, yet not fully explained by conventional risk factors. Current evidence shows higher coronary heart disease (CHD) prevalence among South Asians, and greater stroke but lower CHD rates in African-Caribbeans despite higher blood pressures and more frequent diabetes compared to Europeans.

Interestingly, South Asians and African-Caribbeans with more pigmented skins are known to have lower levels of vitamin D compared to their European counterparts. Given current evidence on association of vitamin D deficiency and cardiovascular disease, we aimed to study the relationship between vitamin D with CIMT as a subclinical structural marker of atherosclerosis in large arteries and an independent predictor of stroke. In this context, we also studied similar associations of CIMT with aldosterone, a related hormone of RAAS pathway speculated to be up-regulated in vitamin D deficiency.

Methods

Study participants were 40-80 year-old men of three ethnic groups: African-Caribbean, South Asian and European living in Manchester, North England. The participants had already been enrolled in the European Male Ageing Study (EMAS), through advertising for volunteers in community centres and media for the minority ethnic groups and by general practice register random sampling for Europeans. The participants did not have active malignancy or severe renal or liver chronic disease. Ethnicity was determined by participants’ self-reporting, and 3 out of 4 grand-parents being of the same ethnic origin.

Carotid ultrasound

Carotid ultrasound images were taken according to Mannheim Carotid Intima-Media Thickness Consensus with a 7.5 MHz linear wide-band probe using a Philips/ATL HDI-5000 ultrasound scanner, with the participant supine, and the neck extended and rotated 45° to the opposite side. Images were taken from left and right common carotids.
by the same trained cardiologist (SB), and stored anonymously on a PC hard drive for later blinded offline analysis.

A single observer (MR) conducted image analysis based on Mannheim consensus. Mean IMT was measured on the far wall of the distal 10 mm of both right and left common carotid arteries using semi-automatic edge-detection software (Philips QLab, Philips, Netherlands). As arterial wall thickness and IMT varies with vessel distension during each pulse (cardiac) cycle, the measurements were repeated at maximal and minimal vessel diameters, and the results were averaged. This practice has been recommended to increase reproducibility.

The same observer repeated image analysis on 26 randomly selected series blinded to previous results. Coefficient of variation (intra-class correlation) of intra-observer variability were 3.8%(0.98) for right, 1.4%(1.0) for left, and 2.3%(0.99) for average CIMT.

Other measurements

Standard sitting blood pressure was measured within 40 minutes of the carotid ultrasound using an Omron semi-automatic device. Circulating 25(OH)D was measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS) as previously described, and aldosterone by radio-immunoassay (Coat-A-Count aldosterone; Siemens Medical Solutions Diagnostics, Los Angeles, CA) in frozen serum samples.

Statistical analysis

The R statistical package (version 2.11.1) was used for data analysis. ANOVA and chi-square tests were used to compare continuous and categorical variables by ethnicity, respectively. Ethnic difference in non-parametric variables (e.g. CIMT) was investigated by Kruskal-Wallis test in univariate analysis. Robust regression models were used to test the association of CIMT with hormones and/or ethnicity controlling for covariates. Bivariate correlations were measured by Pearson r for parametric, and Spearman ρ for non-parametric variables. P value significance threshold in ethnic
Results

Carotid ultrasound was available from 195 participants: 64 African-Caribbean, 68 South Asian and 63 Europeans. General characteristics of the participants and hormonal levels have been published before \(^\text{21}\), and is not significantly different from here. Compared to that paper, 3 African-Caribbean participants did not have carotid ultrasounds. In brief, age and BMI did not significantly vary across the groups, but there were more diabetics among South Asians than two other groups. South Asians and African-Caribbeans had significantly lower circulating levels of 25(OH)D than Europeans after/before adjusting for age, weight, season and supplement use. Aldosterone levels were significantly lower in African-Caribbeans than two other groups after/before adjusting for BMI and anti-hypertensive use \(^\text{21}\).

CIMT values were not normally distributed even after log transformation. Left, right or average (of left and right) CIMT were not significantly different across ethnic groups by Kruskal-Wallis test in univariate analysis (Table 1 and Figure 1). Within group differences between left and right CIMT values revealed significantly higher left than right CIMT among South Asians, but left-right CIMT differences in the other two groups were insignificant (Table 1).

Age, systolic blood pressure (SBP) and body mass index (BMI), but not TC/HDL ratio and diabetes were found as significant covariates of CIMT in regression models. Adjusting for age, SBP, and BMI; borderline associations (p=0.1) were found between average CIMT and 25(OH)D, and aldosterone when tested in separate linear regression models (Table 2). Similar models revealed that 25(OH)D was inversely related with left, but not right CIMT (Table 2). In contrast, right CIMT showed borderline correlation with aldosterone levels, but not 25(OH)D (Table 2).

Ethnic differences in CIMT were further tested in regression models with and without hormones, and separately for average, left and right CIMT (Tables 3-5). Average CIMT
differences among ethnic groups did not reach statistical significance (Table 3 – Model A). Right CIMT was higher in African-Caribbeans than South Asians (p=0.03) (Table 4, Model A), but with aldosterone in the model this difference became more significant (p=0.01) (Table 4, Model C). Correlation between aldosterone and right CIMT was significant in the model adjusted for ethnicity (Table 4, Model C). Left CIMT was marginally higher in African-Caribbeans than Europeans, but did not reach statistical significance (p=0.06) (Table 5, Model A). Ethnicity in the model weakened 25(OH)D correlation with left CIMT (Table 5, Model B).

Left-right CIMT differences were independently associated with aldosterone [B(SE): -0.16(0.06) microns, p=0.006] and South Asian ethnicity [41(19) microns, p=0.03] adjusted for the same covariates as above.

Discussion

Overall, average of left and right CIMT did not reveal statistically significant differences across ethnic groups, or associations with hormones. African-Caribbeans had higher right CIMT than South Asians. The right and left CIMT also showed different relations with circulating hormonal levels. South Asians had significantly higher left than right CIMT, with South Asian ethnicity and aldosterone being independent correlates of the left-right CIMT differences.

Previous studies with larger samples found rather similar results regarding higher CIMT in African-Caribbeans. Two UK studies found higher mean CIMT in African-Caribbeans than Caucasian Europeans (0.81±0.20 vs. 0.75±0.18 mm) [22], or South Asians (0.64±0.14 vs. 0.61±0.13 mm) [23]. A US study reported higher maximal CIMT in African-Americans (0.865 mm) than Caucasian Europeans (0.808 mm) [24]. However, a population-based study in Canada found maximal CIMT higher in Europeans (0.75±0.16 mm) than in South Asians (0.72±0.16 mm) [25]. Using different approaches in CIMT image analysis (e.g. maximal vs. mean) may explain part of such differences. None of previous studies separately considered left and right CIMT differences.
Compared to rather borderline CIMT results here, our previous reports from same participants suggests that aortic pulse wave velocity (aPWV), an index of arterial stiffness, may be a more sensitive risk marker describing CHD risk by ethnic group as aPWV paralleled known ethnic differences in CHD risk in the UK. Current evidence suggests that CIMT is more consistently and slightly more closely related with stroke than CHD. Higher CIMT in African-Caribbeans found here and previous studies is also consistent with their higher stroke incidence rates.

Data on association of vitamin D and CIMT is sparse and limited to cross-sectional studies as here. One study in general population and others in diabetes and HIV-infected patients also revealed inverse correlation between CIMT and circulating 25(OH)D. Positive correlation of circulating aldosterone and CIMT has been found in a few studies. Aldosterone was weakly but positively correlated with CIMT in patients with essential hypertension. Higher levels of CIMT were found in primary aldosteronism patients compared to essential hypertension or controls. Trials using ACE inhibitors and spironolactone, both known to counter RAAS and aldosterone effects, have reported reduction in CIMT progression. To our knowledge, none of these hormonal studies on vitamin D and aldosterone reported left or right CIMT associations separately.

Differential associations here between right and left CIMT with vitamin D, aldosterone, SBP and BMI are interesting, but they are exploratory and difficult to explain. Studies investigating different correlates of right and left CIMT are rare. A cross-sectional study by Luo et al in 447 participants of wide age range attending annual physical check-ups suggested that right CIMT was more related with hemodynamic indices measured within carotid arteries (e.g. flow velocity) while left CIMT was better correlated with biochemical indices (e.g. LDL cholesterol) and BMI. Their results also suggested that right CIMT thickening was delayed compared to the left. In our data, right CIMT showed closer correlation with SBP and BMI than left CIMT (Table 2). Such results may be fortuitous and their replication in larger samples is necessary. The cross-sectional design also prevents any cause-effect conclusions and the associations found here may be due to unmeasured confounders.
Our data showed aldosterone and South Asian ethnicity as independent predictors of right-left CIMT differences in two different directions. It is already known that left CIMT is generally higher than right \cite{14}. Anatomical differences between right and left carotid trees and their interaction with hemodynamic factors such as pressure and flow could be a possible explanation, also suggested by Luo et al \cite{33}. Whether such right-left differences reflect possible different flow/pressure dynamics in two carotid trees, or they have cardiovascular risk prediction value is also unknown.

