Non - invasive assessment of vascular function in healthy ageing and disease

Thesis submitted for the degree of

Doctor of Medicine

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by

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List of Abbreviations.

PWA – Pulse wave analysis

PWV – Pulse wave velocity

BP – Blood pressure

SBP/PSBP – Systolic blood pressure/Peripheral systolic blood pressure

DBP/PDBP – Diastolic blood pressure/Peripheral diastolic blood pressure

PP/PPP/ - Pulse pressure/Peripheral pulse pressure

CPP – Central pulse pressure

MAP – Mean arterial pressure

AT – Applanation tonometry

AI/CAI/PAI – Augmentation Index/Central Augmentation Index/Peripheral Augmentation Index

BMI – Body Mass Index

LDL – Low density lipoprotein

HDL – High density lipoprotein

ACR – Albumin/creatinine ratio

Tr – Time to reflected wave

dP/dT- Change in pressure with time

TTI – Tension time integral

QC – Quality control

ECG – Electrocardiograph

Hb A1c – Glycosylated haemoglobin

bpm – beats per minute

GP – General Practitioner

List of Abbreviations (cont.).

ACE – Angiotensin converting enzyme

GTN – Glyceryl Trinitrate

ED – Ejection duration

SVI – Sub-endocardial viability index (Buckberg Ratio)

DTI – Diastolic time integral

GTF – Generalised transfer function

DM – Diabetes Mellitus

LVH – Left ventricular hypertrophy

CVA – Cerebrovascular accident

MI – Myocardial infarction

BHS – British Hypertension Society

WHO – World Health Organisation

ASCOT – Anglo-Scandinavian Cardiac Outcomes Trial

CAFÉ Conduit Artery Functional end-point study

MMP – Matrix metalloproteinase

TIMP – Tissue inhibitor of matrix Metalloproteinases

E – Young's Modulus

h – vessel wall thickness

ρ - Density of blood

Acknowledgements

I would like to acknowledge all the staff at the Clinical Sciences Building and Clinical Research Unit, for making my time in Leicester such a happy and memorable one.

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I am indebted to Professor Bryan Williams for his tireless enthusiasm, encouragement and guidance in the completion of this thesis, not forgetting his invaluable critical appraisal of the final manuscript.

Finally, I could not have completed this work without the ongoing support and understanding of my fantastic wife, Kathryn and two beautiful daughters, Grace and Ella, and it is to them, with much love, that I dedicate this thesis.

Statement of Originality

This work was conducted at the Cardiovascular Research Institute, University of Leicester, under the supervision of Professor Bryan Williams and working in collaboration with Dr. Peter Lacy. I confirm that I was personally involved in the design of the project, submission of requests for ethical approval, recruitment and study of patients, and the collection, analysis and interpretation of data. I acknowledge that I worked as part of a team who assisted in the recruitment and study of patients for this and many ongoing clinical studies. I confirm that all work recorded in this thesis is original work.

1.0 Introduction

1.1 Background

In westernised societies, the average age of the population is steadily increasing, and it appears inevitable that the incidence of cardiovascular disease will rise proportionally. Indeed, alarming reports of global health predict that cardiovascular disease will shortly become the number one cause of death worldwide[1]. Interestingly, cardiovascular disease associated with this seemingly "normal ageing" has largely been a phenomenon of the developed world. Studies in rural African communities have now shown that hypertension, for instance, does not have to accompany old age[2-6]. Increased weight[7-9], salt[10] and alcohol intake[11-13] and reduction in levels of exercise[14] have all been implicated in the aetiology of hypertension in the elderly.

Whether genetic, environmental or more likely a combination of factors is responsible is not clear, more worrying is the observation that the developing world is now rapidly catching up with its industrialized neighbours in terms of cardiovascular disease. [12;15]This has profound implications for global health provision, and such is its importance that the World Health Organization has now given high priority into research in this field.[16]

With increasing age, the probability of co-morbid conditions increases. Hypertension, atherosclerosis and diabetes, to name but a few, have a profound influence on cardiovascular structure and function and tend to cluster in "at risk" individuals.

In addition to this, the well-recognized polypharmacy we now see in an elderly cohort of individuals serves only to muddy the water further when we attempt to study alterations in cardiovascular structure and function with age. Although this poses a potential problem for studying the effects of true "normal ageing" in isolation, we are never the less presented with a "real-world" situation, in which new techniques and research must be applicable.

Both cross sectional and longitudinal studies are now available to assist with our understanding of the changes associated with "normal ageing". Data from Framingham[17] suggests that with progression through middle age, a gradual increase in systolic, diastolic and mean arterial pressure is seen (Figure 1). This cohort of Caucasian, predominantly middle class Americans were observed prior to the recognition of the benefits of treatment of mild hypertension, and provided an opportunity to study individuals in the absence of pharmacotherapy. The rise in these blood pressure parameters with age was seen at all levels of starting blood pressure (including normotensives) and in both men and women. Although females initially had lower blood pressures than their male counterparts, they would later be seen to equal them at around the sixth decade, before concluding with higher pressures in the seventh and eighth decade.

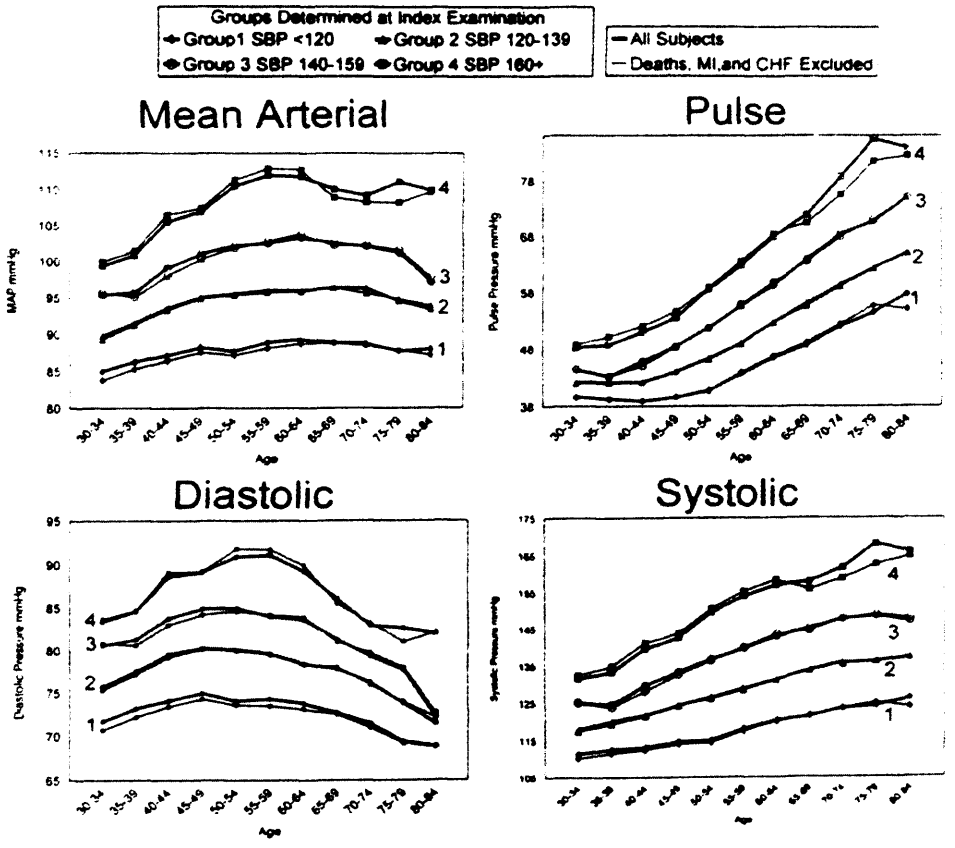


Figure 1: Relationship of blood pressure parameters to age [17]

The changes in blood pressure observed in middle age are likely to represent an increase in peripheral vascular resistance. At or around the fifth decade, diastolic blood pressure levels off, before even falling with advancing age despite a continuing rise in systolic blood pressure. These changes led researchers to suspect that blood pressure changes after middle age may not be driven by continuing elevations in peripheral vascular resistance, which one would have expected to further increase diastolic pressure.

Continuing rise in systolic pressure with a falling diastolic pressure results in an increasing pulse pressure. This phenomenon was again seen at all blood pressure levels, although the steepest rise was seen in those with hypertension. The fall in diastolic pressure and elevation in pulse pressure is representative of an increase in underlying large artery stiffness. As the large arteries become less compliant, peak systolic pressure rises and there is less of an elastic reservoir effect, causing increased diastolic run-off to the peripheries and a drop in diastolic pressure. It was postulated therefore, that peripheral vascular resistance was not the dominant cause of blood pressure changes seen after the sixth decade, which was more sensibly explained by an increase in large artery stiffness and increased wave reflection from the periphery. This may also explain why epidemiological studies have linked diastolic and MAP with cardiovascular risk in those aged less than 45 years, whilst systolic blood pressure is more strongly correlated to outcome in those aged greater than 45 years[17].

Pulse pressure, as a surrogate marker of large artery stiffness, has continued to gain strength as an independent predictor of outcome, not only in patients with established cardiovascular disease but even in normotensive populations[18-27]. Despite this fact, systolic and diastolic blood pressure remain the main stay in terms of clinical evaluation of hypertensive individuals.

What then is the role for assessing stiffness of the large arteries versus absolute blood pressure parameters in a population already established to

be 'high risk' in terms of cardiovascular disease? As noted above, arterial stiffness appears to increase with advancing age[28-30] and this is likely to be related to structural and functional changes throughout the cardiovascular system. These changes obviously form the basis of much the cardiovascular alterations we see clinically with advancing age and will therefore be considered in more detail below.

What remains to be elucidated is whether or not structural and functional changes develop in parallel, or whether a change in function, for example, leads to a compensatory alteration in structure?

The main thrust of this Thesis is to concentrate on vascular alterations with age and disease, and in particular the potential clinical role of non-invasive assessment of "arterial function" using applanation tonometry. For completeness however, a brief overview of the cardiac changes seen with advancing age is included below.

1.2 Structural and functional *cardiac* alterations with age.

Excluding the obvious increase in atheromatous coronary artery disease with all its sequelae, animal and human studies provide extensive evidence of both structural and functional changes in the heart with age. These include a reduction in myocyte number[31;32] with consequent hypertrophy of

remaining cells[33-35], in addition to an associated increase in matrix connective tissue, fibrous tissue[31;36] and even amyloid. Functionally this results in prolonged myocyte action potentials and subsequent prolonged myocyte contraction, diminished response to adrenergic stimulation and a generalized increase in myocardial stiffness. The underlying mechanisms for these changes are not fully understood but are likely to be due to a complex interplay between alterations in ionic transport, stimulation of cardiac natriuretic peptides, and possibly altered myocardial gene expression[37-39]. These changes are summarised in table 1 below.

Relationship of Cardiac Human Aging in Health to Cardiac Diseases

Age-Associated Changes	Plausible Mechanisms	Possible Relation to Human Disease
Cardiac structural remodeling		
↑ LV wall thickness	↑ LV myocyte size with altered Ca^{2+} handling	Retarded early diastolic cardiac filling
	↓ Myocyte No. (necrotic and apoptotic death)	↑ Cardiac filling pressure
	Altered growth factor regulation	Lower threshold for dyspnea
	Focal matrix collagen deposition	↑ Likelihood of heart failure with relatively normal systolic function
		LVH
↑ Left atrial size	↑ Left atrial pressure/volume	↑ Prevalence of atrial fibrillation and other atrial arrhythmias
Cardiac functional changes		
Reduced threshold for cell Ca^{2+} overload	Changes in gene expression of proteins that regulate Ca^{2+} handling; increased $\omega 6$:$\omega 3$ polyunsaturated fatty acids ratio in cardiac membranes	Lower threshold for atrial and ventricular arrhythmia
		Increased myocyte death
		Increased fibrosis
		Reduced diastolic and systolic function
↓ Cardiovascular reserve	↑ Vascular load	Lower threshold for, and increased severity of heart failure
	↓ Intrinsic myocardial contractility	
	Ventricular-vascular load mismatch during stress	
	↑ Plasma levels of catecholamines	
	↓ β adrenergic modulation of heart rate myocardial contractility and vascular tone due to post-synaptic signaling deficits	
Reduced physical activity	Learned lifestyle	Exaggerated age changes in some aspects of cardiac structure and function, eg. impaired LV ejection reserve capacity
	Frailty	Negative impact on atherosclerotic vascular disease, hypertension and heart failure

Table 1 Changes seen in human cardiac ageing[39].

Progressive left ventricular hypertrophy has been documented with age even in the absence of systolic hypertension[40]. In the Baltimore longitudinal study, left ventricular posterior wall thickness was seen to increase by 25% between the second and seventh decade, in the absence of any significant rise in systolic blood pressure[41]. Despite ventricular hypertrophy, resting left ventricular ejection fraction appears to be relatively well preserved with age probably due to an associated degree of chamber dilatation[40]. Diastolic relaxation is, however, frequently, abnormally prolonged consistent with the degree of hypertrophy[39]. Functionally this does not appear to be problematic as heart rate, even during exercise tends to be lower in the elderly[42;43] allowing more time for diastolic filling and coronary artery perfusion.[44]

Figure 2 below outlines some of the changes seen in cardiac function with age, both at rest and during exercise.

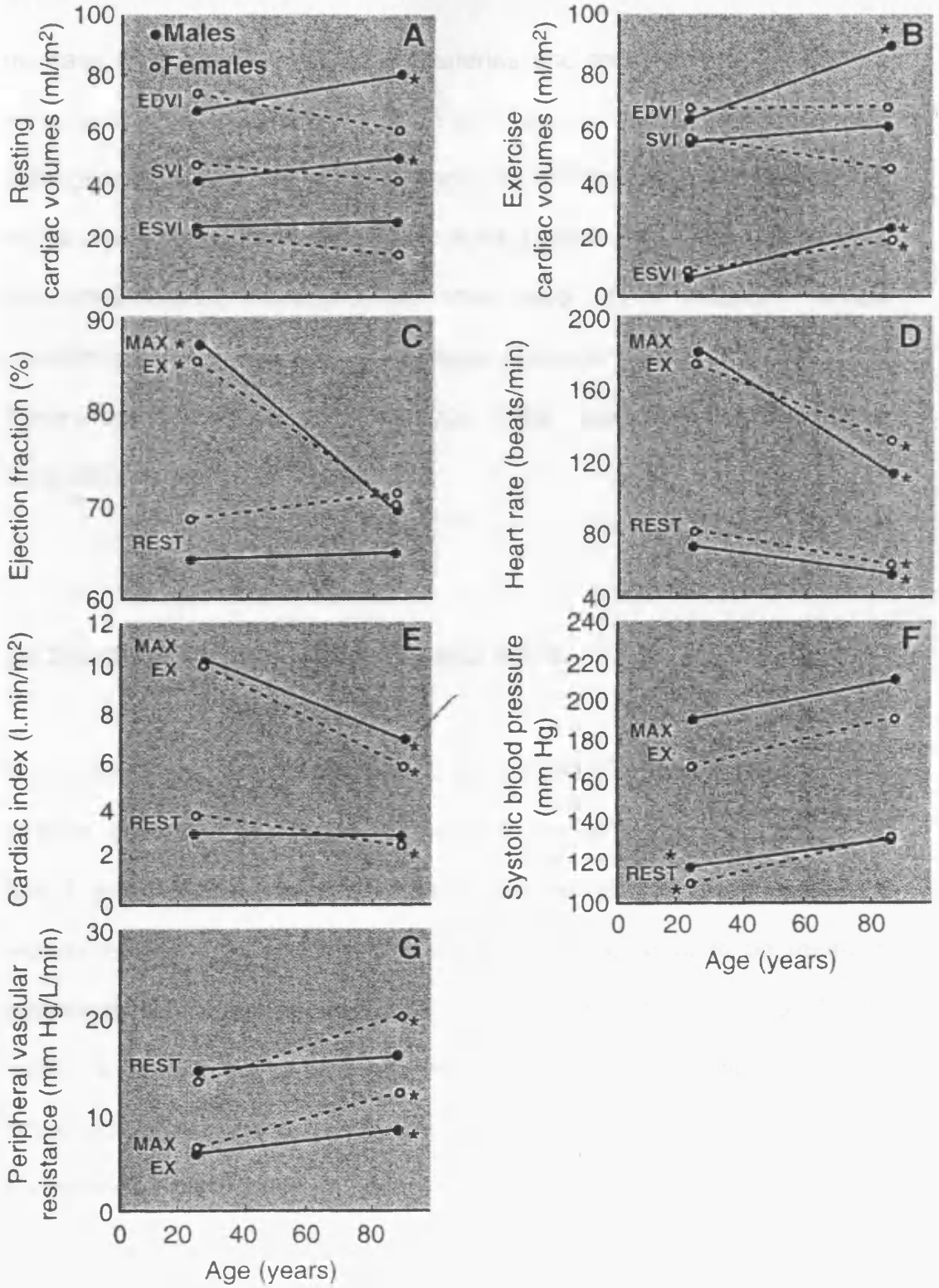


Figure 2: Linear regressions of age and cardiovascular indices.[43]

Increase in stiffness of the central arteries and consequent early systolic wave augmentation is thought to be an important causative factor in the pathogenesis of left ventricular hypertrophy[45-49] seen with increasing age, and is discussed in more detail later. Ageing hearts are also prone to other pathophysiological processes that may also affect function, namely conducting system and valvular disease. Although undoubtedly important, these processes are out with the scope of this discussion and shall not be considered further.

