

APPENDIX 1
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**THE EFFECT OF DIABETES, ETHNICITY, IMPAIRED
FASTING GLUCOSE AND EXERCISE
ON ARTERIAL STIFFNESS**

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A thesis submitted for the degree of

Doctor of Philosophy

Cardiff University,

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September 2007

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ABSTRACT

Although conventional risk factors such as hypertension, diabetes, smoking and dyslipidaemia are all established risk factors for cardiovascular disease, they do not explain the total risk. Arterial stiffness has emerged as a major independent risk factor for cardiovascular disease and measurement of arterial stiffness in clinical practice should improve the diagnosis and management of patients with cardiovascular disease.

Study 1, compared 3 methods of assessing arterial stiffness and found that: each method of assessment was comparable to the other and that reproducibility was similar throughout the systems. Within-observer variation was also very low, suggesting that high quality and accurate recordings of arterial stiffness measurements were obtained.

Study 2. Since there are conflicting data associated with arterial stiffness and type-2 diabetes, **study 2a and 2b** therefore assessed arterial stiffness, using pulse wave analysis and pulse wave velocity and found there to be increased arterial stiffness in a group of type-2 diabetics compared to healthy controls.

Study 2c and 2d. Since there are also ethnic variations in cardiovascular risk, the second study also investigated differences in arterial stiffness between South Asians and Caucasians, with and without diabetes and found that South Asians had significantly lower arterial stiffness in the femoral and aortic vascular bed compared to the Caucasians, whilst having higher arterial stiffness in the radial vascular bed.

Study 3. Although diabetes is known to increase arterial stiffness, the effect of pre-diabetes (impaired fasting glucose) on arterial stiffness is unclear. The effect of impaired fasting glucose on arterial stiffness has therefore been investigated in the *third study* and the findings demonstrate that individuals with impaired fasting

glucose have increased arterial stiffness compared to individuals with normal fasting glucose. Similar findings were observed when comparing diabetics and individuals with normoglycaemia.

Study 4. Finally, therapeutic intervention targeted at increased arterial stiffness should be of benefit in reducing the prevalence of cardiovascular disease. The *fourth study* has therefore also examined the effect of regular aerobic exercise on arterial stiffness and found that in older individuals, arterial stiffness was significantly lower in a group of individuals who exercised regularly compared to sedentary controls. Therefore, suggesting the potential benefit of aerobic exercise as a non-pharmacological intervention to decrease arterial stiffness and cardiovascular disease.

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LIST OF ABBREVIATIONS

ACCT – Anglo Cardiff Collaborative Trial
ACh – Acetylcholine
ADA – American diabetes association
AIx – Augmentation index
ANOVA – Analysis of variance
AP – Augmentation pressure
aPWV – aortic pulse wave velocity
BMI – Body mass index
BP - Blood pressure
CAD – Coronary artery disease
CCIMT – Common carotid intima media thickness
CDBP - Central diastolic blood pressure
CF PWV – Carotid - femoral pulse wave velocity
CHD – Coronary Heart Disease
CHD – Coronary heart disease
CI – Cardiac index
CMAP –Central mean arterial pressure
CNP – C natriuretic peptide
CNP - C-type natriuretic peptide
CO – Cardiac output
CPP – Central pulse pressure
CR PWV – Carotid - radial pulse wave velocity
CRP – C-reactive protein
CSBP – Central systolic blood pressure
CV – Cardiovascular
DIABS - Diabetics
ECG – Electrocardiogram
ED – Ejection duration
EDRF– endothelium derived relaxing factor
EGFR – Estimated glomerular filtrate ratio
ENOS – Endothelial nitric oxide synthase
ESRF – End stage renal failure

FD PWV – Femoral - dorsalis pedis pulse wave velocity

FMD – Flow mediated dilatation

GLUC - Glucose

GTN – Glyceryl trinitrate

HA – Healthy south asians

HC – Healthy caucasians

HDL – High density lipoprotein

HLTPA – High leisure time physical activity

HR – Heart rate

HRv – Heart rate variability

HT - Height

IDF – International diabetes federation

IHD – Ischemic heart disease

I-IFG – Isolated impaired fasting glucose

I-IGT – Isolated impaired glucose tolerance

IMT – Intima media thickness

ISH – Isolated systolic hypertension

K - Potassium

LDL – Low density lipoprotein

LLTPA – Low leisure time physical activity

LTPA – Leisure time physical activity

LVM – Left ventricular mass

MAP – Mean arterial pressure

MI – Myocardial infarction

NA - Sodium

NG – Normal glucose

NGT – Normal glucose tolerance

NO – Nitric oxide

NOS – nitric oxide synthase

PA – Physical activity

PAD – Peripheral arterial disease

PDBP – Peripheral diastolic blood pressure

PGI₂ – Prostacyclin

PMAP – Peripheral mean arterial pressure

PP – pulse pressure
PPP – Peripheral pulse pressure
PSBP – Peripheral systolic blood pressure
PVR – Peripheral vascular resistance
PWV – Pulse wave velocity
SD - Standard deviation
SEM – Standard error of mean
SNP – Sodium nitroprusside
SNS–Sympathetic nervous system
SV – Stroke volume
TCHOL – Total cholesterol
TP – Total power
Tr – Time for reflected wave to return
TRIGS - Triglycerides
UKPDS – United Kingdom project in Diabetes states
WHO – World health organisation
WT – Weight

CHAPTER 1

INTRODUCTION

1.1 Historical perspectives

1.1.1 *History of arterial stiffness*

The ancient Greeks believed that the blood vessels were in fact air tubes used for transporting air through the body. The word “Artery”, originating from the Greek words of *aer* and *terein*, meaning air-duct. This was the general consensus up until the early 17th century when Harvey published a book entitled “An Anatomical Essay on the Movement of the Heart and Blood in Animals”, in which he described the circulation of the blood vessels and the heart ¹ and laid the foundations of modern cardiovascular medicine. However, the blood vessels themselves were still thought to be simple conduits with the sole function of conveying blood from the heart to the vital organs and back again.

Therefore, despite Harveys’ seminal work, research in cardiovascular medicine was slow to progress and it was not until one hundred years later that Reverend Stephen Hales recorded the arterial pressure in a horse. Using only a water manometer at the carotid artery, arterial pressure was recorded along with simultaneously demonstrating the effects of exsanguinations ². The height of the water in the manometer equated to the arterial pressure being the first early equivalent of the mercury column sphygmomanometer used today.

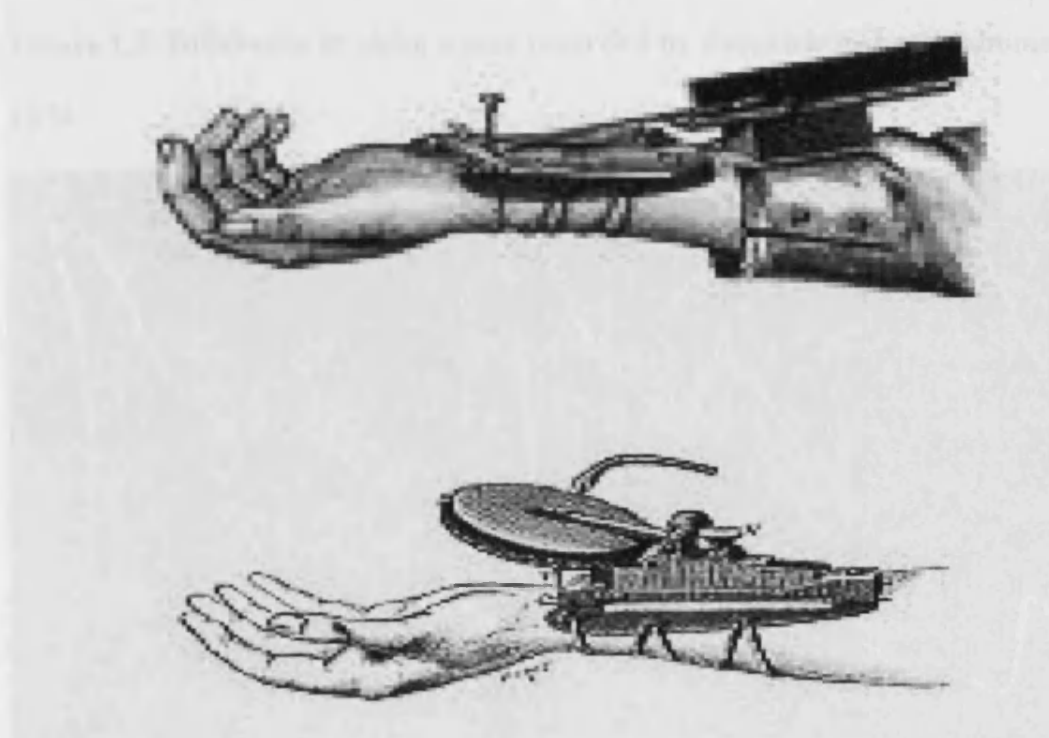
Hales further compared the elastic arterial system to that of a contemporary fire engine. Termed the “Windkessel” model, Hales described the air-filled chamber as the buffer that converts pulsatile flow at the input to the steady state flow at the nozzle.

However, this model proved too simplistic, as it did not totally consider wave travel and reflection.

Indeed, all the early studies measured pressure and it was not until much later that flow was measured. The French physicist Poiseuille, investigated the factors responsible for the resistance to flow in tubes of capillary dimensions³ which lead to the identification of pressure fluctuations during each heart beat. These and the findings of others encouraged early researchers to search for more objective and sensitive methods of assessing the arterial pulse.

Eventually Marey developed a series of devices which he termed sphygmographs, essentially consisting of a base plate over a radial artery connected to a smoked drum driven by clockwork on which the fluctuations in the arterial pulse were recorded⁴.

Figure 1.1. Sphygmographs developed by Marey (taken from O'Rourke. The arterial pulse. 1992)⁵



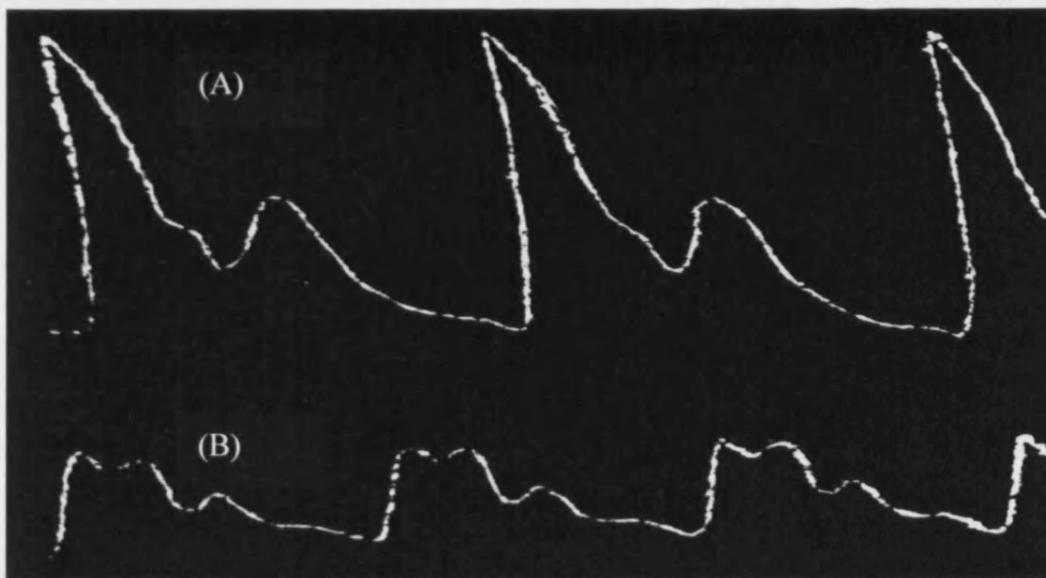
(A) Illustrates the normal wave form recorded in young individuals.

(B) Illustrates the different wave forms recorded in older individuals.

Such devices were further refined over the ensuing years. However, it was Frederic Akbar Mahomed, when a medical student at Guy's hospital London who was to make one of the most significant contributions ⁶. At the time Mahomed was working at Guy's, the effects of renal disease on arterial pressure had just been noted by Richard Bright ⁷. Mahomed used the sphygmograph to record pulse waveforms from patients with Bright's disease and compared them to healthy subjects. He was able to correctly identify the characteristic hypertensive pressure pulse waveform with a late systolic peak and little or no diastolic fluctuation. Using the sphygmograph, Mahomed was also able to study the pre-albuminuric stages of Bright's disease. Mahomed therefore promoted the use of pulse waveforms in clinical practice.

Mahomed was also able to show differences in waveforms between old and young subjects (Figure 1.2). Although at the time he himself failed to fully appreciate the relationship between changes in the arterial wave and arterial stiffness.

Figure 1.2. Difference in pulse waves recorded by Fredrick Akbar Mahomed, 1874.



(A) Illustrates the first waveforms measured in young individuals.

(B) Illustrates the different waveforms measured in older individuals.

“The pulse ranks the first among our guides; no surgeon can despise its counsel, no physician can shut his ear to its appeal”

(Frederick Akbar Mahomed 1874) taken from ⁵.

Early physicians continued to regard stiffening of the arteries as an inevitable consequence of aging, and its clinical significance was not fully appreciated by many. Nevertheless, a physiologist by the name of C.S. Roy, in the early 1880's, suggested that with increasing age, the stiffness of the arteries changed, and that this might have a consequence for the health of the individual. He stated that:

“Only in the case of young children do we find that the elasticity of the arteries is so perfectly adapted to the requirements of the organism as it is in the case of the lower animals”⁸

A major problem for Mahomed was that although he was able to identify clear qualitative differences in the arterial waveform between health and disease he was unable to quantify them. This precluded the widespread introduction of the sphygmograph into clinical practice and subsequent work by Riva-Rocci (1896) and Korotkov (1905) led to the introduction of the forerunner of the modern sphygmomanometer. However, the sphygmomanometer only provided information as to the extremes of blood pressure, systolic and diastolic and all the information contained in the pressure waveforms of Mahomed was ignored.

Despite such reservations, the sphygmomanometer superseded the sphygmograph and was introduced into widespread clinical practice.

However, a number of researchers remained interested in the arterial waveform and subsequently a number of devices to record the arterial waveform were developed that unlike the original sphygmographs, were able to provide quantitative data. One such device the vasculograph⁹ was used to assess the effect of age, exercise, hypertension and drugs on the arterial waveform in the 1950s and 1960s.

Furthermore, the widespread introduction of the sphygmomanometer was accompanied by clinicians focusing on diastolic pressure as being of major importance as the rise in systolic pressure was regarded as part of the natural ageing process and not as predictive of CV risk. Indeed many early physicians regarded a high systolic pressure as a good feature being representative of “cardiac strength”.

Subsequently this has been shown to be misguided, as age related arterial stiffening has been clearly demonstrated to be a major risk factor for cardiovascular events. Indeed, pulse pressure (the difference between systolic and diastolic pressure) has been shown in older individuals to be a better predictor of CV events than either systolic or diastolic pressure¹⁰⁻¹³. Furthermore, with the increased longevity of many modern societies isolated systolic hypertension (ISH), the result of age related arterial stiffening is increasing exponentially, such that individuals who are normotensive at age 50yrs have a lifetime risk of developing hypertension of 90%.

Modern appreciation of the deleterious impact of arterial stiffening on vascular haemodynamics and ventricular-vascular interactions, coupled with improved technology has lead to the development of a number of devices for measuring arterial stiffness in clinical practice. These developments have enabled clinicians and researchers to improve diagnosis, risk stratification and therapeutic management of patients with premature arterial stiffening, due to CV risk factors. Indeed, arterial

stiffness has emerged many years after Mahomed's original experiments as a major independent risk factor for CV disease in the 21st century.

1.2 Definition and measurement of arterial stiffness

1.2.1 Arterial stiffness

"In extreme old age, the arteries themselves, the grand instrument of the circulation, the continual apposition of earth, become hard, and as it were bony, till, having lost the power of contracting themselves they can no longer propel the blood, even through the largest channels, in consequence of which death naturally ensues."

(John Wesley, 1703-1791)

Although John Wesley appreciated the importance and predictive value of arterial stiffness in terms of mortality there was no way of defining or measuring arterial stiffness available. The generic term "arterial stiffness" in simple terms defines how elastic, stiff, distensible or compliant an artery is. This term will be used throughout the thesis.

Terms used to describe the properties of the vessel walls are defined in Table 1.1

Important indices and methods of assessment include compliance, elasticity, distensibility and vascular impedance. However, interpretation of these indices remains complicated since many are dependent on blood pressure. Thus stiffness index may be more useful clinically as it is less dependent on blood pressure. In assessment of vascular impedance ¹⁴ the circulatory system is compared to an electrical circuit. Impedance then describes the relationship between forces acting in

the circulation to the motion of the blood. However, the introduction of impedance measurements into clinical practice has been limited by the necessity to measure pressure and flow simultaneously at a fixed point in the circulation. Therefore characteristic impedance is probably the most useful clinically, as it relates pressure and flow. However, this makes an assumption that there is no pressure wave reflection within the system which is an over simplification. Input impedance on the other hand describes the impedance of the vascular bed as a whole and is therefore influenced by wave reflection.

Table 1.1. Methods used in the determination of arterial stiffness. (Modified from Woodman et al, 2003)

Term	Definition	Methods of Assessment
Elastic Modulus Ψ	Pressure change required for a theoretical 100% stretch from resting diameter: $(\Delta P \cdot D) / \Delta D$ (mmHg)	MRI Ultrasound
Young's Elastic Modulus Ψ	Elastic modulus per unit area: $(\Delta P \cdot D) / \Delta D \cdot h^2$ (mmHg/cm ²)	MRI Ultrasound
Arterial Distensibility Ψ	Relative change in diameter (or area) for a given pressure change; inverse of elastic modulus: $\Delta D / (\Delta P \cdot D)$ (mmHg ⁻¹)	MRI Ultrasound
Arterial Compliance Ψ	Absolute diameter (or area) change for a given pressure step: $\Delta D / \Delta P$ (mmHg) or (cm ² /mmHg)	MRI Ultrasound
Pulse Wave Velocity	Velocity of travel of a pulse along a length of the artery: Distance/Δt (m/s)	Pressure waveform Volume waveform MRI, Ultrasound
Augmentation Index	Difference between second and first systolic peaks as a percentage of the pulse pressure: $((P_2 - P_1) / PP) * 100$	Pressure Waveform
Stiffness Index (β)Ψ	Ratio of logarithm (systolic/diastolic pressures) to (relative changes in diameter): $\beta = \ln(P_s / P_d) / (D_s - D_d) / D_d$	Ultrasound
Capacitative compliance	Relationship between pressure change and volume change in the arteries during the exponential component of diastolic pressure decay: $\Delta V / \Delta P$ (cm ³ /mmHg)	Pressure waveform
Oscillatory Compliance	Relationship between oscillating pressure change and oscillating volume change around the exponential pressure decay during diastole: $\Delta V / \Delta P$ (cm ³ /mmHg)	Pressure waveform

P: pressure, **D:** diameter, **V:** volume, **h:** wall thickness, **t:** time, **v:** velocity, **s:** systolic, **d:** diastolic, Ψ : also requires pressure measurements.

1.2.2 Measurement of arterial stiffness

There are several methods in use for the measurement of arterial stiffness. Some methods are more widely used than others, due to their easy operational procedure, relative low cost and mobility. However, whilst some techniques give information on systemic arterial stiffness, others only give information on local stiffness of the vessel being studied. It is therefore important that different techniques are not used interchangeably when comparing data.

In this thesis, pulse wave analysis (PWA) and pulse wave velocity (PWV) are used to assess arterial stiffness.

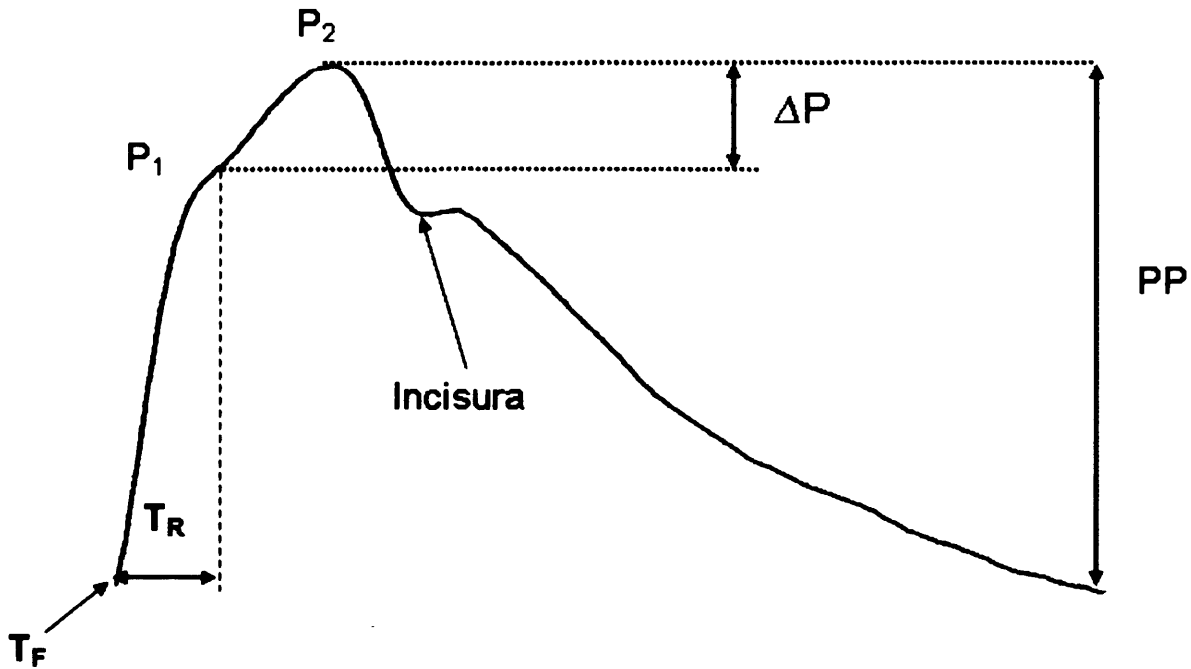
1.2.2.1 PWA

To better understand the technique of PWA it is vital to appreciate that the arterial pressure wave form wherever it is measured in the arterial tree will be a composite of a forward going pressure wave (produced by ventricular ejection) and a reflected wave. The reflected wave travels back from the periphery and sites of reflection, usually arterial bifurcations or sites of impedance mismatch. Therefore, the arterial waveform varies along the arterial tree¹⁵. The velocity at which the wave will travel along the arterial wall is dependant on how stiff the artery is. The greater the arterial stiffness of the vessel, the higher the pulse wave velocity. The more elastic the artery is, the slower the pressure wave will travel along the arterial tree. Furthermore, the slower the reflected wave will return to the aortic root. If the reflected wave returns slowly, the wave will reach the heart during diastole. Thus, augmenting diastolic pressure and increasing coronary perfusion. In the case of stiff arteries, the reflected

wave will return earlier to the aortic root, thus augmenting central systolic pressure with a consequent decrease in diastolic pressure. This early return of the reflected wave can also lead to left ventricular hypertrophy (LVH).

Non-invasive analysis of central arterial wave forms has been made possible with the development of the SphygmoCor system by O'Rourke and colleagues ¹⁶. Using the SphygmoCor system, peripheral pressure waveforms are recorded non-invasively with a high fidelity applanation tonometer ¹⁷. The tonometer with a Millar micromanometer at its tip, is used to flatten but not occlude a peripheral artery (radial, carotid). Circumferential pressures are thus equalised and accurate recording of the pressure waveform is obtained. The peripheral waveform is then transformed into the corresponding central arterial waveform, using Fourier analysis, and a validated generalised transfer function based on data obtained from invasive measurements ^{18,19}. Augmentation index (AIx), a measure of systemic arterial stiffness can then be calculated as the difference between the second and first systolic peaks expressed as a percentage of the pulse pressure (Figure 1.3), and derived central pressure¹⁶. In addition, by gating the measurements to the R wave of an ECG, and recording at two sites, aortic (carotid to femoral) PWV can also be measured.

Figure 1.3. A typical aortic pressure waveform constructed by the SphygmoCor software.



T_f : Foot of the forward travelling wave, T_r : time difference between the foot of the wave and the point at which the reflected wave has an effect (the inflection point), P_1 & P_2 : first and second systolic peaks, PP: pulse pressure.

1.2.2.2 PWV

Pulse wave velocity describes the speed at which the pressure wave generated by left ventricular contraction travels down the arterial tree from the aorta to the site of measurement in the peripheral vasculature. It is calculated by measuring the time taken for the arterial pressure wave to travel between two points a measured distance apart, and thus requires measurements to be made at two different sites within the arterial system. This can be achieved either by simultaneous measurement or by gating recordings to a fixed point in the cardiac cycle, often the R wave on the ECG. It is important to have a good reference point on the recorded waveforms and the majority of systems employ a foot-to-foot methodology, as this has been shown to avoid confounding by wave reflection within the system.

PWV can be measured by a variety of methods, either invasive or non-invasive, and can be applied to either flow or pressure waves. PWV provides information on the distensibility of the local vessel being studied, rather than on systemic arterial stiffness. Distensibility being inversely related to stiffness. The relationship between PWV and distensibility is defined in the Bramwell Hill equation as:

$$PWV = \sqrt{1/D\rho}$$

Where D = distensibility and ρ = density of blood.

*Other methods of assessing arterial stiffness***1.2.2.3 MRI**

Magnetic resonance imaging (MRI) is being increasingly used in modern clinical practice and can be adapted to measure arterial distensibility non-invasively. The majority of studies to date have measured distensibility in the aorta. This is important, as aortic distensibility is influenced by pressure within the aorta and this will be different from pressure in the brachial artery due to the phenomenon of pressure wave amplification. Since many of the MRI studies published to date have used brachial artery pressure to calculate distensibility they remain difficult to interpret. In addition, MRI is expensive, immobile, time consuming, needs well-trained staff and can only be applied to large arteries. For this reason, its place in clinical practice remains speculative.

1.2.2.4 Ultrasound

Ultrasound can also be used in assessment of arterial stiffness (compliance and distensibility). Using ultrasound is limited to the larger and more accessible arteries. Therefore this technique has been used to assess compliance or distensibility at the carotid, brachial, abdominal aorta and femoral artery sites.

Although ultrasound has the advantage of being non-invasive, imaging equipment is expensive and not very portable. The technique is very operator dependent as it is highly reliant on accurate determination of vessel wall diameters.

1.2.2.5 Photoplethysmography

The technique of Photoplethysmography (digital pulse contour analysis) is another method of assessing arterial stiffness. This technique utilises the transmission of

infrared light through the finger to detect changes in flow and produce a volume waveform. Developers of the system have devised a reproducible parameter termed “stiffness index” (SI), by measuring the time delay between direct and reflected waves in the digital volume pulse²⁰. One disadvantage of this method is that it uses height to estimate arterial path length. However, SI has demonstrated to be significantly correlated to the “gold standard” method of assessing arterial stiffness, CF PWV. Therefore, it may be used as a valid surrogate measure for aortic PWV.

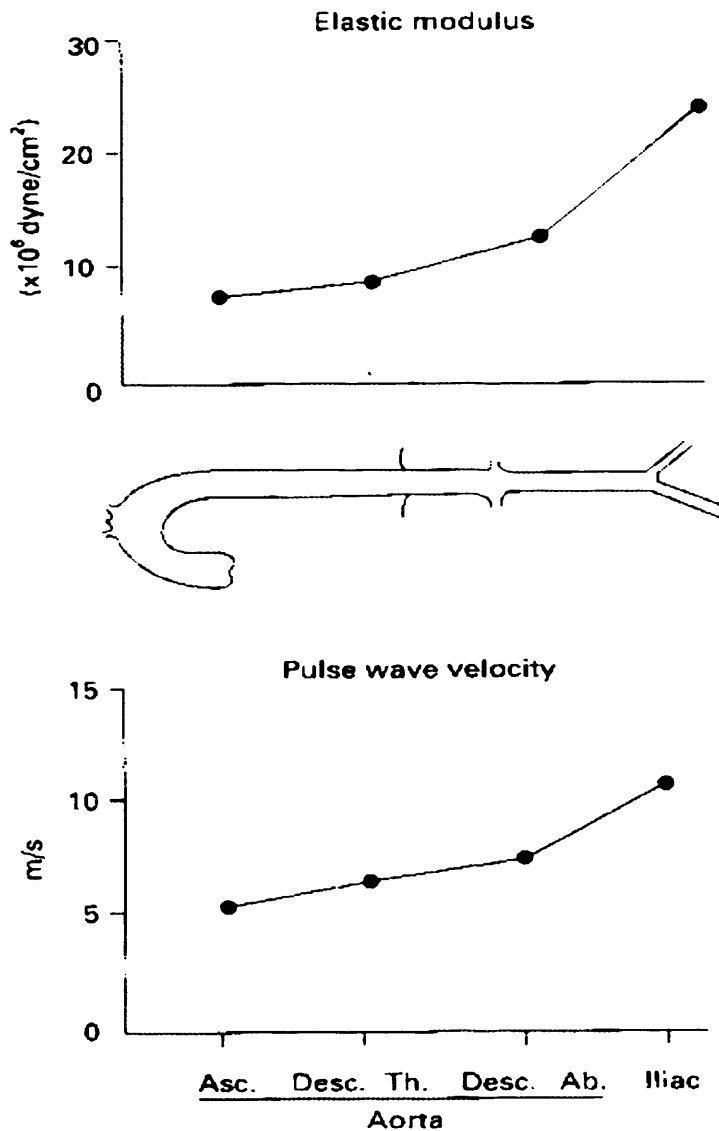
1.3 Variations in arterial stiffness throughout the arterial tree

Arteries vary in size from the largest most elastic section of the arterial tree known as the aorta to the smaller branched muscular arteries of the arterial tree. Arterial diameters range from a few centimetres in the aorta to less than 200 μ m in the tiny resistance vessels known as arterioles. Structural properties of the vessels vary from the conduit arteries to the arterioles and will thus influence local PWV and elastic modulus (Figure 1.4). The aorta comprises of predominantly elastin and collagen fibres along with smooth muscle, whilst the iliac and femoral arteries are more muscular. Changes in the composition of the arterial wall, predominantly the balance between elastin and collagen, will effect the pressure flow relationships at various points in the arterial tree (Figure 1.5).