This study has some limitations. The ethnic group samples are not randomly selected and thus may not be representative though their blood pressure and metabolic profiles are similar to known levels from population. The study is cross sectional and cause-effect relation between hormonal levels and CIMT can not be interpreted from such results. It is noteworthy that the results on right-left CIMT differences, and unilateral analyses presented here were of exploratory nature. The average of left and right CIMT did not reveal statistically significant ethnic differences, or association with 25(OH)D and aldosterone.
References


10


## Results

### Table 1. CIMT in three ethnic groups

<table>
<thead>
<tr>
<th></th>
<th>AFC N=64</th>
<th>SA N=68</th>
<th>Eu N=63</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>Average CIMT (mm)</strong></td>
<td>0.64(0.12)</td>
<td>0.60(0.12)</td>
<td>0.61(0.16)</td>
<td>0.4</td>
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<tr>
<td><strong>Left CIMT (mm)</strong></td>
<td>0.64(0.14)</td>
<td>0.66(0.16)</td>
<td>0.61(0.14)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Right CIMT (mm)</strong></td>
<td>0.64(0.15)</td>
<td>0.58(0.13)</td>
<td>0.61(0.17)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>CIMT difference: Left – Right (mm)</strong></td>
<td>0.01(0.14)</td>
<td>0.07(0.15)</td>
<td>0.02(0.09)</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

* Significant p values; AfC: African-Caribbean, SA: South Asian, Eu: European

### Table 2. Regression models showing hormonal relations with right and left CIMT

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D (nmol/L)</th>
<th>Aldosterone (pmol/L)</th>
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<tbody>
<tr>
<td></td>
<td>Average CIMT (micron) B(SE) P</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>4.75(0.73) &lt;0.001*</td>
<td>4.6(0.72) &lt;0.001*</td>
</tr>
<tr>
<td>SBP</td>
<td>1.49(0.63) 0.02 *</td>
<td>1.3(0.59) 0.03*</td>
</tr>
<tr>
<td>BMI</td>
<td>5.8(2.28) 0.01 *</td>
<td>5.2(2.29) 0.02*</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>-0.6(0.40) 0.13</td>
<td>-</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>-</td>
<td>0.0(0.05) 0.10</td>
</tr>
</tbody>
</table>

|                  | Left CIMT (micron) B(SE) P |            | B(SE) P |
| Age              | 3.8(0.7) <0.001*           | 3.5(0.7) <0.001* |
| SBP              | 1.2(0.6) 0.06              | 1.1(0.6) 0.08  |
| BMI              | 3.5(2.8) 0.2               | 3.4(2.9) 0.2   |
| 25(OH)D          | -0.9(0.4) 0.03*            | -             |
| Aldosterone      | -                | 0.0(0.07) 0.6   |

|                  | Right CIMT (micron) B(SE) P |            | B(SE) P |
| Age              | 4.3(0.9) <0.001*            | 0.9(5.0) <0.001* |
| SBP              | 1.97(0.7) 0.01*             | 0.7(2.5) 0.01*  |
| BMI              | 5.1(2.2) 0.02*              | 2.2(2.1) 0.03*  |
| 25(OH)D          | -0.3(0.4) 0.5              | -             |
| Aldosterone      | -                | 0.08(1.6) 0.1§ |

§ Borderline p values ≤0.1, * Significant p values
### Table 3. Three models showing ethnicity and hormonal relations with average (of left and right) CIMT (in microns)

<table>
<thead>
<tr>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td>Ethnicity + 25(OH)D</td>
<td>Ethnicity + Aldosterone</td>
</tr>
<tr>
<td></td>
<td>B(SE)</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>4.5(0.8)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SBP</td>
<td>1.2(0.6)</td>
<td>0.03*</td>
</tr>
<tr>
<td>BMI</td>
<td>5.5(2.1)</td>
<td>0.01*</td>
</tr>
<tr>
<td>AfC vs. SA‡</td>
<td>12.3(18.1)</td>
<td>0.5</td>
</tr>
<tr>
<td>Eu vs. SA‡</td>
<td>-14.4(16.4)</td>
<td>0.4</td>
</tr>
<tr>
<td>AfC vs. Eu‡</td>
<td>26.7(16.6)</td>
<td>0.1§</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

‡ Significant P value threshold in ethnic differences is <0.02 after multiple testing adjustment
§ Borderline p values: ≤0.05 for ethnic differences and ≤0.1 for the rest

### Table 4. Three models showing ethnicity and hormonal relations with right CIMT (in microns)

<table>
<thead>
<tr>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td>Ethnicity + 25(OH)D</td>
<td>Ethnicity + Aldosterone</td>
</tr>
<tr>
<td></td>
<td>B(SE)</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>4.5(0.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SBP</td>
<td>1.5(0.7)</td>
<td>0.02*</td>
</tr>
<tr>
<td>BMI</td>
<td>5.5(2.1)</td>
<td>0.01*</td>
</tr>
<tr>
<td>AfC vs. SA‡</td>
<td>40.1(18.9)</td>
<td>0.03§</td>
</tr>
<tr>
<td>Eu vs. SA‡</td>
<td>19.7(17.2)</td>
<td>0.3</td>
</tr>
<tr>
<td>AfC vs. Eu‡</td>
<td>20.4(19.5)</td>
<td>0.3</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

‡ Significant P value threshold in ethnic differences is <0.02 after multiple testing adjustment
§ Borderline p values: ≤0.05 for ethnic differences and ≤0.1 for the rest

### Table 5. Three models showing ethnicity and hormonal relations with left CIMT (in microns)

<table>
<thead>
<tr>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td>Ethnicity + 25(OH)D</td>
<td>Ethnicity + Aldosterone</td>
</tr>
<tr>
<td></td>
<td>B(SE)</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>3.8(0.7)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SBP</td>
<td>1.0(0.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>BMI</td>
<td>2.6(2.6)</td>
<td>0.3</td>
</tr>
<tr>
<td>AfC vs. SA‡</td>
<td>7.7(20.1)</td>
<td>0.7</td>
</tr>
<tr>
<td>Eu vs. SA‡</td>
<td>-28.1(18.0)</td>
<td>0.1</td>
</tr>
<tr>
<td>AfC vs. Eu‡</td>
<td>35.8(18.5)</td>
<td>0.06</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

‡ Significant P value threshold in ethnic differences is <0.02 after multiple testing adjustment
§ Borderline p values: ≤0.05 for ethnic differences and ≤0.1 for the rest
Figures

Figure 1. CIMT across ethnic groups.
F.5 Paper 5: Pulse wave velocity by MRI and Arteriograph device

More Precise Central Pulse Wave Velocity Measurement by Improving Aortic Length Estimation via Magnetic Resonance Imaging
More Precise Central Pulse Wave Velocity Measurement by Improving Aortic Length Estimation via Magnetic Resonance Imaging

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Abstract

**Background:** Simple reproducible methods of measuring arterial stiffness, a powerful index of prognosis, are becoming available.

**Objectives:** (i) To compare aortic pathway length, pulse wave transit time (TT) and velocity (PWV) between magnetic resonance imaging (MR) and an arm cuff-based oscillometric method, the Arteriograph (AG), and (ii) investigate improving AG measurements by combining them with pathway length from MR.

**Methods:** MR phase-contrast data were acquired at the aortic arch and just above the aortic bifurcation in 49 men (age: 53±6 yr). Supine left-arm AG measurements were made after MR, using the surface sternal notch to symphysis-pubis pathway length.

**Results:** MR TT and PWV covered 86% of aortic root-bifurcation length omitting a mean 4.7 cm of proximal ascending aorta. (i) AG TT (71±9ms) was 6.6(95%CI: 3.9-9.4) ms or 10% higher than MR (64±10 ms). (ii) AG PWV (7.9±1.3m/s) was 1.33(0.95-1.70) m/s higher than MR (6.6±1.2m/s) primarily because the surface aortic length was 70(59-81) mm longer than MR. The PWV difference between two methods decreased to 0.31(0.01-0.61) m/s when AG PWV was calculated using the MR aortic path length, and to 0.25(-0.05-0.55) m/s after correcting MR PWV for the aortic segments omitted in MR method. (iii) A regression model using age and AG sternal notch-pubis surface length from 27 cases, predicted MR length within 0.5(-14-15) mm in the remaining 22 cases.

**Conclusion:** TT estimations by AG and MR are close. More accurate length estimation from MR improves Arteriograph PWV measurement.

**Key words:** Aortic pulse wave velocity; aorta length; magnetic resonance imaging; Arteriograph
Introduction

Aortic pulse wave velocity (PWV) is a powerful independent predictor of cardiovascular events \(^1\) in both normal populations, \(^2, 3\) and those at increased risk \(^4, 5\). It is gaining popularity for risk stratification following its inclusion in the European hypertension guidelines \(^6\). However, most PWV methods require significant user expertise and for technical reasons may not be optimised for the outpatient setting \(^1\).

A novel oscillometric method (Arteriograph, TensioMed, Hungary) has been described with potential for general use \(^7\). Based on invasive validation, it claims to estimate the central aortic pulse wave transit time (TT) between the aortic root and bifurcation from the forward and reflected pressure waves recorded by an arm cuff \(^7\). The corresponding aortic path is approximated by the sternal-notch to pubis distance measured on the body surface.

Three non-invasive validation studies have demonstrated PWV agreement between the Arteriograph and the more widely used devices, SphygmoCor (AtCor Medical, Australia) and Complior (Artech Medical, France) \(^8-10\). However, SphygmoCor and Complior measure TT over an arterial path which combines both the central aortic and carotid and iliofemoral segments.

The main difference in PWV estimates by non-invasive devices may be due to differences in length estimation rather than the measurement of TT \(^10\) and this issue has been highlighted recently in a pooled analysis of 12 studies of over 13,000 participants \(^6\). “Standardizing” arterial path length measurement \(^11\) has been addressed for carotid-femoral PWV using magnetic resonance imaging (MR) \(^12\) and invasive studies \(^13\), but similar efforts to standardize the length measurement exclusive of the carotid and iliofemoral segments are not available.