1.3 Structural *vascular* alterations with aging.

In addition to the now often expected age related increase in atheromatous disease, a number of other disturbances in vascular structure appear with age. A generalized increase in systemic vascular resistance is documented with increasing age[42], and since cardiac output appears to be relatively preserved, it remains unclear what the stimulus to this rise may be. In reality, a number of processes are likely to be responsible including increased adrenergic drive, capillary rarefaction and remodelling, and alteration in responsiveness of the renin-angiotensin-aldosterone system.

Age related stiffening of the vessels and consequent increase in pulsatile pressure, results in repetitive cyclical mechanical strain that is likely to contribute to significant continuing damage to the arterial system if left

unchecked. Indeed pathological studies in aged aortas have demonstrated fragmentation of elastin with disruption of elastin lamellae, in addition to increased deposition of collagen (with increased cross linking), calcium and extra cellular matrix proteins at the expense of smooth muscle cells. This ultimately results in the pathological entity recognized as cystic medial necrosis. [45;50]. A table summarizing these vascular changes with age is shown below (Table 2).

Relationship of Vascular Human Aging in Health to Vascular Diseases

Age-Associated Changes	Plausible Mechanisms	Possible Relation to Human Disease
Vascular structural remodeling		
↑ Vascular intimal thickness	↑ Migration of and Ý matrix production by VSMC	Promotes development of atherosclerosis
	Possible derivation of intimal cells from other sources	
↑ Vascular stiffness	Elastin fragmentation	Systolic hypertension
	↑ Elastase activity	Left ventricular wall thickening
	↑ Collagen production by VSMC and ↑ Cross linking of collagen	Stroke
		Atherosclerosis
	Altered growth factor regulation/tissue repair mechanisms	Left ventricular hypertrophy
Vascular functional changes		
Altered regulation of vascular tone	↓ NO production/effects	Vascular stiffening; hypertension
		Early atherosclerosis
Reduced physical activity	Learned lifestyle	Exaggerated age changes in some aspects of vascular structure and function, eg, arterial stiffening
	Frailty	Negative impact on atherosclerotic vascular disease, hypertension and heart failure

VSMC indicates vascular smooth muscle cell.

Table 2. Changes in human vascular ageing[50]

These changes are thought to occur after the fourth decade and be steadily progressive thereafter. Large arteries which rely on elastin to maintain their integrity are obviously at greatest risk of degeneration over time, rather than

small muscular arteries relying largely on the muscular component of their wall for structural support. [28;51;52]

Such damage can be reproduced in the laboratory by observations on natural rubber subjected to repetitive mechanical strain. With an increasing life expectancy, it may be that we have quite literally reached a point where biological systems are wearing out!

In vitro experiments on cultured human vascular smooth muscle cells subjected to cyclical pulsatile mechanical strain also demonstrate stretch related increase in vascular extra cellular matrix proteins such as fibronectin and collagen, the substances largely responsible for vascular stiffening. Interestingly this is seen both at physiological as well as pathological levels of simulated stretch[53].

In health, the extra cellular matrix milieu is in dynamic equilibrium and animal models have suggested that vascular matrix remodelling occurs at a rate of approximately 0.6% per day[54]. This dynamic process of deposition and degradation is under the control of numerous regulatory enzymes such as Matrix Metalloproteinases (MMPs) and their Tissue Inhibitors of Matrix Metalloproteinases (TIMPs). Currently at least sixteen members of this group of zinc dependent endopeptidases have been identified, each one with a preferred specific substrate. Of particular relevance to the cardiovascular system, MMP-1 and MMP-3 degrade types I and III collagen while MMP-2 and MMP-9 degrade type IV collagen and Elastin[55].

Experimental evidence in human subjects now suggests that this balance may be upset in the presence of hypertension, and that serum markers of matrix turnover may provide a relatively non-invasive assessment of this dynamic biological process.[56-58]

Whether this matrix equilibrium can be favourably influenced with pharmacotherapy, or whether matrix turnover could be used to monitor disease progression and treatment remains to be evaluated?

1.4 Functional *vascular* alterations with ageing

Age related functional changes are also seen in the arterial system and may be in part related to a reduction in endothelium-mediated vasodilatation[59;60]. It is still not clear whether such changes are primary or secondary phenomena. The exact mechanism for this observation is poorly understood, but may be a combination of decreased activity and/or accelerated destruction of nitric oxide, possibly in response to excess free radicals and super-oxides.

Elevation in systemic vascular resistance with age may be due to the sympathetic over-activity seen with ageing. Reduction in the number of β_1 adrenoceptors with age, and increased levels of circulating noradrenaline, may both play a part in what is essentially reduced effectiveness of adrenergic modulation with age.[61 62;63]

Age related reduction in arterial compliance may interfere with carotid sinus function with resultant blunting of the baroreceptor response, allowing excess sympathetic outflow to continue unchecked, although again whether or not this is a primary or secondary effect remains unclear.

With progressive rise in mean arterial blood pressure with age, it is likely that distension of the large conduit arteries may force the artery away from relying on its elastic properties towards dependency on more collagenous elements of the artery wall. This results in "functional" stiffening of the artery that in its early stage may be potentially reversible by reduction in distending pressures using antihypertensive therapy.

One can therefore postulate that if functional stiffness (whatever its aetiology) remains untreated it will act as a stimulus for structural change that may well then become irreversible. This poses considerable concern over current clinical practice which advocates delayed treatment of mild hypertension until the emergence of target organ damage.[64] This will be discussed further in a subsequent chapter dedicated to assessment of patients with hypertension.

1.5 Arterial stiffness and ageing

As alluded to above, arterial stiffening appears to be an inevitable consequence of ageing in Westernised societies[28;45;65;66]. This is often

clinically apparent by the high prevalence of isolated systolic hypertension in the elderly population. Moreover, age related arterial stiffening has been documented in populations known to have a low prevalence of atherosclerotic disease suggesting that there is stiffening of the vasculature independent of coexistent atheromatous disease [67](figure 3). It is important to note however that age related changes in these populations are much less marked that those seen in industrialized societies.

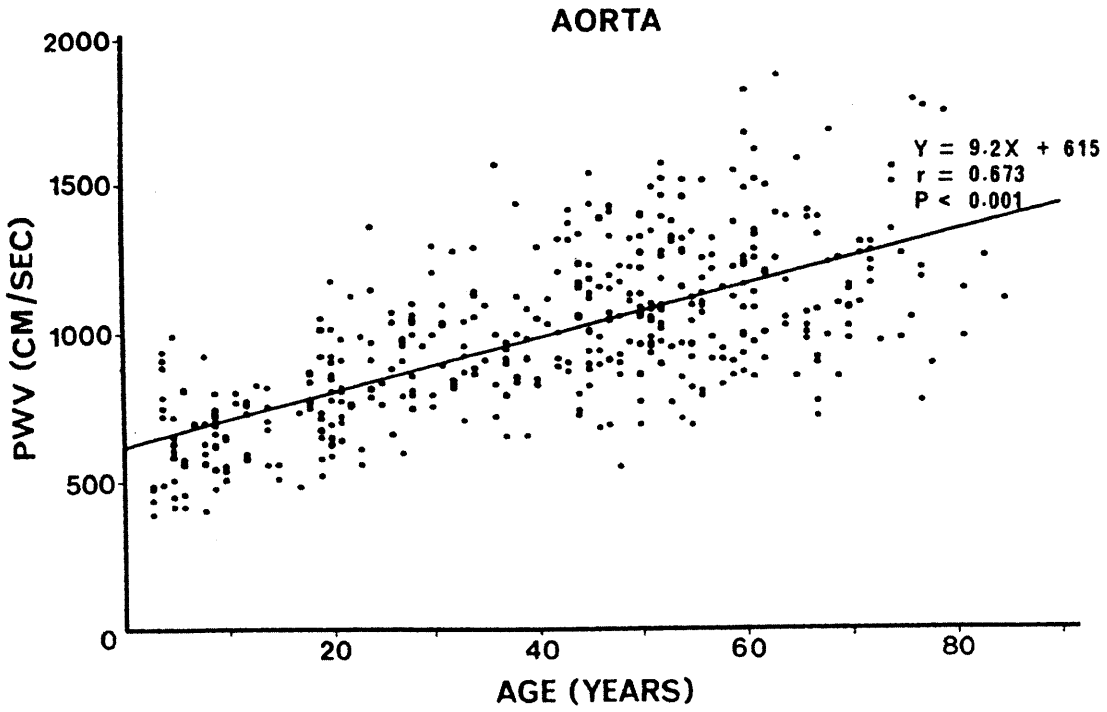


Figure 3: Age related changes in aortic PWV in an urban Chinese population. [67]

As noted previously, there are certain rural tribes with no apparent rise (possibly even a small fall) in blood pressure or arterial stiffness with age[2-4;6]. It is highly probable therefore that a collection of western risk factors such as smoking, salt intake, lipid level, alcohol use, obesity and sedentary

lifestyle to name but a few have a profound effect on so called *age* related changes in the cardiovascular system.

Arterial stiffening per se however is likely to be one of the major factors contributing to increased cardiovascular morbidity and mortality seen in the elderly[18-21;23;25-27;68-73]. Reduced vascular compliance and resulting arterial stiffening is responsible for the progressive rise in systolic and pulse pressure seen with ageing, and in turn the subsequent development of target organ damage and associated adverse cardiovascular events. (Both coronary and cerebrovascular disease.) The stiffening of large conduit arteries results in an increased pulse wave velocity, early reflection of the arterial wave from the periphery and subsequent summation of central arterial pressure referred to as *Augmentation*.

Elevation in central arterial pressure secondary to systolic wave augmentation obviously results in a considerable increase in cardiac after load. As well as stimulating hypertrophy of the left ventricle and reducing coronary filling in diastole, elevated central pressures expose the arterial tree to a cycle of continuing and excessive haemodynamic stress with resultant compensatory structural arterial changes and further increased vascular stiffness. The ageing arterial system acts as a positive feedback system leading to relentless arterial injury. Termination of this cycle is unlikely without therapeutic intervention.

Elevated pulsatile pressure generated as a result of reduced vascular compliance is now considered an important factor in the development of the target-organ damage. Such is the importance of vascular stiffening that it could be considered as target organ damage in its own right. Indeed recent studies have indicated that an elevated pulse pressure [19;21;70](a surrogate marker of arterial stiffening), and pulse wave velocity itself [69;69;72;74;75] (a more direct measure of arterial stiffness) are independent risk factors/predictors for cardiovascular disease (See later chapters)

Although this concept of "vascular stiffness" is basic and describes a simplified integration of the complex pathological processes involved in vascular ageing, it does provide a concept which can be further researched.

A detailed description of the changes in arterial pressure waveforms with ageing will be considered in a following chapter dedicated specifically to the effects of normal ageing on these parameters.

1.6 The role of the arterial system.

When studying the arterial system it is important to consider its primary role. The aim of the arterial system is to provide a continual flow of blood transporting oxygen and nutrients to the tissues. This steady flow has to occur in the face of a pulsatile generating pressure source. Conversion of a pulsatile pressure into steady flow is paramount to protect fragile capillary beds. Auto regulation at an arteriolar level obviously plays a fundamental

role in protecting the micro-vascular beds from high pressures but a significant amount of "damping" also occurs in the large elastic conduit arteries. This stored energy can be expended during cardiac diastole via vascular relaxation to perpetuate a steady forward flow.

Pulsatile blood flow generates a pressure wave front, which travels from the heart to the peripheral vascular beds. At these arterial: arteriole interfaces (and therefore regions of impedance mismatch), whilst a proportion of the outgoing pressure wave continues distally, a degree of reflection of the outgoing wave occurs to interact with subsequent more central and forward propagating waves.[45;52] This can be demonstrated by the simplified diagram below (figure 4). At the top of the figure are shown amplitude and contour graphs of generated pressure waves. The tubes represent a simplified arterial system, (a) represents a normally compliant system with normal pulse wave velocity. As "stiffness" increases with no increase in pulse wave velocity, (b) pressure wave amplitude increases. If stiffness increases with an associated increase in pulse wave velocity (c), then the returning wave front arrives during ventricular ejection rather than ventricular diastole and augments the outgoing wave, therefore augmenting pressure. This model assumes the same ventricular ejection in each case.[76]

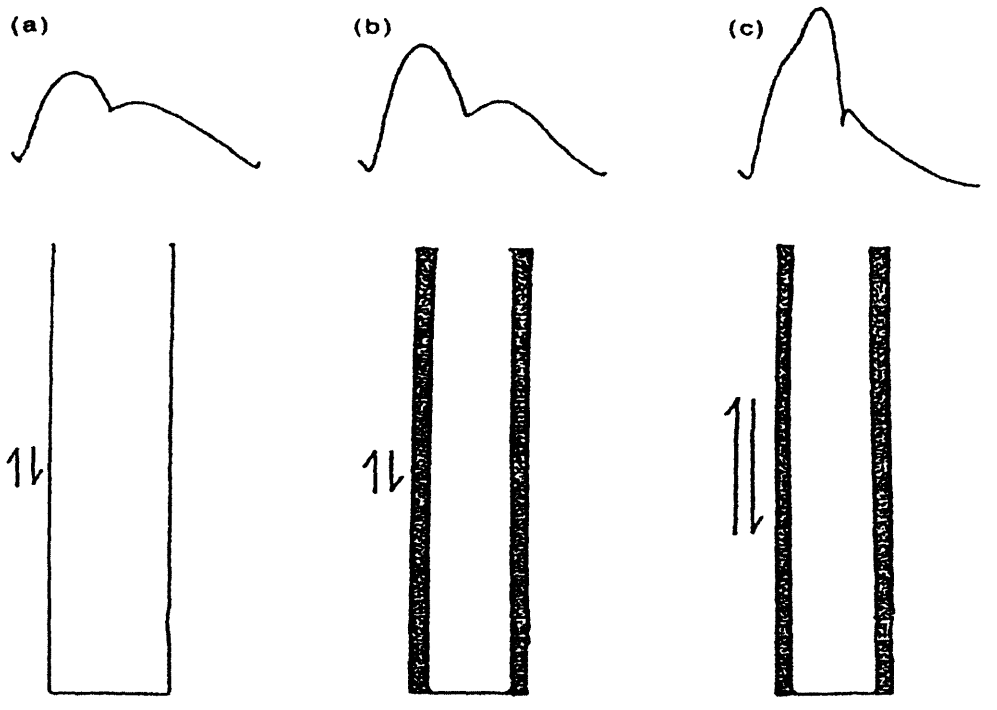


Figure 4: A simplified model demonstrating concepts of arterial stiffness and altered pressure wave contours[76]

Despite this potentially more realistic 'pressure wave' concept of arterial blood flow, it is still numerical values representing systolic and diastolic blood pressure (as derived by brachial cuff sphygmomanometry) that are routinely used in the cardiovascular assessment of patients. Clinicians have long been under the misconception that systolic and diastolic blood pressures remain constant throughout the arterial tree. This is plainly not the case, and the examples below are used to illustrate how a greater understanding of differing haemodynamic parameters in the cardiovascular system leads to a greater understanding of frequently observed outcomes seen in clinical practice.