Structural differences, as the pulse travels from the elastic central arteries to the more muscular peripheral arteries have an important role to play in wave reflection. As portrayed in Figure 1.4, pulse wave velocity increases as the pressure wave travels along increasingly muscular arteries as it moves down the arterial tree. This increased PWV together with branching of the aorta and narrowing of the lumen, creates an

impedance mismatch, resulting in partial reflection of the forward travelling pressure waveform²¹. An increased number of these sites of impedance mismatch can increase the velocity of the reflected wave, resulting in augmented central pressure.

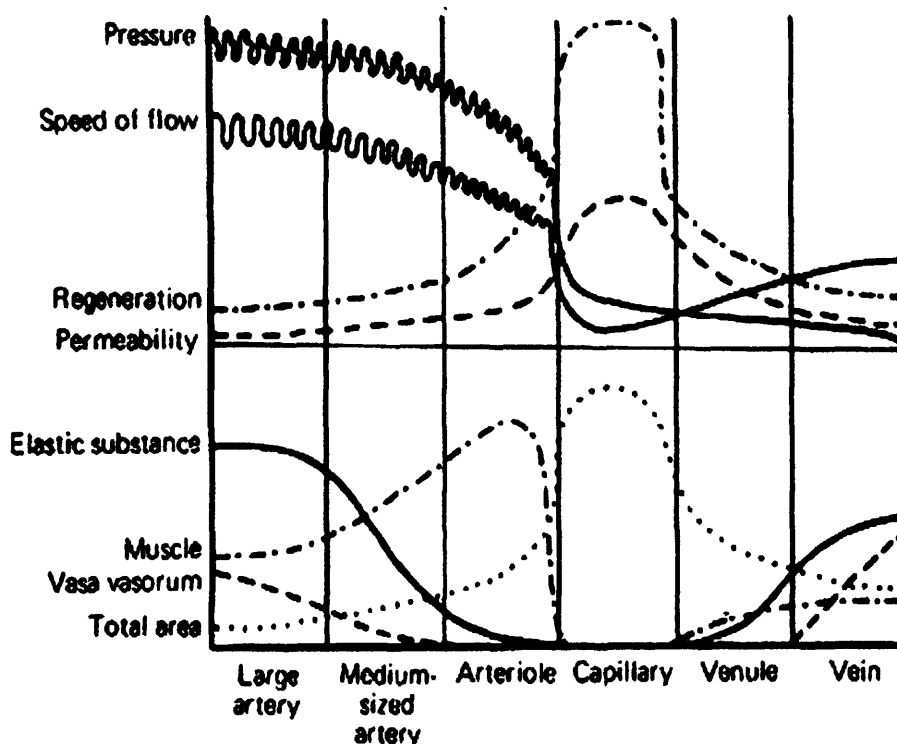
Figure 1.4. Variations in arterial stiffness throughout the arterial tree. Taken from²¹.



As the aorta and large vessels stiffen with age impedance mismatch decreases, leading to less wave reflection. This is supported by the finding that AIx plateaus²² and in

some cases decreases²³ after age 55yrs. This may be an explanation for the link between arterial stiffness and microvascular disease in that the decrease in wave reflection may thus expose the microvasculature to an increased direct pulsatile load. This is especially true for high flow, low pressure, circulations such as the brain and the kidney. Indeed, a recent study showed that PP, a surrogate for arterial stiffness was the best correlate of deterioration in renal function in a group of hypertensive subjects with normal renal function at baseline²⁴

Figure 1.5. Haemodynamic and structural differences observed between the different sections of the vasculature.



Taken from: "Heart Development", Harvey, R.P. and Rosenthal, N. 1999. Academic Press, San Diego, p. 240.

1.4 Haemodynamic consequences of arterial stiffness

1.4.1 *Increased central blood pressure*

Arterial stiffening and enhanced wave reflections from the periphery cause systolic blood pressure to rise and diastolic pressure to fall in the central arteries. This results in a widening of the central pulse pressure. Moreover, it is the pressure in the central, not peripheral arteries to which the major organs are exposed. Since there are now a range of techniques with which to assess central blood pressure including the SphymoCor system as used in the current studies, there has been increased interest in central blood pressure in the pathogenesis of cardiovascular disease. This interest in central blood pressure has been re-enforced by a recent large population based study demonstrating that central aortic blood pressure was a better predictor of CV outcome than blood pressure measured conventionally in the brachial artery²⁵.

Pulse pressure in the central arteries is influenced by three major haemodynamic mechanisms: stroke volume, aortic stiffness, and wave reflections. The large elastic arteries normally buffer the cyclical changes in pressure which occur during each cardiac cycle. However, as elastic arteries become stiffer, due to ageing or disease, this buffering capacity is reduced and central systolic pressure increases. The rise in systolic pressure is augmented further by a faster return of reflected pressure waves from the periphery. In addition, diastolic blood pressure falls, because stiffened arteries can no longer recoil as effectively. Although a marker of increased arterial stiffness, these changes in central pulse pressure may also promote cardiovascular disease.

The function of the heart is to pump blood through the arterial system to the organs and tissues in an amount sufficient to meet their metabolic needs at rest and during

periods of stress. The amount of oxygen used by the left ventricle to produce the cardiac output is dependent not only on the contractile properties of the myocardium, but also on the physical properties of the large arteries and resistance vessels. As such, it is the central, not peripheral blood pressure, which constitutes the afterload placed on the ventricle during cardiac ejection.

If the heart is ideally matched to its afterload, there is optimal coupling between the ventricle and the vascular system and cardiac efficiency is high. However, any increase in central systolic blood pressure directly influences ventricular afterload and reduces the efficiency of cardiac ejection. Indeed, a heart ejecting blood into a stiffened arterial system must generate higher end-systolic pressures for a given stroke volume, resulting in a greater energy requirement for a given level of blood flow . A sustained increase in central systolic blood pressure promotes the development of LVH, itself, a major cardiovascular and cerebrovascular risk factor, ultimately leading to diastolic dysfunction and heart failure and predisposing to myocardial ischaemia. The close relationship between central pulse pressure and LVH is underlined by the fact that left ventricular mass is more closely related to pulse pressure in the aorta than in the brachial artery .

As discussed previously, the major components of the arterial wall that regulate vessel stiffness are elastin, collagen and smooth muscle. With ageing, there is progressive disruption and fatigue-fracture of the elastic load-bearing elements of the arterial wall, resulting in a transfer of stress to the more collagenous fibres and an increase in arterial stiffness. The resulting rise in central systolic blood pressure aggravates this degenerative process, by further increasing the level of cyclical stress and altering the absolute and relative amounts of elastin and collagen within the arterial wall , thus creating a vicious cycle. Moreover, the widened central pulse pressure is transmitted

to other arteries such as the carotid, which then undergo a process of remodelling to reduce wall stress, leading to intima-media thickening .

1.4.2 Isolated Systolic Hypertension

As noted by Bramwell and Hill in 1922, the difference between systolic and diastolic blood pressure, the pulse pressure, is directly related to the compliance of the artery and the associated change in arterial volume ²⁶:

“Hence the difference between systolic and diastolic pressure, that is the pulse pressure, other things being equal will vary directly as the rigidity of the arterial walls.” ²⁶

Isolated systolic hypertension is defined generically as a wide pulse pressure. The BHS definition being: (systolic pressure ≥ 160 mmHg and a diastolic pressure ≤ 90 mmHg). When arteries are young and elastic, the reflected wave returns during diastole. As large arteries stiffen with age and cardiovascular risk factors, PWV increases and the reflected wave returns earlier during systole, resulting in increased systolic pressure augmentation and increasing central PP. This shift of the reflected wave out of diastole results in a simultaneous lowering of diastolic pressure. Changes in reflection coefficient (the proportion of forward to backward travelling waves) and the major sites of reflection may also contribute to altered magnitude and timing of wave reflection and therefore central pressure augmentation. Since there is proportionality between cardiac ejection time and the duration of the cardiac cycle, the peak of the forward travelling wave will be relatively delayed at slower heart

rates. Therefore, even with a fixed reflection site and speed of wave transmission, there will be an altered relationship between forward and backward waves. Central PP will therefore be amplified at lower heart rates²⁷. Therefore, accounting for heart rate differences between groups, when assessing the effects of certain medications (β -blockers) on central PP becomes very important.

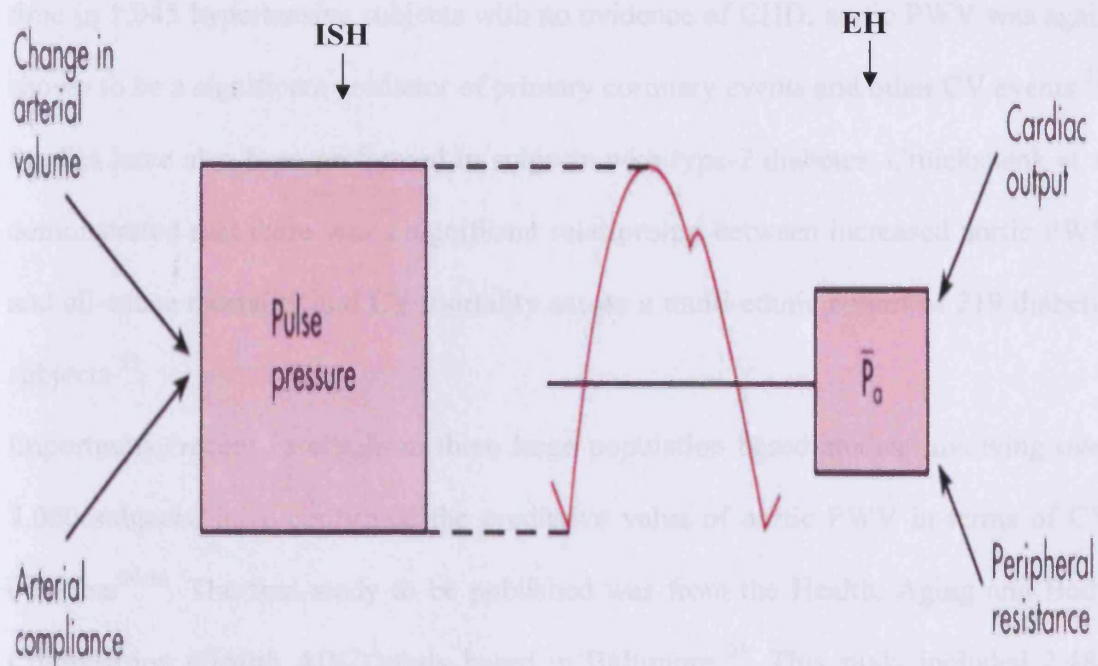
Interestingly, increased pulse pressure may also be associated with aortic diameter change. Traditional views suggest that ISH is associated with increased elastin breakdown with secondary collagen deposition and therefore stiffening of the arterial wall, leading to a dilated noncompliant vessel. However, a more recent theory suggests that ISH may be a result of a reduction in aortic lumen diameter and resultant increase in wall tension with normal elasticity²⁸. Therefore, suggesting a functional rather than a structural role of the aorta in the pathophysiology of ISH.

Isolated systolic hypertension can be regarded haemodynamically as a different condition to essential hypertension. Essential hypertension is a disease characterised by a rise in the static component of blood pressure MAP and DBP and is thus influenced by cardiac output and peripheral vascular resistance (PVR). The majority of subjects exhibiting increased PVR. Essential hypertension is therefore a disease of the smaller resistance vessels. It is more common in younger individuals (<55yrs) and is relatively easy to treat as the majority of anti-hypertensive agents are targeted at lowering peripheral resistance. In contrast, ISH is a disease of the larger arteries and is associated with an increase in the pulsatile component of blood pressure, the pulse pressure (PP), which is influenced by stroke volume and large arterial stiffness (Figure 1.6). The majority of subjects exhibiting increased large artery stiffness and

relatively normal peripheral vascular resistance. Again in contrast to essential hypertension ISH is more common in older individuals (>55yrs) and is often difficult to treat, as available drugs are not specifically targeted at large arterial stiffness. Providing a plausible explanation as to why patients with ISH are resistant to a number of current therapies ²⁹. Traditional antihypertensive medication appears to reduce arterial stiffness by indirectly reducing mean arterial pressure. The medication predominantly acting on PVR to reduce mean arterial pressure. However, medication is urgently needed to act on large arteries and reduce large artery stiffness. Nitric oxide donors and phosphodiesterase inhibitors have been suggested as possible agents for reducing large arterial stiffness. Breakers of advanced end glycation end products and collagen cross-links between elastin and collagen proteins have also been suggested as potentially beneficial to large artery stiffness ²⁹.

Clinically this is important, as increased PP has been demonstrated in the large Framingham study, to be a better predictor of cardiovascular events than systolic or diastolic pressure alone in people over 50 years ¹⁰. This has been confirmed in a number of other population-based studies¹¹⁻¹³. In the MRC Mild Hypertension Trial, PP was shown to be a strong risk factor for coronary events in untreated hypertensive male subjects ¹¹. In addition, another major population based study in 2,311 subjects, the PIUMA study, after a multivariate analysis and adjustment for confounding factors, showed that for every 10mmHg increase in PP, there was a 30% increase in risk of cardiac events ¹². Indeed, a meta-analysis of three large studies, in 7,929 subjects with ISH, clearly showed PP and not MAP, to be the major determinant of cardiovascular risk, in older hypertensive subjects ¹³.

Figure 1.6. Cardiovascular determinants associated with ISH and EH. (adapted from Physiology; Berne & Levy).



ISH: Isolated Systolic Hypertension; EH: Essential Hypertension.

1.5 Arterial stiffness and cardiovascular (CV) outcome

To date, the majority of studies investigating the relationship between arterial stiffness and CV outcome have involved measurement of PWV using a variety of different methodologies. Furthermore, these studies have been undertaken in defined patient populations such as subjects with end stage renal disease (ESRD)³⁰, hypertension^{31,32} and diabetes³³.

Blacher et al demonstrated that aortic PWV and age were significantly associated with all cause mortality and CV mortality in a group of patients with a mean age of 52yrs with ESRD³⁰. In a cohort of 1,980 hypertensive individuals of 50±13yrs old, a significant association between aortic PWV, age, heart rate and history of myocardial

infarction was observed with all-cause mortality and CV mortality, during a 9 year follow up³¹. Furthermore, in a subsequent study by the same group, conducted this time in 1,045 hypertensive subjects with no evidence of CHD, aortic PWV was again shown to be a significant predictor of primary coronary events and other CV events³². Studies have also been performed in subjects with type-2 diabetes. Cruickshank et al demonstrated that there was a significant relationship between increased aortic PWV and all-cause mortality and CV mortality across a multi-ethnic cohort of 219 diabetic subjects³³.

Importantly, recent results from three large population based studies involving over 7,000 subjects, have confirmed the predictive value of aortic PWV in terms of CV outcome³⁴⁻³⁶. The first study to be published was from the Health, Aging and Body Composition (Health ABC) study based in Baltimore³⁴. This study included 2,488 individuals and after 4.3 yrs of follow up, 616 CV events had been recorded. In addition, there were 265 deaths, of which 111 were cardiovascular. The investigators divided the cohort into quartiles of aortic PWV and showed that there was a significant relationship between aortic PWV and both total and CV mortality. There was also a relationship between aortic PWV and stroke. The associations remained significant after adjustment for age, race, systolic BP, and known CV disease. The following year, results from two further population based studies were reported. The Rotterdam study involved 2,835 subjects and followed up for 4.1 yrs. In that time 174 CV events were detailed. Again, aortic PWV was measured to assess arterial stiffness and a significant association was shown between aortic PWV and both coronary heart disease (CHD) and stroke. After adjustment for confounding factors aortic PWV remained as an independent predictor of heart disease and stroke³⁵. Finally, results from a Danish study involving 1,678 subjects who were followed for 9.4 yrs, during

which time there were 317 CV events and 171 deaths, of which 62 were cardiovascular. They also demonstrated a significant relationship between aortic PWV and cardiovascular outcome. Most importantly, in contrast to the other population based studies, this study was also able to show that aortic PWV was a better predictor of CV outcome than traditional CV risk factors including 24hr MAP³⁶. Indeed, a recent analysis reviewed 97 studies where PWV had been measured in order to assess the clinical value of PWV in terms of CV risk prediction. The findings showed that 1 standard deviation increment in PWV is equivalent to 10 yrs of ageing, or 1.5 to 2 times the risk of a 10mmHg increase in systolic pressure, suggesting that PWV can be used effectively for risk stratification in clinical practice³⁷.

Other Studies investigating the predictive value of arterial stiffness have used AIx as a surrogate for arterial stiffness. London et al studied subjects with ESRD and showed that carotid AIx was a predictor of both all-cause and CV mortality³⁸. More recently, Weber et al studied patients undergoing coronary angiography and PCI and demonstrated that aortic AIx predicted the occurrence of severe CV events³⁹

1.6 Arterial stiffness and coronary artery disease (CAD)

A number of studies have been performed relating indices of arterial stiffness to the presence of coronary artery disease (CAD). Since the detection of CAD requires coronary angiography, the majority of these studies have used invasive methodology. A number of small studies have demonstrated a relationship between central PP and angiographic CAD^{40,41}. These findings have recently been confirmed in a large multi-centre study involving 1,337 patients at high risk of CAD from 75 centres⁴² which demonstrated a significant relationship between invasively determined central aortic PP and both the presence and extent of angiographic CAD. The relationship between aortic stiffness and angiographic CAD has also been investigated. Both invasive⁴³ and non-invasive⁴⁴ measurements of aortic stiffness have been shown to correlate significantly with CAD. Indeed, Hirai et al demonstrated that aortic stiffness was related to the number of atherosclerotic stenoses of the coronary arteries. This data has recently been supported by a study demonstrating a significant correlation between non-invasive measurement of carotid radial PWV and atherosclerotic plaque load as determined by intravascular ultrasound⁴⁵. Interestingly, in this study by McLeod, AIx measured non-invasively did not correlate with plaque load. Although another study has shown a significant association between invasively measured AIx and the risk of CHD⁴⁶. However, non-invasive measurements are much more applicable to the assessment of large patient groups in clinical practice and a recent study has shown that non-invasive measurement of AIx does correlate with cardiovascular risk⁴⁷, although CAD was not assessed in this group of patients. Weber et al examined the relationship between aortic AIx and augmentation pressure, measured non-invasively with the SphygmoCor system and CAD assessed

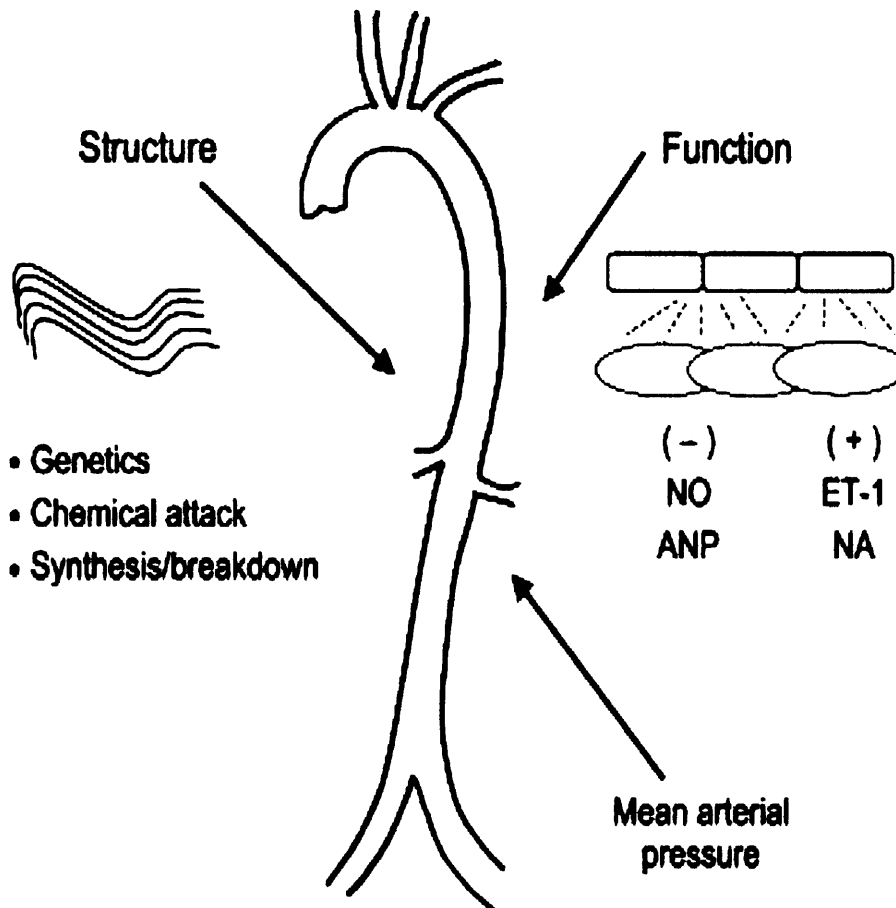
angiographically. 465 consecutive male patients undergoing coronary angiography for symptoms of suspected CAD were studied. There was a strong association between the presence and severity of CAD and AIX⁴⁸. Taken together the results from the studies to date are consistent with the hypothesis that central aortic stiffness may promote the development of coronary artery disease and that therapeutic intervention targeted at reducing arterial stiffness may be of benefit in patients with CAD.

1.7 Factors regulating arterial stiffness

The major factors regulating arterial stiffness can be considered as relating to structure, function and mean arterial pressure (MAP) (Figure 1.7).

Until recently, arterial stiffness was thought to be largely dependant upon distending pressure and structural components like elastin and collagen. However, it is now recognised that smooth muscle tone also regulates arterial stiffness. A number of locally produced circulating factors may contribute to the short term and functional regulation of smooth muscle tone and hence large artery stiffness. These include Nitric oxide (NO), endothelin-1 and natriuretic peptides⁴⁹. Of these, NO is probably the most important as many conditions exhibiting endothelial dysfunction, characterised by decreased bioavailability of NO, are also associated with increased arterial stiffness.

Figure 1.7. Major determinants of arterial stiffness. Structure, Function and MAP. (Taken from Wilkinson and McEniery 2004)



NO, *Nitric Oxide*; ANP *Atrial Natriuretic Peptide*; ET-1, *Endothelin-1*; NA, *Noradrenaline*.

1.7.1 Nitric oxide and arterial stiffness

Endothelial dysfunction, characterised by decreased bioavailability of NO, in resistance and conduit arteries is a predictor of cardiovascular risk and outcome⁵⁰. Conditions associated with endothelial dysfunction such as hypercholesterolaemia⁵¹⁻⁵³ and diabetes⁵⁴⁻⁵⁶ are also associated with increased arterial stiffness. Furthermore, a

number of therapeutic interventions that improve endothelial function also reduce arterial stiffness⁵⁷, suggesting that NO may itself regulate large arterial stiffness.

Systemic infusions of drugs that promote or inhibit NO release have been used to investigate the role of NO in regulating large artery stiffness. Many studies have clearly demonstrated that NO donors, such as glyceryl trinitrate (GTN), reduce AIx, a composite measure of arterial stiffness and wave reflection, independently of any effect on blood pressure in healthy subjects, and in those with a range of cardiovascular risk factors including hypertension and hypercholesterolaemia⁵⁸. NO donors also reduce large artery stiffness in hypertensive individuals^{59,60}, and in animals⁶¹, but not always independently of changes in distending pressure. Although GTN reduces aortic PWV in animals, again independently from changes in blood pressure, such observations have not been repeated in humans.

The contribution of basal NO to resting large artery stiffness has been assessed by infusion of various inhibitors of nitric oxide synthase (NOS) including L-N^G-monomethyl-L-arginine (LNMMA) and N^G-nitro-L-arginine methyl ester (L-NAME). Systemic infusion of LNMMA increases augmentation index in healthy normal volunteers⁶², and L-NAME has been shown to decrease small artery compliance⁶³. However, since these studies employed systemic infusions of NOS inhibitors, the accompanying rise in MAP made the results difficult to interpret. Stewart et al attempted to overcome these limitations by infusing LNMMA, but also noradrenaline and dobutamine to control for changes in MAP⁶⁴. They showed that systemic inhibition of NO had no effect on carotid-femoral PWV over and above that attributable to a rise in MAP *per se*.

Stewart's observations demonstrated that the increase in aortic PWV following chronic NO inhibition was even greater, which suggests some degree of vascular remodelling may have occurred. Therefore, conditions associated with decreased NO bioavailability, such as diabetes and hypercholesterolaemia, may affect aortic stiffness in the long term by structural modification, thus providing a mechanism linking endothelial dysfunction to an increased risk of cardiovascular events.

More definitive evidence for the role of NO in regulating large artery stiffness comes from local intra-arterial infusion of LNMMA and GTN. Such techniques overcome many of the methodological limitations of systemic infusions because the drug doses used are much lower, and if infusion periods are relatively short, MAP and heart rate are unusually unaffected. Such an approach can be further enhanced by direct, high fidelity, intra-vascular measurement of PWV, using pressure or flow waveforms, or distensibility or compliance, using ultrasound. Using such techniques, endothelium-derived NO has been shown to regulate large arterial stiffness in the ovine iliac artery⁶⁵. Similar findings have recently been demonstrated in the human iliac arterial bed⁶⁶.

1.7.2 Arterial Structure

The constant fracturing and repair of the structural elements of the arterial wall ultimately result in stiffening. Therefore age will have a significant effect on arterial stiffness as the stress on the arterial wall will be influenced by pulsatile load and heart rate. By the time an individual has reached 60yrs, the heart will have beat over two billion times. Age is therefore one of the major factors influencing structural changes leading to increased arterial stiffness.

Large arteries are made up of a number of different structural proteins. As described earlier, it is elastin and collagen that make up the majority of these proteins. Changes in the balance between elastin and collagen will therefore influence vascular stiffness. Such changes may involve increased synthesis or degradation of elastin or collagen. Recent evidence suggests that these proteins are susceptible to chemical attack and degradation by a number of proteolytic enzymes, especially serine proteases and matrix metalloproteases (MMPs) ⁶⁷. MMP-9 is particularly involved in the degradation of elastin and other basement membrane proteins including fibronectin, laminin, and type IV collagen ⁶⁷. Recently, increased levels of MMP-9 have been shown to predict a worse outcome in patients with cardiovascular disease ⁶⁸. MMP-9 is also associated with increased arterial stiffness in healthy individuals and risk of cardiovascular disease. Support for the involvement of MMP-9 in the regulation of arterial stiffness comes from a study which has shown a relationship between MMP-9 levels and increased arterial stiffness as assessed by carotid femoral PWV in normal subjects ⁶⁷. In addition, a further study by the same group also showed that subjects with ISH, a condition characterised by increased arterial stiffness, had increased levels of MMP-9 compared to matched controls ⁶⁷.

It is now clear that arterial stiffness is, in part, a heritable and dynamic trait ⁶⁹ suggesting that genetic make up may influence arterial stiffness. This is supported by studies that show a relationship between polymorphisms of a number of genes that are involved in the synthesis and breakdown of elastin. A recent study demonstrated that polymorphisms of the MMP-9 gene are associated with increased arterial stiffness in healthy individuals ⁶⁷. Studies have also shown an association between polymorphisms in the angiotensin-II gene and increased arterial stiffness ⁷⁰. Similar associations have been reported for polymorphisms within the angiotensin-II receptor gene ⁷¹

Finally, hypertension *per se* has been shown to alter the absolute and percentage of elastin to collagen within the arterial wall, leading to further increases in arterial stiffness and thus establishing a vicious cycle ⁴⁹.

1.7.3 MAP

Elasticity of an artery is determined by the mean arterial pressure it is exposed to, and its consequent deformation for that applied pressure. The force per unit area that produces the deformation is called the stress, the deformation, described as the ratio of the deformation to its original form, is called the strain. The pressure exerted upon the artery has to be considered when assessing arterial stiffness, as the stress strain relationship of an artery is not linear. The relationship between stress and strain is known as an elastic modulus and defined as, the pressure change required for theoretical 100% stretch from resting diameter $(\Delta P \cdot D) / \Delta D$ (mmHg) ⁷². Where P=Pressure, and D=diameter.