Anatomical MR imaging provides accurate non-invasive measurement of aortic length and phase-contrast imaging (PCI) provides pulse wave TT, thereby allowing accurate calculation of PWV between any two points in the aorta \(^14-17\).
In this study we used MR to answer the following questions:

1) How well does Arteriograph TT and PWV agree with the MR gold standard?
2) Does more accurate length estimation from MR improve Arteriograph PWV estimation?
3) If so, could a model be suggested to standardize or improve length estimation for central aortic PWV?

Methods

Participants were 49 asymptomatic community-dwelling males already recruited into the European Male Ageing Study who were free from active chronic disease or malignancy, with no history of cardiovascular events or diabetes, and not taking any vasoactive medications such as anti-hypertensives or statins.

The study was approved by the local ethics committee and informed consent was obtained from all participants. Participants were asked to avoid caffeine, heavy meals and tobacco for 3 hours before the MR scan.

MR protocol

MR scans were performed on a 1.5T Philips Intera (Philips Medical Systems, Best, The Netherlands). The protocol included both cardiac (not described here) and aortic acquisitions taking about 45 minutes.

Standard planning scouts were followed by an ECG-gated breath-held para-sagittal steady-state free procession (SSFP) cine of the aorta from the aortic root to the proximal thoracic aorta (flip angle 60º, thickness 8mm, matrix 176x256, field of view (FOV) 320mm, repetition/echo time (TR/TE) 2.8/1.4ms, reconstructed voxel size 1.25x1.25mm). A further 10-20 contiguous sagittal slices were acquired from the aortic arch to the bifurcation to define aortic geometry and allow length to be measured (flip angle 50º, thickness 5mm, matrix 179x256, FOV 500mm, TR/TE 2.8/1.2ms, reconstructed voxel size 1.95x1.95mm). Finally, the aortic bifurcation was imaged with a coronal non-breath-hold PCI cine (flip angle 15º, thickness 60mm, matrix 128x256,
Results

**Pulse wave velocity by MRI and Arteriograph device**

FOV 450mm, velocity encoding (VENC) 90cm/s in three separate directions: right-left, anterior-posterior and head-foot, TR/TE 7.5/4.4ms, providing 16 heart phases per cardiac cycle.

Two free-breathing through-plane PCI acquisitions were planned orthogonal to the aorta at 1) the aortic arch at the level of the pulmonary artery bifurcation, and 2) approximately 2cm above the aortic bifurcation (Figure 1) (flip angle 10º, thickness 8mm, matrix 160x256, FOV 320mm, reconstructed voxel size 1.25x1.25mm, TR/TE 5.4/3.1 ms, and VENC 200cm/s to give 75 heart phases per cardiac cycle. These locations were chosen to measure PWV over most of the arch and descending aorta in the minimum total scan time. The scans were acquired sequentially at the conclusion of the MR protocol to minimise the effect of any heart rate or blood pressure changes and to minimise the interval to the post-scan Arteriograph measurements. Residual eddy currents were corrected in the image acquisition phase by the “local phase correction” filter of the scanner.

**MR image analysis**

Image analysis was performed offline by a single analyst blinded to participant identity and Arteriograph results using Philips Extended MR Workspace Software. Aortic flow and cross-sectional area for all phases of the cardiac cycle were measured from the PCI images in three regions of interest - ascending (P1), proximal descending (P2) and bifurcation (P3). Contours were automatically traced by the software with minimal supervision by the analyst, and the flow and area data for the cardiac cycle (Figure 1) exported to an Excel spreadsheet.

(i) Transit time: The arrival time of the velocity wave was determined using the “foot” algorithm (10% of the wave height) on each of the interpolated flow/time curves. By subtraction, three aortic transit times (TT) could be calculated: arch to proximal descending aorta (TT_Arch; from P1 to P2), proximal descending aorta to bifurcation (TT_Desc; P2 to P3), and arch to bifurcation (TT_Arch+Desc; P1 to P3).

(ii) Path length: Aortic path length was measured using the sagittal images and the 3D volumes rendered from the sagittal aortic slices. Points were identified at the aortic
valve (AV) and at the ascending (P₁), proximal descending (P₂) and bifurcation (P₃), with the latter three co-incident with the PCI imaging planes above. Path lengths were then calculated as AV-P₁, LENₐ=rch=P₁-P₂, LEN₅=Desc=P₂-P₃, and LENₐ=rch+Desc=P₁-P₃. Total aortic length (LENₜ₉=Total) was calculated by summing all segmental lengths from AV to Bif including AV-P₁ and P₃-Bif.

(iii) PWV: The three MR PWV values were calculated by dividing the regional path lengths by their corresponding TT (e.g. PWVₐ=rch=LENₐ=rch/TTₐ=rch).

(iv) Distensibility: Local aortic distensibility was calculated with the formula:

\[ D = \left( \frac{A_{\text{max}} - A_{\text{min}}}{A_{\text{min}}} \right) \times \frac{1}{PP} \]

where \( A_{\text{max}} \) and \( A_{\text{min}} \) (mm²) are the maximum and minimum cross-sectional areas, and PP (mmHg) is central pulse pressure provided by the immediate post-MR Arteriograph measurement. Three aortic distensibility values could be calculated at P₁, P₂ and P₃.

Arteriograph measurement

The Arteriograph measurements were performed immediately after the MR scans by a single trained operator. The device could not be taken into the scan room due to the presence of the magnetic field. To minimize changes in blood pressure and heart rate between the MR and Arteriograph measurements, participants rolled gently from the scanner bed onto a non-magnetic trolley of the same height and were moved outside the scan room where 2-3 consecutive left arm Arteriograph measurements were made. The surrogate surface measurement for aortic length was measured from sternal-notch to symphysis pubis using a flexible tape. Recorded waves were automatically analysed by the Arteriograph Software 10.1.1, and the results averaged. The Arteriograph output also included the aortic (central) systolic blood pressure (cSBP), augmentation index (aoAIX) as an index of wave reflection, standard peripheral arm pressures (using a previously validated algorithm ²⁰) and heart rate.
**Results**

**Pulse wave velocity by MRI and Arteriograph device**

*Repeatability*

Intra-observer variability of the MR analysis was measured by the same observer repeating the analysis of 10 randomly selected subjects, blinded to participant identity and previous measurements, two months after the initial analysis.

Repeat measure variability of Arteriograph measurement was obtained by comparing the study measurement in 20 participants with one performed by the same operator in the preceding 5±3 weeks.

*Statistical analysis*

All three TTs and the PWV for the entire aorta were compared between MR and the Arteriograph. The MR aortic length \( \text{LEN}_{\text{Total}} \) was divided by the Arteriograph TT to create a new PWV which was then compared with the MR PWV \( \text{Arch+Desc} \).

Bivariate correlations and multiple regression were used to investigate the correlates of the three MR lengths. Paired t-tests were used to obtain mean differences (95%CI), Pearson’s r for correlations, and Bland-Altman plots for agreement with SPSS 16.0. P-values <0.05 were considered significant. Repeatability statistics by coefficient of variation (CV) and intra-class correlation (ICC) was performed as previously described.

**Results**

*Repeatability*

Participant characteristics are shown in Table 1. Two cases were excluded from the PWV and TT comparisons - one because the heart rate between the arch and bifurcation PCI acquisitions was greatly different, and the other because the bifurcation PCI plane was positioned lower than required by the MR protocol. The results shown therefore include 47 cases. The aortic length comparison is reported for all 49 participants.
Differences in heart rate between the two PCI sequences were 0.5 bpm (95%CI -0.3–1.2; p=NS). The CV for repeat Arteriograph measures was <5% for TT and PWV.

Intra-observer CV for MR TT and PWV measurements for P2-P3 (descending) and P1-P3 (arch + descending) segments were <3%, and for P1-P2 (the arch) <8%. Similar values for MR LEN_Total was <1%. Repeatability details are listed in the supplementary file, Table A.

The aortic segments for which PWV and TT were calculable by MRI covered (on average) 72% of the AV-P2 (proximal aorta), 93% of P2-Bif (descending aorta), and 86% of AV-Bif (total) aortic regions (Figure 1). Therefore, AV-P1 and P3-Bif regions were not included in the PWV measurements by MR.

**MR and Arteriograph comparison**

PWV, TT and length measurements by MR and the Arteriograph are summarized in Table 2. Of the three MR-measured transit times, \( TT_{Arch+Desc} \) was the closest to (i.e. 10% lower than) Arteriograph TT (Table 3 and Figure 2-a). The 6.6ms difference in TT by the two methods could be attributed to the aortic path length being measured between the two PCI slices (P1-P3) which did not include the aortic valve to the first slice (AV-P1), and the 2cm space from the second slice and the bifurcation (P3-Bif) from where the reflected wave measured by the Arteriograph is assumed to be generated. To investigate this, it was assumed that the PWV from the valve to the first slice (AV-P1) could be approximated by the adjacent arch segment (PWV_Arch). Dividing AV-P1 aortic path by PWV_Arch resulted in a TT of 6.4±1.8ms. By a similar approach and using PWV_Arch+Desc, a TT of 3.5±1 ms was resulted for P3-Bif segment. After correcting for both missed segments, the difference between MR TT for total aorta (TT_Total) and Arteriograph TT reduced to 3.2 (0.2 – 6) ms.