Previously, during exercise or treatment with vasoactive drugs, alterations in brachial/radial blood pressure, whether measured either non-invasively or invasively, have been taken to represent change in pressure in the central arteries i.e. the aorta. We are now aware that significant changes in central arterial haemodynamics can occur with little perceptible change in peripheral blood pressure parameters. It may be that by relying solely on brachial blood pressure we have failed to fully appreciate the action of certain pharmacological agents and this may go some way to explain why we have failed to see anticipated outcomes in response to therapy[77;78].

This is no more apparent than in the regression seen in left ventricular hypertrophy with antihypertensive drugs. We can now offer some explanation in terms of wave reflection and augmentation of central arterial pressure for the observation that certain pharmacological agents produce a greater degree of left ventricular hypertrophy regression than others, for similar falls in brachial blood pressure. It is predominantly those with vaso-relaxant therapies, (ACE Inhibitors and calcium channel blockers) which reduce wave reflection, augmentation and therefore cardiac after load, that have the most beneficial effects.[79]

To further illustrate this point, studies using GTN can be seen to have little effect on peak brachial arterial pressure, but due to their vasodilatory properties markedly reduce central systolic augmentation and subsequently reduce central aortic peak pressure.[77] (figure 5)

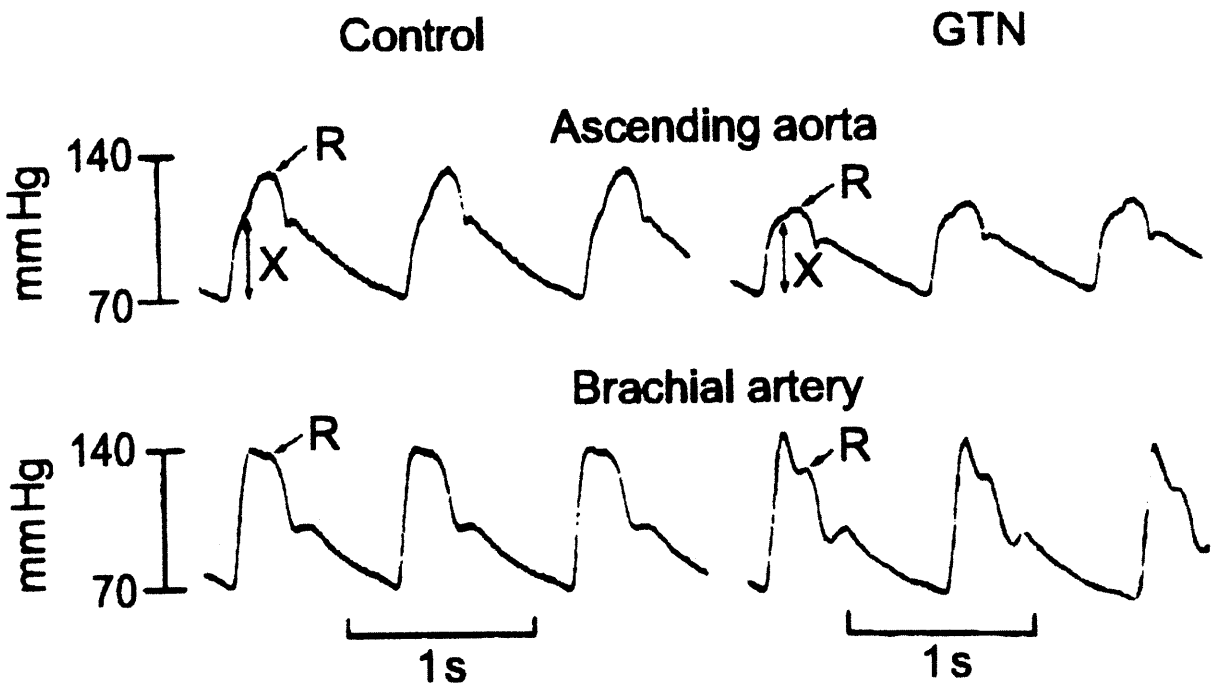


Figure 5. Effect of nitroglycerin on central and brachial waveforms due to alteration of reflected wave (R).[77]

Clinical trials are now in progress to assess various central haemodynamic parameters in response to various pharmacological agents and the relationship of these changes to those of conventional brachial blood pressure[80]. The results of these trials are awaited with much interest.

A better understanding of the principles of wave reflection and harmonics has therefore led to substantial progress in the field of arterial haemodynamics and a number of important principles required for subsequent discussion are outlined below.

1.7 Models of arterial wave propagation.

A number of models of arterial wave propagation have been suggested over the years to facilitate further study.

In the late 1930's, Hamilton and Dow suggested a simplistic model of arterial wave propagation in which the aorta was regarded as a blind ending tube with the heart at one end and the high resistance arterioles at the other [81](cited in O'Rourke [52]). (figure 6)

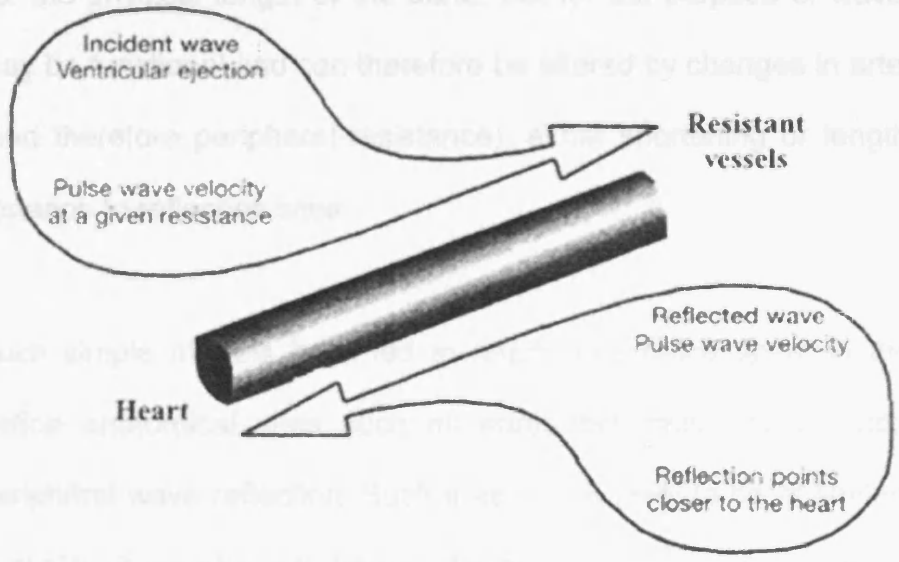


Figure 6: Determinants of the arterial pulse pressure[82]

A pressure wave generated by the heart travels via the aorta to the periphery. It is reflected back towards the heart, and subsequently augments the outgoing wave, depending on where in the cardiac cycle it

returns. Return can be influenced therefore by several fundamental factors namely speed of transmission (Pulse wave velocity), length of the conduit and cardiac cycle time (predominantly heart rate). Speed of transmission (Pulse wave velocity), is obviously determined in part by the degree of stiffness in the tube, stiffer tubes allowing less damping of the pressure wave and therefore greater velocities. The correlation to 'stiffness' is by the relationship to *Young's Modulus* (and therefore indirectly arterial distensibility) as defined by the Moens-Korteweg equation ($PWV=\sqrt{Eh/2\rho R}$), where E=Young's Modulus, h=wall thickness, ρ=blood density and R=arterial radius at end diastole.[45] Length of the conduit may be structural i.e. the physical length of the aorta, but for the purpose of wave reflection may be functional and can therefore be altered by changes in arteriolar tone (and therefore peripheral resistance), either shortening or lengthening the distance to reflection sites.

Such simple models have led to much time being spent in the quest to define anatomical sites such as aortic bifurcation as principal sites of peripheral wave reflection. Such a search is likely to be of limited value, as in reality, it is unlikely to be one distinct point, but more a summative wave front propagated by different peripheral sites. It is also likely to be influenced by a number of factors in any individual at any one time, as peripheral resistance and arteriolar tone is a dynamic and not static measure.

By the late 1950's McDonald and co-workers postulated that wave reflection was too low and attenuation too high to make this simple bouncing of waves

from aortic valve and periphery and back a realistic model [83;84] (again cited in O'Rourke[52]). They suggested a quantitative frequency analysis approach to assessing arterial pressure waves, which has led to a far greater understanding of vascular haemodynamics than ever before. This model looked at pressure waves by interrogating them in the same wave as we do sound waves. Any given sound can be broken down into its component harmonics for subsequent analysis and pressure waves are no different in this regard (Figure 7). Given that each harmonic of pressure has a specific amplitude (known as the *modulus*) and delay from a set reference point (known as *phase*); from any given peripheral wave, we are able to re-synthesize the original central wave[52].

In addition, it is accepted that for any given point in an artery there is a linear relationship between pressure and flow. By utilizing this relationship, we are able to calculate vascular impedance in specific vessels and also subsequent co-efficient of reflection. Studies have suggested that in humans, the coefficient of reflection is approximately 0.8 (that is to say the reflected wave from the periphery has an amplitude of 80% of the incident wave), and that the majority of wave reflection occurs from the lower body. Infusion of vasodilators reduces calculated co-efficients of reflection to zero and conversely infusion of vasoconstrictors raises the co-efficient towards one. Using these co-efficients one can estimate the distance of the cumulative reflecting sites from the source in an individual for any given frequency and velocity of an arterial pressure wave[52].

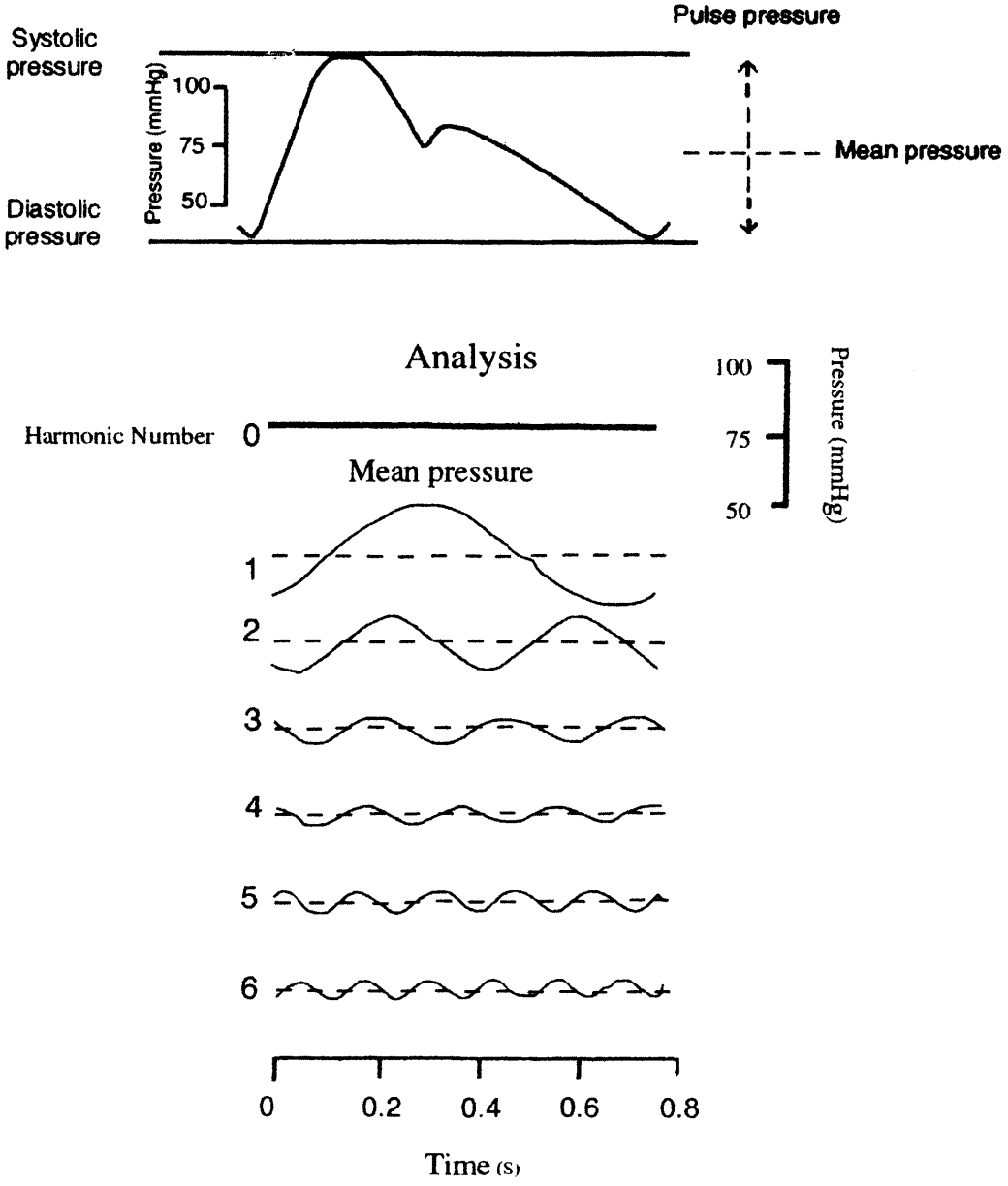


Figure 7: Fourier Analysis of arterial pressure waves [82]

Utilizing these concepts, it has been modelled that the vascular system in humans can be divided roughly into two functionally distinct reflecting sites at different distances from the heart.