For an ideal elastic body, there is a linear relationship between stress and strain. However, arteries are not linear elastic bodies. Figure 1.8, illustrates the elastic nonlinearity of an artery, where change in arterial stress (Mean arterial pressure) and strain (change in diameter) are expressed in a typical human aorta.

Figure 1.8. The Stress Strain relationship of an artery ²⁹.

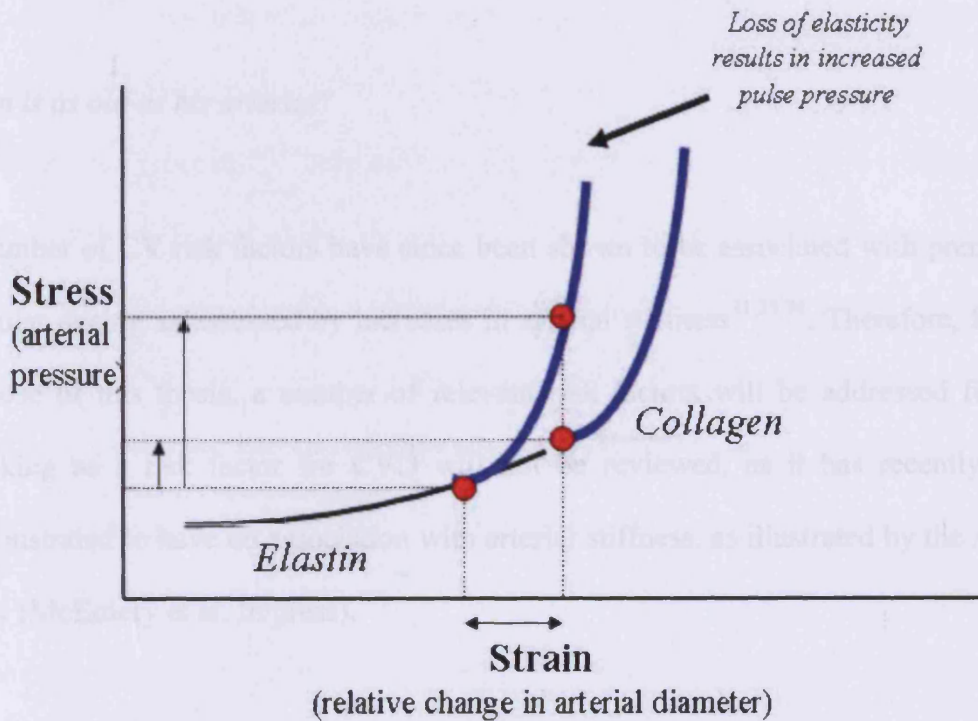


Figure 1.8, demonstrates a sequential aspect of the loading of the individual wall components that leads to a non-linear response. At lower mean arterial pressures, contractile-elastic components are preferentially loaded, whereas as MAP increases the load is transferred progressively to collagen. The shift from elastin to collagen components of the arterial wall leads to an exponential rise in the stress strain relationship due to collagen having little or no buffering capacity on the pressure.

1.8 Cardiovascular risk factors and arterial stiffness

As we age our arteries get stiffer. However, subjects with cardiovascular risk factors exhibit premature vascular ageing. Indeed, vascular age may be a better predictor of cardiovascular morbidity and mortality than chronological age as pointed out over 100 years ago by William Osler.

“Man is as old as his arteries”

73.

A number of CV risk factors have since been shown to be associated with premature vascular ageing as assessed by increases in arterial stiffness^{31,33,74}. Therefore, for the purpose of this thesis, a number of relevant risk factors will be addressed further. Smoking as a risk factor for CVD will not be reviewed, as it has recently been demonstrated to have no association with arterial stiffness, as illustrated by the ACCT study (McEniery et al. In press).

1.8.1 Diabetes mellitus and arterial stiffness

The relationship between diabetes and arterial stiffness has been investigated in a number of both large and small studies. Despite the fact that the methodologies for assessing arterial stiffness have varied, the findings have been very consistent in showing a positive relationship between diabetes and arterial stiffness.

The ARIC study used ultrasound to assess carotid artery stiffness in 4,700 type-2 diabetics and demonstrated a positive association between diabetes and increased arterial stiffness. Furthermore, subjects who were not diabetic, but had impaired glucose tolerance also exhibited significant increases in carotid artery stiffness⁷⁵. The Hoorn study also measured carotid intima media thickness (CIMT) in 1,193

individuals with either normal glucose levels, impaired glucose regulation or diabetes. The study included 301 subjects with type-2 diabetes and in agreement with the ARIC study the investigators were able to show a significant relationship between glucose tolerance and CIMT. The Hoorn study investigators also measured PP, as a surrogate for arterial stiffness in 2,484 subjects of which 208 were type-2 diabetics ⁷⁶. They demonstrated a positive association between PP and CV mortality. This has been confirmed in another study in 2,911 subjects with diabetes in which PP was shown to be a better predictor of CV outcome than either SBP or DBP ⁷⁷. Subsequently a larger study, the FinnDianne study ⁷⁸, also examined the relationship between type-1 diabetes and PP. This study compared 2,988 type-1 diabetics and 5,486 non-diabetic controls across a wide age range. Again, there was a significantly higher PP in the diabetic group. Also, the age related increase in pulse pressure was greater in the diabetic group and consequently there was a greater prevalence of ISH in the diabetic group. Furthermore, the age related rise in PP was more pronounced in diabetic individuals with nephropathy. However, although these studies included large numbers of patients and were consistent in their findings, they were non the less cross sectional and do not provide adequate insight into cause and effect. However, a study by Hopkins et al in 1996, using doppler ultrasound as a measurement of arterial stiffness demonstrated that family history of type-2 diabetes is associated with decreased aortic distensibility in normal healthy young adults ⁷⁹.

A number of smaller studies have also measured arterial stiffness in subjects with diabetes. Early studies performed by Wahlqvist et al in 1988 used ultrasound to measure aorto-iliac PWV and showed it to be significantly higher in the diabetics relative to controls. These findings were confirmed in a later study employing similar

methodology^{80,81}. A more recent study measured aortic PWV in Japanese men and showed that aortic PWV was significantly higher in diabetics compared to healthy controls⁸².

Differential effects of diabetes on central elastic, and peripheral, more muscular arteries has also been the focus of investigation. Kimoto et al 2003, measured PWV in the aortic, carotid, brachial, and femoral artery beds in diabetic subjects and controls and demonstrated that the central arteries of diabetics stiffen at an accelerated rate compared to peripheral arteries. Furthermore, they showed that diabetes was only significantly associated with PWV in the central arteries⁸³. A similar study by Cameron et al 2002, has investigated the differential stiffening of central and peripheral arteries in diabetics and controls and also shows a significant difference in the rate of age-related increase in vascular stiffness in the elastic arteries of diabetics compared to non-diabetic controls. The diabetic arteries appearing to age at an accelerated rate from an earlier age and then reach a functional plateau⁸⁴.

However, not all studies have shown a positive relationship between diabetes and arterial stiffness. Initially, Scarpello et al 1980, demonstrated that carotid artery, brachial artery and femoral artery distensibility were not significantly different between diabetics and non-diabetics, as assessed by doppler ultrasound. More recently, investigators have demonstrated no difference in large artery stiffness (carotid, brachial or femoral) using ultrasound and stiffness index (SI)^{85,86} and also no significant difference in CF PWV between diabetics and controls⁸⁶.

The introduction of the sphyMoCor system has made it possible to use PWA to study arterial stiffness in a variety of patient populations and a number of investigators have employed this technique to compare diabetics and controls. One of the first studies

assessed AIx and central pressure as a measure of arterial stiffness in a group of type-1 diabetics and controls, and demonstrated a significant increase in both AIx and central pressure in the diabetic group ⁸⁷. This was subsequently confirmed in another study using similar methodology ⁵⁵. In this study, AIx was also significantly higher in the type-1 diabetic individuals. In addition the investigators measured the time to return of the reflected wave (Tr), as a surrogate for aortic PWV and showed that this too was increased in the diabetic group. PWA has also been used to investigate arterial stiffness in subjects with type-2 diabetes. A study by Brooks et al compared 88 subjects with type-2 diabetes and 85 controls. As with type-I diabetes, the subjects with type-2 diabetes also exhibited a significantly higher AIx when compared to controls ⁸⁸

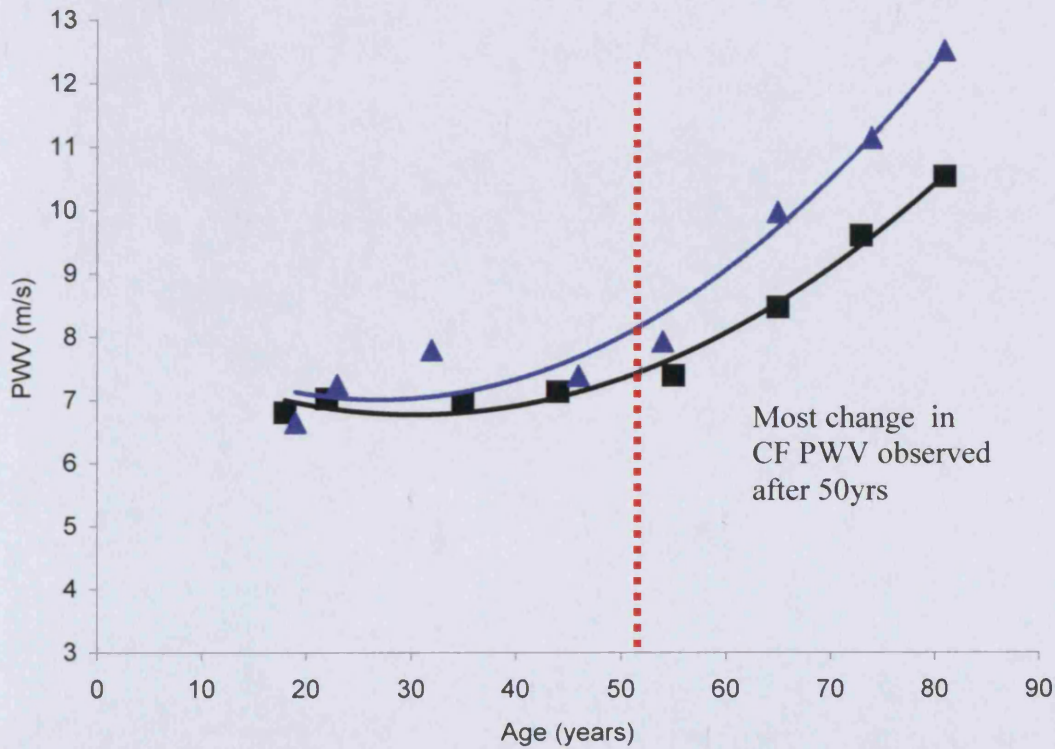
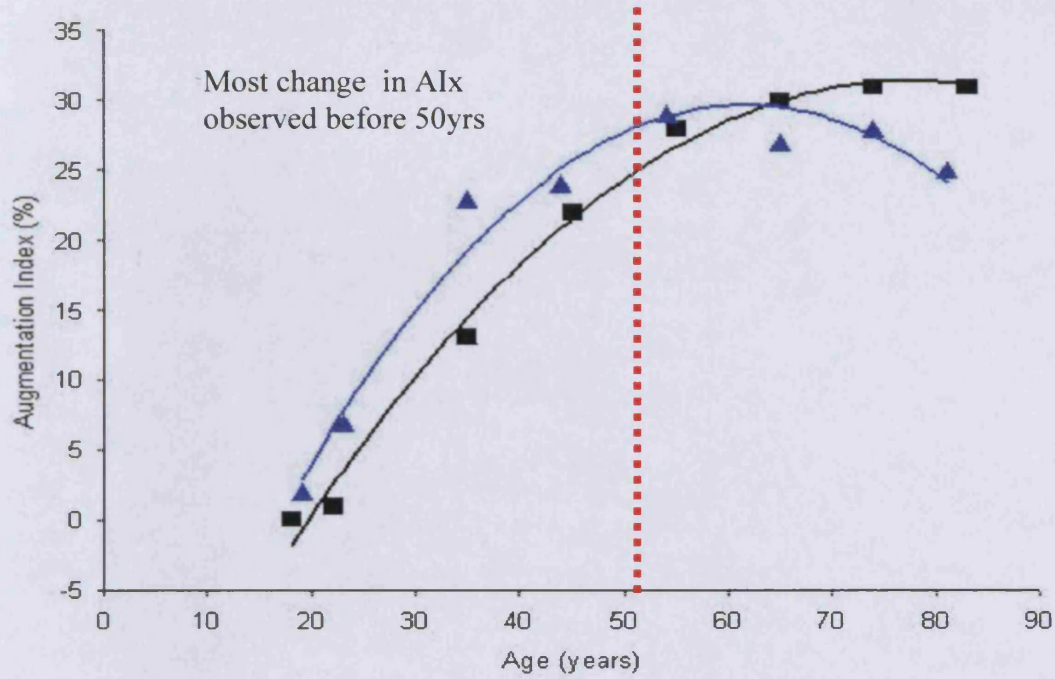
Recently Lacy et al, in 2004, also used PWA to study differences between a mixed cohort of type-1 and type-2 diabetics ⁸⁹. CF PWV was increased in the diabetics relative to controls, in agreement with previous studies. However, AIx was not significantly different between diabetics and non-diabetics. A potential explanation for this, is, the age of the subjects studied. Subjects of Lacy et al's were 55yrs or older, whereas the subjects in both the previous studies ^{87,88} were younger. Unpublished data from our own study group and others have recently demonstrated that in normal individuals, AIx tends to plateau at around age 55yrs (Figure 1.9). Indeed one study has shown a decrease in AIx after age 55 yrs ⁹⁰, making it more difficult to demonstrate changes in AIx in younger age groups. However, CF PWV increases relatively little to age 55yrs and then rises steeply making it more likely that changes in CF PWV due to diabetes will be more easily distinguishable. Diabetes induces little shift in the age AIx relationship over 55yrs but produces large shifts in

the CF PWV age relationship in subjects over 55yrs (Figure 1.9). This is important as it suggests that AIx will be more predictive of risk in younger subjects with diabetes whereas CF PWV should be more predictive in older subjects.

Although a considerable number of intervention studies have demonstrated decreased arterial stiffness in different patient populations, there is less published data in subjects with diabetes. In general therapeutic interventions that have been shown to improve endothelial function have been successful in reducing arterial stiffness. One early study showed that 6-weeks treatment with fish oil decreased arterial stiffness as assessed by an improvement in oscillatory compliance in 20 patients with type-2 diabetes⁹¹. Vitamin C therapy also decreases arterial stiffness, assessed by PWA, in type-2 diabetics treated for 1 month⁹². Furthermore, type-2 diabetics treated with atorvastatin also exhibited a decrease in femoral PWV⁹³. Esposito et al in 2004 have also illustrated that control of postprandial hyperglycaemia but not fasting hyperglycaemia was associated with a reduction of CIMT using repaglinide and glyburide over a 12 month period, in type-2 diabetics⁹⁴. Finally, Laurent et al were able to demonstrate that ACE inhibition with perindopril was able to decrease carotid distensibility independently of changes in blood pressure in a group of hypertensive type -2 diabetic subjects⁹⁵.

Overall the majority of data are consistent in showing that diabetes is associated with increased arterial stiffness. However, there are as yet no large longitudinal studies so that cause and effect remain unclear. Furthermore, the increasing incidence of obesity and the pre-diabetic metabolic syndrome make it important to study the relationship between states of abnormal glucose metabolism and arterial stiffness.

Figure 1.9. The general trend of AIx and CF PWV with age, in diabetics and non-diabetics. Taken from the ACCT study group database, 2007.



▲ = Diabetics, ■ = Normal Healthy Controls

1.8.2 Impaired glucose metabolism and arterial stiffness

In contrast to overt diabetes, far less studies have investigated the relationship between abnormal glucose metabolism and arterial stiffness. The large Rotterdam study, involving 2,987 elderly normal subjects has shown a positive relationship between impaired fasting glucose (IFG) and arterial stiffness, as assessed by carotid arterial distensibility. However, this observation was only apparent after the age of 75yrs ⁹⁶. The Hoorn study has also examined the association between impaired glucose metabolism, defined as a combination of IFG and impaired glucose tolerance (IGT) and arterial stiffness, assessed by PP and CIMT, in 1,193 individuals with either normal glucose levels, impaired glucose regulation or diabetes. However, this study only demonstrated an association between brachial and femoral IMT and impaired glucose metabolism and did not observe any association between these groups in the carotid vascular bed. Unfortunately, the study did not report findings in the IFG as separate group in their analysis. ⁹⁷

Subsequently, the same group employed PWA to assess arterial stiffness within the same cohorts as their previous study. Again, subjects with IFG were considered together with subjects with IGT. There was no difference in AIX between the impaired glucose metabolism group (IFG and IGT) and controls ⁹⁸. Again the mean age was 68yrs so that as suggested previously differences in AIX would be difficult to demonstrate. The investigators did not measure CF PWV or CV outcome. A study by Cruickshank et al in 2002, examined the relationship between arterial stiffness and CV outcome in subjects with normoglycaemia, abnormal glucose metabolism and type-2 diabetes. Again no distinction was made between IFG and IGT. Arterial stiffness was assessed by measuring aortic PWV. Aortic PWV was raised in both the

diabetic and impaired glucose metabolism groups and was shown to correlate positively with mortality³³.

To date, few studies have examined the relationship between arterial stiffness and IFG with conflicting results. In one study 2,080 Japanese men were investigated and exhibited a positive relationship between brachial-ankle PWV and IFG⁹⁹. However, the results of this study are difficult to interpret, as subjects were hypertensive and PWV was assessed using brachial-ankle PWV, which unlike CF PWV has not been shown to predict outcome¹⁰⁰. A similar smaller study in 282 Japanese, also measured brachial-ankle PWV and showed a positive correlation with IFG¹⁰¹.

Further studies failed to show any association between IFG and arterial stiffness, as assessed by CIMT¹⁰² and PWA¹⁰³. Furthermore, a reduction in post prandial hyperglycaemia but not fasting hyperglycaemia in diabetics was associated with CIMT reduction⁹⁴ and in a study which assessed the second derivative of plethysmogram as a measure of arterial stiffness, 2hr post challenge hyperglycaemia and not fasting glucose was associated with increased arterial stiffness¹⁰⁴. Impaired glucose metabolism is an important component of the metabolic syndrome which has been shown to correlate significantly with increased arterial stiffness^{105,106}. However, no studies apart from the ones just cited and discussed in chapter 5 have separated IFG from impaired glucose metabolism or attempted to analyse the components of the metabolic syndrome separately in terms of the individual effects on arterial stiffness. Since obesity is strongly associated with abnormal glucose metabolism and the metabolic syndrome, it has been difficult to tease out the relationship between various components of the metabolic syndrome and arterial stiffness. This is especially true for insulin resistance and obesity.

1.8.3 Obesity, insulin resistance and arterial stiffness.

Studies examining the relationship between obesity and arterial stiffness are difficult to fully interpret as they are confounded by a number of factors. Including whether obesity is defined by BMI or waist circumference. In addition, some studies have made distinctions between visceral and abdominal fat. Clustering of other risk factors such as hypertension, insulin resistance and inflammation also make accurate interpretation problematic. Finally, the degree of insulin resistance will vary within obese populations.

CF PWV was measured in 1,014 middle-aged subjects, with no clinical evidence of CV disease, enrolled in the SU.VI. MAX study ¹⁰⁷. CF PWV was significantly correlated with obesity assessed by increased waist circumference. A further large study involving 2,488 older adults showed in a multivariate regression analysis that obesity defined by abdominal visceral fat was positively associated with aortic PWV ¹⁰⁸. Van Popele et al measured carotid distensibility in 180 non-diabetic women. There was a significant correlation between BMI, waist hip ratio and carotid distensibility. This relationship remained significant after adjusting for MAP ¹⁰⁹. Smaller studies examining the relationship between obesity and arterial stiffness have been conflicting. This may be due in part to the different subject characteristics. In subjects with hypertension, brachial PWV was significantly increased in obese subjects with increased BMI's relative to lean controls. Furthermore, there was a positive relationship between obesity and PWV, which remained significant after adjustment for glucose, lipids and triglycerides (TG's) ¹¹⁰. A study in severely obese children has demonstrated increased arterial stiffness, assessed by ultrasound. This was also associated with endothelial dysfunction ¹¹¹. However, in a study that

measured BMI and aortic PWV in 524 healthy young adults, multiple linear regression analysis failed to show an association between BMI and aortic PWV ¹¹².

A number of intervention studies have also reported the effect of weight loss on arterial stiffness. A study involving 152 individuals followed for 2 years, showed that a weight gain of > 4.5 Kgs was associated with a significant increase in aortic PWV. In contrast subjects who lost > 4.5 Kgs in weight exhibited a significant decrease in aortic PWV ¹¹³. A study in 38 obese patients with type-2 diabetes showed that weight loss after a period of 1 year was associated with a significant decrease in aortic PWV ¹¹⁴. Weight loss in a small study of overweight adults was associated with a significant improvement in brachial artery compliance. This was associated with an accompanying improvement in lipid profile and insulin sensitivity ¹¹⁵. An association between obesity and insulin resistance has been reported in adults and children. Studies in children by Caprio et al in 1996 employing a hyperinsulinaemic euglycaemic clamp technique, demonstrated that insulin resistance and hyperinsulinaemia co-exist with various extremes of obesity ¹¹⁶.

The ARIC study measured serum insulin levels and demonstrated a graded relationship between fasting insulin and arterial stiffness ⁷⁵. Similar findings were reported from the Health ABC study in which insulin levels were correlated with increased aortic PWV in 2,488 subjects ¹⁰⁸. A large Danish population study measured fasting insulin levels in 2,420 men and women subjects. Hyperinsulinaemia was associated with increased aortic PWV which remained significant in a multiple regression analysis controlling for conventional risk factors ¹¹⁷. A number of studies have used the hyperinsulinaemic euglycaemic clamp to investigate the effects of insulin on arterial stiffness in normal subjects, insulin resistant subjects and type-2 diabetics. In normal subjects, insulin at physiological levels decreases wave reflection

and AIx¹¹⁸. Furthermore, the same investigators demonstrated that this effect of insulin was blunted in obese insulin resistant subjects¹¹⁹ and in subjects with type-1 (Westerbacka et al Hypertension 2000) and type-2 diabetes¹²⁰.

1.8.4 Physical Activity and arterial stiffness

Lack of physical activity is an independent risk factor for CVD^{121,122}. Although it has been demonstrated that increased physical activity decreases blood pressure, its effect on arterial stiffness remains unclear¹²³.

Major large-scale studies have focused on the beneficial effects increased physical activity has on arterial stiffness. One such study, performed by the Northern Ireland Young Hearts Project involving 405 young subjects (12-15yrs) showed an inverse relationship between estimated cardio respiratory (CR) fitness and self reported physical activity (PA) levels with arterial stiffness, assessed by photoplethysmography which measured aorto-iliac and aorto-dorsalis pedis PWV¹²⁴. These findings have been confirmed in a recent similar sized population based study of 432 healthy young to middle age subjects. This study measured CIMT and carotid stiffness and showed a significant inverse correlation between habitual physical activity and both CIMT and carotid stiffness¹²⁵. However, not all large studies are in agreement with this data. The ARIC study involving 10,644 older individuals who performed habitual exercise demonstrated no association with decreased arterial stiffness, as assessed using CIMT and habitual physical activity. However, vigorous PA was shown to have a weekly positive association with decreased arterial stiffness, again measured by CIMT¹²⁶. A much smaller study has confirmed these findings, showing that a habitual recreationally active group of men did not exhibit any

significant differences in arterial stiffness, measured at the carotid artery using B mode ultrasound and applanation tonometry, compared to a sedentary control group. However, a significant difference in arterial stiffness was observed between an endurance trained group and both recreationally active and sedentary groups, assessed again using B mode ultrasound and applanation tonometry ¹²⁷.

Interestingly, earlier smaller studies have shown that leisure time physical activity (LTPA) was associated with decreased arterial stiffness, assessed by doppler ultrasound and Young's elastic modulus. However, it was also established that higher LTPA had significantly lower arterial stiffness compared to the lower LTPA ¹²⁸. Suggesting that CR fitness may, in part, have a role to play in decreasing arterial stiffness. Indeed, it has been demonstrated that increased CR fitness (measured by VO₂max), is associated with decreased arterial stiffness as measured by AIx and aortic PWV, in 146 men aged 54-75 yrs ¹²⁹. Cardiorespiratory fitness was also shown to be inversely associated with AIx in a group of 201 men, aged approximately 50yrs without CHD. However, the subjects used in this study were a mixture of hypertensives, diabetics and smokers, making interpretation of the results very difficult ¹²⁹. Similar findings have been demonstrated in females. Both moderate and vigorous PA levels in postmenopausal women were associated with decreased arterial stiffness, assessed by carotid β stiffness index ¹³⁰. These studies were however, cross sectional and thus provide no data on cause and effect relationships.

Longitudinal studies, like the DANSCO study have shown that regular low to moderate intensity aerobic exercise is associated with a 40% lower 6-year progression of CIMT compared to a group taking little or no exercise ¹³¹. The ACT study demonstrated that increased PA was associated with a significant decrease in aortic PWV in 555 men and women, aged 35-75yrs after 24 months of walking intervention

¹³². A much smaller interventional study, of 16-weeks low to moderate exercise was performed on 17 sedentary men, and showed a significant decrease in arterial stiffness of the central elastic arteries and no difference in the peripheral muscular arteries, assessed using PWV. Suggesting that like age, physical activity may have a differential effect on the central arteries compared to the peripheral arteries. Interestingly, findings by Tanaka et al 1998, had already observed that age related increases in arterial stiffness, as measured by aortic PWV, were not apparent in 53 highly PA women ¹³³. Interestingly, although ISH is associated with increased large arterial stiffness, a small study failed to show benefit of aerobic exercise on large arterial compliance ¹³⁴. However, the same group was able to show that cholesterol reduction did decrease arterial stiffness in a similar group of subjects with ISH, so this result is perhaps surprising as exercise has been shown to decrease cholesterol levels ¹³⁴.

Indeed, a major potential limitation of these interventional studies is that they may be confounded by the effect of exercise on other CV risk factors, including blood glucose . Especially since exercise has been shown to increase insulin sensitivity and therefore improve glucose levels ¹³⁶. Indeed, the effect of short-term aerobic exercise was shown to decrease arterial stiffness, assessed by β stiffness index at the carotid and femoral site, in type-2 diabetics. Furthermore a number of other studies have demonstrated that physical inactivity is associated with adverse metabolic effects such as increased fasting glucose and raised triglycerides (TG's) ¹³⁷⁻¹³⁹. Increased PA, especially via aerobic exercise has also been shown to alter gene expression e.g. Prostaglandin, C-type Natriuretic Peptide (CNP) and endothelial Nitric Oxide Synthase (eNOS). Expression of these genes, especially nitric oxide in the aorta, may

be in part an explanation for the beneficial effect seen by aerobic exercise on aortic stiffness¹⁴⁰.

Finally, the majority of data suggests the beneficial impact that increased physical activity has on arterial stiffness and overall CV risk. However, further longitudinal and interventional studies are needed to demonstrate cause and effect. Various ranges of intensity levels and forms of PA need to be considered, along with biochemical measures relating to endothelial function need attention in these proposed longitudinal studies to demonstrate the causal determinants of decreased arterial stiffness and CV risk.

1.8.5 Ethnicity and arterial stiffness

To date, although there is some published data on differences in ethnic background and arterial stiffness, data is mostly confined to studies comparing Black Afro-carribeans and Caucasians. African Americans have been demonstrated to exhibit accelerated large arterial stiffening when compared to Caucasians¹⁴¹. Furthermore, in a separate study they show a greater pressure-dependant increase in aortic stiffness¹⁴². These results suggest that there are major differences in the mechanical properties of the large arteries between the two ethnic groups.

In contrast, very little data has been published comparing South Asians and Caucasians when assessing arterial stiffness and vascular haemodynamics. Although a recent multiethnic study measuring aortic PWV in subjects with diabetes and impaired glucose metabolism included a group of South Asians, but no data for the South Asians were presented separately³³.

Studies have however, examined differences in CV disease between South Asians and Caucasians. Following an 11 year follow up study by Mather et al, 1998, investigators demonstrated that 77% of all South Asian deaths are caused by circulatory disease compared to 46% in Europeans ¹⁴³. Furthermore, it has also been shown that South Asian men have a 60% greater rate of CHD related death compared to white European men. However, 50% of all deaths in South Asians are associated with diabetes mellitus compared to only 13% of deaths associated with diabetes in white Europeans ¹⁴⁴.

Interestingly, although South Asians have similar prevalence of ischaemic heart disease (IHD) to Caucasians, the prevalence of PVD is considerably lower. A study of clinical features and vascular complications of diabetes between migrant Asians and Caucasians in Leicester illustrated the prevalence of PVD as 3% in the South Asians *versus* 9.3% in the Caucasian, $P < 0.05$ ¹⁴⁵. This remains a consistent finding even in the presence of classical risk factors such as diabetes ¹⁴⁶. Furthermore, a recent study has demonstrated that South Asians have a lower risk of amputation compared to Europeans and that this was related to lower prevalence of neuropathy and PVD ¹⁴⁷. In this study by Chaturvedi et al, investigators demonstrated that South Asian diabetics have about a quarter of the risk of amputation of Europeans.