The surface Arteriograph length over-estimated the MR aortic valve to bifurcation distance by 15%. Given that the transit times were similar; this led to a significantly higher PWV (Table 3). Using the MR length in the Arteriograph calculation produced a PWV of 6.9±1.1 m/s, reducing the difference with MR from 1.33±1.3 (p<0.001) to 0.31±1.0 m/s. It also increased the correlation from \( r=0.49 \) to 0.62 (Table 3), and
narrowed the limits of agreement (Figure 2(c) and (d)). Finally, estimating MR PWV$_{\text{Total}}$ as MR LEN$_{\text{Total}}$ divided by MR TT$_{\text{Total}}$ (i.e. corrected for the AV-P$_1$ and P$_3$-Bif missing segments) further reduced the difference between the methods to 0.25 (-0.05 – 0.55) m/s (P=NS) (see Bland-Altman graph in Supplementary file: Figure A).

The PWV measured by MR in the arch was 1.3(0.8–1.9) m/s higher than in the descending aorta (p<0.001). The Arteriograph PWV showed stronger correlations with MR stiffness indices of the descending aorta than that of the arch (Table 4).

*Linear model for aortic length prediction*

We investigated how well a regression model developed from significantly related and conveniently available correlates of aortic length (e.g. age, and height) predicted the aortic valve to bifurcation distance obtained from MR (LEN$_{\text{Total}}$) in mm. Such a model might have the potential to improve the accuracy of Arteriograph.

First, in a randomly selected *training-dataset* (n=27), we derived the following regression equations, with age (years) and Arteriograph length (cm) -

\[
\text{MR LEN}_{\text{Total}} = 157 + 1.8 \times \text{age} + 0.4 \times (\text{Arteriograph length}) \quad (R^2=0.54) \quad (1)
\]

and age and height (cm) -

\[
\text{MR LEN}_{\text{Total}} = -119 + 2.9 \times \text{age} + 2.5 \times \text{height} \quad (R^2=0.46) \quad (2)
\]

These models were then tested in the remaining participants (test-dataset, n=22), and the predicted and measured LEN$_{\text{Total}}$ were compared using a paired t-test. The mean difference (95%CI) between MR-measured aortic length and that by equation (1) was 0.5(-14–15) mm (r=0.56) (Figure 2(b)), and by equation (2) -6.0(-20–8) mm (r=0.63) with neither difference being statistically significant.

Finally, the two aforementioned regression equations derived from the “whole” data to predict aortic length in mm were (R$^2$=0.41 for both equations) -

\[
\text{MR LEN}_{\text{Total}} = 160.4 + 2.05 \times \text{age} + 0.40 \times (\text{Arteriograph length}) \quad (3)
\]

\[
\text{MR LEN}_{\text{Total}} = -109.7 + 2.9 \times \text{age} + 2.5 \times \text{height} \quad (4)
\]
Discussion

*Arteriograph and MR*

We found that transit time measured by the Arteriograph were close to that measured by MR. Differences in PWV were primarily due to differences between the Arteriograph’s external surface estimate of aortic root to bifurcation length and that measured by MR, a problem common to any device using external length measures. Using the MR length with the Arteriograph transit time to calculate PWV reduced the mean difference to less than 0.5 (“excellent”) and standard deviation to 1.5 m/s (“acceptable”) as suggested by the Artery Society guideline for validation of non-invasive hemodynamic measurement devices. Our data also suggest that predicting the aortic length using age and body size correlates (e.g. surface measurement or height) may be practical, and so may improve Arteriograph PWV in practice.

To date, most comparison studies compared the Arteriograph with carotid-femoral PWV which has a longer arterial path and is not “central” aortic PWV. One report on invasive validation of the Arteriograph (with the device inventor among the authors) showed good agreement with invasive catheterisation. In that report, we did not find data comparing invasively-measured aortic length and the surface measurement. Apparently, the authors used the surface length to calculate both the invasive and non-invasive PWV and showed very close agreement. If the term with the greatest error is in fact the length, then this represents a serious limitation of this study. It essentially only validates that the transit time or denominator in the equation. In contrast, our study directly compared both the numerator (length) and denominator (time) in the calculation of PWV, finding that it is in fact the length that contributes the greatest error compared to MR.

The average total aortic length in our study (479 mm) is similar to that we calculated from Sugawara et al’s MR study (469mm) by adding their reported ascending (74mm) and descending (395mm) aortic lengths, and to that reported by Weber et al (499 mm) using invasive catheterisation. Weber et al also reported overestimation of aortic length by the external sternal notch-pubis measurement though their average difference
(42 mm) is lower than our finding (70 mm). Both Weber and Sugawara recruited both male and female participants.

This study provides some clarification on the TT estimation range provided by the Arteriograph. To our knowledge, no other study has compared Arteriograph TT with that measured over different aortic regions. Our results show that Arteriograph TT is closest to MR TT for the aortic region spanning from near the aortic root to bifurcation which is in keeping with the only invasive study available \(^7\). However, Trachet et al’s model-based study \(^{23}\) suggested that Arteriograph measures TT between the axillary artery and where the cuff is wrapped on the brachial artery. Their study was a “numerical validation”, based purely on mathematical modelling of the cardiovascular system rather than direct measurements on human participants as we did. We did not measure axillary-brachial TT, and so could not test their theory. Whether the TT of the “axillary” and “aortic root-bifurcation” pathways are accidentally close is what future studies should clarify.

Recalculated Arteriograph PWV using MR-measured length is closer (6.9 m/s) to MR PWV\(_{Arch+Desc}\) (6.6 m/s), though still slightly (0.31 m/s) higher. This 0.31 m/s is mainly accounted for by the ascending aortic length (AV-P\(_1\)) and the last bit of descending segment (P\(_3\)-Bif) missing from the range covered by MR.

There are other factors influencing TT and PWV agreement between the two methods. Arteriograph uses a “simultaneous” approach to TT measurement on pressure waveforms recorded at the arm whereas MR “sequentially” records flow waveforms in proximal and distal aorta at two different time points gated by ECG. This is similar to other sequential methods such as SphygmoCor, and quality of measurement could be influenced by ECG quality during MR image acquisition, as well as the constancy of hemodynamic factors between sequential recordings. Other differences lie in the algorithms used for TT calculation: “wave-peak detection” by Arteriograph versus “10% of the wave amplitude” method used for MR. Observer variability from MR image acquisition/analysis or Arteriograph length measurement may be inevitable. Finally, measurement conditions such as ambient temperature, light and patients’ emotions in the scanner may have varied between the two methods despite the efforts made to minimise them.
Study limitations

The acceptable accuracy of the aortic length prediction equations in our data are based on a sample of middle-aged to elderly men; extrapolation of our aortic length prediction results to women or other age groups may be inappropriate. MR studies of wider age ranges and in women could improve these models.

The order of the two methods compared here was the same for all participants. One might suggest that the order of the Arteriograph and MR measures could have been randomly allocated. However, the MR protocol was long, significant time was needed for image planning, and having Arteriograph first would create longer intervals between the two PWV measurements. Another related limitation was that a non-magnetic MR-safe sphygmomanometer was unavailable to measure the blood pressure during MR scans. Nevertheless, the change in blood pressure is expected to be minimal between the two tests as the subjects had been supine for more than 40 min during the scan and their supine position did not change before Arteriograph measurement. Finally, the mean difference of 2 bpm (r=0.92, p<0.001) between average heart rate measured during MR (by ECG) and that by Arteriograph was small.

Conclusions

This study was the first to test the central aortic stiffness indices measured by a device such as the Arteriograph from a MR perspective and suggest the potential for central aortic length standardization. We found that the Arteriograph’s transit time is close to that measured by MR. Improved length estimation for the Arteriograph significantly enhances the agreement between its PWV and that by MR. Accuracy of the aortic length estimation by the linear equations based on age and height or sternal-pubis length in our data was acceptable and suggests such an approach for practical use. Validation with both genders may be necessary to achieve more accurate equations.
Acknowledgement

The authors wish to acknowledge the support of the Wellcome Trust Clinical Research Facility (WTCRF), Manchester where the MRI scans took place, and to thank WTCRF radiographers and all the participants who took part in the study.

Sources of Funding

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Disclosures

The study used an Arteriograph device donated by the manufacturer. The authors have no other disclosures or conflicts of interest.
References


Results

**Pulse wave velocity by MRI and Arteriograph device**

### Tables

#### Table 1. Participant characteristics (n=49).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>53±6</td>
<td>43 – 67</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83±12</td>
<td>58 – 116</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175±6</td>
<td>162 – 192</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27±3</td>
<td>21 – 34</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>128±14</td>
<td>108 – 168</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80±9</td>
<td>65 – 106</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>61±8</td>
<td>46 – 77</td>
</tr>
</tbody>
</table>

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate

#### Table 2. Mean±SD of the length and pulse wave transit time and velocity by Arteriograph and MRI for different aortic segments (n=47)

<table>
<thead>
<tr>
<th></th>
<th>Aortic length (mm)</th>
<th>TT (ms)</th>
<th>PWV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic Resonance*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending (AV-P₁)</td>
<td>46±8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arch (P₁-P₂)</td>
<td>116±17</td>
<td>16.0±3.9</td>
<td>7.7±2.2</td>
</tr>
<tr>
<td>Descending (P₂-P₃)</td>
<td>298±28</td>
<td>47.9±8.3</td>
<td>6.4±1.2</td>
</tr>
<tr>
<td>Arch-Descending (P₁-P₃)</td>
<td>413±34</td>
<td>63.9±10.1</td>
<td>6.6±1.2</td>
</tr>
<tr>
<td>Bifurcation (P₃-Bif)</td>
<td>20±15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total (AV-Bif)</td>
<td>479±36</td>
<td>73.8±11.5‡</td>
<td>6.7±1.2‡</td>
</tr>
<tr>
<td>Arteriograph</td>
<td>549±44 †</td>
<td>70.5±8.7</td>
<td>7.9±1.3</td>
</tr>
</tbody>
</table>