These sites are thought to be representative of the upper and lower body in parallel and have been described as a simple T-tube model (figure 8). This model helps to explain the contour of the central wave including wave reflection in human subjects. In normal subjects, the tidal wave initially returns early from the reflecting sites in the upper body to indent on the outgoing wave. The main reflected wave from the lower body sites returns to the central vessels to create what we know as the diastolic wave. With ageing (and hypertension), predominantly increased aortic pulse wave velocity results in earlier reflection from the lower body and the tidal wave is now a result of the combined reflection from upper and lower body reflection sites, which in turn summate (*Augment*) the outgoing or incident wave.[85]

1.8 Generalized transfer function.

Several validation studies have now confirmed that by using the above principles, in terms of wave contour at least, derivation of central arterial waves from both carotid and radial traces via a generalized transfer function give comparable results to waveforms derived invasively (with >90% accuracy). [86-89]

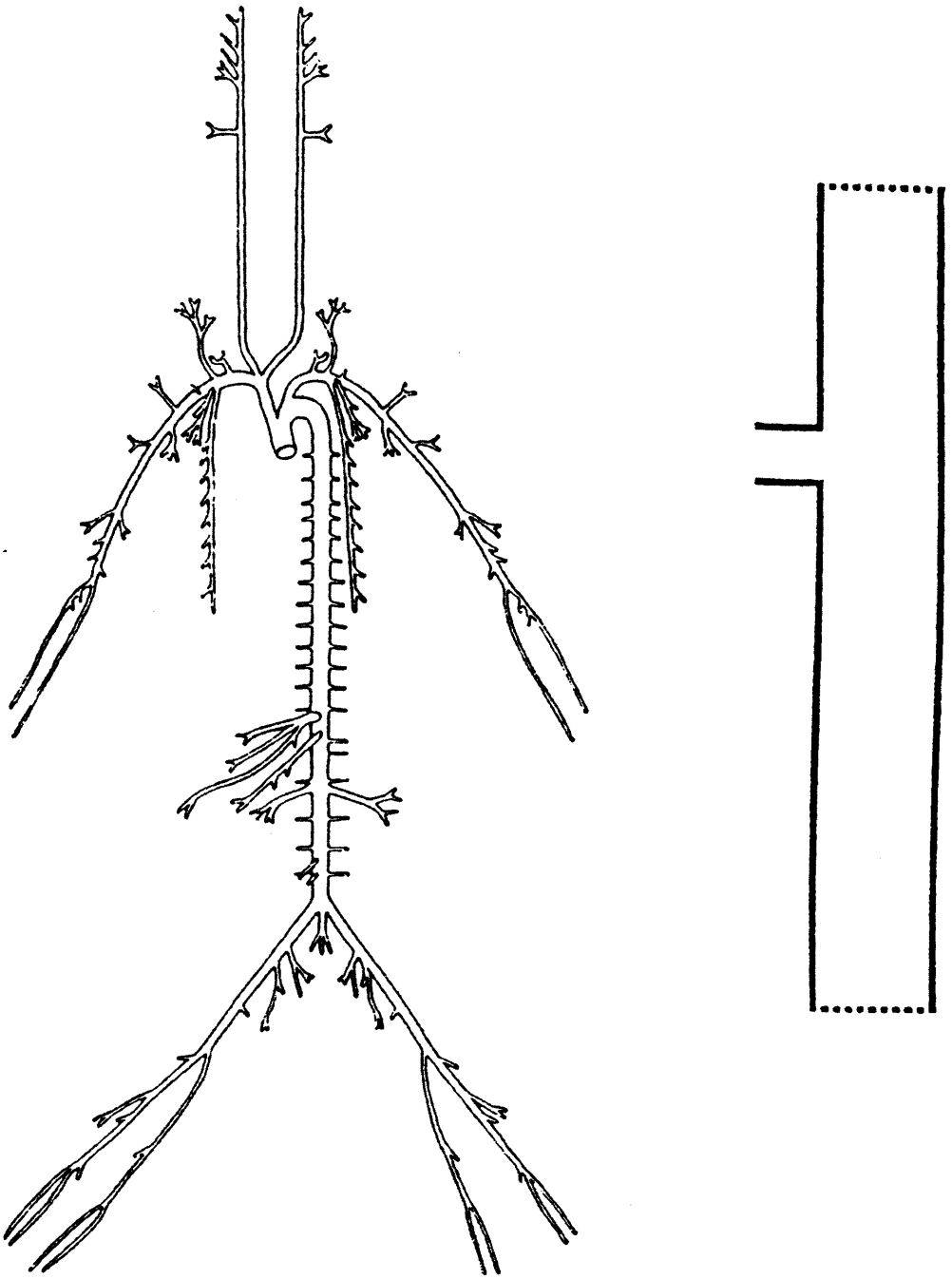


Figure 8:T-tube model of the arterial circulation in man.[85]

Limitations occur in measurement as even in experienced hands, carotid applanation tonometry is difficult and accurate applanation is unlikely to be achieved due to the anatomical position of this artery. For this reason internal calibration and estimation of pressure values is not recommended and external calibration of the trace by brachial blood pressure is suggested to enable estimation of central haemodynamic parameters[90].

Mean brachial arterial pressure is assumed to roughly equate to mean radial and carotid arterial pressure for these calculations and obviously, the system relies on cuff sphygmomanometry for its calibration with all its inherent inaccuracies. Of all the stages in this non-invasive assessment it is the inaccuracies in brachial blood pressure measurement that are likely to introduce one of the greatest sources of error[91;92]. Despite these potential problems, non-invasively derived central pressures reflect invasively measured parameters reasonably well[86-88].

Much debate has been raised over the use of a generalized transfer function, as potentially individual transfer functions derived for each subject would be preferable[87;93]. This however would be impractical, and as suggested above a generalized transfer factor produces acceptable values not only at resting states but also during alterations in wave reflection (e.g. GTN infusion to decrease or handgrip exercises known to increase wave reflection).

In terms of radial artery pulse wave analysis, reasonable approximation is likely to be achieved due to the fact that brachial pulse wave velocity remains largely unchanged with age, upper limb length differs little between adults and that the brachial artery is seldom affected by pathological processes responsible for altered vascular stiffness, namely athero/arteriosclerosis. The fact that a generalized transfer function appears to approximate individual transfer functions has lead authors of validation studies to suggest that body morphology, age and sex may not be as strong determinants of transfer function as the pressure amplification due to vascular branching in the upper body, which occurs in all subjects [88].

Caution would obviously be advised however in using generalized transfer functions to synthesize central waveforms using lower limb pulses such as from femoral arteries. Caution needs also to be advised in using generalized transfer functions in patients with disease processes that may affect the cardiovascular system e.g. diabetes, as patients with these conditions were not included in validation studies[94;95].

Concern has been raised however regarding the derivation of Central augmentation Index from peripheral waves using the technique of applanation tonometry and generalised transfer function, when calibrated non-invasively[91;95-100]. Indeed recent publications show little relationship between central aortic waveforms and augmentation index 'generated' from peripheral arterial traces and those directly measured invasively[93;101;102]. Augmentation Index in particular relies heavily on high frequency

information, which may be prone to considerable artefact and therefore error. Calculation of central blood pressure parameters per se do not rely so heavily on high frequency information and may therefore provide more robust information[103].

It is important to note that the aforementioned concerns about the validity of the transfer function, Augmentation Index and other derived indices emerged only after I began the work contained in this thesis. As such, a more detailed review of these issues is confined to the discussion chapter.

Recently, some researchers have questioned the use of a generalized transfer function at all, and have explored whether similar haemodynamic information can be gathered instead from peripheral pulse applanation alone. Initial results would suggest that this may be the case and further results are awaited[99;103]

Whilst estimation of central arterial pressure by means of this technique is by no means perfect, it is a step forward to better understanding human arterial haemodynamics than the current clinical practice of assuming no difference in pressure between peripheral and central arteries!

Although invasive techniques have remained the gold standard for measurement of central haemodynamic parameters, they are not without considerable disadvantage. In addition to all the well-recognized complications of invasive vascular procedures, intuitively the presence of a

foreign body within the vessel lumen and traumatic damage to the endothelium must alter vascular function. Moreover, the majority of invasive techniques are not practical for either routine clinical use or indeed large-scale clinical trials. More importantly, they are not applicable to use in the community where the majority of patients with cardiovascular disease are cared for.

There are now a number of non-invasive technologies available to interrogate the arterial system. These techniques commonly utilize principles of Doppler ultrasound, digital pulse contour analysis and applanation tonometry (AT).

For this research, vascular structure and function was assessed using applanation tonometry via a commercially available system (Sphygmocor TM).

Although enjoying a resurgence, the principles of applanation tonometry are not new however, and a little historical background of this technique is provided below.

1.9 Historical perspective.

Since the mid nineteenth century, doctors and scientists have recognized the importance of analysing the arterial pulse in the diagnosis of disease. Long before the introduction of the cuff sphygmomanometer, researchers

were interrogating the pulse using cumbersome devices which were difficult to calibrate and prone to artefacts (figures 9 and 10). Never the less, for all their simplicity and problems, these early sphygmograms were able to give doctors invaluable information regarding the clinical state of their patients.

Frederick A Mahomed performed some of the most significant work in this field[104;105]. Following on from Marey's original work in Paris[106], Mahomed, then a young doctor working a Guy's (London), produced a sphygmogram which allowed not only calculation of the "hold down" force applied to an artery but also a graphical trace of the arterial pulse pressure wave. Using this early instrument, he was able to categorize a group of patients he recognized as having elevated arterial pressure and a "sustained tidal wave" on their arterial trace. This group is now recognized as having essential hypertension. Unfortunately, a combination of Mohamed's premature death and the arrival of the Riva-Rocci cuff sphygmomanometer in the early nineteen hundreds led to a gradual demise in interest in the technique until fairly recently[107].

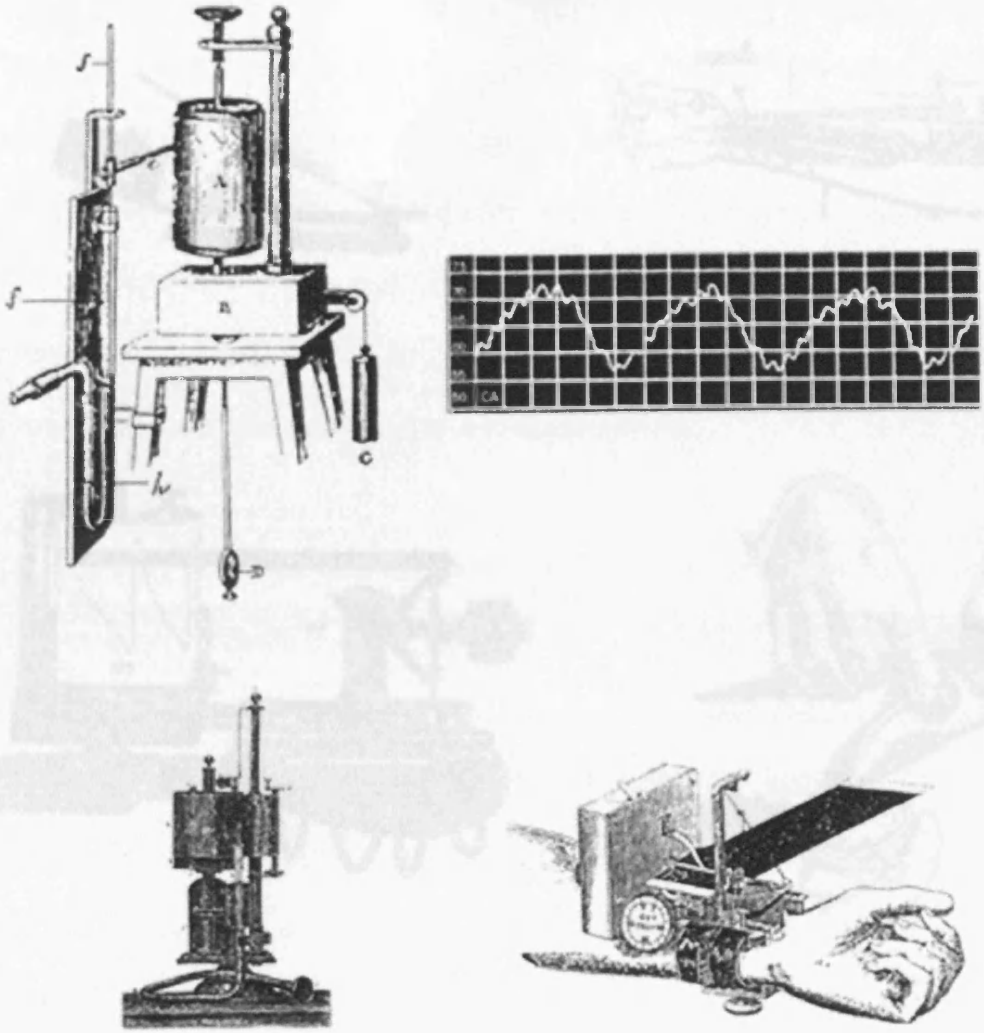


Figure 9: Some historical Sphygmographs [82]

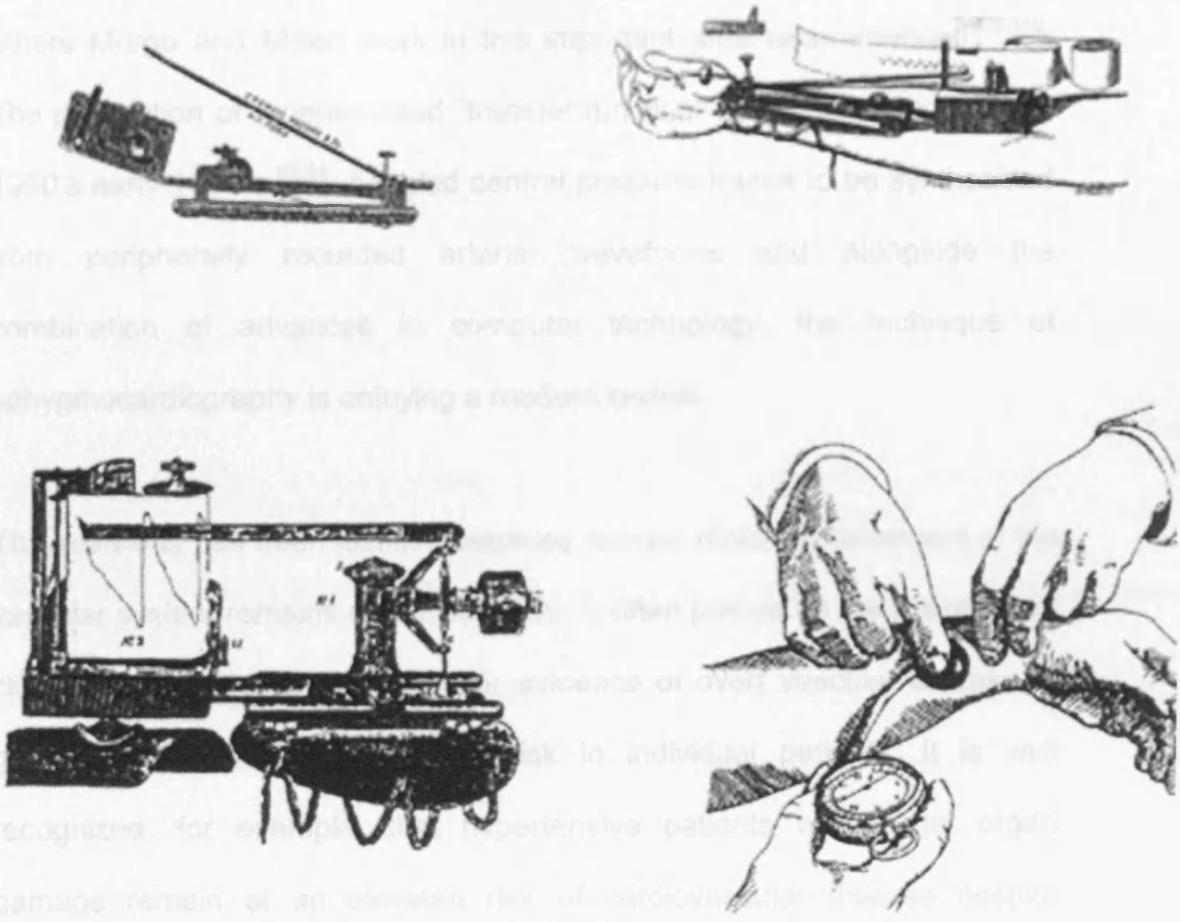


Figure 10: More Historical sphymographs including that used by Mahomed [82]

With the development of accurate piezo-electric manometers by amongst others Murgo and Miller, work in this important area recommenced[108-110]. The publication of a generalized "transfer function" by McDonald in the late 1950's early 1960's [83;84], enabled central pressure traces to be synthesized from peripherally recorded arterial waveforms and alongside the combination of advances in computer technology, the technique of sphygmocardiography is enjoying a modern revival.

This revival is has been justified because current clinical assessment of the vascular system remains crude. Reliance is often placed on the presence of clinically overt structural damage or evidence of overt vascular disease to guide the estimation of vascular risk in individual patients. It is well recognized, for example, that hypertensive patients with target organ damage remain at an elevated risk of cardiovascular disease despite optimal treatment aiming to normalize blood pressure[64;111]. It is conceivable that structural changes may not be completely reversed by drug therapy once they are fully established.

Consequently, new techniques are required to define occult vascular damage and thereby more accurately stratify risk. It is conceivable that the use of non-invasive technologies to simply detect disturbances in vascular function prior to the development of overt cardiovascular disease could offer us a potentially important window of opportunity for earlier therapeutic intervention. It was the purpose of this thesis to fully evaluate applanation tonometry, and pulse wave analysis, in this context and define whether this

technique was sufficiently robust and free from confounding factors to allow its simple use in the clinical assessment of patients with cardiovascular disease.

1.10 Applanation tonometry

Modern pulse wave analysis utilizes the principal of applanation tonometry and high fidelity pressure sensors (tonometers) to measure peripheral arterial waves. Applanation tonometry is not a new technique and as noted previously the basic principle has been around for many years. In fact, the technique has been used successfully for decades in the practice of ophthalmology where direct orbital tonometry is used to estimate intra-ocular pressure for the diagnosis of glaucoma.