The mechanisms associated with these findings are unclear. However, if arterial stiffness precedes atherogenesis, then a plausible explanation would be that the femoral vascular bed of South Asians exhibits a lower rate of age related stiffening than that of Caucasians. This hypothesis will be investigated in this thesis.

1.8.6 Cholesterol and arterial stiffness

Unlike diabetes, the relationship between cholesterol and arterial stiffness is less consistent. This may be due to a number of factors including differences in the methodologies used to assess arterial stiffness and the vascular bed that the measurements were made in. Studies have also reported results for different lipid components ranging between LDL, total and HDL cholesterol. In addition, some of the patient populations studied had additional risk factors such as hypertension. Finally, there is also considerable age variation between the studies reported. Indeed, one of the earliest studies performed in children with familial hypercholesterolaemia (FH), Lehmann et al, 1992, demonstrated a positive relationship between low density lipoprotein (LDL) and decreased arterial stiffness, assessed by measuring aortic distensibility using ultrasound. The study also demonstrated an inverse relationship between arterial stiffness and HDL,¹⁴⁸. Interestingly, when the investigators measured the same parameters in an adult cohort, the reverse was found with there now being a positive relationship between LDL and total cholesterol (TChol) and increased arterial stiffness⁸¹. These studies were small and a number of larger more recent studies have examined the relationship between cholesterol and arterial stiffness. Pitasavos et al, 1998, demonstrated a positive correlation between LDL cholesterol and increased arterial stiffness in 60 subjects with FH, compared to 20 normocholesterolaemic controls¹⁴⁹. However, these findings were not confirmed by a smaller study using similar methodology in 25 patients with FH and 10 controls, which demonstrated no significant difference in aortic or carotid stiffness between groups⁵¹. These studies assessed aortic stiffness using ultrasound. A further study by Giannattasio et al, 1996, assessed arterial stiffness in the radial artery and demonstrated that radial artery stiffness, assessed by ultrasound derived radial compliance, was significantly greater

in a group of subjects with FH compared to controls ¹⁵⁰. Studies involving subjects with FH have reported on extremes of cholesterol. Other studies have been undertaken in subjects with cholesterol more representative of those found within the general population.

Recent studies have used PWA to study arterial haemodynamics in hypercholesterolaemic subjects and controls. Interestingly, investigations carried out by Dart et al, 2004, in a cohort of 868 hypertensives demonstrated no relationship between TChol and arterial stiffness, when measured by AIx ¹⁵¹. Interestingly, this study was conducted in a population over 65yrs. The group was also hypertensive, making interpretation of the results difficult. In contrast to this study, a further study performed by Wilkinson et al, in 2002, demonstrated that arterial stiffness assessed by AIx, and central blood pressure were significantly higher in a hypercholesterolaemic group compared to matched healthy controls, despite no differences in peripheral blood pressure. In addition, there was also a positive relationship between AIx and LDL cholesterol ⁵². Overall, the majority of studies demonstrate a positive relationship between raised cholesterol levels and increased arterial stiffness, suggesting that cholesterol reduction may decrease arterial stiffness.

Indeed, a study by Ferrier et al was able to show that cholesterol reduction in subjects with ISH, a condition characterised by increased arterial stiffness, not only improved systemic arterial compliance but also resulted in small but significant reductions in peripheral systolic pressure ¹⁵². Interestingly these patients were not hypercholesterolaemic. A number of further studies have addressed the effects of cholesterol lowering therapy, mostly with statins, and arterial stiffness, in cohorts with hypercholesterolaemia. The results having been conflicting. Whilst Kool et al were unable to demonstrate any effect of statin therapy on either carotid, brachial or

femoral arterial distensibility¹⁵³, Muramatsu et al were able to show decreased aortic PWV in hypercholesterolaemic subjects after 6 months statin therapy. Possible mechanisms for the reduction in aortic PWV were suggested as the decrease in cholesterol levels, which resulted in an improvement in peripheral endothelium-dependant vasodilation disorder associated with hypercholesterolaemia. Moreover, this effect was still present at 5 years¹⁵⁴.

To date the majority of studies would tend to support the benefits of cholesterol reduction on arterial stiffness. A number of questions however remain unanswered. There is a clear association between stiffening of the arterial wall (arteriosclerosis) and atheromatous deposition (atherosclerosis). Therefore stiffening may reflect atherosclerosis rather than a direct effect of cholesterol on the arterial wall. Future studies designed to better understand causality will need to include much larger cohorts than those studied to date and particularly include younger individuals free from clinical evidence of CV disease in order to minimise the confounding effects of pre-existing atheromatous disease and CV risk factors. As with diabetes and IFG, there is a major need for large longitudinal studies.

CHAPTER 2

METHODOLOGY

2.1 Introduction

This chapter briefly describes the methods used for recruiting study volunteers and their selection, but more details are provided in the relevant chapters. However, the various techniques used to assess arterial stiffness are addressed in more detail in this chapter.

2.2 General recruitment process

Recruitment of volunteers was performed through a self-referral risk factor clinic. A constant throughput of volunteers enabled recruitment of an appropriate number of subjects needed for each study. Specific recruitment procedures and ethical issues are addressed in the methodology section of each chapter accordingly.

2.3 Measurements

2.3.1 Blood Pressure (supine and seated measurements)

Blood pressure and other haemodynamic measurements were performed after ten minutes rest in a temperature controlled room. Blood pressure was measured at the brachial artery using a validated semi-automated oscillometric device¹⁵⁵. All measurements were taken on the dominant arm and in duplicate to rule out any discrepancies and mean values were used in the subsequent analysis. All measurements were obtained according to BHS guidelines¹⁵⁶.

2.3.2 Measurement of vascular haemodynamics, using three non-invasive systems.

The SphygmoCor, Gaon21A and PP-1000 were used to measure various indices of arterial stiffness. The SphygmoCor and Gaon21A systems individually use applanation tonometry and PWA to derive the augmentation index (AIx). The SphygmoCor and PP-1000 systems were used to measure regional artery PWV.

2.3.2.1 SphygmoCor system

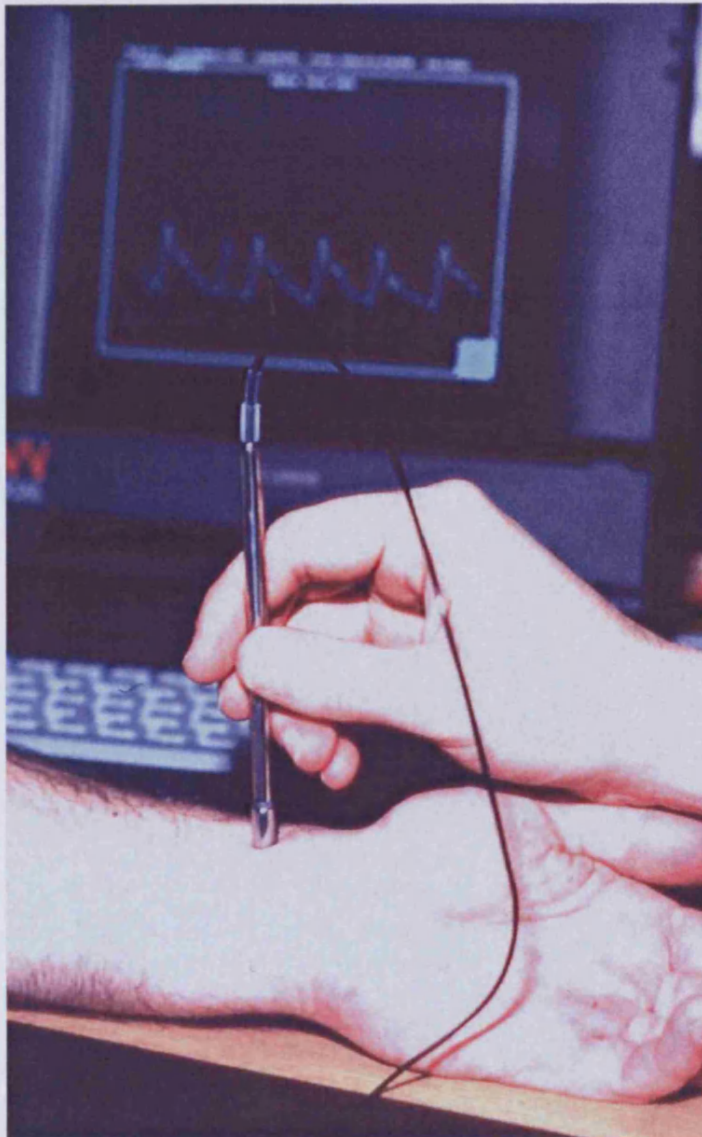
2.3.2.1.1 Applanation tonometry.

Arterial stiffness was measured using the method of applanation tonometry. Applanation tonometry was initially used by Mac Kay and Marg in 1960, for the investigation of intraocular pressures¹⁵⁷. This theory relied on the elimination of circumferential wall stresses by flattening a portion of the chamber and allowing a central cantilever beam to respond directly to intraocular pressure. The principles of this were used by O'Rourke and Gallagher in 1996, to record high fidelity waveforms from peripheral arteries and termed pulse wave analysis (PWA)¹⁶. The investigators showed that once the artery is flattened, circumferential pressures are equalised and transluminal pressures can be recorded accurately. In fact, Kelly¹⁷ demonstrated intra-arterial pressures can be recorded with a high degree of accuracy. Radial artery waveforms are recorded with a high-fidelity micromanometer (SPC-301; Millar Instruments, Texas, Houston) from the radial pulse at the wrist of the dominant arm. The hand held Millar tonometer consists of a pencil shaped probe with a high fidelity micromanometer at its tip. The tip consists of a 0.5 x 1mm piezoelectric crystal with a frequency response rate greater than 2kHz, which is co-planar with a 7mm face allowing direct applanation of the artery under study. (See Figure 2.1).

2.3.2.1.2 Pulse wave analysis (PWA)

Peripheral artery waveforms are recorded using the Millar hand-held tonometer at the radial artery and analysed using the SphygmoCor software (Figure 2.1).

Figure 2.1. The Millar hand-held tonometer, applanating the left radial artery with the obtained waveform being demonstrated in the background



Calibration of the device is performed using the brachial blood pressure taken from the dominant arm, using a validated automated oscillometric sphygmomanometer

(HEM-705CP, Omron Corporation, Japan). Two measurements are made and an average used for later analysis. The pulse trace is recorded for ten consecutive beats, with a subsequent pulse train being averaged and processed by the inbuilt software package.

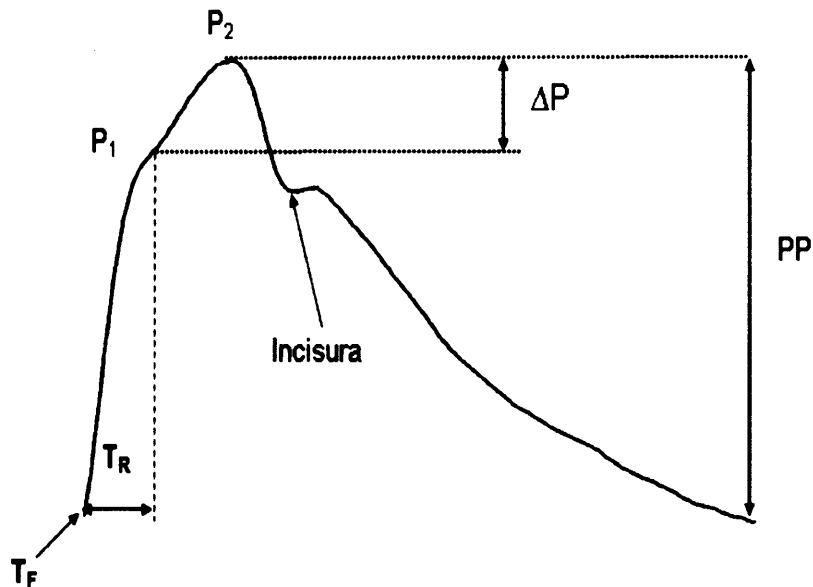
The system uses a method known as Fourier analysis to break down the averaged radial pulse wave recorded, split it into its component harmonics and uses a transfer function to recreate a central aortic waveform. (Figure 2.2). Although the use of a generalised transfer function has been the subject of some criticism, a number of studies have proven its validity ^{18,19,158}. Pauca et al, demonstrated in an invasive comparison, that the absolute difference between estimated (via transfer function) and actual central pressures was less than 1mmHg ¹⁸.

Figure 2.2 Data recorded by the SphygmoCor system (version 8.1), displaying results of PWA.



For the purpose of this thesis, the most relevant parameters recorded from the waveform include central blood pressure, heart rate (HR), ejection duration (ED), augmentation index (AIx), HR adjusted AIx, augmentation pressure (AP) and time for the reflected wave to return (Tr). Figure 2.3 describes the morphology and sites of relevant information of a typical aortic waveform constructed by the SphygmoCor system.

Figure 2.3 A typical aortic pressure waveform constructed by the SphygmoCor software.



T_f : Foot of the forward travelling wave, T_r : time difference between the foot of the wave and the point at which the reflected wave has an effect (the inflection point), P_1 & P_2 : first and second systolic peaks, PP : pulse pressure.

A_{Ix} is measured as the difference between the first (P_1) and the second (P_2) of the systolic peaks and recorded as a percentage of the pulse pressure (PP). The first systolic peak is the maximum pressure the forward travelling wave expresses and the second systolic peak, is the peak generated after the influence of the forward and backward travelling waves are combined. A_{Ix} is also a measure of wave reflection. One of the main sites of wave reflection is the aortic bifurcation. Since A_{Ix} is influenced by aortic PWV and the site and magnitude of wave reflection from the periphery it can be considered as an indirect measure of arterial stiffness. Further analysis of the recorded wave forms can be made to obtain values of central blood pressure, HR and T_r , which is the time taken for the reflected wave to return and is

therefore a reasonable surrogate for aortic PWV ¹⁵⁹. Guidelines for the use of PWA in research and clinical practice have recently been published ¹⁶⁰. The guidelines addressed in this paper refer to the importance of a standardised procedure for measuring PWA. The investigators also discuss the importance that confounding factors like blood pressure, age, gender, BMI, heart rate and medication have on PWA.

2.3.2.1.3 Pulse wave velocity (PWV)

Although PWA and indices such as AIx have been measured in a large number of studies, as yet, AIx has not been shown to be a major predictor of CV outcome. PWV has been established as the “gold standard” for assessing arterial stiffness in a variety of arterial beds. However, it is aortic PWV (usually assessed as carotid-femoral PWV) that has been shown to be predictive of outcome (See Chapter 1, Section 1.5) and not PWV in other vascular beds such as the brachial and femoral ¹⁰⁰.

Using the SphyMoCor system aortic, brachial, and femoral PWV can be measured as the time taken for the pulse to travel from the R-wave of a three lead ECG-gated signal, to the first upstroke of the pulse wave at the carotid, radial, femoral and dorsalis pedis arterial site. To calculate PWV it is necessary to determine the path length. This is achieved by surface measurement of the distance between the carotid brachial or carotid femoral measurement sites. (In practice this is done by measuring the distance from the supra-sternal notch to the site of measurement, over and across the surface of the body). A three lead ECG is attached to the subject in this instance to obtain an ECG signal for software analysis.

Waveforms are measured sequentially at each location, whilst the signal is simultaneously being gated from the R-wave of the ECG. The measurement is

therefore recorded from the R-wave of the ECG to the foot of the forward travelling pulse wave. The foot of the pulse is used as the identification point of the wave because the foot is the only area of the wave that is not influenced by wave reflection. The intersecting tangent algorithm is used in the determination of where the upstroke or point of recording ends.

2.3.2.2 Gaon21A system (Hanbyul Meditech Co., Korea)

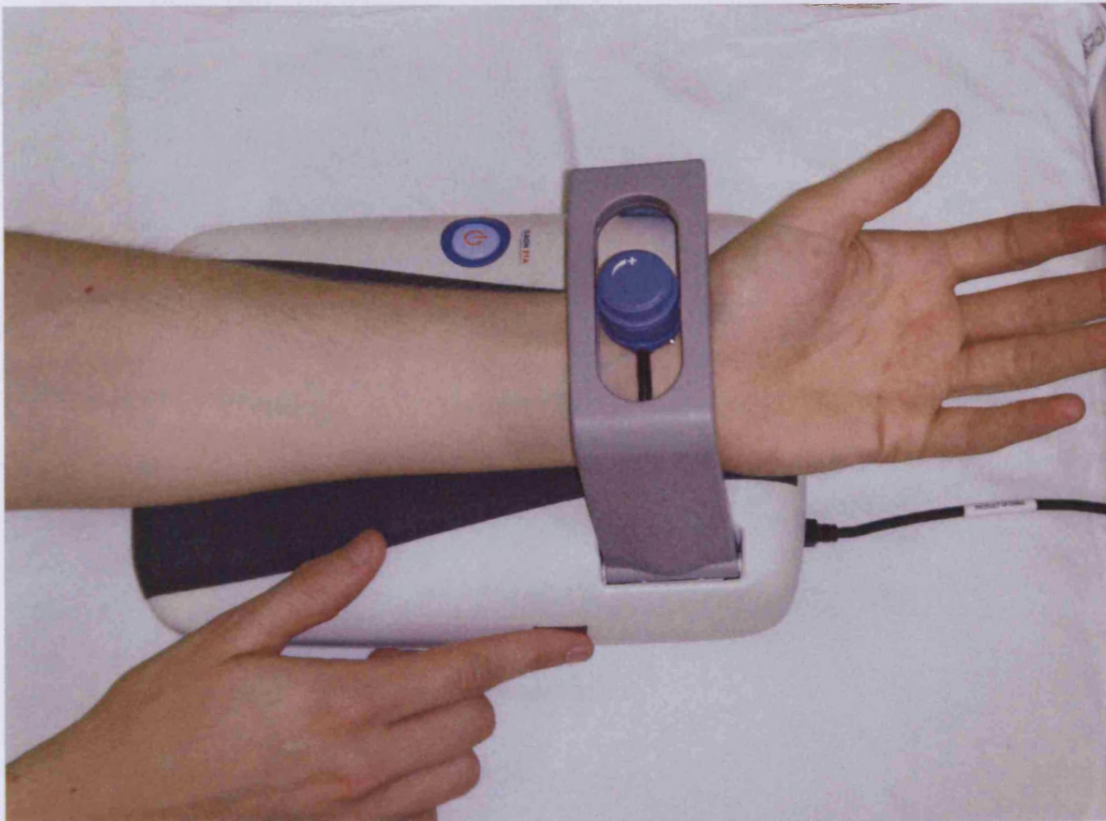
2.3.2.2.1 Applanation tonometry

Like the SphyMoCor system this system also uses applanation tonometry to measure peripheral pulse waveforms and generate the AIx. However, unlike the SphyMoCor system this method of applanation tonometry uses a larger pressure sensor mounted onto a radial wrist clamp (Figure 2.4). To date, this system has yet to be validated against the SphyMoCor system in terms of AIx.

2.3.2.2.2 Pulse wave analysis (PWA)

This simple to use method of measuring AIx has only recently been developed. Applanation of the radial artery is performed and the pressure sensor located directly over the artery (Figure 2.4). Various degrees of pressure can then be applied to obtain clear waveforms. The pulse trace is recorded for ten consecutive beats, with a subsequent pulse train being averaged and processed by the inbuilt software package. The Gaon21A uses the method of Fourier transformation analysis to break down the averaged radial pulse wave recorded, similar to that performed by the SphyMoCor. This system can only be used to measure AIx as there is no data on the validity of the integral transfer function to measure central arterial pressure.

Figure 2.4 Measurement of Aix, using the Gaon21A system.



2.3.2.3 PP-1000 system (Hanbyul Meditech Co., Korea)

2.3.2.3.1 Applanation tonometry

Again, this system utilises applanation tonometry to accurately record peripheral arterial waveforms. However, unlike the SphygmoCor system which uses the Millar hand-held tonometer, the PP-1000 uses gel-filled semiconductor pressure sensors, designed to measure the applied pulse pressure from the artery. Sensors are attached to elastic, Velcro attaching bands, which were easily strapped around the approximate artery area, with the sensor directly over the artery bed. Unlike the SphygmoCor and Goan21A systems, the PP-1000 does not incorporate an integral transfer function and so cannot be used for measuring AIx from a generalised central aortic waveform.

2.3.2.3.2 Pulse wave velocity (PWV)

Using the PP-1000 (Hanbyul Meditech Co., Korea), regional PWV values based on measurements of electrocardiography (ECG), phonocardiography (PCG), and pulse waves from four different artery sites (carotid, femoral, radial, and dorsalis pedis) can be obtained simultaneously. ECG signals are acquired from both right and left forearms, and PCG sensor, which incorporates a piezopolymer film contact microphone, is placed on the chest. Sensors are attached to elastic, Velcro attaching bands, which are easily strapped around the approximate artery area, with the sensor directly over the artery bed. The sensor applied over the carotid artery is applied using a spring-mounted clamp. Similar to the Velcro straps on the peripheral arteries, increased pressures can be applied to the clamp in order to obtain a clear waveform.

Figure 2.5 shows the sensors and the Velcro strapping used by this apparatus.

Cut-off frequency of analogue filters for pulse waves is set at 0.05~20Hz. ECG and four pulse waves from carotid, radial, femoral, and dorsalis pedis arteries are measured on the left side of the body and recorded simultaneously for the duration of 10 seconds. Like the SphygmoCor system, the PP-1000 uses foot to foot methodology. PWV is recorded as the velocity of the pulse wave from the R wave of the ECG gated signal to the upstroke of the simultaneously recorded pulse waves at each artery site. However, unlike the SphygmoCor system, the PP-1000 records pulse waves at all four arterial sites simultaneously (Figure 2.6).

Figure 2.5 Sensor and Velcro strap used by the PP-1000 system.

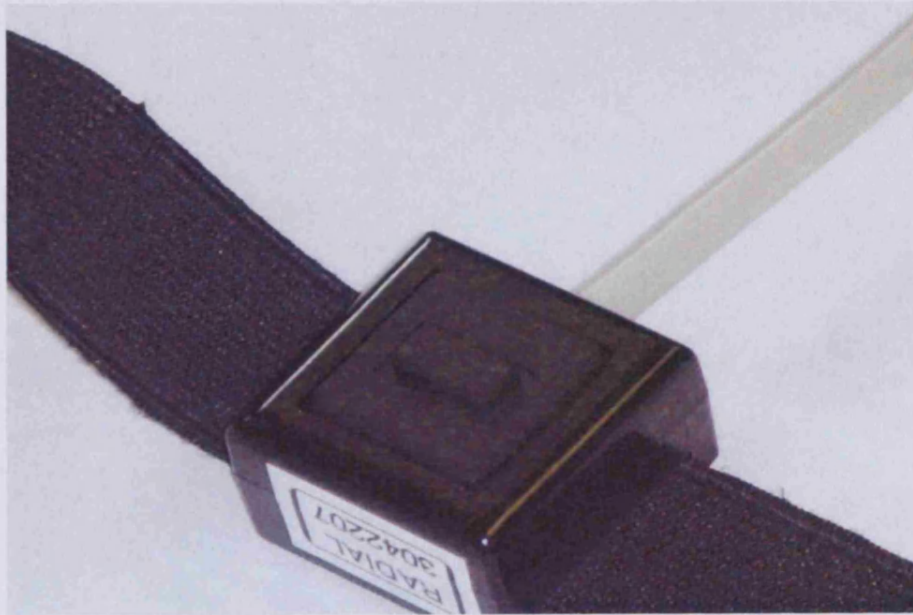


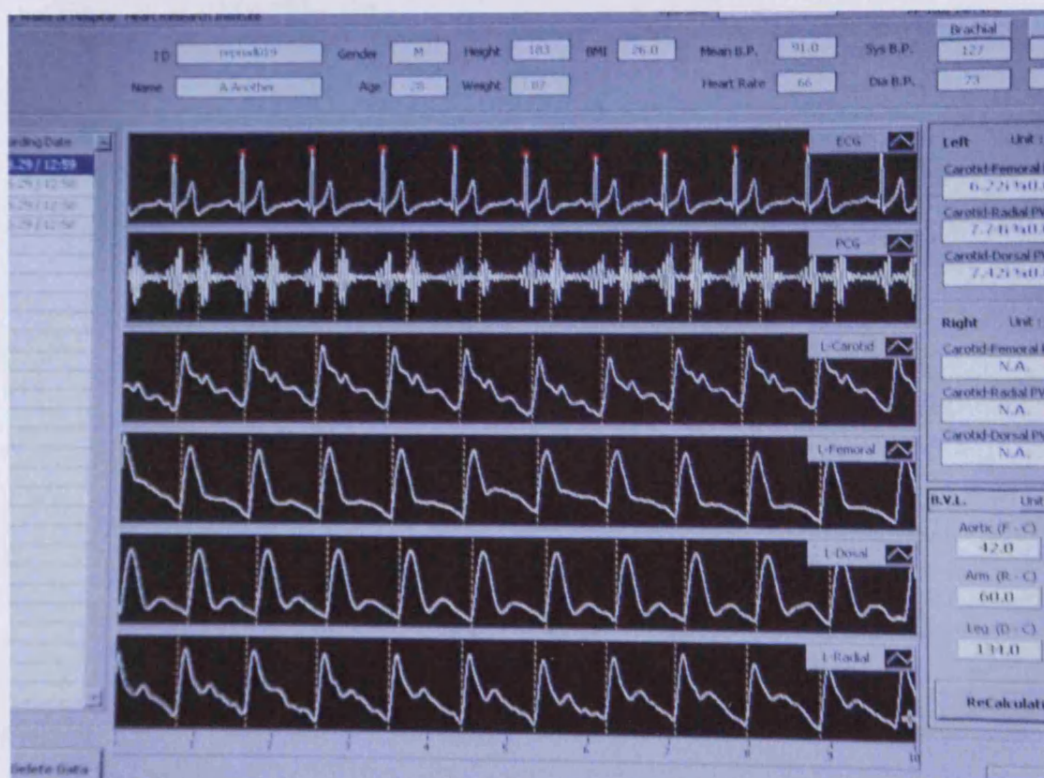
Figure 2.6 PWV measurements recorded simultaneously by the PP-1000.



For an automatic determination of PWV values, surface distances between the two recording pulse waves are measured and inputted to the system to allow the calculation of PWV values. $PWV = \text{Distance} / \Delta\text{Time (m/s)}$.

Once data collection is finished, the system extracts characteristic points from each signal. R-peaks of ECGs are detected using a time division adaptive threshold algorithm. Based on the time-domain scales obtained from the above values, upstroke points of pulse waves at the carotid, radial, femoral, and dorsalis pedis arteries are detected using intersecting tangent methods (similar to SphygmoCor method). Time differences of upstroke points between two different sites are then used to calculate regional PWV values. Four different regional PWV values can be derived: Carotid to Radial (CR), Carotid to Femoral (CF) Carotid to Dorsalis pedis (CD) and Femoral to Dorsalis pedis (FD). The simultaneously measured pulse wave velocities with their pressure waveforms using PP-1000 systems are illustrated in Figure 2.7.

Figure 2.7 Simultaneous PWV recordings displayed on screen of PP-1000 system.



The reproducibility of the PP-1000 system for measuring PWV has not been assessed against other systems. It has therefore been compared to the SphyMoCor system in the following Chapter. In addition, measurement of AIx has been compared between the SphyMoCor system and the Goan21A.

2.4 Statistics and power calculations

In order to test the study hypothesis, power calculations were performed to ensure the correct number of subjects to be recruited:

$$N = \frac{10.5 \times 1.17^2 \times 2}{0.7^2}$$

$$N = 59 \text{ people}$$

10.5 = Constant used to obtain a significance level of 0.05 with a power of 0.90,

1.17 = SD from repeated measure aortic PWV, **0.7** = a clinically significant difference in aortic PWV.

Based on a standard deviation of differences between repeated measurements of aortic pulse wave velocity of 1.17 metre/second (taken from the Anglo Cardiff Collaboration Trial, ACCT study database of over 2500 subjects), 59 subjects in each group will provide a 90% chance of detecting a 0.7 metres/second difference in aPWV between the groups, at the 0.05 significance level. A difference observed below 0.7 metres/second is unlikely to be clinically or physiologically meaningful.

CHAPTER 3

**PULSE WAVE VELOCITY AND AUGMENTATION INDEX,
MEASURED BY THE PP-1000/GAON21A SYSTEM AND
COMPARED TO THE SPHYGMOCOR SYSTEM
(STUDY 1)**

3.1 Introduction

Large arterial stiffness is a major independent risk factor for cardiovascular disease³⁴⁻³⁶. Large artery stiffness is a consequence of premature vascular ageing due to cardiovascular risk factors such as hypertension³¹, diabetes³³ and hypercholesterolemia⁷⁴. The aetiology and progression of these changes are due to structural and functional alterations (see Figure 1.7, Chapter 1). Clinically overt atheromatous disease may be preceded by increased arterial stiffness¹⁶¹, thus, making early detection extremely important in the diagnosis and management of impending cardiovascular disease. Recognition of this has led to the development of a number of different techniques to measure arterial stiffness¹⁰⁰. The majority of published studies have validated and used the SphygmoCor system to perform pulse wave analysis and measure carotid-femoral PWV^{16,162,163}. This study therefore examines the reproducibility of the PP-1000/Gaon21A system (Hanbyul Meditech, Korea) and compares it with the SphygmoCor system as one of the most widely used and “gold standard” method for measuring aortic stiffness¹⁰⁰. This is important, as subsequent studies will use the PP-1000 to measure PWV in the femoral-dorsalis pedis vascular bed.