TT: transit time, PWV: pulse wave velocity, AV: aortic valve, Bif: aortic bifurcation
* See Figure 1 for specific aortic segmentation boundaries (P₁, P₂ & P₃) as measured by MR.
† Sternal-notch to pubic-symphysis distance on body surface, recommended by the manufacturer as the corresponding aortic path for Arteriograph transit time.
‡ Estimated values after correcting for AV-P₁ and P₃-Bif segments not directly measured by MR (see text).
Table 3. Comparison of pulse wave transit times and velocity, and aortic lengths by MR and Arteriograph (n=47). See Figure 1 and text for definition of aortic segments

<table>
<thead>
<tr>
<th></th>
<th>Mean difference (95%CI)</th>
<th>p</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aortic length (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteriograph§ – MR LEN&lt;sub&gt;Total&lt;/sub&gt;</td>
<td>70 (59 – 81)</td>
<td>&lt; 0.001</td>
<td>0.55*</td>
</tr>
<tr>
<td><strong>TT (ms)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteriograph – MR TT&lt;sub&gt;Arch&lt;/sub&gt;</td>
<td>54.5 (52.0 – 57.1)</td>
<td>&lt; 0.001</td>
<td>0.23</td>
</tr>
<tr>
<td>Arteriograph – MR TT&lt;sub&gt;Desc&lt;/sub&gt;</td>
<td>22.7 (20.2 – 25.1)</td>
<td>&lt; 0.001</td>
<td>0.52*</td>
</tr>
<tr>
<td>Arteriograph – MR TT&lt;sub&gt;Arch+Desc&lt;/sub&gt;</td>
<td>6.6 (3.9 – 9.4)</td>
<td>&lt; 0.001</td>
<td>0.51*</td>
</tr>
<tr>
<td>Arteriograph – MR TT&lt;sub&gt;Total&lt;/sub&gt;‡</td>
<td>3.2 (2.2 – 6)</td>
<td>0.04</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>PWV (m/s)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteriograph – MR PWV&lt;sub&gt;Arch+Desc&lt;/sub&gt;</td>
<td>1.33 (0.95 – 1.70)</td>
<td>&lt; 0.001</td>
<td>0.49*</td>
</tr>
<tr>
<td>Arteriograph† – MR PWV&lt;sub&gt;Arch+Desc&lt;/sub&gt;</td>
<td>0.31 (0.01 – 0.61)</td>
<td>0.04</td>
<td>0.62*</td>
</tr>
<tr>
<td>Arteriograph† – MR PWV&lt;sub&gt;Total&lt;/sub&gt;‡</td>
<td>0.25 (-0.05 – 0.55)</td>
<td>0.11</td>
<td>0.62*</td>
</tr>
</tbody>
</table>

* Significant correlations
§ Arteriograph’s sternal notch to pubis surface distance
† Arteriograph pulse wave velocity by dividing MR LEN<sub>Total</sub> by Arteriograph TT
‡ Corrected for AV-P<sub>1</sub> and P<sub>3</sub>-Bif segments not directly measured by MR (see text).

Table 4. Correlations (r) between the arterial stiffness indices from Arteriograph and MR.

<table>
<thead>
<tr>
<th></th>
<th>Arteriograph PWV</th>
<th>Arteriograph PWV (using MR length)</th>
<th>aoAIx</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR PWV (m/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arch</td>
<td>0.30*</td>
<td>0.39**</td>
<td>0.37*</td>
</tr>
<tr>
<td>Descending</td>
<td>0.48**</td>
<td>0.61**</td>
<td>0.15</td>
</tr>
<tr>
<td>Arch + Descending</td>
<td>0.49**</td>
<td>0.62**</td>
<td>0.25</td>
</tr>
<tr>
<td>MR distensibility (10&lt;sup&gt;3&lt;/sup&gt; mmHg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascending (P&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>-0.29</td>
<td>-0.44**</td>
<td>-0.38**</td>
</tr>
<tr>
<td>Descending (P&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>-0.41**</td>
<td>-0.43**</td>
<td>-0.26</td>
</tr>
<tr>
<td>Bifurcation (P&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>-0.36*</td>
<td>-0.49**</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01
aoAIx: aortic augmentation index by Arteriograph in %. Other abbreviations are as in Table 3.
Figure 1. Anatomic position of the phase-contrast slices recording aortic flow at \( P_1 \) (ascending aorta), \( P_2 \) (proximal descending aorta) at the level of pulmonary artery bifurcation, and \( P_3 \) approximately 2cm above aortic bifurcation (Bif); AV: aortic valve.
Results

**Figure 2.a-b.** Bland-Altman plots comparing a) Arteriograph versus MR $TT_{Arch-Desc}$, b) aortic lengths predicted from age and Arteriograph length (equation (1)) versus MR in the test dataset. Dashed lines are $±2SD$ of the mean difference.

a

b
**Figure 2.c-d.** Bland-Altman plots comparing MR $PWV_{\text{Arch+Desc}}$ and the Arteriograph’s c) original and d) MR-length calibrated PWV.
Web Supplement

**Table A.** Intra-observer repeatability results for repeated MR image analysis and Arteriograph different-session measurements.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>CV (ICC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MR</strong></td>
<td></td>
</tr>
<tr>
<td>$PWV_{Arch}$</td>
<td>7.8% (0.91)</td>
</tr>
<tr>
<td>$TT_{Arch}$</td>
<td>7.9% (0.83)</td>
</tr>
<tr>
<td>$PWV_{Desc}$</td>
<td>3.4% (0.96)</td>
</tr>
<tr>
<td>$TT_{Desc}$</td>
<td>3.2% (0.96)</td>
</tr>
<tr>
<td>$PWV_{Arch+Desc}$</td>
<td>2.5% (0.98)</td>
</tr>
<tr>
<td>$TT_{Arch+Desc}$</td>
<td>2.4% (0.97)</td>
</tr>
<tr>
<td>DistP1</td>
<td>9.4% (0.97)</td>
</tr>
<tr>
<td>DistP2</td>
<td>8.1% (0.94)</td>
</tr>
<tr>
<td>DistP3</td>
<td>10.5% (0.95)</td>
</tr>
<tr>
<td>LENTotal</td>
<td>0.7% (0.99)</td>
</tr>
<tr>
<td><strong>Arteriograph</strong></td>
<td></td>
</tr>
<tr>
<td>PWV</td>
<td>5.1% (0.87)</td>
</tr>
<tr>
<td>TT</td>
<td>4.5% (0.88)</td>
</tr>
</tbody>
</table>

CV: coefficient of variation, ICC: intra-class correlation, mrPWV, mrTT & mrDist: transit time, pulse wave velocity and aortic distensibility measured by MR for aortic regions (levels) defined in Figure 1, agPWV & agTT: Arteriograph’s pulse wave velocity and transit time.
**Figure A.** Bland-Altman graph showing the agreement between the MR length calibrated Arteriograph PWV and MR PWV<sub>total</sub> estimated for total aorta (AV-Bif).
Calibration Mode Influences Central Blood Pressure Differences between SphygmoCor and Two Newer Devices, the Arteriograph and Omron HEM-9000
Calibration mode influences central blood pressure differences between SphygmoCor and two newer devices, the Arteriograph and Omron HEM-9000

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* Previous publication surname: Rezailashkajani
Abstract

**Aims:** To compare central systolic blood pressure (cSBP) and augmentation index (AIx) from two recently introduced devices Omron HEM-9000 (OM) and Arteriograph (AG) not using a transfer function with that of the widely used SphygmoCor (SC) calibrated on brachial BP like OM.

**Methods:** Random-order manufacturer-recommended measurements using SC and OM by radial tonometry and AG were taken on the left arm in 35 men (54±10 yr) after 5 minutes supine rest. Results are means (95%CI) of differences using paired t-tests.

**Results:** cSBP by OM was 4.1(1.0–7.1) mmHg higher than by AG. Both OM and AG estimated mean cSBP significantly higher than that of SC (114.8 mmHg) by 12.5(10.3–14.7) and 8.6(4.9–12.3) mmHg, respectively, while closely correlated (r=0.9). Calibrating SC with DBP and more accurate mean arterial pressure (as DBP+0.4*PP) resulted in significantly higher cSBP statistically not different from AG’s cSBP: 0.9(-1.1–+2.9)mmHg, and closer to OM’s: 5.1(3.4–6.8)mmHg.

*Radial* AIx from SC and OM disagreed by 3(0.7–5.4)%, and correlated (r=0.8) with AG’s *brachial* AIx. AG’s aortic AIx was 7.9(5.7–10.2)% higher than SC’s, but closely correlated (r=0.9).

**Conclusion:** Clinically significant, higher cSBP measured by AG, OM and more accurately calibrated SC add to previous data suggesting that SC measurements by classic calibration underestimate cSBP. Invasive studies involving all 3 devices would be more illuminating.
Central blood pressure (cSBP) and indices of wave reflections have become relevant to cardiovascular risk stratification and hypertension management. The primary organs targeted by hypertensive damage (heart, brain and kidneys) may be exposed more closely to aortic blood pressure (BP) and pulsations rather than that estimated by traditionally measured brachial pressures \(^1\). Moreover, antihypertensive medications may affect central and peripheral pressures differently \(^2\) which could influence drug efficacy and risk reduction \(^3\). Our previous data from the CAFE study suggested that one drug class and combination produced greater central but not peripheral BP change based on SphygmoCor \(^2\).