The principle relies on the fact that if the wall of a vessel is flattened perpendicular to the surface of a probe then the circumferential pressures within the wall will be equalized and the pressure from within the vessel will be transmitted directly to the probe. Obviously if insufficient applanation is achieved then inaccurate results will be obtained. Similarly, if too much hold down pressure is applied then the artery will be distorted and abnormal pressures generated within it.

Whilst in ocular tonometry direct visualization can confirm adequate applanation, this is not possible with arterial tonometry, which is usually

performed at a peripheral site such as the radial artery. An additional complicating factor in vascular applanation tonometry is that tissue overlying the vessel provides a physical barrier between the tonometer and the vessel being studied which can distort recordings. Arterial sites are chosen where the artery is relatively easily accessible and can be applanated by compressing against underlying tissues or bone-in this context, the radial artery has been the preferred site for most studies.

Whilst the carotid artery has the distinct advantage of being nearer to the central arteries it is often deep and compression against underlying tissues is not always easy and potentially hazardous, making AT at this site difficult without considerable practice. There are also the theoretical problems of stimulating carotid bodies with subsequent changes in heart rate and blood pressure in addition to the concern of potentiating an embolic cerebrovascular event in high-risk individuals. This has never yet been reported in the literature, but even so, most individuals using this technique would be cautious in applanating the carotid artery of a patient with documented carotid artery disease, cerebrovascular disease or carotid bruits.

The radial artery has therefore been adopted as a preferred site at which to perform the applanation tonometry technique. It is readily assessable, easy to applanate with only a little training and confers little risk or discomfort to the patient. Since direct visualization to confirm adequate applanation is not available, most operators rely on a combination of maximal amplitude of

waveform, predictable waveform shape and consistency between waveforms as a marker of optimal applanation. Several waveforms are recorded before being signal averaged to give a representative waveform for that arterial site. A computer is then used to analyze the waveform to identify various points as well as re-synthesizing a central waveform, with corresponding pressure recordings calibrated from brachial blood pressure as described above. An example of such a waveform is given below (figure 11).

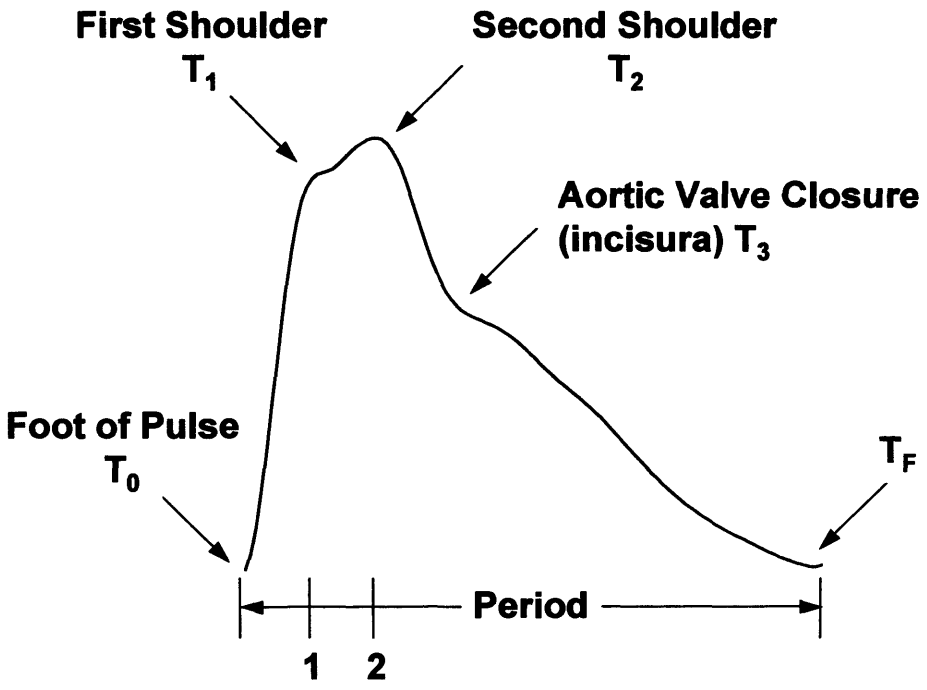


Figure 11. The basic features of the arterial pulse.

(After the onset of ejection (T_0), the pressure wave rises to an initial shoulder (T_1), which relates to timing of peak flow, and then proceeds to the

second shoulder (T_2) relating to the reflected waves. The end of ejection (T_3) is associated with closure of the aortic valve (incisura)).

To orientate the data to be presented in this thesis, additional examples of pulse wave traces are shown below (figure 12 A). To reiterate, during left ventricular systole, an increase in pressure is generated resulting in a maximum peak pressure P1. This maximum pressure decays into diastole and is notched by aortic valve closure as indicated by T3. As the systolic pressure wave reaches the periphery, it is reflected back towards the heart where it ordinarily augments the diastolic wave to aid coronary perfusion and left ventricular filling.

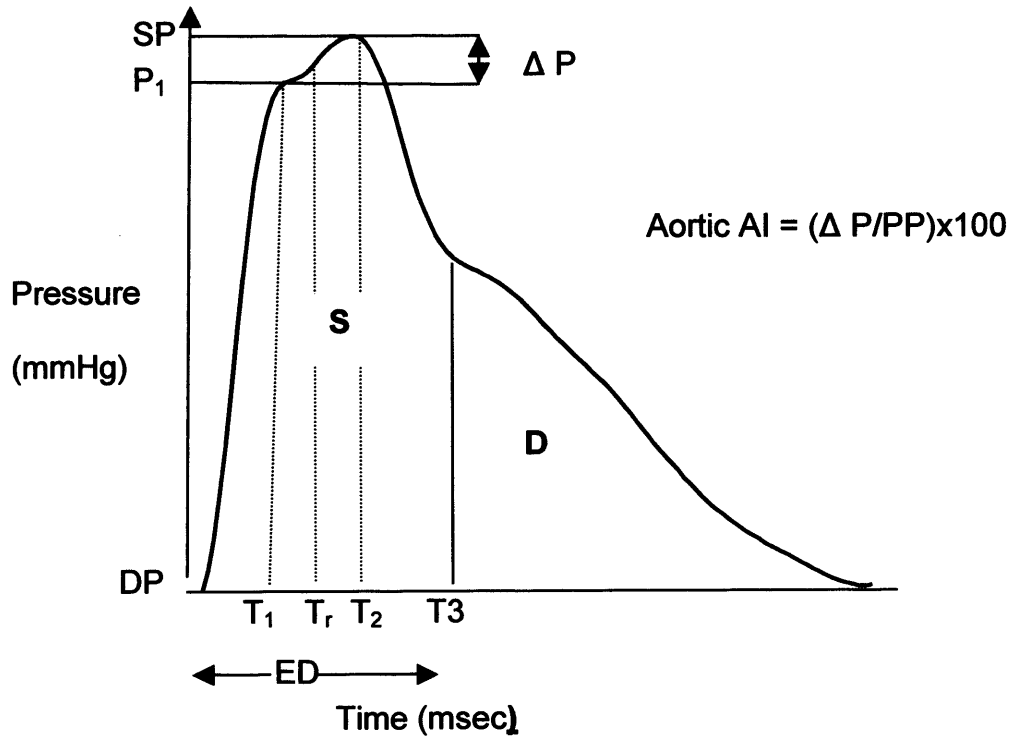
An increase in vascular stiffness results in an increased velocity of the outgoing pulse wave and hence reduces time for peripheral wave reflection to occur. The reflected wave therefore arrives earlier in the cardiac cycle and returns towards the end of systole rather than during diastole. This produces systolic wave summation and an elevation in maximal generated pressure (P2), a process known as *systolic wave augmentation*.

The augmented wave is indicated on the figure as P2. When augmentation is expressed as a percentage of pulse pressure, it is referred to as the *Augmentation Index.* (AI) and is annotated on the figure as ΔP.

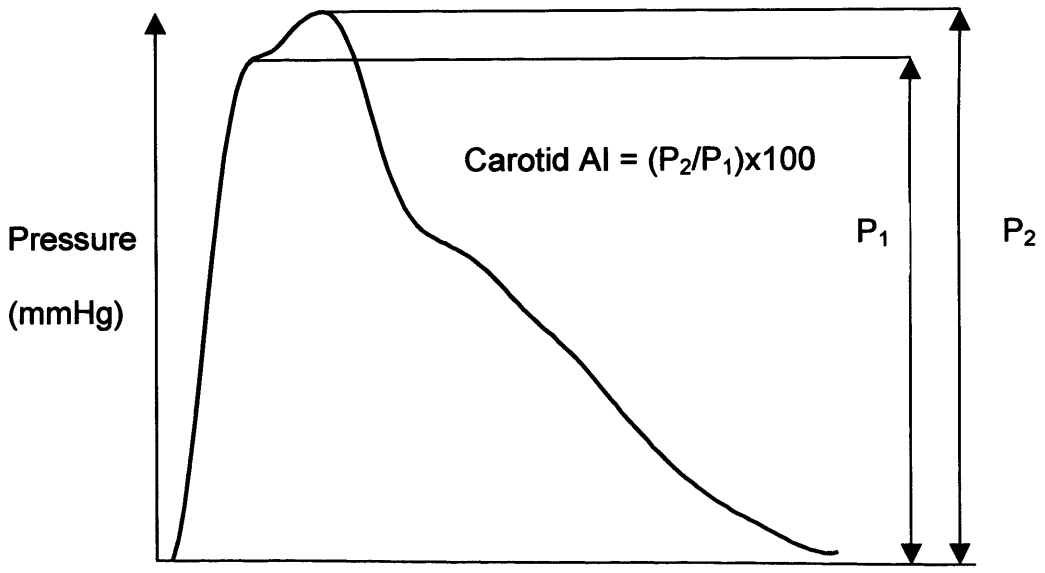
A number of other parameters can also be derived from the arterial trace, namely the time to reflection of the returning wave, known as Tr which may

be indicative of pulse wave velocity in the arterial tree. The rate of change in pressure over time (dp/dt) can also be calculated and provides an indication of the strength of cardiac contraction.

A.



B.



C.

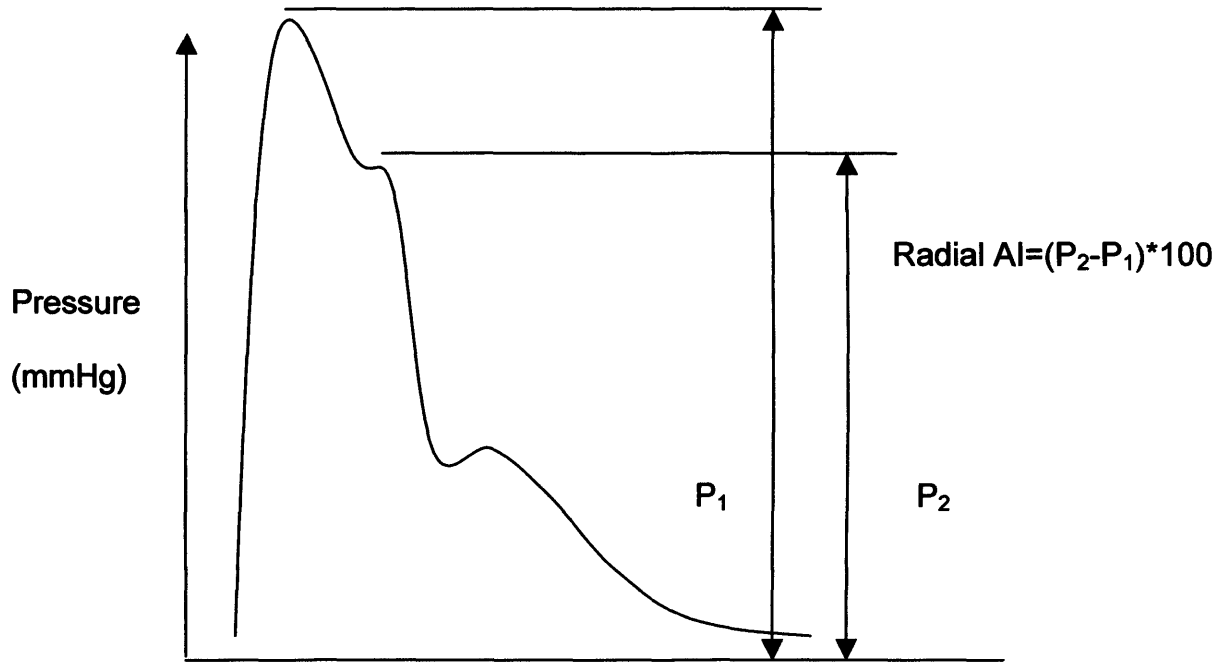


Figure 12. Augmentation index and tonometry parameters derived from (A) the central aortic pressure pulse, (B) the peripheral carotid pressure pulse and (C) the radial pulse.

Figure 12 legend: (A) SP, systolic pressure, DP, diastolic pressure, PP, pulse pressure (SP-DP), P_1, pressure of the outgoing pressure wave, ΔP, augmentation, AI, augmentation index, T_1 time to the peak of the outgoing pressure wave, Tr, time to the foot of the reflected wave, T_2, time to the peak of the reflected wave, ED, ejection duration, solid vertical line represents the incisura, area under the pressure waveform during systole represents tension time index (TTI), area under the pressure waveform

during diastole represents diastolic time index (DTI). (B) (C) P_1, pressure of the outgoing pressure wave, P_2, pressure of the outgoing plus the reflected wave, AI, augmentation index.

Waveforms are different in contour depending on the arterial site that they are recorded[112]. The nearer the periphery, the greater the contribution of the reflected wave to augmentation of the outgoing wave. This phenomenon is responsible for the well recognised amplification of the pulse from central to peripheral arteries. This is most obvious in youth with compliant elastic large arteries but gradually reduces with age and increasing vascular stiffness. As stiffness increases, so does pulse wave velocity, and subsequent increases in central augmentation raises central aortic pressures toward those of peripheral vessels and the amplification 'gradient' falls[112]. Examples of pulse waveforms derived from various arterial sites are shown in figure12 below.

Even in health, the waveform is profoundly altered by a number of physiological factors such as blood pressure, heart rate, height and age to name just a few[90]. It is therefore necessary to have a full understanding of the alteration in wave contour and subsequent haemodynamic parameters with normal ageing and in a control population, before the technique can be used to compare differing pathological processes and therapeutic interventions between patient groups.

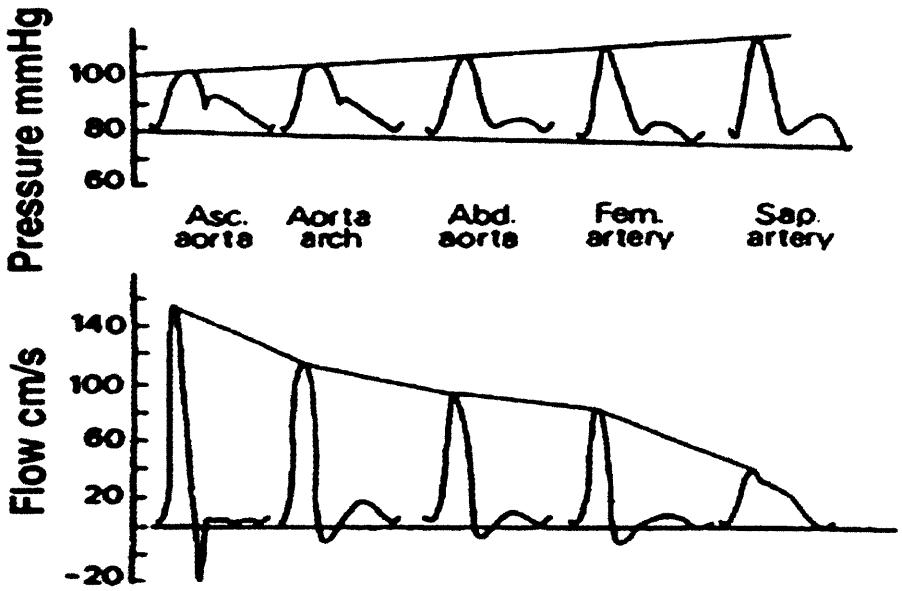


Figure 13. Contour and amplitude of pressure and flow waves in arteries as they travel away from the heart. [84]

Determination of inflection points on arterial pressure waves is carried out automatically by computer software. This relies on complex mathematical algorhythms to correlate points from the first derivative of the individual harmonics of the arterial pressure trace in relation to the zero line. Although largely accurate, detection of particular points on the pressure trace, and in particular the inflection point on the outgoing pressure wave (annotated T1 on figure 12 A), may be inaccurate, inappropriately labelled or incalculable, and may therefore introduce significant error or result in erroneous augmentation indices or Tr values (time to return of the reflected wave).