3.2 Methodology

3.2.1 *Subject Recruitment*

19 subjects (10 healthy participants, aged 24 ± 3 and 9 Type-2 diabetics, aged 50 ± 7 years) participated in the study. Participants were recruited for the study, as part of a self-referral risk factor clinic, in the Heath Hospital, Cardiff. Identical protocols were performed on each of the participants.

3.2.2 *Blood Pressure Measurement*

BP was recorded after ten minutes rest at the brachial artery of the dominant arm using a validated semi-automated oscillometric device and according to BHS guidelines¹⁵⁶. All haemodynamic measurements were made in a quiet, temperature-controlled room of 22°C. Duplicate readings were performed on each occasion, and an average recorded.

Table 3.1. General group characteristics (N=19)

	Mean \pm SD	Min - Max
Age (yr)	40 \pm 18	21 - 67
Height (cm)	177	164 - 192
Weight (kg)	78	57 - 115
BMI (kg/m ²)	24	20 - 32
SBP (mmHg)	119	94 - 144
DBP (mmHg)	72	55 - 89
MBP (mmHg)	103	87 - 122

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure

3.2.3 Pulse Wave Analysis (PWA) Using SphygmoCor

PWA was performed using the SphygmoCor system as described in Chapter 2. For the purpose of this study, only AIx was recorded for comparison with Gaon21A.

3.2.4 Pulse Wave Velocity (PWV) Using SphygmoCor

Palpation of the radial, carotid, femoral and dorsalis pedis pulse were located and marked before the distance to each site was measured from the sternal notch, using a measuring tape. In brief, carotid radial, carotid femoral and carotid dorsalis pulse wave velocities were measured sequentially as the time taken for the pulse to travel from a three lead ECG-gated signal to the first upstroke of the pulse wave at the carotid, radial, femoral and dorsalis pedis site. A simple 3 lead ECG was attached in order for the pulse traces obtained from the tonometer to be gated against the R wave of the ECG and determine pulse wave transit time (velocity = distance/ Δ time), which along with the measured distances, allowed PWV to be calculated. Two separate readings were obtained, with approximately two minutes duration between each measurement, for each regional segment (e.g. CR PWV). Waveforms and readings were assessed visually to ensure good quality pulse traces and minimize any user induced artefacts.

Upon completion of all the measurements using the SphgymoCor system, the ECG was removed and the PP-1000 device was set up.

3.2.5 Pulse Wave Analysis (PWA) Using Gaon21A

Pulse wave analysis was performed and AIx measured in the same way as described in Chapter 2.

At the screening visit, participants proceeded to lie in a supine position for at least ten minutes before any other measurements were obtained. Duplicate supine blood pressure readings were obtained using the Omron device before PWV measurements obtained.

3.2.6 Pulse Wave Velocity (PWV) Using PP-1000

Forceps were placed over the subject's arms to obtain an ECG signal, and a PCG sensor placed over the chest. Semi conductor pressure sensors were then attached to each arterial site using velcro straps, illustrated in Chapter II. In similar fashion to the SphygmoCor system, PWV was measured as the time taken for the pulse to travel from the R wave of the simultaneously recorded ECG to the upstroke of the pulse wave at the site of assessment. As mentioned earlier, the difference between the two systems is that the PP-1000 system records all regional PWV measurements simultaneously, compared to sequential readings of the SphygmoCor.

Once all the Velcro straps and pressure sensors are applied over all the sites, recording of each regional PWV was obtained.

Measurements were repeated and assessed visually to ensure a good quality trace was recorded. Previously measured supine blood pressure and anatomical distances were used for the PP-1000 system.

3.2.7 Statistical Analysis

Data were analysed using Bland-Altman plots and reproducibility was expressed in terms of the mean difference \pm SD between paired measurements. Paired sample *t*-tests were used to test for significant differences where appropriate and a P-value of <0.05 was accepted as significant.

3.3 Results

3.3.1 Comparison of PWV Using Three Systems

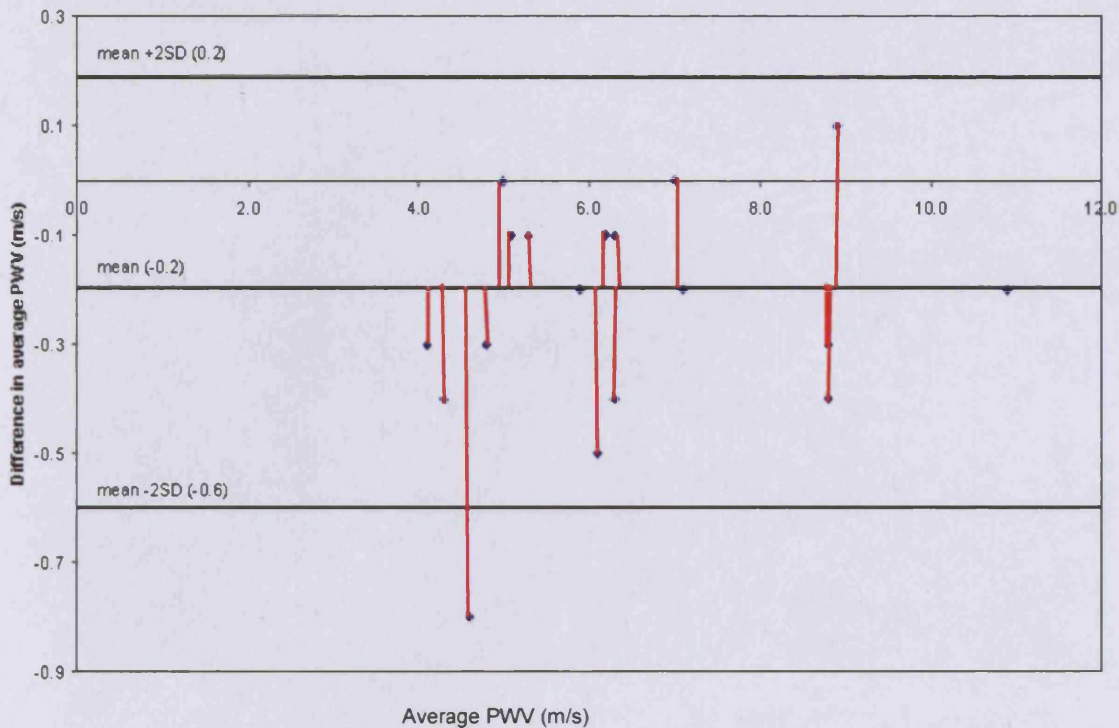
Table 3.2 presents the results obtained from the SphygmoCor, PP-1000 and Gaon21A systems. Results for each regional PWV, AIx and heart rate are shown as mean \pm SD. A P-value of <0.05 was considered as significant. CF PWV measured by the SphygmoCor system and the PP-1000 was significantly different (7.2 ± 2.1 m/s compared to 6.4 ± 1.8 m/s respectively ($P=0.022$)). CR PWV was also significantly different between the SphygmoCor and the PP-1000 (7.8 ± 1.1 m/s compared to 7.0 ± 1.6 m/s respectively ($P=0.036$)). However, results for the Carotid-Dorsalis pedis PWV (CD PWV) and FD PWV were not significantly different between the SphygmoCor and the PP-1000 systems (NS).

Table 3.2. Differences observed between SphygmCor and the PP-1000 or the Goan 21A systems

	SphygmCor	PP1000/Gaon 21A	P-value
Carotid–Femoral PWV (m/s)	7.2 ± 2.1	6.4 ± 1.8	0.022
Carotid–Radial PWV (m/s)	7.8 ± 1.1	7.0 ± 1.6	0.036
Carotid-Dorsalis-pedisPWV (m/s)	7.3 ± 1.1	6.9 ± 1.1	0.065
Femoral-Dorsalis-pedis PWV (m/s)	7.6 ± 1.0	7.9 ± 2.1	0.34
Supine Heart Rate (bpm)	59.5 ± 8.7	59.9 ± 9.1	0.842
Seated Augmentation Index (%)	15.1 ± 9.4	14.5 ± 7.9	0.645

Using Bland-Altman plots, within observer variation for the CF PWV reading for PP-1000 showed a SD of 0.02m/s, (Figure 3.1). Similar results were obtained for within observer variability for the SphygmCor system (SD 0.02 m/s), demonstrating a high degree of reproducibility for measurements of CF PWV within the two systems.

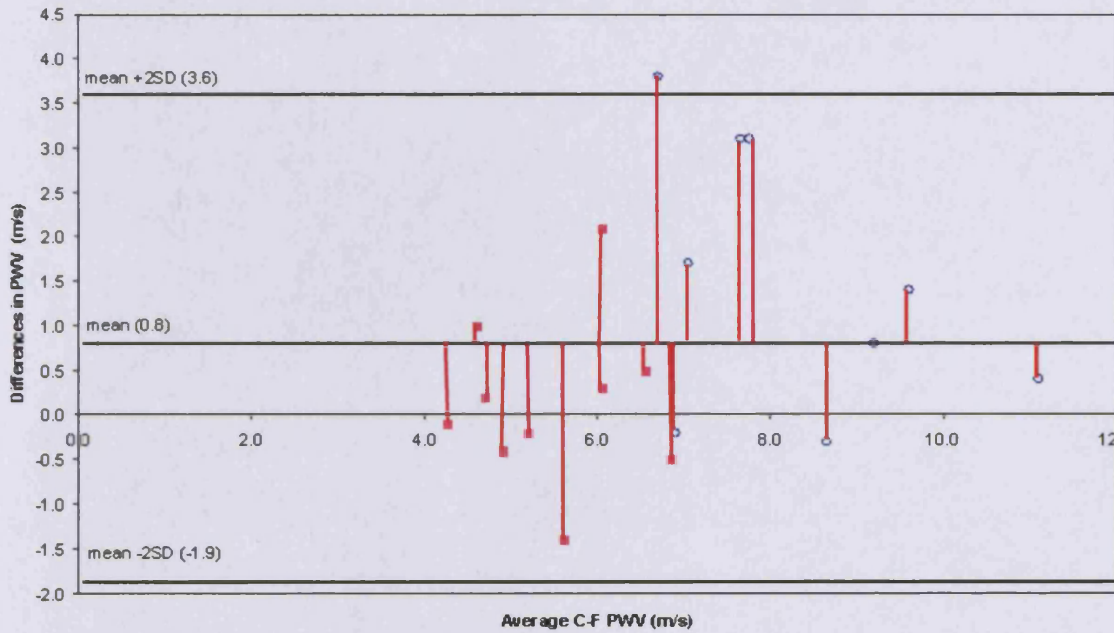
Figure 3.1. Within observer variation of CF PWV for the PP-1000 apparatus shown here as a Bland Altman plot.



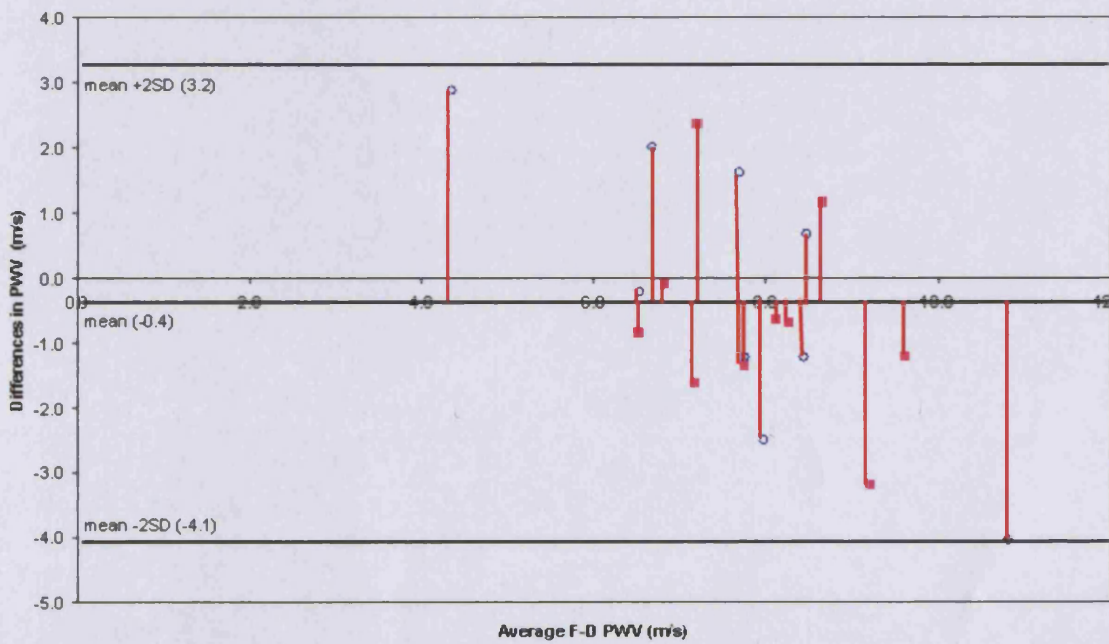
On the next page, between-apparatus variability was also assessed using Bland-Altman plots. Bland-Altman plots were constructed for differences between systems using (a) CF PWV and (b) FD PWV (Figures 3.2a and b). CF PWV showed a SD of 1.5 m/s between systems. The between system variability for the FD PWV showed a SD of 1.9 m/s (see Table 3.3). We examined correlation coefficients for the two systems. Between systems, the CF PWV showed a correlation coefficient of 0.76. When measuring FD PWV the observed correlation coefficient was 0.47.

Figure 3.2. Bland-Altman plots demonstrate differences observed between SphygmoCor and PP-1000 when measuring CF PWV and FD PWV.

(a) Carotid-Femoral PWV



(b) Femoral-Dorsalis pedis PWV



■ = Non-diabetic controls, ○ = Diabetic individuals.

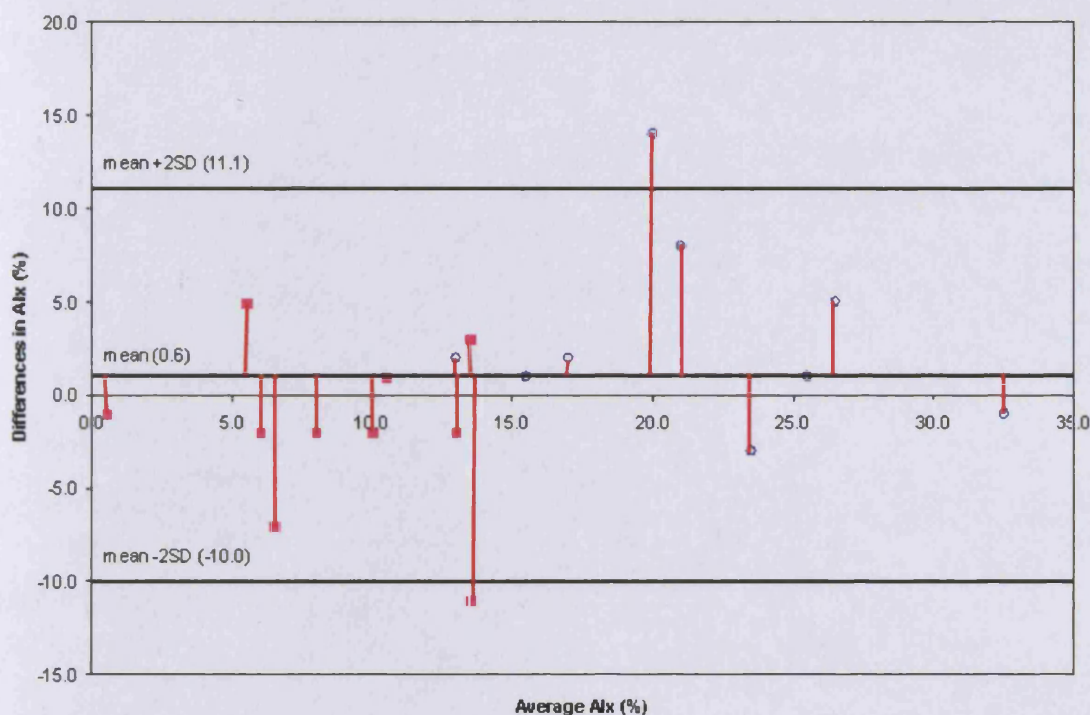
Table 3.3. Between apparatus differences (PP-1000 and Gaon21A versus SphygmoCor) for each parameter throughout the entire group.

	Mean	SD	r value	P-value
Carotid – Femoral (m/s)	0.8	1.5	0.76	<0.001
Carotid – Radial (m/s)	0.8	1.4	0.52	0.023
Carotid – Dorsalis (m/s)	0.4	0.9	0.65	0.003
Femoral – Dorsalis (m/s)	0.4	1.9	0.47	0.041
Aix (%)	0.6	5.4	0.82	<0.001

3.4.2 Comparison of Aix Using SphygmoCor and Gaon21A

A comparison was also performed between the SphygmoCor system and the Gaon21A for Aix. Figure 3.3 on the next page illustrates the variation observed between the two systems, as demonstrated in a Bland-Altman plot. A non-significant difference of $P=0.645$ was observed after completion of a paired sample t-test, suggesting there is no significant difference observed between the two apparatus involved. A correlation coefficient of $r= 0.82$, with $\text{mean}\pm\text{SD}$ of 0.6 ± 5.4 was also observed.

Figure 3.3. Bland-Altman plots describe differences observed between SphygmoCor and Gaon 21A, when measuring AIx.



■ = Non-diabetic controls ○ = Diabetic individuals.

3.4 Discussion

Findings from this study show that there is a between apparatus variation of 1.4m/s between the SphygmoCor system and the PP-1000 device when measuring CF PWV. The results of this study do however show that there is a significant difference observed between the means of each apparatus when assessed using a paired sample t-test.

However, correlation coefficients (shown in Table 3.3) demonstrate the relationship observed between CF PWV results. An r value of 0.76, with a P value of <0.001 , suggests that there is a good relationship between apparatus. See Table 3.3 for details

of all the regional correlations observed in PWV and AIx for the two devices under investigation.

FD PWV was also assessed in order to assess the reproducibility between apparatus. Again, Bland-Altman plots were used to demonstrate the overall reproducibility of the two devices used. A significant correlation was observed between the two systems ($r=0.47$, $P = 0.041$). A paired sample t-test was used to assess the differences in means between the two apparatus. No significant difference was observed between devices.

Previous studies have compared SphygmoCor to another similar apparatus devised for measuring PWV. The study described by Milleasseau et al, 2005, compares the Complior system, which measures PWV simultaneously, similar to that of the PP-1000, to the SphygmoCor¹⁶⁴. This study demonstrated a variability of 1.07 m/s between the SphygmoCor and the Complior, which is in broad agreement with the current study comparing the SphymoCor system to the PP-1000. Potential reasons for this agreement are that the two systems used the same intersecting tangent algorithms to identify the foot of the pulse waveform. However, the differences observed between comparisons of the Complior and PP-1000 with the SphygmoCor system may be due to differences in the pressure transducers used to record the arterial waveforms.

In the current study, some degree of variability may be due to the fact that the SphygmoCor system measures PWV sequentially, whereas the PP-1000 measures PWV simultaneously. However, the fact that the two systems exhibit the same within observer variation, make this unlikely.

Possible limitations of the study: The femoral artery pulse waveform can sometimes be difficult to record using the PP-1000 system due to the design of the apparatus. Having the transducer applied over the artery at the femoral site by a Velcro strap sometimes does not allow enough pressure to obtain a clear waveform in overweight subjects, due to increased adiposity. Therefore, inaccurate femoral pulse waveforms may have resulted in some degree of variation when recording CF PWV and FD PWV when using the PP-1000. However, the average BMI of the group investigated was not considered obese, therefore minimal obstruction in obtaining a clear femoral waveform would have been witnessed.

CHAPTER 4

DIABETES, ETHNICITY AND ARTERIAL STIFFNESS**(STUDY 2)****4.1 Introduction**

Diabetes mellitus is a major risk factor in cardiovascular disease (CVD) with CVD being the most common cause of death in patients with type-2 diabetes mellitus ¹⁶⁵.

The increased risk of CVD is due in part to clustering of other risk factors such as obesity, hypertension and dyslipidaemia with type-2 diabetes.

However, this does not explain all the risk. Indeed, diabetics without additional risk factors have increased cardiovascular risk compared with non diabetics. Arterial stiffness is emerging as an independent risk factor for CVD ³⁴⁻³⁶ and this has lead a number of investigators to examine the relationship between diabetes and arterial stiffness. Studies have shown increased arterial stiffness in both type-1 and type-2 diabetics compared with controls ^{55,75,76}.

These findings were confirmed in the large population based study Atherosclerosis Risk In Communities (ARIC) study, which used carotid intima media thickness as a measure of arterial stiffness and demonstrated increased arterial stiffness in patients with type 2 diabetes mellitus. Investigators also showed a positive correlation between glucose levels and arterial stiffness and an inverse relationship between insulin and arterial stiffness ⁷⁵.

Recent studies have extended these findings, showing PWV to be “a powerful independent predictor of later mortality across an entire spectrum of glucose tolerance, with or without type-2 diabetes”³³.

Although most studies have demonstrated an increased AIx in diabetics compared to non-diabetics ^{55,87,88} a recent study by Lacy et al in 2004 ⁸⁹ failed to show any differences in AIx between diabetics and controls although there was a significantly

higher aortic PWV in the diabetics. Other studies using carotid intima media thickness (CIMT) as a measure of arterial stiffness have shown no differences between diabetic subjects and controls^{86,166}. Suggesting that diabetes may have a differential effect on large arteries such as the aorta and carotid. Interestingly, an interventional study by Esposito et al 2004 illustrated that a reduction in CIMT was associated with a reduction in postprandial hyperglycaemia but not fasting hyperglycaemia in diabetics⁹⁴. Furthermore, recent data suggest that in type-2 diabetics, central artery stiffening is more pronounced than that of the peripheral arteries⁸³. Similar findings were reported by Cameron et al, in 2002, where they showed a differential stiffening of the central and peripheral arteries in diabetics compared to controls⁸⁴.

Mortality and stroke rates of South Asians are 1.5 times that of the general population in the UK¹⁶⁷ and glucose intolerance, central obesity, fasting triglycerides and insulin are all elevated compared to Europeans. Interestingly, although South Asians have a similar prevalence of Ischaemic Heart Disease (IHD) to Caucasians, their prevalence of peripheral vascular disease (PVD) is considerably lower¹⁴⁵. This remains a consistent finding even in the presence of classical risk factors such as diabetes. A recent study (Chaturvedi et al in 2002), showed that South Asians have a lower incidence of amputation compared to Europeans and that this was related to a lower prevalence of neuropathy and PVD compared to Caucasians¹⁴⁷.

The reasons that PVD is less prevalent in South Asians remains unclear. However, if increased arterial stiffness predisposes to atherogenesis, then a plausible explanation would be that the femoral vascular bed of South Asians exhibits a lower rate of age related stiffening than that of Caucasians.

In order to investigate these possibilities further, this study has examined arterial stiffness in a number of vascular beds by measuring carotid-femoral, carotid-radial and femoral-dorsalis pedis PWV in South Asians and Caucasians, with and without diabetes.

Aims and Objectives

In this chapter, the results of four studies undertaken to address the following hypothesis are presented.

(Study 2a) Comparison of arterial stiffness in all subjects with type-2 diabetes and matched controls.

(Study 2b) Comparison of arterial stiffness in Caucasian type-2 diabetics and matched controls.

(Study 2c) Comparison of arterial stiffness in non diabetic South Asians and Caucasians, and

(Study 2d) Comparison of arterial stiffness in type-2 diabetics of South Asian and Caucasian origin.

Table 4.0. Diabetes and Arterial Stiffness.

Reference	Population	Measurements	Main Finding
ARIC [75]	4,700 T-2 diabetics	Carotid artery stiffness	+ve association between diabetes and diabetes
Hoorn [76]	1,193 with normoglycaemia, impaired gluc. regulation or diabetes	CIMT	Diabetics had a sig. relationship with CIMT
Hoorn [76]	208 T-2 diabetics	Pulse Pressure	+ve association between PP and CV mortality
Finne Dianne [78]	2,988 T-1 diabetics 5,486 non-diabetics	Pulse Pressure	Sig. higher PP in the T-1 diabetics
Wahlqvist [80]	Small numbers. <100.	Aorto-iliac PWV (Ultrasound)	Higher aorto-iliac PWV in diabetics compared to controls
Taniwaki [82]	Small numbers. <100.	Aortic PWV	Higher aPWV in diabetics compared to controls
Cameron [84]	Small numbers. T-2 diabetics and controls	PWV of central and peripheral arteries	Central arteries age at an accelerated rate in diabetics compared to controls
Brooks [87][88]	Small numbers <100. T-1, T-2 diabetics and controls	AIx and central pressure	Higher AIx and central pressure in diabetics compared to controls
Wilkinson [55]	Small numbers. <100. T-1 diabetics and controls	AIx, Tr	AIX and Tr were greater in diabetics compared to controls.
Emoto [85] and Kool [86]	Small numbers. Diabetics and controls	Carotid, brachial and femoral. Ultrasound and SI.	No difference between diabetics and controls
Kool [86]	Small numbers. Diabetics and controls	CF PWV	No difference between diabetics and controls
Lacey [89]	Small numbers. <100 T-1, T-2 diabetics and controls	AIx and CF PWV	CF PWV was >in the diabetics compared to the controls, however, AIx was not.

4.2 Methodology

4.2.1 *Recruitment of Participants*

Diabetic volunteers were recruited from diabetic outpatient clinics in the Heath Hospital and Llandough Hospital, in South Wales. An advertisement was written for the health section of the South Wales echo (a local news paper), to increase people's awareness of the prevalence of diabetes and cardiovascular disease within the South Wales area, particularly in the South Asian community, whilst advertising for this study. In addition, a telephone contact number was provided with the advertisement in the paper for those individuals interested in taking part or who wanted further information. South Asian volunteers were recruited from the general population, *via* links with the Asian professional association and the Barefoot Health-Workers Project, in Cardiff. A family, general health promotion day was held during the year to once again, increase awareness of diabetes and CVD in South Wales and especially in the South Asian community. From this event, we also promoted our study. Due to ethical / religious issues involved in the part-removal of clothing during the study, only male volunteers were chosen for this study.

4.2.2 *Study Population*

347 healthy, male volunteers, aged 20-70, mean age 39.56 ± 12.3 years, of South Asian (N=65) and Caucasian (N= 282) ethnicities, were recruited for the study. Participants were generally from the local population of Cardiff city and the surrounding areas. Participants free from any overt cardiovascular disease were recruited for the study. 85 individuals with known type-2 diabetes, aged between 40-70, mean age 58.91 ± 8.3 years, of South Asian (N=36) and Caucasian (N= 49) ethnicities, participated in the study.

A detailed personal medical and family medical history questionnaire was completed. Subjects receiving cardiovascular related medications were also excluded from the control group. Ethical approval was obtained from the local research ethics committee (LREC), our institution's research and development department (R&D) and performed in accordance with the declaration of Helsinki. Informed consent was also obtained from each participant before any measurements were undertaken.

4.2.3 Data Collection

Participants were invited to attend an initial screening visit, lasting approximately one hour. Written informed consent was obtained from all participating subjects, before answering questions about their personal and family medical history. Height, weight, waist and hip circumferences were measured using standard methods.

4.2.4 Blood Pressure Measurement (Seated and Supine)

Blood pressure was recorded as stated in chapter 2, section 2.3.1. Duplicate readings were performed on each occasion, to rule out any discrepancies, and the average was used in subsequent analysis.

4.2.5 Assessment of Arterial Stiffness

Arterial stiffness was compared between diabetic subjects and age sex matched controls. In addition, arterial stiffness was also assessed in South Asians with type 2 diabetes and compared to Caucasian diabetic subjects. Arterial stiffening was also compared across different vascular beds in non-diabetic South Asians and Caucasians. Arterial stiffness was assessed using SphygmoCor and PP-1000 systems, which uses the applanation tonometry principle, described in detail in Chapter 2, section 2.3.

In order to assess Femoral-Dorsalis PWV (FD PWV), the PP-1000 (*Hanbyul Meditech Co., Korea*) was used. PWV was recorded based on the measurements of ECG and pulse waves from four different sites (carotid, femoral, radial and dorsalis pedis) simultaneously.

4.2.6 Data Analysis and Statistics

Data was analysed using SPSS for Windows version 11.5 (SPSS Inc., Chicago, Illinois). Independent sample t-test were used to test for significance and compare means of each group. All values represented as mean \pm SD, and a P value of <0.05 was considered significant. In order to test the study hypothesis, power calculations were performed to ensure the correct number of subjects to be recruited. See calculation in Chapter 2, section 2.4.

4.3 Results

The following sections describe the results of the four studies and presented as Tables 4.1 to 4.8 and Figures 4.1 to 4.4.