There are still few large cohort studies comparing the prospective value of central versus peripheral (brachial) pressures, but a number of small to medium size studies have demonstrated the value of invasively measured central systolic and pulsating pressures prospectively \(^4\). Large trials demand quick non-invasive measurement of central pressures.

In the past decade, the SphygmoCor\(^\circledR\) device has provided a commercial scale non-invasive method for estimation of central pressures and reflection waves. It uses a generalized transfer function (GTF) to estimate the aortic pressure waveform from the radial pulse waveform recorded by a tonometry probe calibrated against non-invasively measured brachial pressures. The GTF has been derived and tested in several invasive studies using “invasive” calibration of radial waveform \(^5-9\). However, there is still controversy over accuracy of the typical outpatient SphygmoCor measurements using “non-invasive” calibration with brachial pressures, and in different patient populations \(^4, 10-14\).

Recently, two other non-invasive devices which do not use a transfer function have been introduced for cSBP and wave reflection measurement, the Omron HEM-9000\(^\circledR\) (Omron Healthcare, Kyoto, Japan) and Arteriograph\(^\circledR\) (TensioMed, Budapest, Hungary). The aim of this study was to compare the cSBP and reflection wave indices from the two newly introduced devices with that of SphygmoCor, and between themselves.
Methods

**Devices**

The SphygmoCor (AtCor Medical, Sydney, Australia) uses a tonometry probe manually applied by a trained user on the radial artery pulse on the wrist. It records the pulse waveform which is calibrated against upper arm cuff BPs. A generalized transfer function is then applied to the radial waveform to derive that in the aorta and calculate cSBP.

The Omron HEM-9000 uses an automatic tonometry probe wrapped onto the wrist to record radial waveforms which are then calibrated against the contralateral brachial BP measured by an arm cuff immediately after tonometry. It then applies an algorithm based on a linear regression model to estimate cSBP from the “late systolic shoulder” (pSBP$_2$) of the radial pulse waveform which has been shown to agree closely with cSBP$^{15,16}$. The device uses the maxima of the “multidimensional derivatives” on the recorded pressure waveforms to detect first and second inflection points corresponding to early and late systolic (pSBP$_2$) pressures$^{15,17}$.

The Arteriograph records the brachial pressure waveform using an oscillometric method after occluding the brachial flow by inflating an arm cuff to about 35 mmHg above the systolic pressure without any calibration. The device estimates cSBP from a commercial algorithm based on the correlation between the pSBP$_2$ on uncalibrated brachial waveforms and aortic systolic pressures derived from the inventors’ invasive studies$^{18}$. The Arteriograph’s algorithm for brachial BP measurement was shown to meet the accuracy criteria from BHS and AAMI$^{19}$.

Each device measures some indices of wave reflection, mainly augmentation index (AIx) peripherally and/or centrally. Peripherally, the SphygmoCor and Omron HEM-9000 both measure radial AIx (rdAIx) on calibrated radial waveforms while the Arteriograph measures brachial AIx (brAIx) from uncalibrated brachial waveform. Centrally, SphygmoCor estimates aortic AIx (aoAIx) from the values on the aortic waveform derived by its transfer function, but Arteriograph calculates aoAIx from its relationship with brAIx by its commercial algorithm. Omron HEM-9000 does not give central AIx values.

Omron HEM-9000 and SphygmoCor both calculate their peripheral AIx as (P$_2$ - DBP)/(P$_1$ - DBP), taking P$_1$ and P$_2$ as the first and second inflection points on the radial
pulse waveform. However, calculation of aoAIx in both SphygmoCor and Arteriograph and that of the brAIx in Arteriograph is performed using \((P_2-P_1)/PP\).

**Participants & Protocol**

The participants were 35 men 40-80 years of age who had already been recruited to European Male Ageing Study (EMAS) in Manchester, UK. The participants were free of active liver and kidney disease or malignancy. All measurements were done in the morning. Participants were advised to avoid caffeine containing beverages and tobacco 3 hours before their visit, and alcohol from the night before. Using a standard protocol, sitting BP was measured using a standard Omron automatic device on the left upper arm, 3 times after 5 minutes of rest in a temperature controlled room. The values from the last two readings were averaged. Afterwards the three device measurements were performed supine in random order on each participant using computer-generated random allocation. The participants were asked to remain still, relaxed and silent during all measurements.

A trained single observer conducted standard SphygmoCor radial tonometry on the left wrist with BP calibrated by average systolic and diastolic BPs from two supine measurements made by an automatic Omron BP measurement device on the left arm immediately before. Three quality measurements were selected and averaged (SCOR-2000 software, version 7.1, AtCor Medical, Sydney, Australia). The Arteriograph cuff was tightly wrapped around the left arm. Only quality measurements were filtered and averaged for each participant (TensioClinic software, version 1.10.0.0). Omron HEM-9000 measurement was conducted with the tonometry probe fastened on the left wrist and the BP cuff on the right arm in order not to interfere with the tonometer measurements on the same side, as recommended by the manufacturer.

**Comparisons & Statistical Analysis**

Central systolic blood pressures from the three devices were compared two by two. Omron HEM-9000’s rdAIx and Arteriograph’s aoAIx were compared with their SphygmoCor counterparts respectively. Omron HEM-9000’s pSBP\(_2\) was also compared with cSBP from SphygmoCor. Comparisons were repeated after cross-calibrating
SphygmoCor by 3 methods using three sets of brachial BPs from Arteriograph and Omron HEM-9000. Method 1 used SBP and DBP, Method 2, DBP and MAP (mean arterial pressure) calculated by the classic formula: DBP+(PP/3), and Method 3, DBP and MAP by a newer formula: DBP+(0.4*PP) derived by Bos et al. comparing non-invasive and invasive brachial BPs. This has been recommended as a more accurate estimate of brachial MAP.

SPSS 16 was used for statistical analysis. Paired t-tests were used to obtain mean differences (95%CI), Bland-Altman plots (i.e. mean±2SD of difference) for agreement, with Pearson’s r for correlation between the device measurements. P values <0.05 were considered significant.

Results

The general characteristics of the participants are shown in Table 1. Three patients used statins one of whom also took antihypertensive medications. Three subjects had known type 2 diabetes.

cSBP comparisons

Both new devices estimated cSBP significantly higher than the SphygmoCor before and after cross-calibration by methods 1 and 2 (Tables 2 and 3). The Bland-Altman plots revealed that cSBP did not agree across the three devices (Figure 1a-b and 2c), with more discrepancy at higher pressures. Omron HEM-9000’s pSBP agreed with SphygmoCor’s (Figure 2d) but was significantly lower than Arteriograph’s and Omron HEM-9000’s cSBP estimations (Table 3).

The calibration methods 1 and 2 did not significantly change the cSBP difference between SphygmoCor and the two other devices, but narrowed limits of agreement and improved correlations (Tables 2 and 3; Bland-Altman plots in Figures A and B in Supplementary Information). However, calibration method 3 resulted in significantly higher cSBP with SphygmoCor, statistically no longer different (p=0.37) from Arteriograph’s cSBP (i.e near agreement; SD difference: 6 mmHg) and closer to Omron HEM-9000 (p<0.001) (Tables 2 and 3, Figures 1c-d). This also increased the pSBP difference between SphygmoCor and Omron (Table 3).
The difference between the two new devices’ cSBP and that of SphygmoCor tended to increase with the average across-device cSBP (Figure 1a and b). In regression models including age, height, heart rate (HR), and MAP, the cSBP difference between original readings of SphygmoCor and Arteriograph was independently associated with HR, MAP and height, and that between SphygmoCor and Omron, with height and HR. The difference between AG and Omron decreased with average cSBP, and was related to younger age and higher MAP (Table A, Supplementary Information).

No significant differences (mean [95%CI]) were found between the standard arm SBP measurements by the Arteriograph and Omron HEM-9000 (-0.3[-3.2 – +2.6] mmHg) or the standard Omron sphygmomanometer used to calibrate the SphygmoCor (0.7[-2.1 – +3.6] mmHg). The two Omron devices’ SBP difference (1[-0.8 – +2.8] mmHg) was also not significant.

**AIx comparisons**

Omron HEM-9000’s and SphygmoCor’s rdAIx disagreed (Figure 2a) but were closely correlated (Table 3). Arteriograph’s aoAIx was significantly lower than but closely correlated with SphygmoCor’s aoAIx (Table 3). The difference between the aoAIx by Arteriograph and SphygmoCor seemed to increase with the average cross-device values (Figure 2b).

The Arteriograph’s brAIx correlated with SphygmoCor’s rdAIx (r=0.84, p<0.001) and Omron HEM-9000’s rdAIx (r=0.75, p<0.001). SphygmoCor’s aoAIx correlated with HEM-9000’s rdAIx (r=0.87, p<0.001), and Arteriograph’s brAIx (r=0.86, p<0.001).

A unified overview of all AIx values across the three devices using a single AIx calculation formula \((P_2-P_1)/PP\) is illustrated in Figure 3. The values are shown from central to peripheral measurement sites. As expected, an ascending gradient exists with the most negative AIx values at the radial artery, slightly less negative at the brachial artery, and positive values estimated for the aorta.