1.11 Key Definitions

In order to facilitate understanding of the data and subsequent discussion, a list of definitions of commonly used PWA parameters is provided below using figure 12 for illustration. (Key terms in bold).

Augmentation = ΔP = SP- P1 (mmHg)

P1 = Pressure of outgoing pressure wave (mmHg)

P2 = Pressure of outgoing pressure wave + reflected wave

T1 = Time to peak of outgoing pressure wave (msec)

T2 = Time to peak of returning pressure wave (msec)

T3 = Aortic valve closure (msec)

Tr = Time to foot of reflected wave (msec)

Aortic Augmentation Index (AI/CAI) = ΔP/PP x 100 (%)

Carotid Augmentation Index = P2/P1 x 100 (%)

Radial Augmentation Index (PAI) = P2/P1 x 100 (%)

Ejection duration (ED) = Time to aortic valve closure (msec) (or as percentage of one complete cardiac cycle)

Diastolic duration (DD) = Time from aortic valve closure to foot of P1 (msec) (or as percentage of one complete cardiac cycle)

Amplification = ratio of PPP: CPP (ratio or %)

Tension Time Integral/TTI/Systolic work = area under curve during systole (S)

Diastolic Time integral/DTI = area under curve during diastole (D)

Buckberg ratio/sub-endocardial viability Index (SVI) = DTI/TTI x 100 (%)

1.12 Rationale for project.

We recognise that there were many variables that could potentially confound the interpretation of arterial wave forms and derived parameters using applanation tonometry. The technique was being uncritically adopted as a measure of the integrity and function of the arterial system and we wished to formally assess the impact of all of these variables and potential confounders.

It was important to conduct a detailed study of the impact of multiple clinical characteristics on pulse waveforms and indices derived from applanation tonometry in a 'normal' control population and in those with evidence of hypertension and vascular disease.

The aim of this study was to initially gain a more complete understanding of not only age related changes, but to study the impact of physiological variables and demographic parameters on arterial wave contour in a normal/control group. This would allow a greater understanding of how different patient groups could be studied and what factors would need adjustment or consideration prior to comparison. Once the technique had been evaluated in a normal cohort then it was applied in patients with various disease states known to be associated with accelerated vascular ageing.

This thesis therefore evaluated a novel measurement technique for the non-invasive assessment of vascular structure/function in the context of "normal ageing". It assessed the impact of various pathological states known to be clinically associated with accelerated vascular ageing, such as hypertension and diabetes. It also discussed the potential for the use of this technology in ongoing clinical research and clinical practice.

The following chapters will therefore concentrate initially on non-invasive tonometrically derived measures of vascular structure and function in 1) a normal control population before addressing 2) the impact of hypertension and 3) diabetes.

Other groups have undertaken this kind of evaluation. However, at the time this study began, there was very little information available with regards the formal evaluation of this technology in a normal control population and in those with cardiovascular disease.

Moreover, what studies were available were small and inconsistent in their findings. Consequently we were concerned that the technique of applanation tonometry was being uncritically accepted to measure "aortic stiffness" without full evaluation of potential confounders when comparing various disease states.

1.13 Hypothesis and summary of aims

Applanation tonometry and pulse wave analysis are increasingly used in clinical practice to non-invasively evaluate vascular function, in particular arterial stiffness. We hypothesised that many of parameters derived from AT and PWA were insufficiently stable and potentially confounded by too many physiological and physical variables to provide accurate assessment of arterial stiffness in patient populations of interest. To evaluate this hypothesis we had 3 specific aims:

To evaluate AT and PWA characteristics in a large healthy volunteer population without clinical evidence of vascular disease, across a wide age range.

To evaluate AT and PWA characteristics in a population of young patients with both borderline and established hypertension to evaluate whether this technique would be able to discriminate between groups and show evidence of increasing 'arterial stiffness' with increasing severity of hypertension in the absence of conventional target organ damage.

To evaluate AT and PWA characteristics in a population of patients with diabetes, to confirm or refute small studies showing elevated 'arterial stiffness' in these patients using this technique.

As our experience with PWA developed, like with application of all new technologies we learned important lessons on the way. Following in house reproducibility studies, we added more direct measurements of vascular stiffness, in the form of pulse wave velocity, to our non-invasive cardiovascular assessment of patients, and these will be discussed in later chapters.

This work therefore chronicles our experience with this novel technique from an initial basic understanding through to incorporation into large clinical trials as mentioned above.

Obviously the vast heterogeneity and extreme bio-variability of the general population make the validity of clinical studies of this nature dependent on random sampling, in addition to recruiting large numbers. To this end, we first set about establishing a large control database of well-characterized people, which would form a comparator group for all our subsequent studies.

2.0 General Methods

Due to the fact that all studies were performed as stand alone studies, detailed descriptions of specific issues relating to methods are noted at the beginning of each chapter. Applanation tonometry techniques have been discussed earlier and are outlined again below in brief.

2.1 Subject recruitment

Subjects were recruited in different ways depending on the particular cohort under investigation.

Normal healthy "Control" subjects were recruited subjects from poster advertisements in a large teaching hospital setting and were a mix of staff, visitors, family members or interested friends. For all studies, only adults over the age of 18 years were approached, in part because the transfer factor used for applanation tonometry has not been adequately validated in children.

Hypertensive subjects were recruited from the Leicester Hypertension Clinic, based at the Leicester Royal Infirmary, from local General Practitioners and from patients with hypertension who had previously been screened for other clinical studies but had failed to meet the entry criteria for these studies.

Diabetic subjects were recruited from local diabetes clinics at the Leicester Royal Infirmary and from interested local General Practitioners

Patients with Chronic Renal Disease were recruited from a local nephrology clinic based at the Leicester General Hospital.

As noted above, more details regarding individual patient groups can be found in the relevant chapters.

Ethical approval for all studies was obtained from the Leicestershire Health Research Ethics Committee and all participants provided written informed consent prior to entry into the studies. (See appendix)

2.2 Blood pressure measurement

In all studies, peripheral blood pressure was measured over the brachial artery of the right arm after a five-minute rest, using a British Hypertension Society approved automated oscillometric sphygmomanometer (Omron 705CP).

Three readings were taken five minutes apart and the mean of the last two readings were used as the measure of peripheral blood pressure. This was used to calculate mean arterial pressure (MAP), which was used to calibrate the arterial wave traces. MAP was calculated as diastolic blood pressure (DBP) + 1/3 pulse pressure (PP) i.e. MAP = DBP + 1/3 PP

2.3 Pulse Wave Analysis

Immediately following blood pressure measurement, applanation tonometry was performed using a commercially available system (Sphygmocor TM) at the right radial artery site. The study subject remained seated for this measurement. Quality control was ensured by adhering to predetermined criteria of pulse height and variability. (Pulse height >100 mV, pulse height variability <10% and diastolic variability<10%). Any recordings not satisfying these criteria were discarded and the measurement repeated.

2.4 Pulse wave velocity

Using the same commercial system described above, carotid to femoral pulse wave velocity (Aortic pulse wave velocity) was calculated by using the "foot of the pulse wave to foot of the pulse wave method". This method uses the time difference (t) between the initial upward deflection of carotid and femoral traces calculated from the R wave of a simultaneously recorded three lead ECG as a reference point. Surface distance between the two arterial points (right carotid and right femoral) was measured from the supra-sternal notch to the carotid pulse (Distance A) and to the femoral pulse (Distance B). Distance travelled was calculated as distance B-distance A. Pulse wave velocity was then calculated from this distance divided by the time interval (t) and expressed in metres per second.

2.5 Laboratory analysis

Analysis of blood and urine samples for all parameters was performed through the local NHS laboratories at the Leicester Royal Infirmary and the Leicester General Hospital. Local laboratory quality control was applied to all samples in the same way as standard NHS samples

2.6 Statistical Analysis

Statistical analysis was performed utilizing commercially available software SPSS (version 11.5) and the specific methods used are detailed in relevant chapters.

3.0 The effects of ageing on vascular structure and function in a normal, healthy population (Results1).

3.1 Introduction

Recommendations of the World Health Organization for future cardiovascular research reinforce the need for further development of both non-invasive assessment and the use of surrogate markers of cardiovascular function in the investigation and management of patients with cardiovascular disease[16].

It is now widely accepted that conventional blood pressure measurement derived from the brachial artery using a cuff sphygmomanometer is an extremely crude marker of cardiovascular structure and function. Although providing a numerical value, it gives no information regarding the character of the pulse waveform and assumes a number of incorrect assumptions. Blood pressure is not the same throughout the vascular system and merely knowing the maximum and minimum pressures in a peripheral artery provides only basic information about the pressure load experienced by the vascular system. This is especially true when considering the effects of antihypertensive therapy on cardiovascular outcome and the development of end-organ damage. As alluded to previously, pharmacotherapy may profoundly affect vascular stiffness and central haemodynamic parameters and yet produce little or no noticeable effect on sphygmomanometrically derived brachial blood pressure[76;77;90;113-119]. Thus, the impact of

pharmacological interventions on blood pressure may underestimate potentially important differences induced by different drug classes on central haemodynamics. This may go some way to explain why alterations in brachial blood pressures have not had the expected beneficial effect on cardiovascular morbidity and mortality (in particular coronary heart disease events) that has been predicted by epidemiological population studies[120].

It remains to be seen whether agents that predominantly alter vascular tone (e.g. ACE Inhibitors and Calcium Channel Blockers) have a more beneficial effect on cardiovascular and stroke outcome than those which achieve blood pressure reduction via other mechanisms such as volume reduction (e.g. diuretics)[121]. Although the effects of specific agents on outcome holds academic interest there is now much evidence and widespread support for the notion that it is a reduction in blood pressure per se, rather than the agent used to achieve this reduction that has the greatest impact on mortality and morbidity reduction.[122]

The effects of various agents on pulse wave analysis parameters are well known, but whether indirect markers of arterial stiffness e.g. Augmentation index or central haemodynamic parameters versus conventional brachial blood pressures provide greater predictive value or guides treatment of hypertension remains to be determined[80].

Vascular stiffness, primarily indicated by elevation in aortic pulse wave velocity, has been shown to be an independent predictor of both

cardiovascular and all-cause mortality not only in patients at high risk of vascular disease such as those with hypertension[69;74], diabetes[123] and chronic renal disease[124] but also in elderly patients seemingly free from clinical disease[73].

With regards to augmentation Index as a surrogate marker of arterial stiffness, outcome data in terms of hard endpoints is limited and largely confined to individuals with end stage renal failure[125].

As noted in the introduction, we decided to evaluate the impact of conditions such as hypertension and diabetes, as well as normal biological ageing on vascular functional parameters as revealed by applanation tonometry. This was important to independently evaluate the impact or otherwise of many potential confounders that may or may not limit the use of this technique to non-invasively assess vascular function and its response to treatment.

Current clinical practice suggests an overall risk-based approach to the management of cardiovascular disease, but in any model of cardiovascular and stroke risk, age is of paramount importance[64].

Age remains a powerful predictor of cardiovascular mortality and morbidity, but despite a wealth of studies attempting to document alteration in vascular structure and function with age, dissimilarities in inclusion criteria, method of measurement and arterial site studied make direct comparison and

subsequent application of results into clinical practice difficult and somewhat limited[28-30;112;126].

As discussed earlier, non-invasive assessment of the vasculature has, in some form, been around for over a century, however, integration of this technology into clinical trials has been lacking. Consequently, use of this technology in clinical practice to date has also been limited. Due to the perceived ease (and accuracy) of recording and interpreting brachial blood pressure, the additional information provided by pulse wave analysis has largely been over looked until recent years. That blood pressure has in fact proven itself to be an extremely important risk factor and predictor of cardiovascular and stroke outcome cannot be denied. This should not however preclude the quest for more robust, sensitive or predictive measures of outcome in the future.

Conceptual difficulties in understanding the principles underlying non-invasive arterial measurements have not made progress to clinical application easy! Arterial haemodynamics are complex and unfortunately, when one attempts to discuss them in any detail, one quickly enters the realm of fluid mechanics and theoretical physics and the average clinician is soon lost before reverting to the safety and simplicity of numerical values as provided by brachial blood pressure.

The technique of applanation tonometry (as discussed earlier) assesses the relationship of pressure with time during pulse wave travel throughout the

arterial system. This is achieved by interrogating accessible points (e.g. radial, carotid and femoral pulses) where the artery is amenable to being applanated (or flattened) against the underlying tissue/bone, thus allowing a peripheral arterial pressure: time trace to be recorded. Using a validated transfer function (as previously discussed), central pressure waves can then be reconstructed and central haemodynamic parameters calculated.

Radial artery applanation tonometry has proven an attractive technique, as it is easy to perform in the clinical setting, and requires relatively little training before reproducible results are obtained [127;128]. The radial artery is easy to applanate (unlike carotid and femoral sites) and requires relatively little preparation or positioning of the patient.

In recognizing the possible potentials of this technique in providing a more complete vascular assessment of patients with cardiovascular disease, we proposed to apply this technology to subjects in a large-scale ongoing clinical trial. Prior to such application however, we decided to fully investigate the variability in measured parameters in a normal population in addition to assessing the impact of various disease states, which will be discussed in turn later.

Although the technique of applanation tonometry is relatively easy to perfect, pioneers of the technology advocate that researchers interested in using these techniques should perform their own in-house studies of

reproducibility as part of their learning curve prior to embarking on formal clinical studies[90].

Not only does this provide a degree of reassurance in terms of quality control, but importantly allows independent operators to compare results. Invaluably it also provides a reference control dataset for subsequent research, which may also be utilized by other investigators in this field.

As documented previously, a number of groups have now shown applanation tonometry to be a simple and reproducible technique[127-129]

Following our own confirmation of reproducibility of radial applanation tonometry[127], we set about studying a normal population to assess the impact of ageing and other variables on central arterial haemodynamic parameters and estimates of arterial function as provided by pulse wave analysis.

It is important to recognize when utilizing PWA, that derived measurements such as augmentation index, often used as a surrogate marker of vascular stiffness[130-134], are in fact composite measurements, comprised of a number of factors.

Speed of wave transmission (PWV), distance to reflecting sites (whether function or structural), peripheral impedance to flow, rate of change in pressure over time (related to force of ventricular contraction in addition to

vascular compliance), mean arterial pressure and cardiac cycle time (namely heart rate) all play an important part.

Documentation of alteration in PWA parameters in any population are therefore a complex interaction between all these variables and make interpretation of indirect measurements hazardous, especially when heterogeneous populations are studied. Whilst ease of application is an important consideration in any new technique, and although producing reproducible measurements, the range of AI values in any given population is large. It is now well recognised that relatively large numbers of patients, or interventions with large perceived effects, are required for clinically significant changes in AI to be noted [129;135].

As discussed previously, the benefits of the radial artery tonometry confer a number of practical advantages over other sites, which is imperative if the technique is to gain widespread clinical acceptance and use.

During normal ageing, alterations in vascular haemodynamics lead to remarkably predictable alterations in wave contour, responsible for a number of the cardiovascular (mal)adaptations we observe clinically. With age, progressive vascular stiffening leads to an elevation in pulse wave velocity and subsequent earlier wave reflection from the periphery. As mentioned earlier, this early wave reflection impacts on the incident wave produced by left ventricular systole to augment the systolic wave. In addition to increasing cardiac afterload and promoting ventricular hypertrophy, this

ventricular-vascular interaction might act to reduce end diastolic pressures and subsequently reduce coronary artery perfusion.