4.3.1. Comparison of arterial stiffness in subjects with type - 2 diabetes and matched controls. (Study 2a)

Three hundred and sixty two men volunteered for this study. Two hundred and seventy eight were healthy controls and eighty four were type-2 diabetics. Seated and supine haemodynamic measurements for the two groups are shown in Tables 4.1 and 4.2.

Seated central pressures were not significantly different between groups. However, supine CSBP and CMAP were significantly different in the diabetic group compared to controls.

Supine PMAP was not significantly different between the two groups. However, there was a significant difference in PPP. Supine A1x was also lower in the diabetic group compared to the healthy controls but when HT and HR and PMAP were adjusted for, this difference was found to be non significant.

CF PWV was found to be higher in the diabetic group compared to the healthy controls. However, there were no significant differences in either CR or FD PWV. (See Figure 4.1)

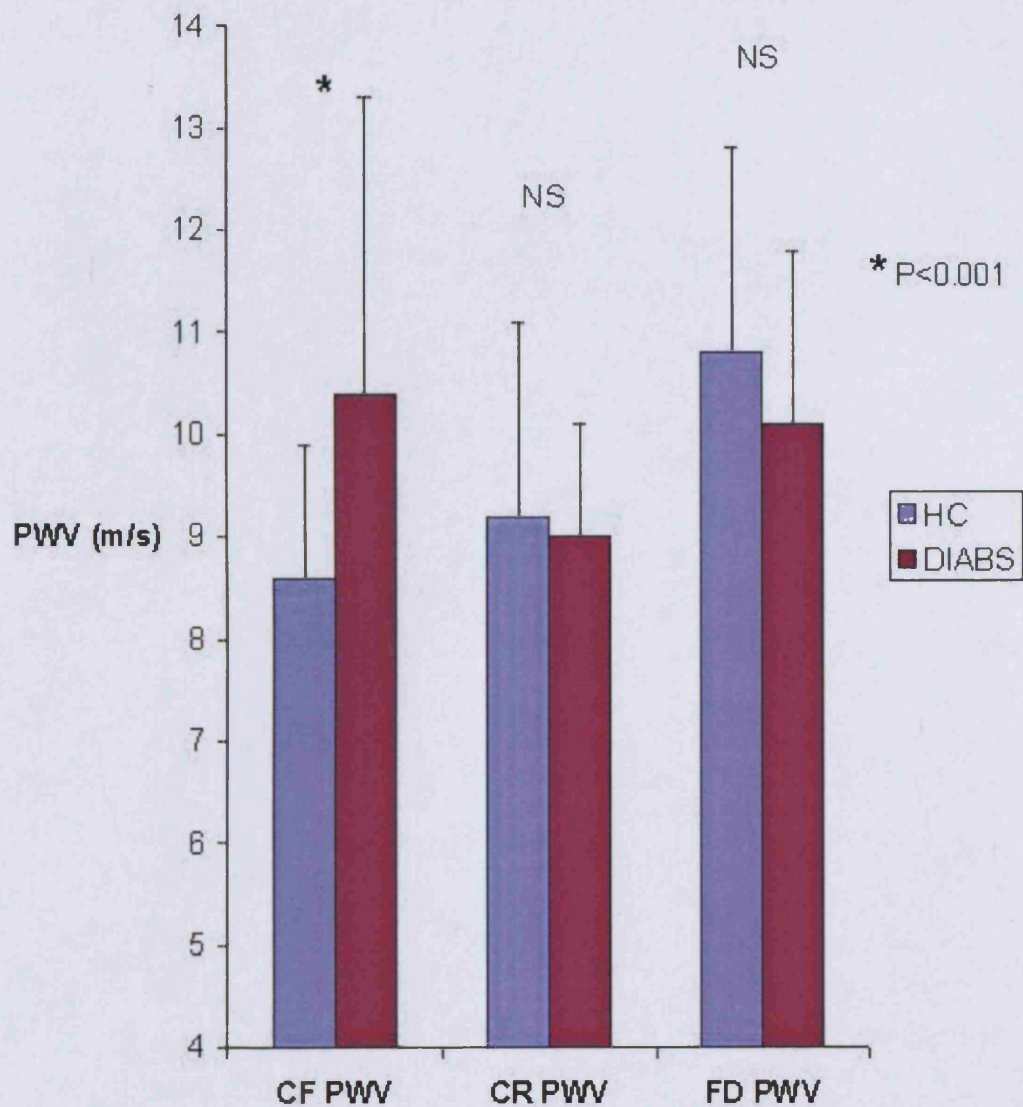
Table 4.1. Group characteristics and seated vascular haemodynamics between healthy controls and diabetics.

	Healthy Controls (N=278)	Diabetics (N=84)	P Value
Group Characteristics			
Age (years)	59±11	59±8	NS
BMI (Kg/m ²)	26.9±3.9	30.0±5.0	<0.001
Height (m)	1.75±0.07	1.69±0.07	<0.001
Vascular Haemodynamics (Seated)			
PSBP (mmHg)	135±18	141±16	0.013
PDBP (mmHg)	82±9	83±11	NS
PMAP (mmHg)	100±13	102±10	NS
PPP (mmHg)	53±13	58±14	0.006
CSBP (mmHg)	123±17	125±14	NS
CDBP (mmHg)	83±11	84±10	NS
CMAP (mmHg)	97±12	98±10	NS
CPP (mmHg)	40±12	40±11	NS
AIx (%)	20±11	15±9	<0.001
AIx (HT & HR adjusted)	20±9	18±8	NS
HR (bpm)	68±11	78±12	<0.001
AP (mmHg)	9±6	7±5	0.013
Tr (ms)	143±14	135±6	<0.001

Table 4.2. Group characteristics and supine vascular haemodynamics between healthy controls and diabetics.

	Healthy Controls (N=278)	Diabetics (N=84)	P Value
<i>Vascular Haemodynamics (Supine)</i>			
PSBP (mmHg)	133±20	134±14	NS
PDBP (mmHg)	79±9	76±8	0.003
PMAP (mmHg)	97±12	95±8	NS
PPP (mmHg)	53±14	58±13	0.01
CSBP (mmHg)	123±17	120±13	NS
CDBP (mmHg)	80±10	77±8	0.003
CMAP (mmHg)	94±12	91±8	0.009
CPP (mmHg)	42±11	43±11	NS
Aix (%)	25±10	21±9	0.003
Aix (corrected)	24±10	22±8	NS
HR (bpm)	64±9	72±10	<0.001
AP (mmHg)	11±6	9±6	0.026
Tr (ms)	144±15	141±11	0.02
Tr (corrected)	145±13	140±11	0.003
CF PWV (m/s)	8.6±1.4	10.3±3	<0.001
CFPWV (corrected)	8.6±1.3	10.4±2.9	<0.001
CR PWV (m/s)	9.0±2.3	8.8±1.2	NS
CRPWV (corrected)	9.2±1.9	9.0±1.1	NS
FD PWV (m/s)	10.2±2.2	9.7±1.8	NS
FDPWV (corrected)	10.8±2.0 (N=66)	10.1±1.7 (N=33)	NS

Figure 4.1. Differences in regional vascular stiffness between all healthy controls and all diabetic participants.



PWV: Pulse wave velocity; CF PWV: Carotid femoral pulse wave velocity; CR PWV: Carotid radial pulse wave velocity; FD PWV: Femoral dorsalis pulse wave velocity; HC: Healthy controls; DIABS: type-2 diabetics.

4.3.2 Comparison of arterial stiffness in Caucasian type-2 diabetics and matched controls. (Study 2b)

Three hundred and thirty one male Caucasian volunteers participated in this study. Forty nine had type 2 diabetes and two hundred and eighty two were non-diabetic controls. Haemodynamic parameters in the two groups are shown in Tables 4.3 and 4.4.

There was no significant difference in PMAP between the groups (Table 4.3). However seated PPP was significantly different (HC:53±13 versus DC:59±13 mmHg, P=0.003). This was mainly due to the significantly higher PSBP of the Diabetic group.

No significant differences were observed in central blood pressures in the seated or supine position between groups.

Seated AIx was also significantly different, with the diabetic group having a lower AIx. However, when seated HR and HT were adjusted for, there was no difference between groups (19±10 versus 17±9 (%), P=0.116).

Results for supine BP were similar with no differences in PMAP but a significant increase in supine PPP in the diabetic group (Table 4.4). There were no differences in Supine AIx and this remained non significant after adjusting for HT, supine HR and supine PMAP.

After adjustment for age and supine PMAP, CF PWV was significantly greater in the diabetics relative to the controls (HC:8.5±1.5 versus DC:10.7±3.3 m/s, P<0.001). No differences were observed in CR PWV between the groups. However, after adjustment for age and supine MAP, FD PWV was higher in the non diabetic subjects (11.1±1.9 versus 10.1±2 m/s, P=0.047). The differences in regional arterial stiffness between the two groups are illustrated graphically in Figure 4.2.

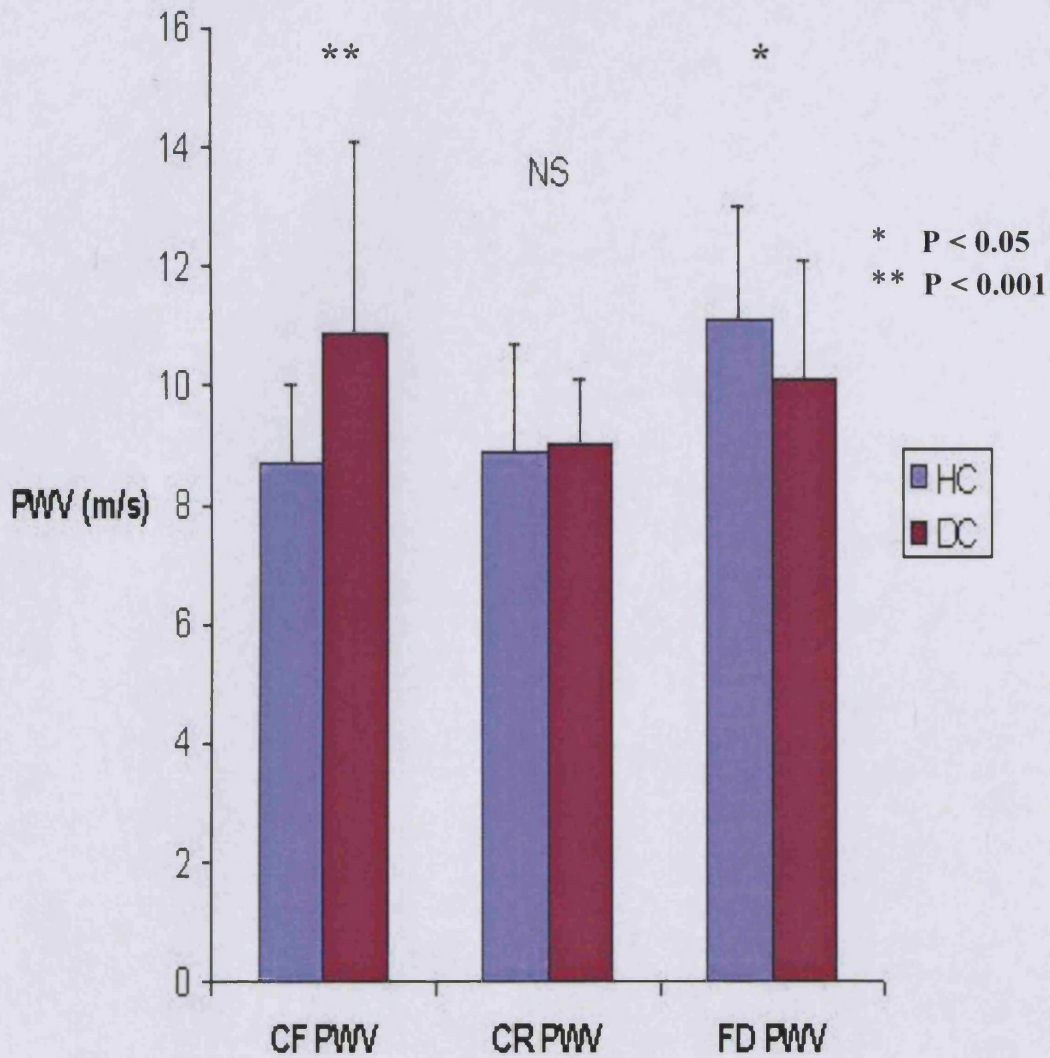
4.3. Group characteristics and seated vascular haemodynamics between healthy Caucasians and diabetic Caucasians.

	Healthy Caucasians (N=282)	Diabetics (N=49)	P Value
Group Characteristics			
Age (years)	58±12	59±7	NS
BMI (Kg/m ²)	26.9±4.0	32.0±5.0	<0.001
Height (m)	1.75±0.07	1.71±0.07	<0.001
Vascular Haemodynamics (Seated)			
PSBP (mmHg)	135±18	143±16	0.006
PDBP (mmHg)	82±11	83±10	NS
PMAP (mmHg)	100±13	103±10	NS
PPP (mmHg)	53±13	59±13	0.003
CSBP (mmHg)	122±17	125±15	NS
CDBP (mmHg)	83±11	84±10	NS
CMAP (mmHg)	96±12	98±11	NS
CPP (mmHg)	39±12	41±11	NS
Aix (%)	20±12	15±9	0.007
Aix (HT & HR adjusted)	19±10	17±9	NS
HR (bpm)	69±11	77±12	<0.001
AP (mmHg)	9±6	7±5	NS
Tr (ms)	144±13	135±6	<0.001

Table 4.4. Group characteristics and supine vascular haemodynamics between healthy Caucasians and diabetic Caucasians.

	Healthy Caucasians (N=282)	Diabetics (N=49)	P Value
<i>Vascular Haemodynamics (Supine)</i>			
PSBP (mmHg)	132±19	137±13	NS
PDBP (mmHg)	79±10	76±7	NS
PMAP (mmHg)	97±12	96±8	NS
PPP (mmHg)	53±14	60±10	<0.001
CSBP (mmHg)	122±17	122±12	NS
CDBP (mmHg)	80±10	77±8	NS
CMAP (mmHg)	94±12	92±8	NS
CPP (mmHg)	42±11	44±9	NS
Aix (%)	24±11	21±9	NS
Aix (corrected)	24±9	23±8	NS
HR (bpm)	64±9	70±10	<0.001
AP (mmHg)	11±6	9±5	NS
Tr (ms)	146±17	142±11	0.022
Tr (corrected)	146±15	141±11	0.009
CF PWV (m/s)	8.5±1.5	10.7±3.4	<0.001
CFPWV (corrected)	8.7±1.3	10.9±3.2	<0.001
CR PWV (m/s)	8.9±2.4	9.2±1.1	NS
CR PWV (corrected)	8.9±1.8	9.0±1.1	NS
FD PWV (m/s)	10.4±2.2	9.8±2.1	NS
FDPWV (corrected)	11.1±1.9 (N=33)	10.1±2.0 (N=66)	0.047

Figure 4.2. Differences in regional arterial stiffness between the healthy Caucasians and diabetic Caucasians.



PWV: Pulse wave velocity; CF PWV: Carotid femoral pulse wave velocity; CR PWV: Carotid radial pulse wave velocity; FD PWV: Femoral dorsalis pulse wave velocity; HC: Healthy controls; DIABS: type-2 diabetics.

4.3.3 Comparison of arterial stiffness in non-diabetic South Asians and Caucasians. (Study 2c)

In total, two hundred and seventy nine men participated in this analysis. 214 Caucasians and 65 South Asians. Table 4.5 shows the general group characteristics and seated vascular haemodynamics, whilst Table 4.6 illustrates the differences between the groups in supine position. Seated peripheral SBP, DBP, MAP, and PP were all significantly higher in the Caucasians relative to the South Asians (Table 4.5). Furthermore, only CPP was significantly higher in the Caucasian groups, both in the seated and supine position.

For supine BP there were no significant differences in PMAP or PDBP. However, there were significant differences in PPP and PSBP which were higher in the Caucasian group. After adjustment for PMAP, HR and HT no significant differences were observed in seated or supine Aix between groups (Table 4.6). Since there was a difference in PMAP between groups, PWV measurements were adjusted for age and PMAP. CF PWV and FD PWV were both significantly lower in the South Asians. However, in contrast, CR PWV was lower in the Caucasian group. (See Figure4.3).

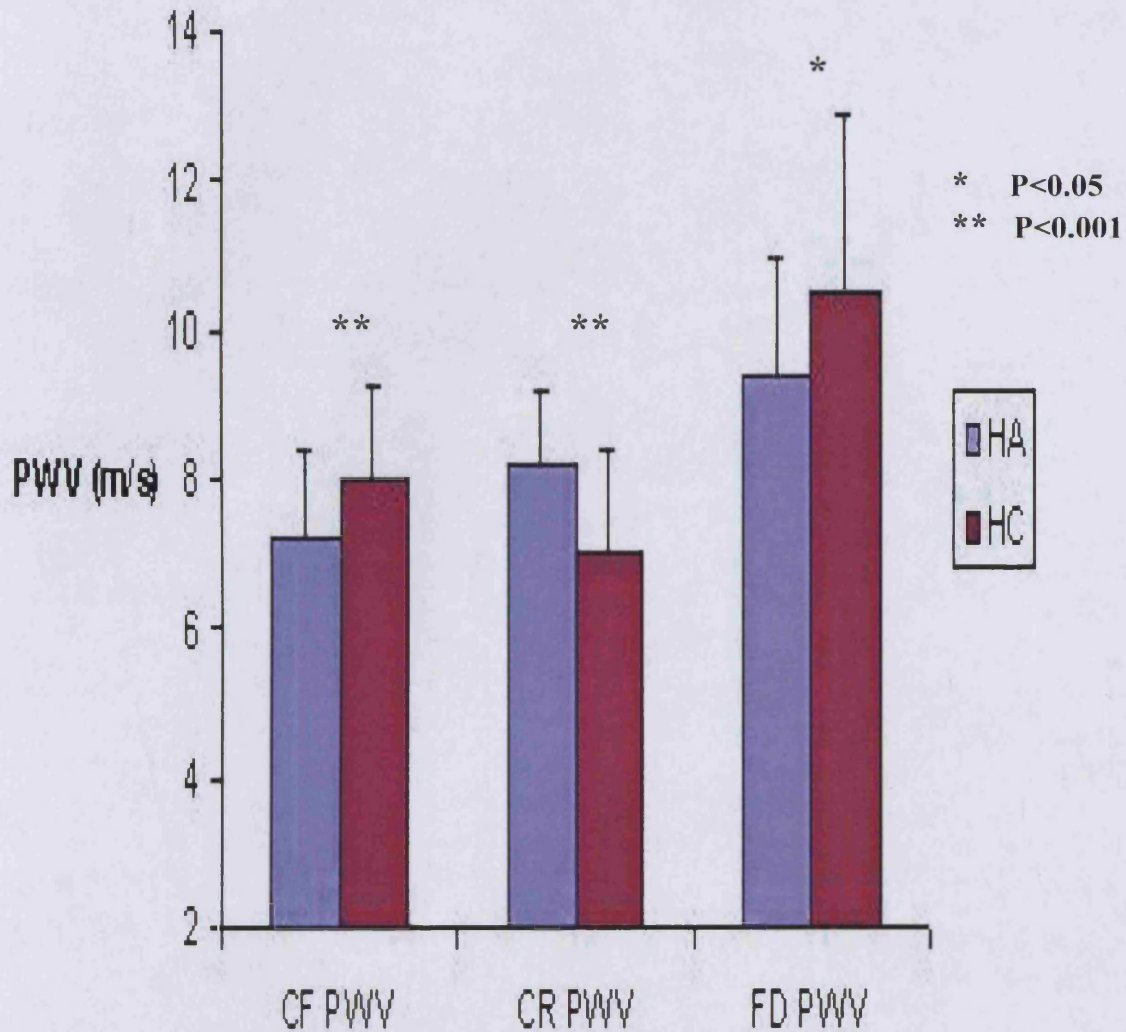
Table 4.5. Group characteristics and seated vascular haemodynamics between healthy South Asians and healthy Caucasians.

	Healthy S. Asians (N=65)	Healthy Caucasians (N=214)	P Value
Group Characteristics			
Age (years)	40±10	39±14	NS
BMI (Kg/m ²)	26.9±3.8	25.9±3.9	NS
Height (m)	1.71±0.07	1.75±0.07	<0.001
Vascular Haemodynamics (Seated)			
PSBP (mmHg)	128±14	133±17	0.015
PDBP (mmHg)	87±9	84±12	0.035
PMAP (mmHg)	101±8	99±13	0.023
PPP (mmHg)	41±11	50±11	<0.001
CSBP (mmHg)	117±13	118±17	NS
CDBP (mmHg)	88±9	85±12	NS
CMAP (mmHg)	98±10	96±13	0.279
CPP (mmHg)	29±8	33±9	0.002
Aix (%)	17±12	9±15	<0.001
Aix (corrected)	13±11	11±14	NS
HR (bpm)	72±11	69±13	NS
AP (mmHg)	6±5	5±7	0.043
Tr (ms)	145±13	147±13	NS

Table 4.6. Group characteristics and supine vascular haemodynamics between healthy South Asians and healthy Caucasians.

	Healthy S. Asians (N=65)	Healthy Caucasians (N=214)	P Value
<i>Vascular Haemodynamics (Supine)</i>			
PSBP (mmHg)	123±13	129±15	0.004
PDBP (mmHg)	78±8	76±12	NS
PMAP (mmHg)	93±9	93±12	NS
PPP (mmHg)	45±10	53±10	<0.001
CSBP (mmHg)	111±12	113±15	NS
CDBP (mmHg)	79±9	77±12	NS
CMAP (mmHg)	90±9	89±13	NS
CPP (mmHg)	32±7	36±7	<0.001
Aix (%)	19±12	11±15	<0.001
Aix (corrected)	13±12	10±14	NS
HR (bpm)	66±10	64±11	NS
AP (mmHg)	6±5	4±6	0.015
Tr (ms)	149±10	157±19	<0.001
Tr (corrected)	149±10	158±17	<0.001
CF PWV (m/s)	7.3±1.4	8.0±1.4	<0.001
CFPWV (corrected)	7.2±1.2	8.0±1.3	<0.001
CR PWV (m/s)	8.3±1.2	7.5±2.2	<0.001
CRPWV (corrected)	8.2±1	7.0±1.4	<0.001
FD PWV (m/s)	9.5±1.6	10.0±2.6	NS
FDPWV (corrected)	9.4±1.6 (N=43)	10.5±2.4 (N=50)	0.019

Figure 4.3. Differences in regional arterial stiffness between healthy South Asians and healthy Caucasians.



PWV: Pulse wave velocity; CF PWV: Carotid femoral pulse wave velocity; CR PWV: Carotid radial pulse wave velocity; FD PWV: Femoral dorsalis pulse wave velocity; HA: Healthy South Asians; HC: Healthy Caucasians.

4.4 Comparison of arterial stiffness in diabetic South Asians and diabetic Caucasians (Study 2d)

Eighty five subjects took part in this study. Thirty six were South Asians with type-2 diabetes and forty nine Caucasian diabetics. The vascular haemodynamic data are presented as Tables 4.7 and Table 4.8 for both seated and supine positions respectively.

No significant differences were observed in any component of blood pressure, either seated or supine, central or peripheral, apart from supine PSBP which was higher in the diabetic Caucasians. Again, after adjustment for PMAP, HR and HT there were no significant differences in AIx, either seated or supine between the groups. Finally, there were no significant differences between CF PWV, CR PWV or FD PWV between the groups.

Table 4.7. Group characteristics and seated vascular haemodynamics between diabetic South Asians and diabetic Caucasians.

	Diabetic S. Asians (N=36)	Diabetic Caucasians (N=49)	P Value
Group Characteristics			
Age (years)	58±9	59±7	NS
BMI (Kg/m ²)	27.2±3.5	32.0±4.9	<0.001
Height (m)	1.68±0.07	1.71±0.07	NS
Duration of Diab (yrs)	12.5±11	9.7±8	NS
No. Microvasc Comp	0.5±0.8	0.5±0.7	NS
No. CV Med	1.6±1.4	2.4±1.7	0.04
Vascular Haemodynamics (Seated)			
PSBP (mmHg)	139±14	143±16	NS
PDBP (mmHg)	83±10	83±10	NS
PMAP (mmHg)	102±9	103±10	NS
PPP (mmHg)	56±15	59±13	NS
CSBP (mmHg)	124±12	125±15	NS
CDBP (mmHg)	85±9	84±10	NS
CMAP (mmHg)	98±8	98±11	NS
CPP (mmHg)	40±12	41±11	NS
Aix (%)	16±9	15±9	NS
HR (bpm)	79±12	77±12	NS
AP (mmHg)	7±6	7±5	NS

Table 4.8. Group characteristics and seated vascular haemodynamics between diabetic South Asians and diabetic Caucasians.

	Diabetic S. Asians (N=36)	Diabetic Caucasians (N=49)	P Value
<i>Vascular Haemodynamics (Supine)</i>			
PSBP (mmHg)	130±15	137±13	0.042
PDBP (mmHg)	75±8	76±8	NS
PMAP (mmHg)	94±8	96±8	NS
PPP (mmHg)	55±15	60±10	NS
CSBP (mmHg)	117±13	121±12	NS
CDBP (mmHg)	77±8	77±8	NS
CMAP (mmHg)	90±9	89±13	NS
CPP (mmHg)	40±13	44±9	NS
Aix (%)	20±10	21±9	NS
Aix (corrected)	22±8	21±7	NS
HR (bpm)	73±10	71±10	NS
AP (mmHg)	9±6	10±5	NS
Tr (ms)	140±12	141±11	NS
Tr (corrected)	139±11	142±11	NS
CF PWV (m/s)	9.8±2.6	10.7±3.4	NS
CFPWV (corrected)	8.5±2.2	8.7±2.8	NS
CR PWV (m/s)	8.4±1.1	9.2±1.1	0.003
CRPWV (corrected)	8.1±1	8.6±1.0	NS
FD PWV (m/s)	9.6±1.3	9.8±2.1	NS
FDPWV (corrected)	9.7±1.3 (N=31)	9.8±1.9 (N=35)	NS

4.4 Discussion

The major positive findings from this series of experiments are, that in agreement with previous studies, subjects with type 2 diabetes had increased CF PWV when compared to matched non-diabetic controls. When Caucasians with type-2 diabetes were compared to matched Caucasian control subjects with diabetes they also exhibited increased CF PWV. In agreement with the original hypothesis that South Asians would have a lower FD PWV than Caucasian controls we were indeed able to demonstrate this. However, interestingly there were no differences in PWV in any of the vascular beds when comparing South Asian diabetics with Caucasian diabetics. These findings are discussed in more detail below.

Study 2a: Comparison of arterial stiffness in all subjects with type-2 diabetes and matched controls

This study demonstrated an increased CF PWV between diabetic subjects and controls but no differences in CR or FD PWV which is in agreement with previous studies^{83,89} showing that there is preferential stiffening of central arteries such as the aorta relative to more peripheral arteries such as the brachial or femoral. The reasons for this remain unclear. However, it may be that diabetes increases breakdown and fragmentation of elastin which would account for its preferential effect on large arteries as they have a higher elastin component. Furthermore, the current study demonstrated no significant differences in AIX between diabetics and controls. Although this is in disagreement with some previous studies^{55,88} a recent study by Lacy et al⁸⁹ despite demonstrating increased CF PWV between diabetics and controls also failed to show differences in AIX. A potential explanation for this is the age of the subjects studied. Our group and that of Lacy et al's were 55yrs or older and we

and others have recently demonstrated that AIx tends to plateau at around age 55yrs making it more difficult to demonstrate changes in AIx in this age group⁹⁰. However, CF PWV increases relatively little to age 55yrs and then rises steeply making it more likely that changes in CF PWV due to diabetes will be more easily distinguishable. Indeed, recent unpublished data from our own group support this hypothesis as diabetes induces little shift in the age AIx relationship under 55yrs but produces large shifts in the CF PWV age relationship in subjects over 55yrs (See Figure 1.9 in Chapter 1)

Study 2b: Comparison of arterial stiffness in Caucasian type-2 diabetics and matched controls.

The previous study compared groups of type 2 diabetics and controls of mixed ethnicity. In this study Caucasian subjects with diabetes were compared to matched non diabetic Caucasian controls. Results were similar in that the diabetics had significantly higher CF PWV compared to control subjects with no significant differences in CR PWV. However, in contrast to the previous study there was a small but significant ($P=0.04$) difference in FD PWV with the diabetics exhibiting decreased FD PWV compared to the controls. This finding was unexpected and the reasons for it are very unclear since as discussed above most studies suggest that central arteries stiffen preferentially in subjects with diabetes. However, it does suggest that ethnic differences may exist in terms of FD PWV, in that, although no differences were seen in FD PWV when comparing mixed ethnicity groups of diabetics and controls there were differences in FD PWV within the Caucasian group. This issue could have been addressed by a comparison between South Asians with type 2 diabetes and matched South Asian controls. However, unfortunately this

comparison was not possible due to the lack of older South Asian non diabetic controls making age matching impossible. In agreement with the first study in groups of diabetics and controls of mixed ethnicity the comparison between Caucasian diabetics and controls also showed no significant differences in AIx. As the age of the subjects was again 55yrs or over this may well be due to plateauing of the age AIx relationship at 55yrs as previously discussed above.