None of the cross-calibration methods above significantly changed AIx differences between SphygmoCor and the newer devices (Tables 2 and 3).
Results

Central blood pressures by three devices

Discussion

To our knowledge this is the first study comparing cSBP and augmentation indices by the three devices at one time. None of the published studies comparing SphygmoCor and Arteriograph\textsuperscript{23-25} reported a comparison of central pressures. Providing uniformly calculated AIx values across devices, and comparing the results using different calibration approaches with SphygmoCor are strong points of this study.

cSBP

Both newer devices estimated cSBP significantly higher than did SphygmoCor calibrated by methods 1 and 2. Richardson et al similarly found higher cSBP (12.2±4.6 mmHg) by Omron HEM-9000 than SphygmoCor in younger subjects\textsuperscript{26}. The key question is whether the SphygmoCor is underestimating or the new devices are overestimating cSBP. Several invasive studies tested SphygmoCor’s GTF for cSBP estimation from radial pressure waveforms\textsuperscript{5-9}. However, they calibrated radial waveforms by “invasive” central DBP and MAP, and not oscillometric arm pressures which is how SphygmoCor measurements are done in practice. Later, other invasive studies consistently showed that cSBP estimated by a GTF with “non-invasively” calibrated radial waveforms significantly underestimated invasively-measured cSBP\textsuperscript{10-12} with the mean difference being 11-13 mmHg in two studies\textsuperscript{10, 27}, 7-8 mmHg in three\textsuperscript{11, 28, 29}, and 1.5-4.2 mmHg in two reports\textsuperscript{13, 14}. The SD of the difference ranged 7-15 mmHg in these studies unacceptable by BHS or AAMI criteria.

Two explanations have been suggested for such discrepancies. First, oscillometric brachial cuff methods are prone to error especially for DBP\textsuperscript{13, 30}. Second, real radial pressures are generally higher than brachial due to the amplification phenomenon\textsuperscript{21, 22}. Calibrating radial waveforms using brachial BPs is therefore a source of pressure underestimation. Such pressure errors can be “transferred” by the transfer function to cSBP estimates\textsuperscript{5, 31}. In line with the first explanation, our results show that when more accurate estimates of peripheral pressures are used to calibrate SphygmoCor (i.e. method 3)\textsuperscript{20-22}, the cSBP estimates are significantly higher, and less or not different from the two newer devices. Given the evidence on SphygmoCor’s traditional calibration, the relatively higher cSBPs by the two new devices and that from SphygmoCor calibrated here by method 3 are likely to be closer to real. However,
invasive studies simultaneously including all three devices can further elucidate this matter.

There are serious consequences in clinical practice and scientific data of these results. At least 3 important studies in the Consensus document used the SphygmoCor, and the data here suggest that re-analysis is appropriate of previous SphygmoCor data recalibrated with recently recommended, more accurate brachial BP estimates.

In contrast with SphygmoCor, initial invasive studies which gave rise to the newer devices, and their standard outpatient measurements both estimate cSBP from non-invasively calibrated radial (Omron HEM-9000) or uncalibrated brachial (Arteriograph) pressure waveforms. Including such errors at the initial development stage could make their measurement less vulnerable, but not immune to oscillometric inaccuracies.

The higher cSBP estimations by the newer devices and the alternatively-calibrated SphygmoCor by method 3 seem closer to peripheral pressures, and so may be questioned considering the concept of central to peripheral pressure “amplification”. In our data, Arteriograph cSBP was 2.5(0.3 – 4.7) mmHg lower (p=0.03) than, and Omron HEM-9000 cSBP statistically not different from, arm SBP (mean difference: 1.14[-1.1 – 3.3], p=0.31) (Table 2). cSBP not statistically different from arm pressures have been previously reported with Omron HEM-9000 and two invasive studies.

In contrast, direct invasive measurements along central-radial arterial path found significant amplification at the peripheral sites. Two points need attention. First, less amplification is expected among older subjects with stiffer arteries, usually comprising a majority of cases in device validation/comparison studies (including ours). Second, it may be inappropriate to judge amplification by comparing invasive cSBP and (subtracting from) error-prone oscillometric brachial SBP. Comparing the newer device’s cSBP with invasively measured peripheral pressures, or those calibrated by invasive DBP and MAP may be more valuable.

Other issues merit attention. The occlusion of the brachial artery by the Arteriograph will create additional wave reflections. To what extent this could alter the recorded waveforms or central pressures has not been investigated. Omron HEM-9000 and
Results

Central blood pressures by three devices

Arteriograph’s cSBP estimation algorithms depend on pSBP, which itself might not be an accurate surrogate of cSBP in lower cSBP ranges.

The 4.1 mmHg difference between cSBP estimations of the Arteriograph and Omron HEM-9000 may reflect the differences in the invasive studies producing their algorithms, (e.g. use of peripheral waveforms in different sites) and Omron’s dependence on calibration by contralateral brachial BPs compared with Arteriograph’s direct recording of pressure waveforms.

Alx

Unlike cSBP where absolute values are important, ranking of Alx or its relative change appears more important. In practice, using the new devices for Alx measurement seems as useful and less complex considering high inter-device correlation, which is still not the same as agreement.

Though HR is an important determinant of Alx, we did not use HR-adjusted Alx values from Omron and SphygmoCor because Arteriograph does not adjust for HR. However, average HR was not statistically different across the three devices (mean differences: SphygmoCor – Arteriograph: -0.1 (r=0.94), SphygmoCor – Omron HEM-9000: 0.7 (r=0.96), Omron HEM-9000 – Arteriograph: -0.8 (r=0.95), p=NS for all).

Here, the rdAlx by Omron HEM-9000 was 3% higher than but closely correlated with SphygmoCor’s rdAlx. Richardson et al found no statistical difference between rdAlx values for the two devices and similar correlations to ours. These suggest that rdAlx by SphygmoCor and Omron HEM-9000 might be used interchangeably with care. However, it is unclear what extent of Alx difference would be of clinical or practical significance.

SphygmoCor’s lower aoAlx than Arteriograph (by 7.9±6.7%) may be due to the differences in cSBP estimation although previous invasive studies showed that a GTF could underestimate invasively-measured aoAlx by 7±9% and 6±20%.
The close correlations between central and peripheral AIX values across devices (e.g. SphygmoCor’s aoAIX and Arteriograph’s brAIX) found here and previously suggest that directly-measured peripheral AIX values might be preferred over the “estimated” central one.

Uniform calculation of AIX across devices as performed here is recommended for future reports. The Arteriograph reports peripheral (brAIX) and central (aoAIX) using $P_2 - P_1 / PP$. The SphygmoCor uses the same formula for aoAIX, but not for rdAIX calculated by $(P_2 - DBP)/(P_1 - DBP)$, as in the Omron. Uniform calculation is possible by $P_2 - P_1 / PP$ as $P_1$ and $P_2$ are available in devices’ outputs. The results range from negative values in peripheral, to predominantly positive values in central sites (Figure 3).

Limitations of this study include few younger and no female participants. As always, a wider range of other variables, (eg: BP, HR, etc) might have been useful but the ranges used here were similar to usual practice.

**Conclusions**

When traditionally calibrated by less-accurate brachial BPs, SphygmoCor estimated cSBP significantly lower than two newer devices. Using more accurate estimates of brachial BPs to calibrate SphygmoCor removed or significantly reduced the between-machine differences. Invasive studies including all the three devices and over a wide range of blood pressures will further clarify this issue.
Acknowledgements

We acknowledge the support of the Wellcome Trust Clinical Research Facility and the donation of the Arteriograph and Omron devices by their suppliers, TensioMed and Omron, respectively. We also thank the reviewers who suggested using a uniform calibration procedure for this paper, presented above.

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Conflicts of Interest/Disclosure(s)

The authors declare no conflicts of interest.
References


Tables

Table 1. General characteristics of the participating men (n=35).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>54±10</td>
<td>41 – 76</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>81 ± 11</td>
<td>53 – 103</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173 ± 6</td>
<td>163 – 184</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27 ± 3</td>
<td>19 – 34</td>
</tr>
<tr>
<td>SBP* (mmHg)</td>
<td>122 ± 17</td>
<td>99 – 172</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79 ± 10</td>
<td>64 – 99</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>61 ± 9</td>
<td>44 – 83</td>
</tr>
</tbody>
</table>

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate
* Measured in sitting position.

Table 2. Mean±SD of the measurements by the three devices and SphygmoCor’s estimates after cross-calibration by three methods

<table>
<thead>
<tr>
<th>mmHg or %</th>
<th>Omron HEM-9000</th>
<th>Arteriograph</th>
<th>SphygmoCor</th>
<th>Calibrated by§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Original</td>
<td>Method 1</td>
<td>Method 2</td>
<td>Method 3</td>
</tr>
<tr>
<td>eSBP</td>
<td>127.3±16</td>
<td>114.8±13</td>
<td>115.3±17</td>
<td>115.5±17</td>
</tr>
<tr>
<td>pSBP₂</td>
<td>112.1±15</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SBP</td>
<td>126.2±14</td>
<td>125.9±18</td>
<td>125.2±14*</td>
<td>NA</td>
</tr>
<tr>
<td>aoAIx (%)</td>
<td>NA</td>
<td>30±13</td>
<td>22±9</td>
<td>22±9</td>
</tr>
<tr>
<td>rdAIx (%)</td>
<td>73±11</td>
<td>NA</td>
<td>76±13</td>
<td>76±13</td>
</tr>
<tr>
<td>brAIx (%)</td>
<td>NA</td>
<td>-15±25</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

§ Cross-calibrated with Arteriograph’s brachial BPs. See text for Methods 1-3. Using Omron HEM-9000 brachial BPs gave similar results.

Table 3. Comparison of central blood pressures and augmentation index between SphygmoCor and Omron HEM-9000 or Arteriograph before/after cross-calibrating “SphygmoCor” with different sets of brachial pressures from Arteriograph and Omron HEM-9000. All Pearson $r$ correlations are significant.