With age, the amplification of pulse pressure from the central arteries to the periphery (seen in a compliant vascular system) is lost as a result of this increasing central systolic augmentation. (see figure 1)

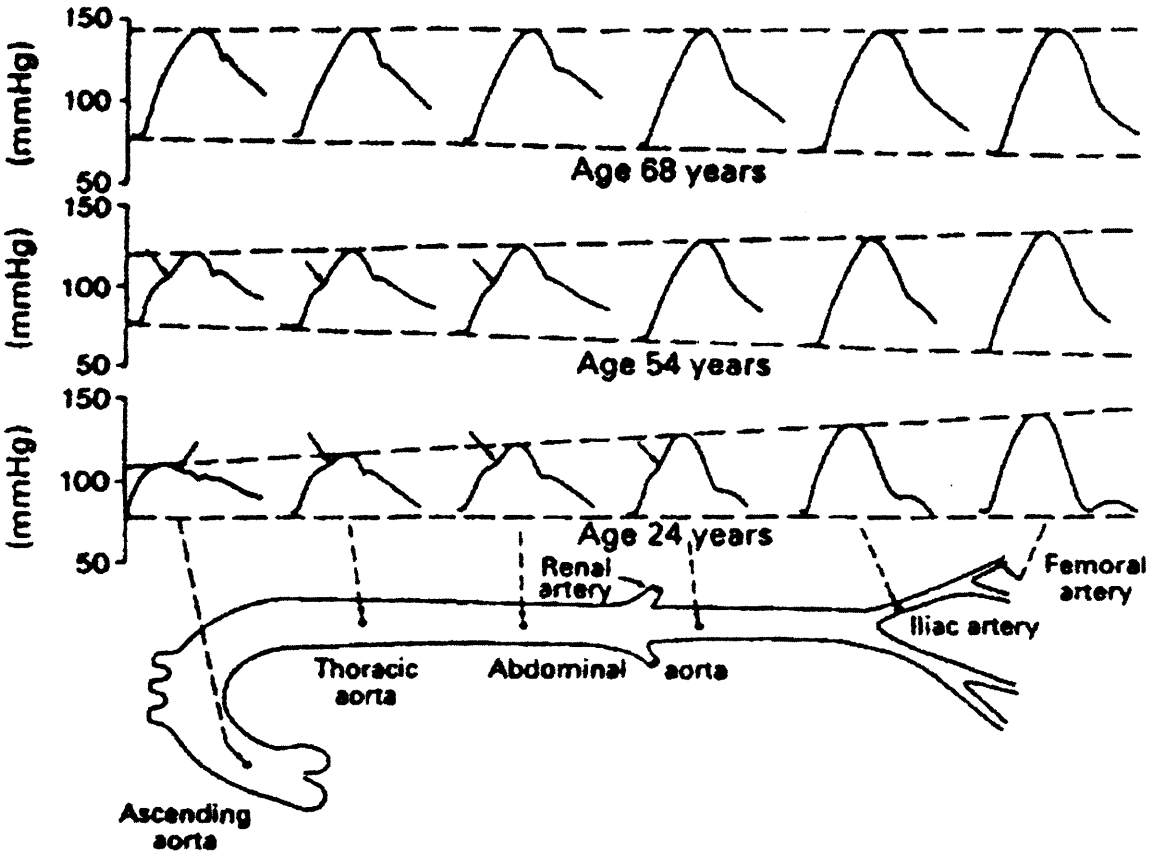


Figure 1. Pressure wave changes with age and distance from heart[136].

As mentioned above, this difference between central and peripheral pressures is most exaggerated in younger subjects in whom central to peripheral amplification is often quite marked[88;137]. Although in old age

therefore, brachial artery pressure is more representative of central arterial pressure, reliance on brachial arterial pressure from cuff sphygmomanometry still underestimates central pressures.

In infants, despite extremely compliant vessels, a combination of short body length and relatively long cardiac ejection period, results in a late systolic peak and resultant central pressure augmentation (not unlike that seen in the elderly). As the cardiovascular system matures and the body develops, the peak of the pressure wave is found in early systole and the late systolic wave moves into diastole to act in a haemodynamically beneficial way (i.e. reduction in cardiac afterload and improved coronary perfusion). In late adolescence, aortic secondary (reflected waves) achieve roughly the same amplitude to the peak systolic wave (i.e. augmentation of zero). With progressive ageing, augmentation of central systolic pressure waves increases as time to reflection falls[90].

Kelly demonstrated the effects of ageing on arterial wave contour using applanation at the carotid arterial site in subjects sub-divided into decades of age[112]. Fig 2 shows the change in radial and carotid artery wave contours with progressive ageing and table 1 documents the average augmentation index and time to reflection seen for each age decade.

Despite respectably large studies documenting the changes in arterial pressure wave contours with "normal ageing", [112]these studies have still allowed patients with significant hypertension to be included. As mentioned

above, blood pressure has a profound influence on both measured and derived haemodynamic parameters and causes considerable distortion of the data. These patients were previously included on the understanding that a degree of hypertension (and in particular isolated systolic hypertension) was a part of the natural ageing process.

The development of hypertension with ageing as commonly seen in western society has been described previously as a natural phenomenon. This is unlikely to be true. Studies from rural Africa have failed to show an age related rise in blood pressure [2-4;6]. This suggests that the rise in blood pressure with ageing is likely to reflect vascular damage and changes in the functional properties of the vasculature leading to enhanced vascular stiffness with age in western societies.

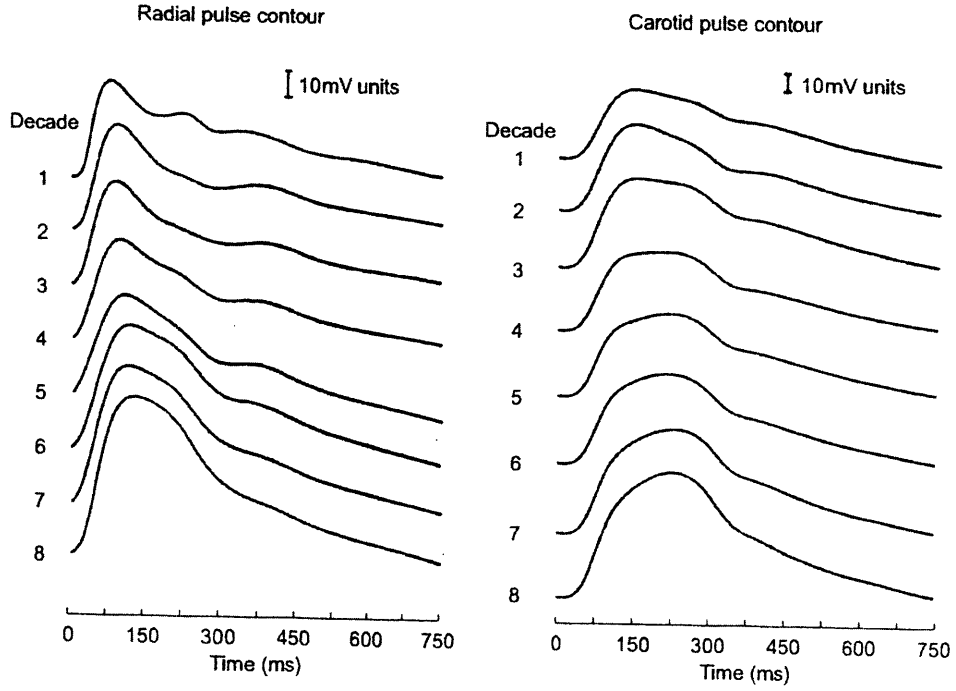


Figure 2: Radial and Carotid pressure waves with age [112]

Table 1 Carotid Augmentation Index for age[112]

Age (years)	Time to shoulder (msecs)	Height of shoulder above foot (mV units)	Height of peak above shoulder (mV units)	Augmentation Index (%)
1-10	116	39.4	0.6	1.6
11-20	116	50.1	1.6	3.0
21-30	110	51.3	2.7	4.9
31-40	110	46.5	3.7	7.4
41-50	102	42.6	8.4	16.5
51-60	102	43.0	11.1	20.5
61-70	102	47.1	14.9	24.1
71+	106	59.0	18.7	24.1

Avolio et al looked at pulse wave velocity using Doppler methods to assess age related vascular stiffening with age in an urban Chinese population and was able to demonstrate an increased pulse wave velocity with age[67] (figure 3).

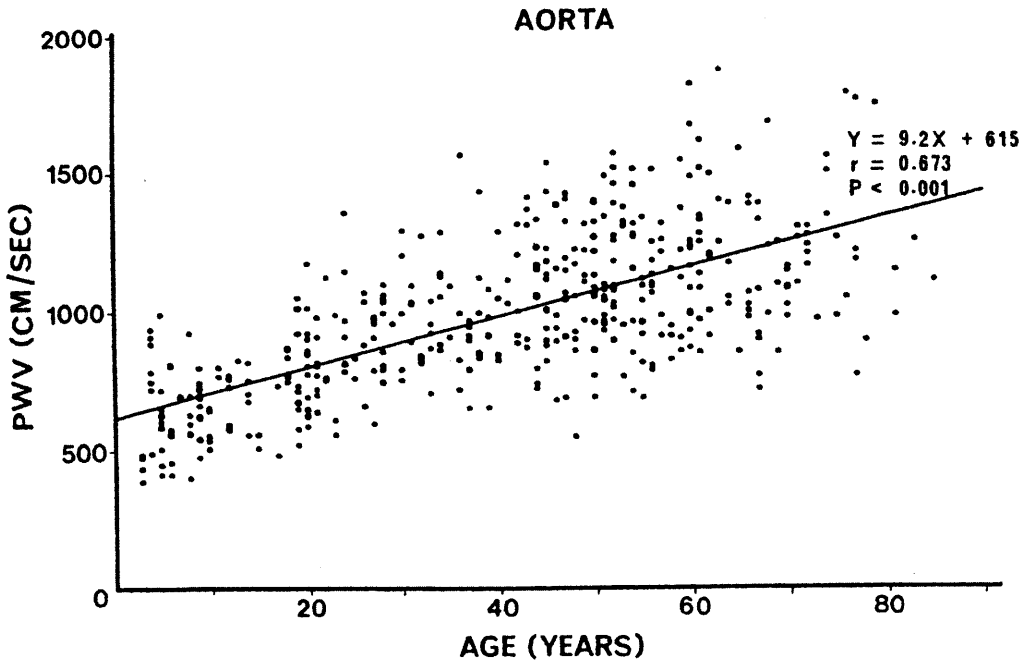


Figure 3. Age related changes in aortic PWV.[67]

Avolio's study population was selected deliberately as having low serum lipid levels and low levels of clinical atherosclerosis. It was therefore postulated that the changes observed in pulse wave velocity were most likely to represent age related arterial medial changes than increased vessel wall thickness secondary to atherosclerosis.

In a subsequent comparison between urban and rural Chinese, the main difference between populations was the degree of hypertension, as expected being much more marked in the urban than the rural group. The rate of this stiffening associated with age, (the gradient of the slope), was much less in the rural group than in the corresponding urban Chinese population. (figure 4) and thought largely to represent the deleterious effects of hypertension[29].

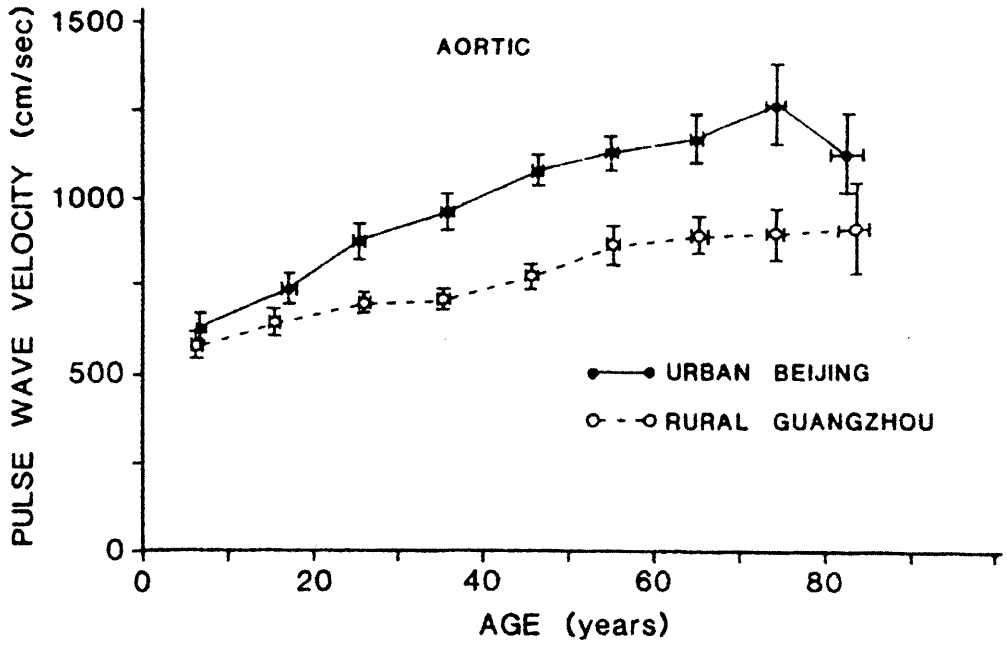


Figure 4: Age vs. PWV (urban vs. rural populations[29])

Recent alterations in the diagnostic criteria for hypertension means that a number of studies using what we would now consider outdated thresholds to define normal blood pressure (systolic>160 mmHg and diastolic >95 mmHg) have resulted in a significant proportion of people in the so-called normal population who would now be regarded as hypertensive.

With this in mind, we decided to limit our control population to patients defined as normotensive by modern criteria, and excluded any patient with an office reading of >140 mmHg systolic and/or >90 mmHg diastolic. We also excluded from our population any volunteer with a personal history of vascular or cardiac disease, those on any regular medication for cardiovascular disease or those drugs prescribed for non cardiovascular disease that might have additional effects on the cardiovascular system (e.g. alpha-blockers prescribed for benign prostatic hypertrophy).

Those with clinically significant abnormal biochemical or haematological parameters, as defined by our local chemical pathology laboratory, were also excluded.

3.2 Methods

General methods are discussed earlier in chapter 2. We recruited subjects from poster advertisements in a large teaching hospital setting. Subjects were a mix of staff, visitors, family members or interested friends. These subjects were likely to be well informed and interested in their cardiovascular health and were therefore possibly not representative of the general population in terms of levels of cardiovascular disease. Nevertheless, despite these concerns this was a practical approach to recruiting a large number of controls in a short period of time. As noted previously, only adults over the age of 18 years were studied, as the transfer factor used in the commercial software is not adequately validated in children.

Ethical approval was obtained from the Leicestershire Health Ethics Committee and all participants gave written informed consent prior to entry into the study (see appendix).

Participants were asked to provide detailed histories for both personal and family history of hypertension, diabetes, ischaemic heart disease or stroke, drug history, smoking status, alcohol intake and level of exercise. Peripheral venous blood samples were taken for routine haematology and biochemistry including lipid profile, glucose and HbA1c. Urinalysis was performed on a spot urine to exclude proteinuria, haematuria or glycosuria and a specimen

was analysed in the laboratory for the microalbumin creatinine ratio. Body mass index (BMI) was calculated from height and weight, and a twelve lead ECG was performed, to exclude left ventricular hypertrophy or changes suggestive of underlying ischaemic heart disease.

We initially recruited almost six hundred "controls". (590). Subsequent careful screening identified 345 subjects who revealed no overt evidence of cardiovascular disease. The majority of subjects were excluded from the normal dataset due to the presence of undiagnosed hypertension, a personal history of cardiovascular disease or abnormal laboratory results. These patients were referred back to their General Practitioner for further assessment and treatment if considered appropriate by their GP. In a few cases, laboratory results were incomplete due to various handling or analytical problems out with our control.