Study 2c: Comparison of arterial stiffness in non-diabetic South Asians and Caucasians.

Despite South Asian residents in the UK having a similar incidence of ischaemic heart disease to Caucasians this group has a significantly lower rate of PVD^{146,168}. Since arterial stiffening may predispose to atherogenesis we hypothesised that an explanation for this interesting finding could be that South Asians had lower FD PWV than Caucasians thus providing some protection against the development of atheroma in this vascular bed. The findings of this study support this hypothesis and demonstrate for the first time ethnic differences in FD PWV. However, it was also observed that the South Asians also had significantly lower CF PWV than the Caucasians. Since the incidence of ischemic heart disease is similar between the two ethnic groups this finding is also somewhat unexpected. Interestingly, CR PWV was observed to be significantly higher in the South Asian group. Again, this finding is unexpected and may well be explained as a chance finding. More studies need to be conducted with much larger numbers to investigate this finding further.

Study 2d: Comparison of arterial stiffness in type-2 diabetics of South Asian and Caucasian origin.

The study above compared indices of arterial stiffness in non diabetic South Asians and a group of matched Caucasians. In the final study we have now compared arterial stiffness in a group of South Asians with type 2 diabetes with age matched Caucasian diabetics. In contrast to the non-diabetics there were no significant differences between the groups. Interestingly, as mentioned earlier, in the presence of diabetes, rates of PVD and amputation are lower in South Asians than Caucasians. However, in this analysis I have illustrated that in the presence of diabetes, there was no significant difference in arterial stiffness observed in any of the vascular beds between the groups. These findings suggest that the development of diabetes has already caused an acceleration of the vascular ageing process associated with diabetes. Further studies are needed and could be addressed by comparing the relationship between age and PWV in the various vascular beds between ethnic groups. This is thus a limitation of the current study but would require very large numbers of subjects to complete. The current findings however may be of clinical importance in that early recognition of pre diabetic states such as metabolic syndrome may be especially important in subjects of South Asian origin. This is supported by findings in Chapter 5 that subjects with IFG already exhibit increased CF PWV compared to controls. The numbers in the study are relatively small and will need confirmation in larger studies.

CHAPTER 5

IMPAIRED FASTING GLUCOSE AND ARTERIAL STIFFNESS

(STUDY 3)

5.1 Introduction

Diabetes mellitus is a major risk factor for cardiovascular morbidity and mortality. Whilst much of this increased risk may be due to clustering of traditional risk factors such as hypertension and dyslipidaemia, diabetes without other risk factors is still associated with increased cardiovascular risk compared to non-diabetic subjects ¹⁶⁹. Increased arterial stiffness is an emerging independent risk factor for cardiovascular disease and thus may explain some of the unaccounted risk in subjects with diabetes. Indeed, diabetes has been shown to be associated with increased arterial stiffness (see Chapter 1, section 1.7.1). A number of studies have demonstrated a relationship between impaired glucose regulation and increased risk of mortality, CVD and development of type-2 diabetes. The DECODE study group in 1999, showed a positive relationship between mortality and impaired fasting glucose (IFG) in men and women (DECODE study). However, this was only observed when levels were above 7mmol/l. However, the relationship between IFG and arterial stiffness remains unclear.

The Rotterdam Study has shown a positive association between IFG and arterial stiffness in 2,987 elderly normal subjects assessed by measurement of carotid arterial distensibility. However, this was only observed in subjects >75yrs ⁹⁶. The Hoorn study also examined the association between abnormal glucose regulation and arterial stiffness in 1,193 subjects with diabetes, impaired glucose metabolism and

normoglycaemia as assessed by PP and IMT in a variety of vascular beds. Although the investigators were able to demonstrate a positive relationship between impaired glucose metabolism and femoral and brachial IMT, they were unable to show a similar relationship for carotid IMT. Furthermore, the authors did not include a specific group with IFG in their analysis⁹⁷. Another study in older hypertensive non-diabetic subjects showed no relationship between IFG and arterial stiffness assessed using pulse wave analysis. However, it was able to demonstrate a significant relationship between insulin resistance and arterial stiffness¹⁰³.

To date, few studies have examined the relationship between IFG and arterial stiffness with conflicting results. The largest of these studies (Tomiyama H et al 2006), has examined the relationship between IFG and increased arterial stiffness using PWV in 2,080 Japanese men. Although the investigators were able to demonstrate a significant relationship between IFG and increased PWV, the results of this study are difficult to interpret, as the subjects were all hypertensive. Furthermore, the investigators used brachial-ankle PWV, which unlike CF PWV has not been shown to predict CV outcome¹⁰⁰. A similar study in only 282 Japanese, measured brachial-ankle PWV and demonstrated a positive relationship between PWV and IFG¹⁰¹. Interestingly, the Rotterdam study investigated IFG and arterial stiffness, assessed by carotid artery distensibility, however, it only demonstrated a significant association in individuals over 75yrs⁹⁶. The remaining studies, failed to show any association between IFG and arterial stiffness using CIMT¹⁰², PWA¹⁷⁰. Furthermore, in a study which assessed the second derivative of plethysmogram as a measure of arterial stiffness, 2hr post challenge hyperglycaemia and not fasting glucose was associated with increased arterial stiffness¹⁰⁴.

This study therefore, examines the relationship between IFG and arterial stiffness using PWV and PWA, in 1,282 subjects divided into groups of normoglycaemia, IFG and type-2 diabetes.

Table 5.0. Impaired Glucose Regulation and Arterial Stiffness.

Reference	Population, N	Measurements	Main findings
Rotterdam Study [96]	2,987 normal subjects	Carotid artery distensibility	+’ve relationship between IFG and distensibility, only after 75years.
Hoom Study [97]	1,193 normoglycaemic, impaired glucose metabolism or diabetes	Pulse Pressure and Carotid, Brachial and Femoral IMT.	Only brachial and femoral IMT was associated with impaired glucose metabolism group
Hoom Study [98]	1,193 normoglycaemic, impaired glucose metabolism or diabetes	PWA (Aix)	No difference in Aix between impaired glucose metabolism group and controls.
Cruickshank [33]	600 diabetic, impaired glucose tolerance or normoglycaemic. Longitudinal study over 10 years	aPWV	Diabetic and IGT group were shown to correlate +’vely with a PWV and mortality.
Tomiyama [99]	2,080 Japanese hypertensive men. Normoglycaemic and impaired fasting glucose groups	BA PWV	+’ve relationship between IFG and BA PWV
Ohnishi [101]	282 Japanese normals	BA PWV	+’ve relationship between BA PWV and IFG
Hanefeld [102]	Small numbers study	CIMT	No association between CIMT and IFG
Sengstock [103]	Small numbers study	PWA (Aix)	No association between Aix and IFG

5.2 Methodology

5.2.1 *Subject Recruitment*

Subjects were recruited from the open access metabolic syndrome risk factor clinic at the WHRI. In order to obtain a large cohort from the general population, individuals who attended our metabolic syndrome risk factor clinic were assessed. Word of mouth and various leaflets and handouts were distributed through various health days, ongoing around Cardiff also proved effective as a means of increasing the numbers attending the clinic. Advertisements in the South Wales Echo were also a helpful tool in increasing knowledge regarding the clinic. Individuals referred themselves to the clinic at times that was most suitable to them.

1,282 individuals aged 18-87 years from Cardiff city and the surrounding area, were recruited for the study. A detailed personal and family medical history questionnaire was completed by each individual. The study group comprised 1074 subjects with NFG (fasting blood glucose <5.6 mmol/L), 94 subjects with IFG (fasting blood glucose >5.6 and <7.0 mmol/L) and 114 subjects with type-2 diabetes (fasting blood glucose >7.0 mmol/L). IFG and type-2 diabetes were defined according to ADA criteria. Ethical approval was obtained from the local research ethics committee. Informed consent was also obtained before any measurements were undertaken.

5.2.2 *Data Collection*

Participants entered the clinic as self-referrals from the general population. All individuals entered the clinic after a 12 hour overnight fast. The screening visit lasted approximately one hour. Informed consent was obtained before personal and family

medical history questionnaire was filled in. Participant's height, weight, waist and hip circumference were all routinely measured.

5.2.3 Blood pressure Measurement

Blood pressure was recorded as described in Chapter 2, section 2.3.1.

5.2.4 Assessment of Arterial Stiffness:

Pulse wave analysis and PWV were recorded using the SphygmoCor system as described in Chapter 2, section 2.3.2.1.1, section 2.3.2.1.2 and section 2.3.2.1.3.

5.2.5 Data Analysis and Statistics

These data were analysed using ANOVA and thereafter, a bonferroni corrected post hoc t- tests, with the statistics software package (SPSS, version 11.5, SPSS Inc., Chicago, Illinois) in order to compare means of each group. All values represent mean \pm SD, and a P value of < 0.05 was considered significant. When adjusting for certain parameters during the analysis. The slope of a linear regression model was used and each parameter adjusted accordingly.

In order to find the power of the test the thesis performed a power calculation to make sure the correct number of subjects were recruited. See Chapter 2, section 2.4 for calculations

5.3 Results

The three groups were matched for age. Tables 5.1 to 5.2 shows the differences between controls and subjects with IFG and type-2 diabetes, in terms of biochemical and metabolic parameters, along with seated and supine haemodynamic parameters. HBA1c was as expected raised in the type-2 diabetics compared to the normoglycaemic subjects and the subjects with IFG. Although HBA1c was higher in the IFG group compared to subjects with normoglycaemia this was not significant. TChol, LDL, HDL and TGs also differed between the groups. TChol was lower in the type 2 diabetics relative to the normoglycaemic group. LDL was significantly lower in both the IFG and diabetic group compared with the normoglycaemic subjects. HDL was only significantly lower in the IFG group compared with normoglycaemic controls. TGs were significantly higher in both the IFG and diabetic groups as expected. Interestingly no differences in CRP were observed between the groups.

As predicted, subjects with IFG and diabetes had significantly greater BMI weight and waist circumference than the normoglycaemic group. There were no significant differences in PMAP between any of the groups. Seated PPP was however significantly greater in the group with type-2 diabetes relative to the normoglycaemic group.

No significant differences were observed between groups in either the seated or supine positions.

CF PWV was significantly different between groups. Subjects with IFG having significantly higher CF PWV than normoglycaemic controls 9.4 ± 1.8 m/s *versus* 8.7 ± 1.8 m/s respectively ($P < 0.01$). subjects with diabetes also exhibited a significantly

greater CF PWV than the normoglycaemic group 9.9 ± 2.0 m/s *versus* 8.7 ± 1.8 m/s respectively ($P < 0.001$, Figure 5.1). There were no significant differences in CF PWV between the IFG and diabetic groups. CR PWV was also assessed and no significant differences were observed between the three groups. AIX after correcting for height, MAP and heart rate was not significantly different between the groups (Table 5.1).

Fasting blood glucose levels for the whole cohort were plotted against CF PWV (Figure 5.2) and a significant relationship was observed ($R^2 = 0.023$, $P < 0.001$). When the graph was split in two, a significant relationship was only observed in the cohort with fasting blood glucose levels < 7 mmol/l ($R^2 = 0.022$, $P < 0.001$). No relationship was observed in the diabetic group with fasting blood glucose levels > 7 mmol/l ($R^2 = 0.001$, $P = 0.83$). See figure 5.3.

A multiple regression analysis with CF PWV as the dependant variable was also performed and showed that fasting glucose levels remained as predictive of CF PWV (Table 5.3).

Table 5.1 Group characteristics and vascular haemodynamics, according to glucmetabolic classification.

	NFG	IFG	DM	P Value
	Mean \pm SD	Mean \pm SD	Mean \pm SD	ANOVA
N=	1074	94	114	
<u>Group characteristics</u>				
AGE (yrs)	53 \pm 19	54 \pm 18	56 \pm 20	0.157
BMI (kg/m ²)	26.0 \pm 4.3	27.5 \pm 5†	27.9 \pm 4.7¶	0.000
HT (cm)	170 \pm 10	170 \pm 10	168 \pm 9	0.056
WT (kg)	75 \pm 14	80 \pm 16†	79 \pm 16‡	0.001
WST CIRC. (cm)	87 \pm 13	95 \pm 15‡	96 \pm 15¶	<0.001
AB CIRC. (cm)	91 \pm 16	96 \pm 11	95 \pm 15	0.218
<u>Vascular haemodynamics (seated)</u>				
PSBP (mmHg)	134 \pm 21	137 \pm 17	138 \pm 20	0.084
PDBP (mmHg)	81 \pm 11	82 \pm 10	81 \pm 11	0.732
PMAP (mmHg)	98 \pm 13	101 \pm 13	99 \pm 14	0.443
PPP (mmHg)	53 \pm 16	55 \pm 14	57 \pm 18‡	0.025
CSBP (mmHg)	121 \pm 20	124 \pm 18	123 \pm 21	0.283
CDBP (mmHg)	82 \pm 11	83 \pm 11	81 \pm 12	0.547
CPP (mmHg)	39 \pm 15	41 \pm 15	42 \pm 17	0.147
AIX (%)	20.6 \pm 16	22.3 \pm 16	19.6 \pm 15	0.511
AIX (corrected)	17 \pm 15	18 \pm 17	22 \pm 15	0.495
HR (bpm)	69 \pm 12	71 \pm 12	77 \pm 15¶#	<0.001
AP (mmHg)	8.9 \pm 9	9.6 \pm 9	9.2 \pm 9	0.846
ED (ms)	299 \pm 27	297 \pm 28	290 \pm 30	0.009
TR (ms)	142 \pm 15	141 \pm 19	137 \pm 13¶	0.008

Groups vascular haemodynamics, according to glucometabolic classification, continued:

<i>(supine)</i>		NFG <i>Mean ±SD</i>	IFG <i>Mean ±SD</i>	DM <i>Mean ±SD</i>	P Value <i>ANOVA</i>
PSBP	(mmHg)	131±20	133±15	134±20	0.250
PDBP	(mmHg)	76±10	77±9	76±10	0.504
PMAP	(mmHg)	94±13	95±13	95±14	0.804
PPP	(mmHg)	55±15	56±12	58±17	0.135
CSBP	(mmHg)	119±20	120±18	121±21	0.497
CDBP	(mmHg)	77±10	78±10	76±11	0.562
CPP	(mmHg)	42±15	42±12	45±17	0.168
AIX	(%)	22±17	22±17	22±16	0.986
AIX	(corrected)	23±12	22±10	24±12	0.603
HR	(bpm)	64±10	67±11	71±13¶#	<0.001
AP	(mmHg)	10±10	9±10	11±10	0.598
TR	(ms)	146±21	143±19	141±19	0.066
TR	(corrected)	146±18	142±17	142±18	0.215
ED	(ms)	327±23	318±25†	316±27¶	<0.001
CF PWV	(m/s)	8.1±2.6	8.7±2.6*	9.5±3.0¶#	<0.001
CF PWV	(corrected)	8.7±1.8	9.4±1.8†	9.9±2.0¶	<0.001
CR PWV	(m/s)	8.1±1.4	8.1±1.4	8.3±1.4	0.502
CR PWV	(corrected)	8.3±1.2	8.1±1.4	8.4±1.3	0.292

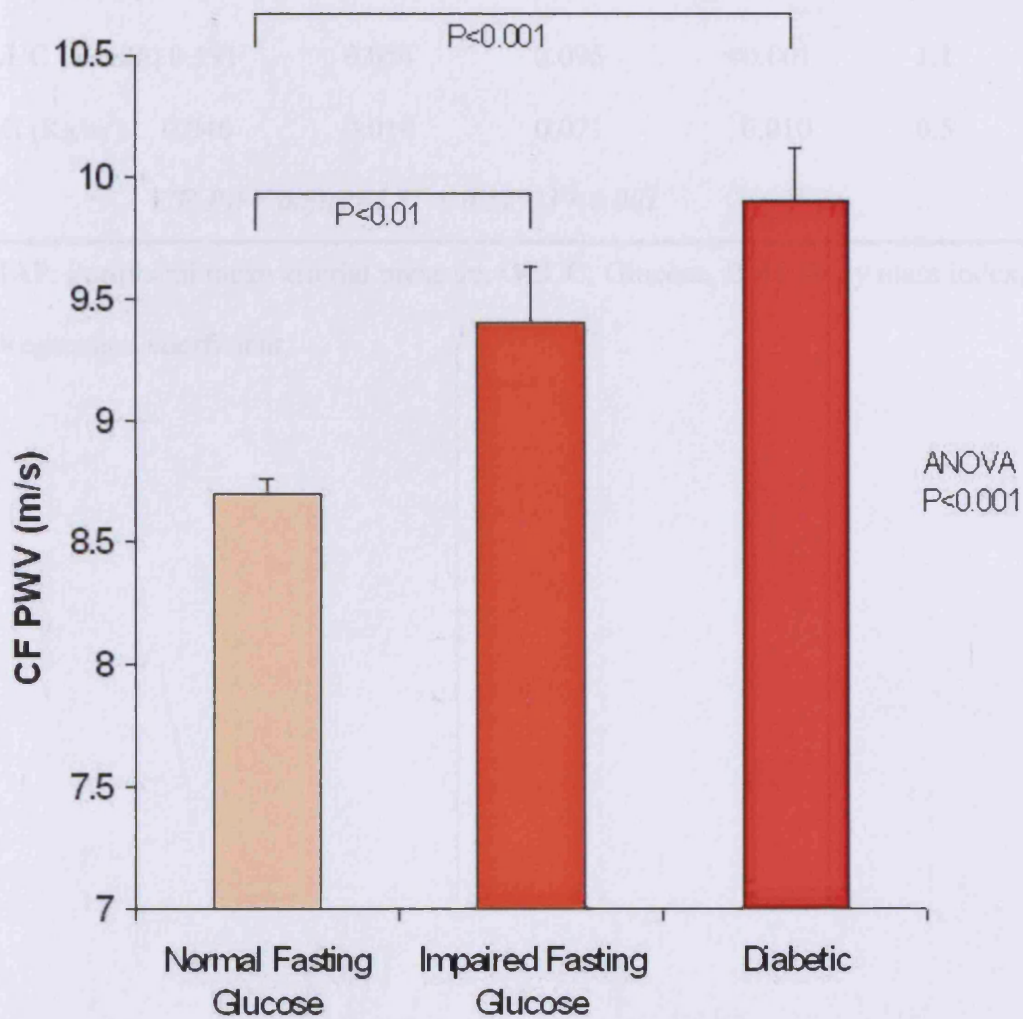
Table 5.2 Biochemical profile, according to glucometabolic classification.

		NFG <i>Mean ±SD</i>	IFG <i>Mean ±SD</i>	DM <i>Mean ±SD</i>	P Value <i>ANOVA</i>
TCHOL	(mmol/L)	5.1±1.1	4.9±1.0	4.7±1.1¶	<0.001
LDL	(mmol/l)	3.06±0.97	2.81±0.96*	2.58±0.86¶	<0.001
HDL	(mmol/l)	1.45±0.43	1.37±0.47†	1.29±0.55	0.001
TRIGS	(mmol/l)	1.43±0.92	1.89±1.19‡	1.93±1.19¶	<0.001
GLUC	(mmol/l) (fasting)	4.7±0.6	6.2±0.4‡	8.4±3.1¶††	<0.001
CRP	(mg/l)	2.56±4.32	3.44±3.94	2.85±3.37	0.395
HBA1c	(%)	5.45±0.42	5.68±0.57	7.02±1.77¶**	<0.001

NFG: Normal fasting glucose, IFG: Impaired fasting glucose, DM: Diabetes mellitus, BMI: Body mass index, HT: Height, WT: Weight, WST CIRC: Waist circumference, AB CIRC: Abdominal circumference, PSBP: Peripheral systolic blood pressure, PDBP: Peripheral diastolic blood pressure, PMAP: Peripheral mean arterial pressure, PPP: Peripheral pulse Pressure, CSBP: Central systolic blood pressure, CDBP: Central diastolic blood pressure, CMAP: Central mean arterial pressure, CPP: Central pulse pressure, AIX: Augmentation index, AIX (corrected): AIX corrected for height, peripheral mean arterial pressure and heart rate, AP: Augmentation pressure, HR: Heart rate, Tr: Time for the reflected wave to return, TR (corrected): Tr corrected for height, peripheral mean arterial pressure and heart rate, ED: Ejection duration, CF PWV: Carotid femoral pulse wave velocity, CF PWV (corrected): CF PWV corrected for age and peripheral mean arterial pressure, CR PWV: Carotid radial pulse wave velocity, CR PWV (corrected): CR PWV corrected for age and peripheral mean arterial pressure, TCHOL: Total cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, TRIGS: Triglycerides, GLUC: Glucose, CRP: C-reactive protein, HBA1c: Glycosolated Haemoglobin.

* P<0.05, †P <0.01, ‡ P < 0.001 between NFG and IFG; ‡P<0.05, ¶P <0.01, ¶¶ P < 0.001 between NFG and DM; # P<0.05, ** P <0.01, ††P < 0.001 between IFG and DM.

Figure 5.1. The effect of fasting glucose levels on arterial stiffness.



CF PWV (m/s): Carotid femoral pulse wave velocity, in metres per second.

Table 5.3. Results of stepwise multiple regression analysis

Model	r	SE	Beta	P	r² Change (%)
AGE (yrs)	0.080	0.004	0.534	<0.001	44.6
PMAP (mmHg)	0.059	0.007	0.269	<0.001	6.8
GLUC (mmol/l)	0.191	0.054	0.095	<0.001	1.1
BMI (Kg/m ²)	0.046	0.018	0.071	0.010	0.5

CF-PWV adjusted R² = 0.529, P<0.001

PMAP: Peripheral mean arterial pressure, GLUC: Glucose, BMI: Body mass index,

r: Regression coefficient.

Figure 5.2. Scatterplot showing the relationship between CF PWV and fasting glucose levels in the whole cohort.

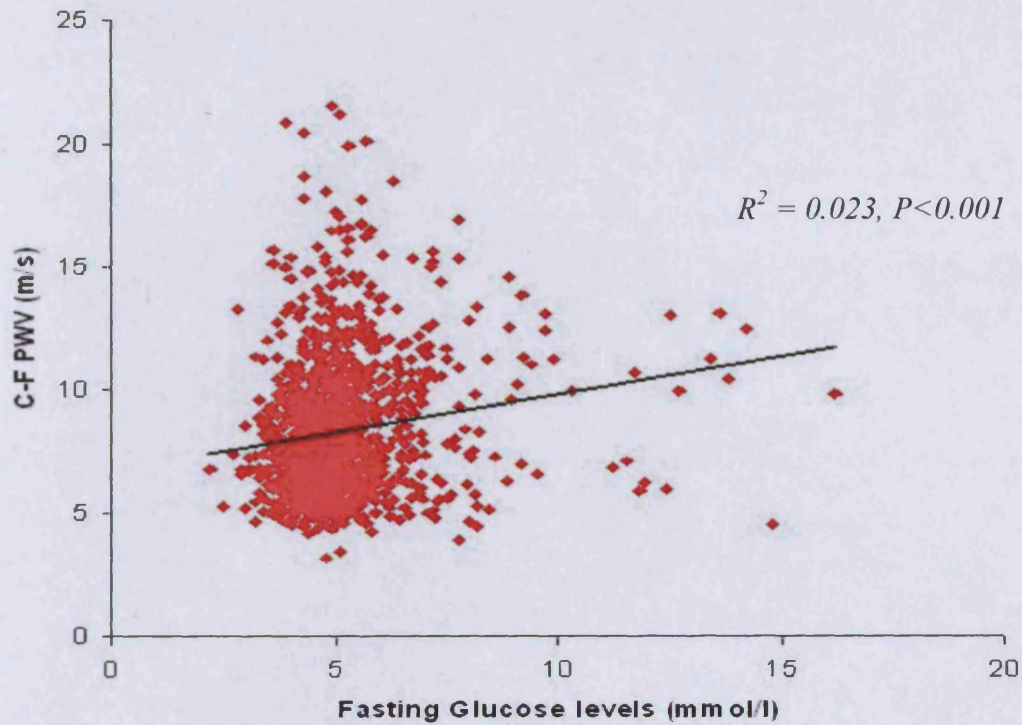
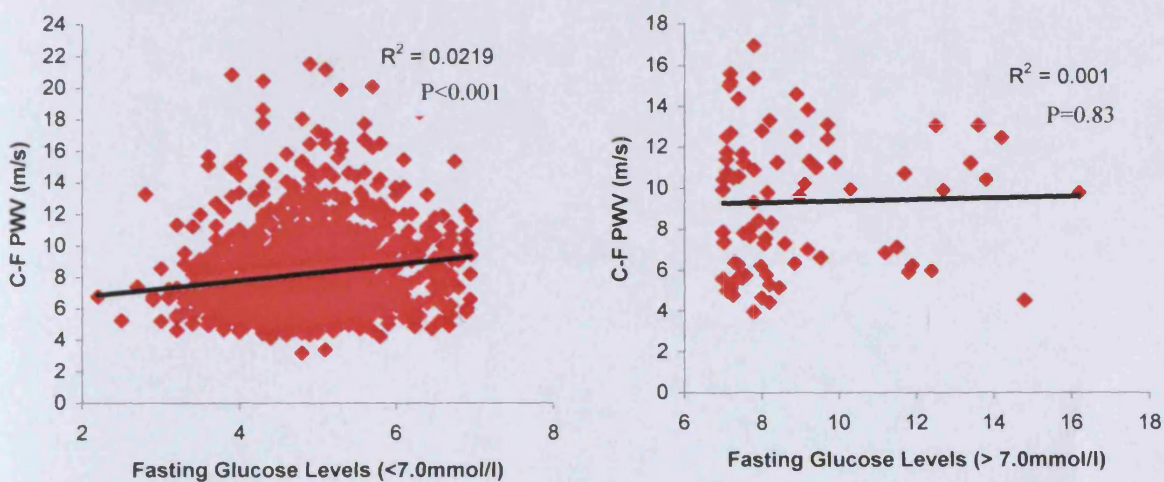


Figure 5.3. Scatterplot showing the relationship between CF PWV and (a) fasting glucose levels <7.0mmol/l and (b) fasting glucose levels > 7.0 mmol/l.



CF PWV (m/s): Carotid femoral pulse wave velocity, in metres per second.

5.4 Discussion

A combination of classical risk factors (hypertension, obesity and dyslipidemia) may account for some but not all of the increased cardiovascular risk associated with type-2 diabetes¹⁶⁹. Increased arterial stiffness is an independent risk factor for CV disease and may mediate some of the increased risk associated with type-2 diabetes. The results of this study confirm previous findings that increased arterial stiffness is associated with type-2 diabetes and extend these findings to show that subjects with IFG also have increased arterial stiffness relative to normoglycaemic controls. Furthermore, a graded relationship was observed between normoglycaemia, IFG and type-2 diabetes. However, these data may be somewhat misleading as most diabetic individuals will have been on medication, which may influence arterial stiffness and mean arterial pressure. Therefore, a graph comparing CF PWV versus fasting glucose levels <7mmol/l (normoglycaemics and IFG group) and a graph comparing the effect of CF PW versus fasting glucose >7mmol/l (diabetics) were described. A graded relationship between fasting blood glucose and CF PWV was only observed in the group with fasting glucose levels <7mmol/l.

Other studies have shown increased arterial stiffness in the early stages of diabetes. However, unlike the current study this was defined as impaired glucose tolerance^{75,171,172}. Few studies have been carried out comparing IFG and arterial stiffness. Of these studies carried out, Tomiyama H et al, examined the relationship between IFG and increased arterial stiffness in 2,080 individuals. However, results of this study are difficult to interpret, as the subjects studied unlike the present study were all hypertensive, which could account for the increased arterial stiffness observed. Furthermore, the investigators used brachial-ankle PWV, which unlike CF PWV has not been shown to predict CV outcome¹⁰⁰. The Rotterdam study also demonstrated an

association between IFG and arterial stiffness, assessed using carotid artery distensibility, however, this was only significant in individuals over 75yrs⁹⁶. Other studies failed to show any association between IFG and arterial stiffness when assessed using CIMT¹⁰² and PWA¹⁷⁰.

CF PWV was significantly higher in IFG group and DM group compared to the normal fasting glucose group. Interestingly, in agreement with other studies no significant differences were observed between the groups in terms of CR PWV, suggesting that increased glucose levels impact more on large elastic arteries such as the aorta rather than more muscular arteries such as the brachial. This may be due to the fact that diabetes predisposes to the development of collagen cross links in elastic tissue. This is supported by the fact that drugs that decrease collagen cross linking have been demonstrated to decrease CF PWV. As mentioned earlier, an increase in various other structural factors e.g. Matrix metalloproteinases (MMP-9), which are associated with increased large artery stiffness⁶⁷ could also have been responsible for this observation. However, MMP-9 levels were not measured on this occasion. When the relationship between fasting glucose levels and CF PWV was assessed there was a significant relationship ($P < 0.001$).