<table>
<thead>
<tr>
<th></th>
<th>Original comparisons</th>
<th>After SC cross-calibration by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method 1) SBP/DBP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean diff. (CI)</td>
</tr>
<tr>
<td>cSBP OM – SC</td>
<td>12.5 (10.3 – 14.7)*</td>
<td>0.91</td>
</tr>
<tr>
<td>cSBP AG – SC</td>
<td>8.6 (4.9 – 12.3)*</td>
<td>0.88</td>
</tr>
<tr>
<td>cSBP OM – AG</td>
<td>4.1 (1.0 –7.1)*</td>
<td>0.91</td>
</tr>
<tr>
<td>cSBP SC – pSBP$_2$ OM</td>
<td>2.0 (-0.1 –4.1)</td>
<td>0.91</td>
</tr>
<tr>
<td>rdAIx OM – SC (%)</td>
<td>-3.0 (-5.4 – -0.7)*</td>
<td>0.84</td>
</tr>
<tr>
<td>aoAIx AG – SC (%)</td>
<td>7.9 (5.7 – 10.2)*</td>
<td>0.86</td>
</tr>
</tbody>
</table>

* Significant $p$ values; $^1$ MAP calculated by classic formula: DBP + (PP/3); $^1$ MAP calculated by newer formula: DBP + (0.40*PP); mean diff. (CI): mean difference (95% CI); OM: Omron HEM-9000, SC: SphygmoCor, AG: Arteriograph, cSBP: central SBP, aoAIx and rdAIx: aortic and radial augmentation indices, pSBP$_2$: late systolic shoulder on the radial pulse pressure waveform.
**Results**

*Paper 6*

**Central blood pressures by three devices**

**Figures**

**Figure 1.a-b.** Bland-Altman plots testing cSBP agreement between original readings of SphygmoCor and Arteriograph or Omron HEM-9000. Dashed lines show limits of agreement.

a)

![Bland-Altman plot a](image1)

b)

![Bland-Altman plot b](image2)
**Figure 1.c-d.** After cross-calibrating by more-accurate estimates of brachial BPs (method 3)
Figure 2.a-b. Bland-Altman plots of a) rdAIx from Omron HEM-9000 and SphygmoCor, and b) aoAIx from Arteriograph and SphygmoCor.
Figure 2.c-d. Bland-Altman plots of c) cSBP from Arteriograph and Omron HEM-9000, and d) SphygmoCor’s cSBP and HEM-9000’s pSBP2.
**Figure 3.** A box-plot comparison of central and peripheral augmentation index (AIx) values measured by Arteriograph (dark gray), SphygmoCor (white) and Omron HEM-9000 (light gray) all calculated by a unified formula (P2-P1/PP). Whiskers are ranges; heavy horizontal line, median and box borders are 25th and 75th percentiles.
Supplementary Information

Table A. Three multiple regression models predicting cSBP differences between 3 devices:

<table>
<thead>
<tr>
<th>Outcome</th>
<th>AG - SC</th>
<th>OM - SC</th>
<th>OM - AG</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-square</td>
<td>0.66</td>
<td>0.38</td>
<td>0.52</td>
</tr>
<tr>
<td>Predictors</td>
<td>B (SE), p</td>
<td>B (SE), p</td>
<td>B (SE), p</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>0.56 (0.09), &lt;0.001*</td>
<td>0.12 (0.08), 0.12</td>
<td>-0.44 (0.10), &lt;0.001*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>-0.39 (0.13), 0.006*</td>
<td>-0.27 (0.11), 0.02*</td>
<td>0.12 (0.13), 0.39</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.39 (0.19), 0.049*</td>
<td>-0.50 (0.16), 0.003*</td>
<td>-0.12 (0.19), 0.55</td>
</tr>
<tr>
<td>Age (year)</td>
<td>0.21 (0.11), 0.07</td>
<td>-0.04 (0.10), 0.67</td>
<td>-0.26 (0.12), 0.04*</td>
</tr>
</tbody>
</table>


Results
Figure A. Bland-Altman graphs comparing cSBP between Arteriograph and Sphygmocor after calibration by the Arteriograph’s BPs using (a) calibration Method 1: SBP/DBP, and (b) Method 2: DBP and traditionally calculated MAP as DBP+(PP/3).
**Figure B.** Bland-Altman graphs comparing cSBP between Omron HEM-9000 and Sphygmocor after calibration by the Omron HEM-9000’s brachial BPs using (a) calibration Method 1: SBP/DBP, and (b) Method 2: DBP and traditionally calculated MAP as DBP+(PP/3).
Summary and Conclusions

G) Summary and Conclusions

G.1 What this study added

In this PhD project, considering the known cardiovascular risk differences among South Asians, African-Caribbean and Europeans in the UK, we described cardiovascular risk in three ethnic groups by non-invasive measurement of some vascular and cardiac intermediate end-points or surrogate biomarkers primarily by imaging methods. We then investigated whether variations in circulating levels of aldosterone and vitamin D are associated with any ethnic differences in surrogate risk biomarkers, and if such hormonal differences might explain part of the risk variations across these ethnic groups.

We found independent associations between vitamin D status as circulating levels of 25(OH)D and both vascular and cardiac risk markers. Among the vascular biomarkers, aortic pulse wave velocity was more consistently related with vitamin D than CIMT. In addition, our results suggested that the ethnic differences in surrogate risk markers could partly be explained by 25(OH)D variation across these groups (Papers 1 and 2). Overall, associations of the cardiovascular risk markers were more consistent with 25(OH)D than aldosterone.

The positive results found here on independent association of vitamin D status and surrogate biomarkers of cardiovascular disease such as aortic pulse wave velocity and left ventricular concentric remodelling are novel findings from a relatively disease-free multi-ethnic community sample, and suggest new perspectives to explain ethnic differences in cardiovascular outcomes.

On the technical side of the project, our introspection of the arterial stiffness methods used in this project (e.g. the Arteriograph device) added more insight into this relatively new method. Our results from aortic path and flow measurements by MRI (Paper 5) was the first independent study directly investigating the Arteriograph’s transit time after the validation paper by Arteriograph’s inventors [31]. This work was also among the first
attempts to standardize aortic length estimation for “central” aortic pulse wave velocity measurement by MRI, paving the way for larger studies leading to more accurate length-estimation methods.

Our study of comparing central blood pressures across three commonly-used devices complemented recent evidence and would contribute to resolve the current controversies over SphygmoCor’s estimation of central pressures (Paper 6).

Figure G-1. Possible causal pathways in our findings
Dashed lines show assumptive pathways.

RFs: Risk factors, RAAS: renin-angiotensin-aldosterone system, PWV: aortic pulse wave velocity

G.2 Limitations

Though discussed within the papers, it is worth underlining some of the study limitation here.

1. The associations between vitamin D and surrogate biomarkers of CVD found here in a cross-sectional design cannot be construed as cause-effect relationships as alternative hypotheses could also explain our findings (Figure G-1).
instance, vitamin D deficiency and the associated unfavourable biomarker
profiles found here (e.g. higher PWV, or LV concentric remodelling) could all
be under influence of an upstream confounder. Alternatively, vitamin D might
affect PWV and cardiac remodelling through unknown intermediary factors
(Figure G-1).

2. The study participants here were not randomly sampled and therefore may be
biased though their metabolic profiles are similar to that known in the
population.

3. Despite compelling evidence on the value of surrogate biomarkers in predicting
cardiovascular events or death in general, it is not yet possible to tell which of
the biomarkers measured in this project are more specifically associated with the
stroke or CHD. Some studies have suggested that higher CIMT seems to be
slightly more closely associated with stroke than CHD risk [102].

G.3 Future Work

The future work will definitely need interventional trials to test the relationships found
here in a cross-sectional design in papers 1 to 4. We suggest that such trials should
include people in wide ranges of vitamin D especially those with very low levels as in
our data. If trials are difficult to perform, then there is still space for larger observational
prospective studies using more representative, less biased samples of the ethnic groups.

In our echocardiographic measurements (Paper 2), we mainly focused on diastolic
markers such as e’ and did not analyze tissue Doppler traces for systolic function
indices. Adding such measurements is worth in future studies as our MR tagging data
(Paper 3) suggested associations between 25(OH)D and tissue-level early markers of
systolic dysfunction (i.e. myocardial strain). Measurement of sensitive early markers of
both systolic and diastolic myocardial function by echocardiography (e.g. speckle
tracking) or MRI (e.g. tagging techniques) are recommended. Using more accurate
cardiac MRI techniques (compared to echocardiography) may help reduce sample sizes needed for future trials.

There is sparse data on comparing proximal versus distal aortic stiffness characteristics in risk stratification, as we showed in our MRI aortic data in Paper 1. We recommend similar, but larger MRI studies on patients at different risk levels of stroke and CHD to see whether pulse wave velocity along proximal (arch) and distal (descending) aortic paths are differentially associated with stroke and CHD. Another related line of work could be to investigate whether aortic stiffening pattern varies by ethnicity. As people age, does aorta stiffen uniformly or more accelerated in proximal region, and whether such patterns differ in the three ethnic groups studied here? MRI studies measuring regional aortic stiffness in different age groups preferably prospectively could be illuminating.

On the technical side and following our work on standardization of aortic length estimate for central pulse wave velocity measurements (Paper 5), we recommend larger MRI studies including both sexes and wider age range to provide more accurate standard methods for estimating pure aortic path using convenient variables such as age and height. Such studies could also be done by pooling data from previous MRI studies where total aortic length is measureable.
H) References

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