Aix, a measure of wave reflection from the periphery was not different between the three groups. This in agreement with a previous study that also showed no difference in Aix between a group of diabetic patients and matched controls⁸⁹. Like the current study the average age of the diabetics was 55 years. A possible explanation for these findings comes from our own data in a normal population showing that Aix tends to plateau at around age 55yrs, whilst CF PWV tends to rise more steeply after age

55yrs. Thus, since the subjects in the current study were 55yrs it may be more difficult to show differences in AIx whereas, as was the case, differences in CF PWV would be more apparent. Support for this hypothesis comes from our own, as yet unpublished data, comparing changes in CF PWV and AIx with age in diabetic and normal subjects (Figure 1.9, Chapter 1). In subjects <55yrs there is a clear shift of the AIx curve to the left in diabetics relative to controls. Similarly the shift in the CF PWV curves occurs after the age of 55yrs (Figure 1.9, Chapter 1).

This increased arterial stiffness associated with IFG and type-2 diabetes, provides an interesting link relating diabetes to CVD. A number of studies have demonstrated endothelial dysfunction, characterised by a decreased bioavailability of endothelium derived nitric oxide (NO) and impaired glucose metabolism as possible mechanisms as to how the vasculature is affected¹⁷³ since NO has been shown to regulate in part large arterial stiffness^{65,66}. Indeed, a recent study showed a relationship between endothelial function and CF PWV¹⁷⁴ in a normal population. Thus decreased bioavailability of NO may account for the increased arterial stiffness observed in subjects with IFG and diabetes. Furthermore, insulin has been shown to produce vasodilation in peripheral arterial beds *via* a NO dependent mechanism. However, the doses of insulin used in these experiments were non physiological. Recently insulin at physiological levels has been demonstrated to have an effect on arterial haemodynamics leading to decreased wave reflection^{118,176} and that these effects of insulin are similar to those of GTN¹⁷⁷ suggesting that NO may well be involved in the haemodynamic effects of insulin. Furthermore, these beneficial effects of insulin are blunted in subjects with insulin resistance¹¹⁹. As yet, the haemodynamic effects of insulin have not been studied in non-diabetic subjects with endothelial dysfunction

such as hypercholesterolaemia. Unfortunately, fasting insulin levels were not measured in this study, hence it is not possible to directly address this issue within the current cohort. However, data from the Caerphilly and Speedwell study have demonstrated that even though glucose concentrations were associated with ischaemic heart disease (IHD), fasting insulin concentrations were not shown to be associated with IHD¹⁷⁸.

This study is limited to an observational design and without fasting insulin levels, we can only speculate as to the mechanisms involved. Further studies should look at fasting insulin levels as a surrogate measure for insulin resistance when performing studies similar to this. Furthermore, it would have been desirable to have more clinical data on all the individuals involved. Therefore, the influence of cardiovascular acting medications could be accounted for and analysed for clearer interpretation of the data. As mentioned earlier, data regarding the use of insulin, blood pressure medication, statins etc, could all have had an effect on the overall results. Unfortunately this data was not available and need for such data should be advised for future research in this area.

CHAPTER 6

EXERCISE AND ARTERIAL STIFFNESS**(STUDY 4)****6.1 Introduction**

Lack of physical activity is an independent risk factor for CVD¹²¹. Moreover, increased physical activity and physical fitness are associated with a decreased incidence of CAD events^{179,180}. The mechanisms whereby exercise reduces CV risk are unclear but may involve improvement in cardiovascular risk factors such as hypertension, hyperglycaemia and hypercholesterolaemia¹⁸¹. Indeed exercise has been shown to be very effective at reducing blood pressure¹²³. However, this may still not account for all the reduction in risk.

Recently increased arterial stiffness has emerged as an important independent CV risk factor and this has led a number of researchers to investigate the relationship between exercise and arterial stiffness. The results have been conflicting partly due to the populations studied and the methodologies employed to assess arterial stiffness. In addition exercise levels have been self-reported and there is a lack of prospective intervention studies on the effects of exercise. Furthermore, as previously stated exercise decreases blood pressure making it difficult to assess whether any observed beneficial changes in arterial stiffness are independent of reductions in MAP. Since arterial stiffening is an inevitable consequence of the ageing process, differences in ages between the populations studied may also account for some of the differing results reported. Finally, exercise may have differential effects on central and peripheral arteries.

The Northern Ireland Young Hearts Project (NIYHP) involving 405 young subjects (12-15yrs) demonstrated an inverse relationship between estimated cardio-respiratory

fitness, and self-reported physical activity levels with arterial stiffness, assessed by photoplethysmographic measurement of aorto-iliac and aorto-dorsalis pedis PWV ¹²⁴. However, the much larger ARIC study involving 10,644 older individuals free from clinical evidence of CVD examined the relationship between physical activity and arterial stiffness assessed using carotid intima media thickness (CIMT). Although the study was able to show that people who performed vigorous physical activity had a weekly positive association with arterial stiffness, there was no such relationship between habitual physical activity and arterial stiffness ¹²⁶. This study was cross sectional and results from the longitudinal DANSCO study have shown that regular low to moderate intensity aerobic exercise is associated with a 40% lower 6-year progression of CIMT compared to a group taking little or no exercise ¹³¹. These findings are supported by a smaller study demonstrating that highly physically active women did not display the age related increases in aortic PWV that usually occur in elderly populations ¹³³.

Results from intervention studies employing increased physical activity have also been conflicting. In a small study of 17 Japanese middle aged men ¹⁸² aerobic exercise of 16 weeks duration was shown to decrease central arterial stiffness, assessed by measuring aortic PWV. Interestingly, no effect was observed in the peripheral, more muscular arteries, of the femoral to dorsalis-pedis region. Suggesting a differential effect of exercise, according to the vascular bed studied. A further intervention in 555 men and women showed that exercise in the form of graded walking, over a 24-month period, also decreased aortic PWV ¹³². A recent study examining the effect of exercise on brachial- ankle PWV showed that exercise prevented deterioration in arterial stiffness .

These and other intervention studies may be confounded by the fact that exercise will improve a number of CV risk factors including blood glucose levels ^{135,183}. Of particular relevance is the increase in insulin sensitivity and consequent improvement in fasting glucose levels associated with exercise ¹³⁶. A number of studies have demonstrated that decreased physical inactivity is associated with adverse metabolic effects such as increased fasting glucose and raised triglycerides ¹³⁷⁻¹³⁹. Indeed, large population based studies have shown regular exercise to slow the onset on diabetes ¹⁶⁹. In Chapter 5, it has already been demonstrated that IFG is associated with increased arterial stiffness, which may be due to increased insulin resistance increasing arterial stiffness in part via a NO dependent mechanism.

Therefore, this study examined the relationship between regular exercise, arterial stiffness and fasting blood glucose levels in a large cohort of men and women with no clinical evidence of CV disease.

Table 6.0. Exercise and Arterial Stiffness.

Reference	Population, N	Measurements	Main Findings
NIYHP [124]	405 young subjects (12-15 years)	Photopleth. Aorto-iliac and aorto-dorsalis pedis PWV.	Inverse relationship between estimated CR fitness, PA levels and arterial stiffness
Kozakova [125]	432 young – middle aged subjects	CIMT and carotid stiffness	Inverse correlation between habitual PA and both CIMT and carotid stiffness
Schmidt-Trucksass [128]	Small study numbers	Youngs elastic modulus.	LTPA was associated with decreased arterial stiffness
Vaitkevicius [129]	146 men (54-75years)	APWV and AIx	Increased CR fitness is associated with decreased AIx and aPWV
Vaitkevicius [129]	201 men (50years) Hypertensives, diabetics and smokers	AIx	Increased CR fitness is associated with decreased AIx
DANSCO [131]	140 middle aged men	CIMT	Regular low to moderate intensity aerobic exercise is associated with a 40% lower 6-year progression of CIMT compared to sedentary group
ACT Study [132]	555 men and women (35-75 years)	aPWV	Increased PA was associated with sig. decrease in aPWV after 24 mths walking intervention
Hayashi [182]	17 sedentary men	PWV	16 weeks low –moderate PA is associated with a decrease in central arterial stiffness but not peripheral artery stiffness
Tanaka [133]	53 women	aPWV	Age related increases in arterial stiffness were not apparent in highly physically active women
ARIC [126]	10,644 older individuals	CIMT	No relationship between habitual exercise and CIMT. A weak but +ve relationship was observed with vigorous physical activity
Tanaka [127]	Small study numbers	Carotid B mode ultrasound and applanation tonometry	Habitual physical activity did not exhibit any sig. difference in arterial stiffness compared to sedentary controls

6.2 Methodology

6.2.1 *Study population*

Two hundred and fourteen healthy male volunteers, free from CV acting medication, aged 63 ± 9 years, were recruited for this study.

Participants were classified as either sedentary (**S**) (*defined as, partaking in absolutely no recreational physical activity whatsoever*) or physically active (**PA**) (*defined as, partaking in aerobic physical activity, at least three times a week, and for at least one hour duration*). Subjects that participated in low to moderate physical activity or high resistance training were excluded from the study.

A detailed medical personal and family history questionnaire was completed. Subjects receiving cardiovascular medications were also excluded. Each participant filled in a detailed physical activity questionnaire, in order to strategically quantify levels of activity. Ethical approval was obtained from the local research ethics committee. Informed consent was also obtained before any measurements undertaken.

6.2.2 *Blood Pressure Measurement*

Blood pressure (BP) was recorded as discussed in chapter 2, section 2.3.1.

6.2.3 *Pulse Wave Analysis*

Pulse wave analysis and PWV were recorded using the SphygmoCor system as described in Chapter 2, section 2.3.2.1.1, section 2.3.2.1.2 and section 2.3.2.1.3.

6.2.4 Biochemical Analyses

Biochemical parameters such as glucose, total cholesterol, LDL, HDL, triglycerides, sodium, potassium, creatinine, urea and C-reactive protein were all measured using standard biochemical procedures in the Heath Hospital, Cardiff.

6.2.5 Statistical Analysis

All data were analysed using SPSS (version 11.5.0) software for windows. Unpaired t-tests were used to determine differences between the two groups' means. Results were expressed as mean±SD. A P-value of less than 0.05 was used to determine significance.

In order to find the power in this study, a power calculation was performed to ensure the correct number of subjects were recruited. See Chapter 2, section 2.4.

6.3 Results

Haemodynamic parameters in seated and supine positions are shown in (Tables 6.1 and 6.2). No significant differences were observed in MAP between the PA and S groups. CF PWV was significantly lower in the PA group compared to the S group (8.5 ± 1.4 versus 9.1 ± 2.0 m/s, $P=0.035$) respectively (Figure 6.1). Although AIX, after correction for height, MAP and heart rate was lower in the PA group this did not reach statistical significance.

Biochemical parameters are shown in (Table 6.3). As expected HDL cholesterol was significantly higher and TG's lower in the PA group. Glucose levels were also significantly lower in the PA group (4.9 ± 1.2 versus 5.6 ± 2.1 mmol/L, $P=0.029$). Importantly there were no significant differences in total and LDL cholesterol between the groups.

Table 6.4. A multiple regression analysis was performed and illustrates that only age, glucose and BMI were the only factors responsible for CF PWV measurements recorded in this cohort ($R^2 = 0.26$, $P=0.031$).

Table 6.1 Differences in baseline characteristics in seated posture between sedentary and physically active elderly groups.

	Sedentary	Active	
	N=(214)	N=(48)	P Value
Group Characteristics			
Age (years)	64±8	61±14	NS
BMI (Kg/m ²)	28±5	25±4	<0.001
Height (m)	167±8	167±9	NS
Vascular Haemodynamics (Seated)			
PSBP (mmHg)	141±20	135±16	NS
PDBP (mmHg)	84±9	83±8	NS
PMAP (mmHg)	104±12	102±11	NS
PPP (mmHg)	56±16	52±12	NS
CSBP (mmHg)	131±19	125±16	0.022
CDBP (mmHg)	86±10	84±6	NS
CPP (mmHg)	45±14	41±12	0.22
AIx (%)	29±11	26±11	NS
HR (bpm)	72±10	69±13	NS
AP (mmHg)	14±8	11±7	NS
Tr (ms)	133±13	140±13	<0.001
ED (ms)	298±25	300±24	NS

Table 6.2 Baseline characteristics in supine posture between sedentary and physically active elderly groups.

	Sedentary	Active	
	N=(214)	N=(48)	P Value
<i>Vascular Haemodynamics (Supine)</i>			
PSBP (mmHg)	130±17	132±16	NS
PDBP (mmHg)	77±8	79±8	NS
PMAP (mmHg)	95±10	97±11	NS
PPP (mmHg)	54±14	53±11	NS
CSBP (mmHg)	121±16	122±17	NS
CDBP (mmHg)	78±8	80±9	NS
CPP (mmHg)	44±13	43±11	NS
AIx (%)	30±9	28±12	NS
AIx (corrected)	34±8	30±11	NS
HR (bpm)	67±10	64±11	NS
AP (mmHg)	14±7	13±7	NS
ED (ms)	324±23	328±20	NS
Tr (ms)	136±13	141±15	NS
Tr (corrected)	138±12	143±12	0.02
CF PWV (m/s)	9.2±2.3	8.5±1.6	0.048
CFPWV (corrected)	9.1±2.0	8.5±1.4	0.035
CRPWV (m/s)	8.4±1.4	8.4±1.2	NS
CRPWV (corrected)	8.5±1.3	8.5±1.1	NS

Table 6.3. Differences in biochemical profile observed between sedentary and physically active elderly groups.

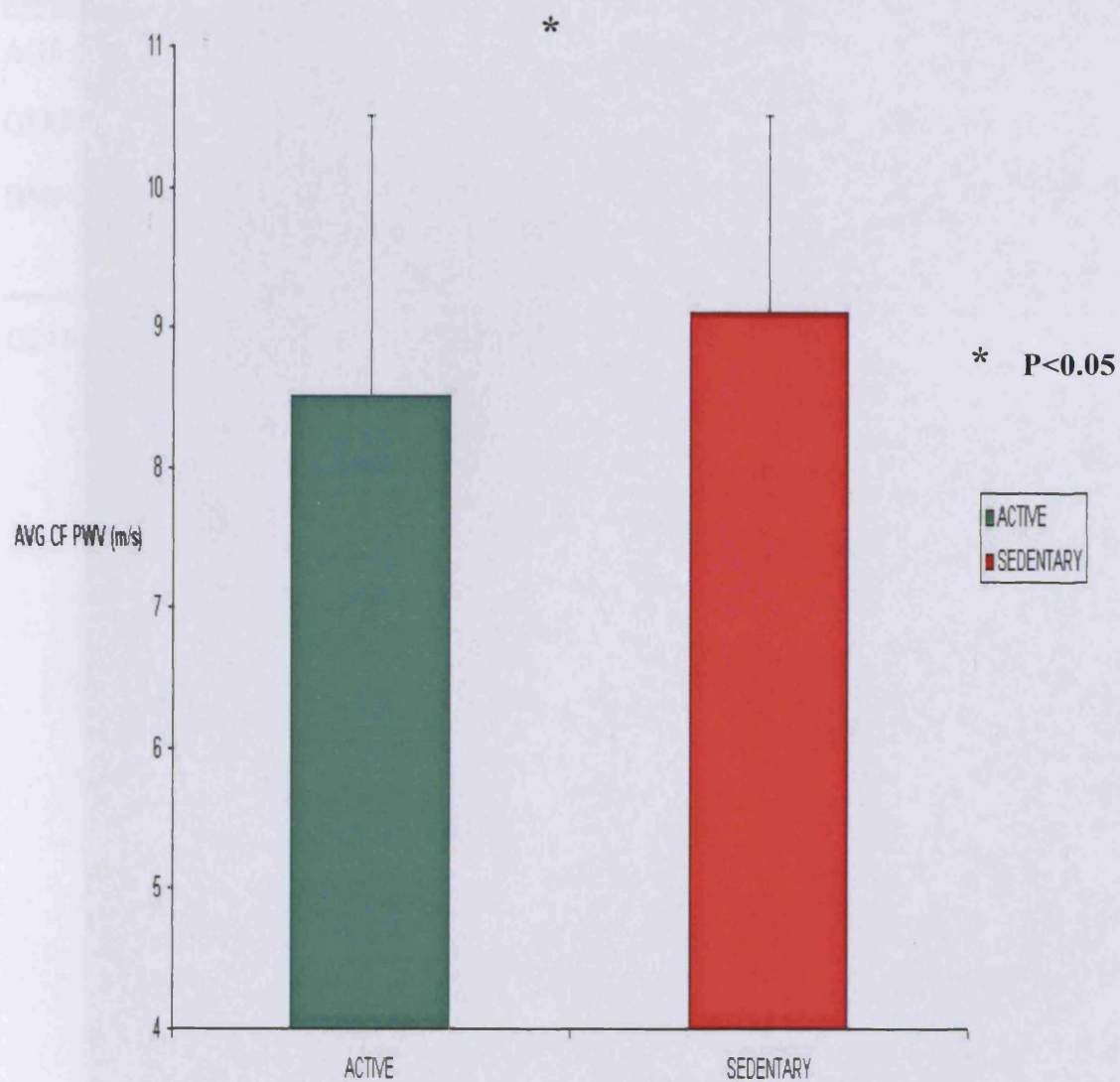
	Sedentary	Active	
	N= (170)	N=(31)	P Value
TCHOL (mmol/l)	5.7±1.1	5.5±1.6	NS
LDL (mmol/l)	3.5±1.0	3.4±1.4	NS
HDL (mmol/l)	1.35±0.4	1.7±0.5	0.003
TRIGS (mmol/l)	2.0±1.1	1.3±0.8	<0.001
GLUC (mmol/l)	5.6±2.1	4.9±1.2	0.029
NA (mmol/l)	141±2.5 (N=53)	141±2.5	NS
K (mmol/l)	4.4±0.4 (N=53)	4.4±0.4	NS
CREATININE (µmol/l)	77.6±17 (N=53)	85±15	0.045
UREA (mmol/l)	5.3±1.0 (N=53)	5.5±1.1	NS
CRP (mg/l)	4.2±3.9 (N=53)	4.9±10.0	NS

TCHOL: Total cholesterol, LDL: Low density lipoprotein, HDL: High density

lipoprotein, TRIGS: Triglycerides, GLUC: Glucose, NA: Sodium, K: Potassium, CRP:

C-reactive protein.(N=): Illustrates the number of measurements recorded for that group.

Figure 6.1. Illustrates the differences observed in CF PWV between physically active and sedentary elderly groups.



AVG CF PWV (m/s); Average carotid to femoral pulse wave velocity, in metre per second.

Table 6.4 Results of stepwise multiple regression analysis of the factors influencing CF PWV in this study.

Model	r	SE	Beta	P value	r² Change(%)
AGE (yrs)	0.084	0.020	0.390	<0.001	17.1
GLUC (mmol/l)	0.304	0.132	0.219	0.024	5.0
BMI (Kg/m ²)	0.090	0.041	0.206	0.031	4.2

CF PWV R² = 0.263, P<0.001

GLUC: Fasting glucose levels, BMI: Body mass index, r: Regression coefficient.

6.4 Discussion

This cross sectional study in 262 men and women demonstrates that CF PWV is significantly lower in the physically active (PA) group compared to sedentary (S) controls. The fact that there were no significant differences in blood pressure or heart rate between the PA and S groups suggest that only moderate increases in physical activity above sedentary are sufficient to produce changes in large arterial stiffness. In agreement with previous data ¹⁸² there was no difference in CR PWV confirming that exercise may have differential effects on central elastic arteries compared to more muscular peripheral ones. This is supported by results from an intervention study which showed that 16-weeks of aerobic exercise decreased central aortic but not femoral-dorsalis pedis PWV ¹⁸². In addition fasting blood glucose levels were also found to be significantly lower in the PA group. The findings of the current study are not in agreement with data from much larger ARIC study. Although the ARIC study did show a positive association between vigorous physical exercise and CIMT, there was no such relationship between habitual physical activity and CIMT ¹²⁶. However, a more recent study in a similar age group to the ARIC participants demonstrated an inverse relationship between habitual physical activity and the age dependent increase in carotid wall stiffness assessed by ultrasound ¹²⁵. This is supported by data from another large study, which, like the current study assessed arterial stiffness by measuring PWV. Although this study assessed aorto-iliac PWV, in agreement with this data on CF PWV, it demonstrated a significant inverse relationship between aorto-iliac PWV and increased levels of physical activity ¹²⁴

The present study is also consistent with results from a number of smaller studies which have shown that aerobic exercise training ¹⁸², increased PA levels ^{124,130},

increased cardio respiratory fitness ^{129,184} and increased leisure time physical activity (LTPA) ¹²⁸ to be associated with decreased arterial stiffness. Methods of measuring arterial stiffness in these studies included, B-mode ultrasound imaging, photoplethysmography, tissue doppler imaging and young's elastic modulus and applanation tonometry.

Explanations for the effect of exercise on arterial stiffness remain unclear. There were no differences in MAP between the PA and S groups so that changes in blood pressure cannot be the explanation as to why the PA group exhibited a significantly lower CF PWV. Interestingly, when a multiple regression analysis was performed, only age, glucose levels and BMI were found to affect CF PWV results. See table 6.4. Other known factors that have previously been shown to be responsible for CF PWV were entered into the regression analysis but had little influence (Gender, Exercise, MAP, TCHOL, LDL, HDL and TRIGS). However, the study has very small numbers in the PA group to perform a reliable regression analysis. Therefore, more numbers are needed in order to identify the effect of exercise on CF PWV.

Furthermore, the PA group had a significantly lower fasting glucose relative to the S group. This may have been responsible for some of the effect of exercise on arterial stiffness. Indeed, studies in the previous chapter have shown that fasting glucose levels have a graded effect on arterial stiffness and that there is a weak but significant correlation between CF PWV and fasting glucose levels. Exercise may have direct or indirect effects on arterial stiffness. Since endothelium derived nitric oxide (NO) is, in part, involved in the regulation of arterial stiffness ^{65,66} changes in the bioavailability of NO could alter arterial stiffness. Indeed, exercise itself increases NO release from the vascular endothelium ^{185,186} and this increase in bioavailability of NO could then

decrease arterial stiffness. Exercise may also indirectly influence arterial stiffness in that as in the current study it reduces fasting blood glucose levels¹³⁶. Increased blood glucose levels are associated with endothelial dysfunction and decreased bioavailability of nitric oxide NO and such effects are reversible on decreasing blood glucose¹⁸⁷. The decreased fasting blood glucose in the PA group may therefore also increase the bioavailability of NO and account for some of the decreased CF PWV seen in the PA group.

In addition exercise has been shown to decrease oxidative stress and levels of oxidized LDL (ox-LDL)¹⁸⁸ Since ox-LDL impairs endothelial function, decreased levels of ox-LDL may also increase levels of NO and reduce arterial stiffness.

Exercise improves insulin sensitivity and decreases insulin resistance. This also provides a possible explanation for the beneficial effects of exercise on arterial stiffness. Recently insulin at physiological levels has been demonstrated to have an effect on arterial haemodynamics leading to decreased wave reflection^{118,176} and that these effects of insulin are similar to those of GTN¹⁷⁷ suggesting that NO may well be involved in the haemodynamic effects of insulin. Furthermore, these beneficial effects of insulin are blunted in subjects with insulin resistance¹¹⁹.

Finally since there is a relationship between arterial stiffening and atherosclerosis it is impossible to exclude the fact that habitual exercise may decrease atherogenesis (perhaps *via* NO mediated mechanisms) and thus a decrease in atheromatous deposition within the arterial wall may also contribute to reductions in structural arterial stiffness with exercise.

There were no differences in AIX between the PA and S groups in the current study. This is in disagreement with a recent study by Binder et al. in men with no clinical evidence of CHD in which there was an inverse relationship between AIX measured, as in this study, using the SphymoCor system and cardio-respiratory fitness assessed by $\text{VO}_2 \text{ max}^{189}$. However, this study, unlike the current one, included only men and more importantly the men were considerably younger than the men and women recruited in this study. The differences in age may provide an explanation for the different findings in that AIX has been shown to plateau with age especially beyond the age of 55yrs. Since the subjects in the current study were all > 60yrs of age differences in AIX may be more difficult to observe when comparing between groups. In contrast CF PWV changes little with age until after 55yrs and then rise steeply thereafter. It would have been interesting to see data on CF PWV in the study from Binder et al. However, this was not measured in that study. This is an important omission as it is CF PWV that has more evidence than AIX to support its predictive value for CV events¹⁰⁰.

Many questions concerning the relationship between physical activity, arterial stiffness and CVD remain unanswered. Based on the current study and the results of others what is required is a large intervention study in subjects across a large age range with accurate haemodynamic measurements including assessment of arterial stiffness. Inclusion of measurements such as endothelial function, fasting insulin and glucose, CRP, ox-LDL would allow analyses to be conducted to study the relationship between the effect of exercise on these parameters and arterial stiffness in order to

better understand how exercise improves arterial function. This will be discussed further in the final Chapter on future directions of research.

CHAPTER 7

FUTURE DIRECTIONS OF RESEARCH

7.1 Future directions of research

The past decade has seen an exponential rise in the incidence of diabetes, such that, by the year 2025 it is estimated that over 50 people in every thousand will have diabetes. This has important health implications as mortality rates for CAD are 2-4 times higher in diabetics compared to healthy controls and overall life expectancy is reduced by 25%¹⁹⁰. Although non-diabetic women have less CHD than men the development of diabetes increases a woman's risk 5 times relative to a man¹⁹¹.

Furthermore, micro-vascular and macro-vascular complications in diabetics have considerable economic cost implications. The Code-2 study, conducted in 8 European countries, estimated that the health service costs of diabetes was 29 billion euros. This study also demonstrated that although routine management of diabetes accounted for 50% of health service costs an estimated 50% came from preventable complications. Despite this very little is spent on interventions to prevent diabetes or slow its onset. Metabolic Syndrome is a pre-diabetic state and individuals with this condition have a 4 times greater chance of developing type-2 diabetes, compared to someone who has not got the syndrome. A major component of the Metabolic Syndrome is impaired glucose tolerance. The current studies (Chapter 5) have shown that IFG is associated with increased arterial stiffness, itself an independent risk factor for CV events. Therefore, interventional studies are needed to assess the mechanisms relating arterial stiffness and IFG or the Metabolic Syndrome. However, the contributions of the various components of the metabolic syndrome to arterial stiffening remain unclear.

It is already well established that exercise is effective at slowing the progression to onset of diabetes in subjects at high risk of its development¹⁶⁹. This thesis has also

demonstrated a positive cross sectional relationship between increased physical activity and decreased arterial stiffness (Chapter 6). However, a number of important questions remain unanswered. Since exercise decreases a number of other CV risk factors such as hypertension and obesity, it is difficult to define the exact mechanisms responsible for the observed benefits of exercise on arterial stiffness and CV outcome. Furthermore, there are few studies examining the effect of exercise on IFG and arterial stiffness that are not confounded by the presence of additional risk factors.

Future studies should therefore be designed to assess the effect of exercise intervention and lifestyle changes on the individual components of the Metabolic Syndrome and their relationship to arterial stiffness. Furthermore, such studies should include male and female subjects across a wide age range and different ethnicities. Results from such studies should allow a better understanding of the factors regulating arterial stiffness and the influence of exercise on these factors. This would then allow a better understanding of the mechanisms contributing to the beneficial effects of exercise. Importantly longitudinal studies are needed to define the factors responsible for the progression of arterial stiffness. Such studies should incorporate detailed information on exercise including duration, intensity, regularity and type of exercise.

On the basis of the current findings, future studies need to take into consideration a study with a research programme of longitudinal design, over a number of years and assess the effect of an exercise intervention on pre-diabetes (IFG). The study should encompass a variety of ethnic groups around the Cardiff area, with the hope of gaining valuable data relating to the differences in vascular physiology. Large numbers will enable the investigator to assess the age related vascular changes

specific to each ethnic group. This will follow on from the original results that have been presented in Chapter 4 (Study 2c and 2d).

When assessing the effects of exercise on arterial stiffness in diabetes and pre-diabetes, the investigator should measure vascular haemodynamics parameters (Blood pressure (central and peripheral, seated and supine), pulse wave velocity (CR PWV, CF PWV, CD PWV and FD PWV) and pulse wave analysis (Aix, Tr, resting HR etc) using the SphygmoCor system) as part of the over all study design. Following the results of Chapter 3, the investigator accepts the good reproducibility of both systems in the measurement of arterial stiffness. However, the SphygmoCor system still remains the “gold standard” method of assessing CF PWV. Cardio respiratory fitness (measured by VO^2_{max}) should also be measured before the intervention is performed, as this is likely to change as a result of increased physical activity. Biochemical analysis will be performed similar to Chapters 5 and 6. However, Insulin levels should also be measured in order to predict insulin sensitivity.

A full personal medical history questionnaire should be completed and all medication detailed appropriately.

After baseline measurements and an exercise intervention of aerobic physical activity performed, subjects will return at 1, 2, 6, 12 and 24 months.

The large numbers recruited throughout the study will enable the investigator to perform a multiple regression analysis to determine the effect of exercise on arterial stiffness measurements. As mentioned earlier, without these large numbers, determination of the effect exercise alone has on arterial stiffness will be difficult to ascertain. Especially, as exercise is known to influence other confounding factors like cholesterol, glucose, insulin, heart rate, blood pressure, and waist circumference.

A study along these lines will therefore help to elucidate the mechanisms responsible for arterial stiffening in diabetic, pre-diabetic and normoglycaemic subject groups derived from various ethnic populations.

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