3.3.1 Results (Descriptives)

The overall descriptives for the control group are shown in table 2 below. Gender split and smoking status are reported in tables 3 and 4 respectively.

Table 2: Descriptive statistics for the control population (cont. overleaf)

	n	minimum	maximum	mean	sd
Age (years)	345	18.0	82.7	42.3	11.8
Peripheral SBP (mmHg)	345	76.0	139.0	114.9	13.2
Peripheral DBP (mmHg)	345	53.0	90.0	74.4	7.3
Peripheral Mean BP (mmHg)	345	62.0	108.0	89.0	8.6
Peripheral PP (mmHg)	345	17.0	71.0	40.5	9.9
Central SBP (mmHg)	344	68.0	133.0	105.5	11.7
Central DBP (mmHg)	344	53.0	91.0	75.4	7.4
Central Mean BP (mmHg)	344	62.0	108.0	88.9	8.6
Central PP (mmHg)	344	11.0	51.0	30.2	7.7
Amplification	344	106.0	188.0	135.9	19.1
Heart rate (BPM)	344	42.0	109.8	68.5	10.4

	n	minimum	maximum	mean	s.d
AI (%)	342	-36.0	47.6	20.3	12.9
Height (cm)	316	146.0	195.0	166.5	9.7
Weight (kg)	315	37.0	130.0	68.6	12.8
BMI	315	17	44	24.7	3.9
HDL (mmol/l)	309	1.0	3.0	1.6	0.5
LDL (mmol/l)	305	1.0	5.0	2.9	0.76
Total Cholesterol (mmol/l)	311	3.0	6.0	5.0	0.84
Triglycerides (mmol/l)	311	1.0	4.0	1.3	0.58
Creatinine (iu/l)	308	49.0	126.0	82.0	12.4
Glucose (mmol/l)	310	3.0	7.0	4.7	0.61
Hb A1c (%)	272	3.0	7.0	5.7	0.5
ACR	299	0	14.0	0.9	1.7

Table 3: Gender distribution for the control group

	Males	Females
Percentage	29.3	70.7

Table 4: Smoking status for the control group

	Smoker	Non-smoker
Percentage	14.5	85.5

The effects of ageing on the measured variables are presented first, before considering their effects and that of age on the various parameters determined by pulse wave analysis.

3.3.2 Results (Ageing, Blood pressure and PWA parameters)

Despite selecting a 'normal population', artificially cut off at a systolic BP of 140 and diastolic BP of 90 mmHg, there remained a significant correlation of systolic, diastolic, mean blood pressure and peripheral pulse pressure with age Although statistically significant, plots of these parameters showed considerable variability and little more than a trend with age was apparent. (See figures 4 to 7).

Figure 4: Age vs. peripheral systolic BP

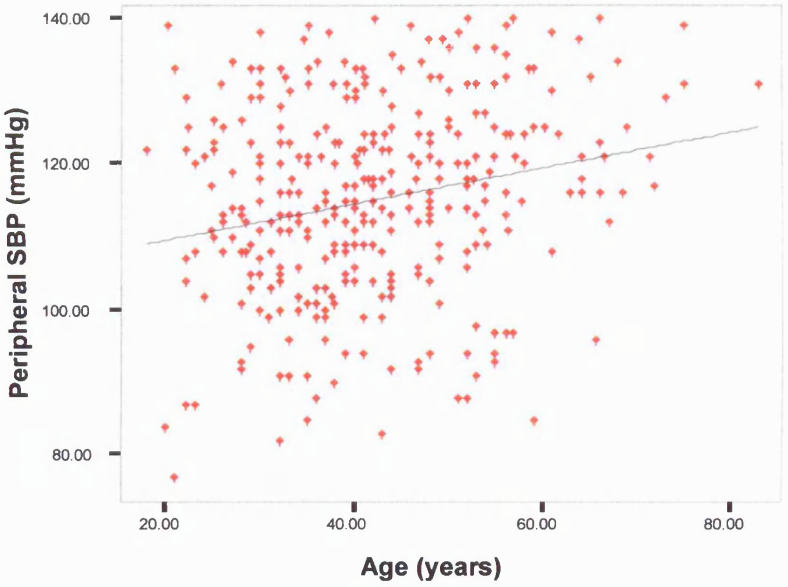
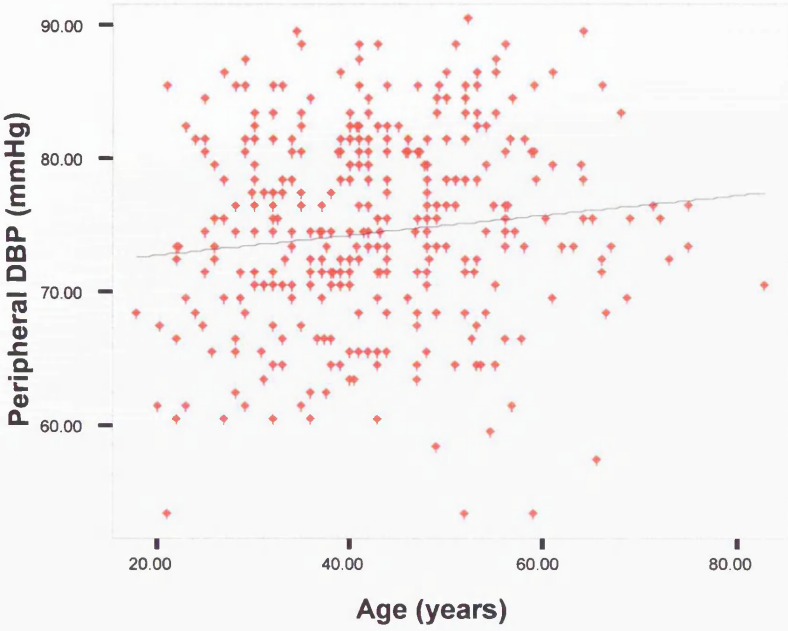


Figure 5: Age vs. peripheral diastolic BP



Interestingly however, Aortic AI displayed a much tighter linear relationship with age within the normal BP range (figure 8). This is despite the wide scatter of results in both standard blood pressure parameters and brachial BP parameters thought to reflect arterial stiffness e.g. Pulse pressure.

Figure 6: Age vs. Peripheral pulse pressure

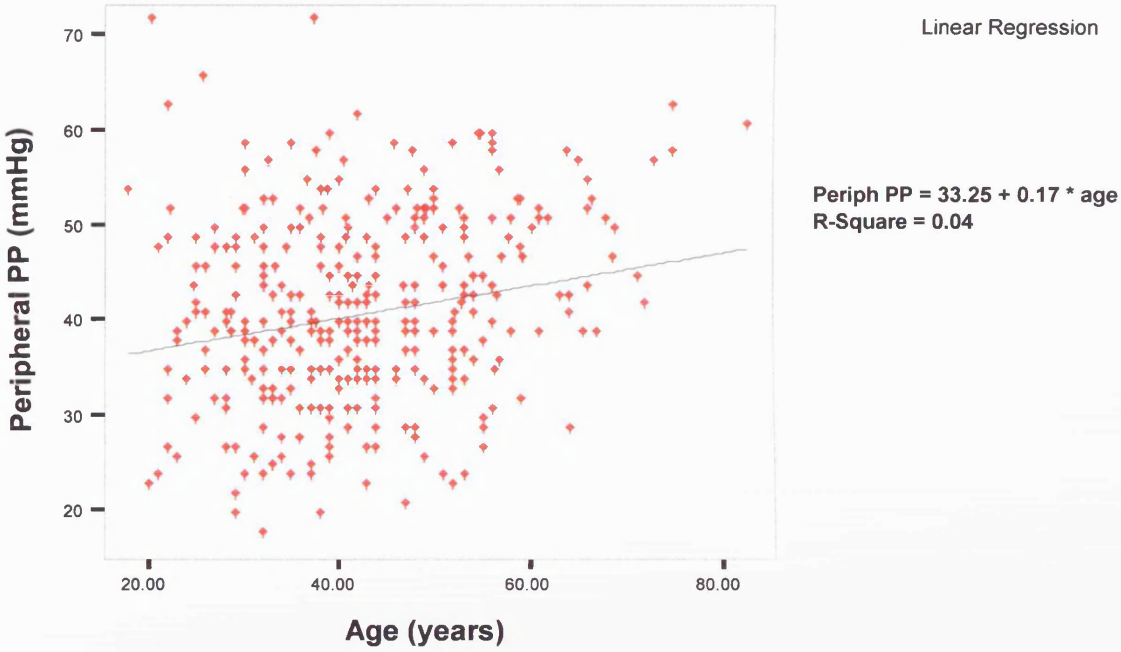


Figure 7: Age vs. MAP

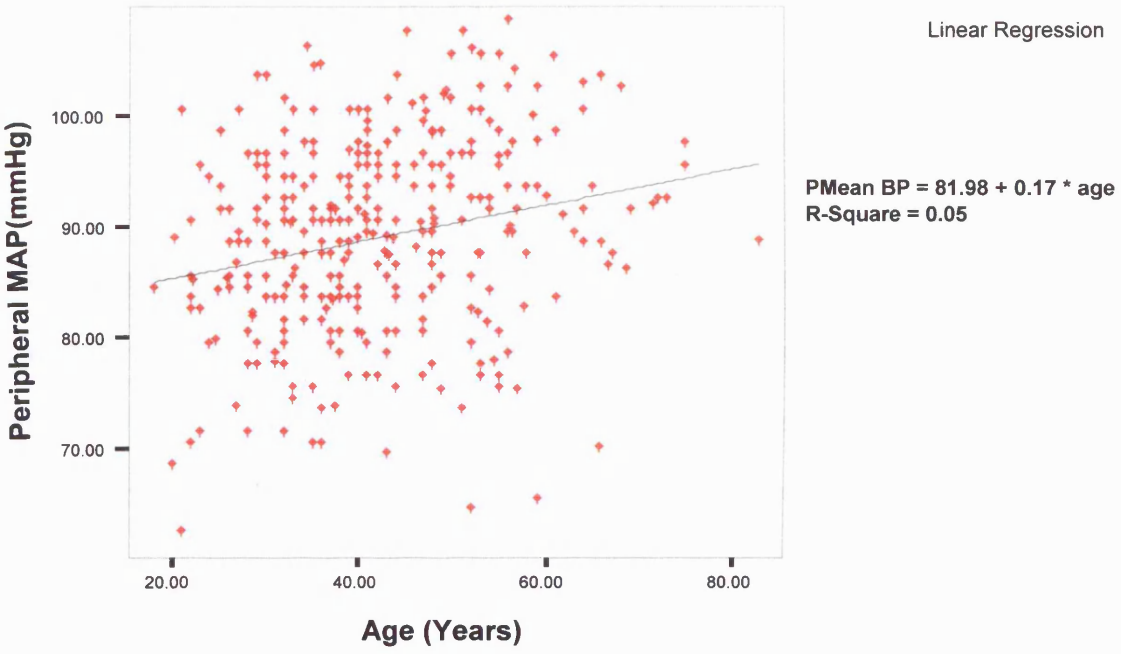
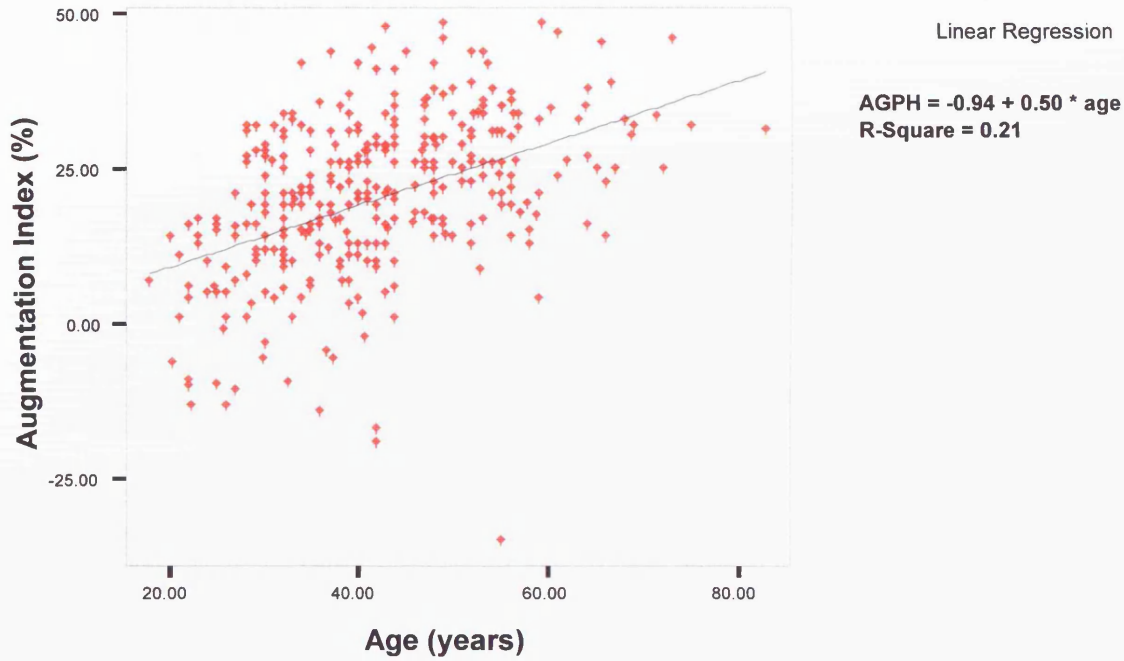


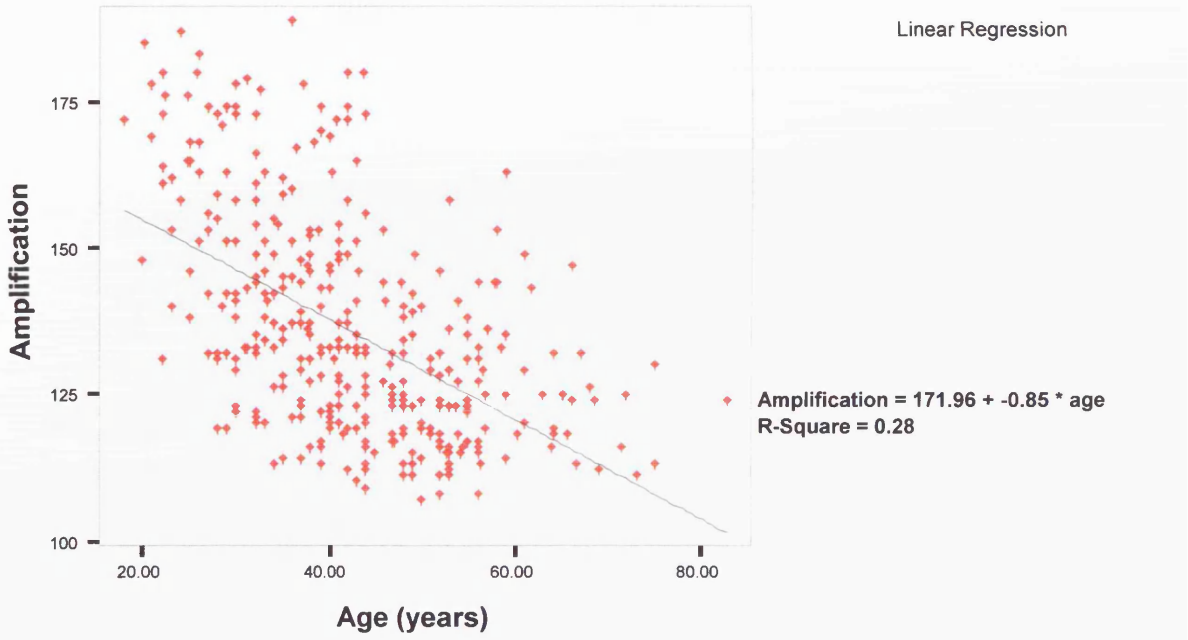
Figure 8: Age vs. Aortic AI



If AI is to be considered a potential surrogate for PWV, then these results would support previously reported findings of age related increase in pulse wave velocity in otherwise normal subjects[67](Avolio).

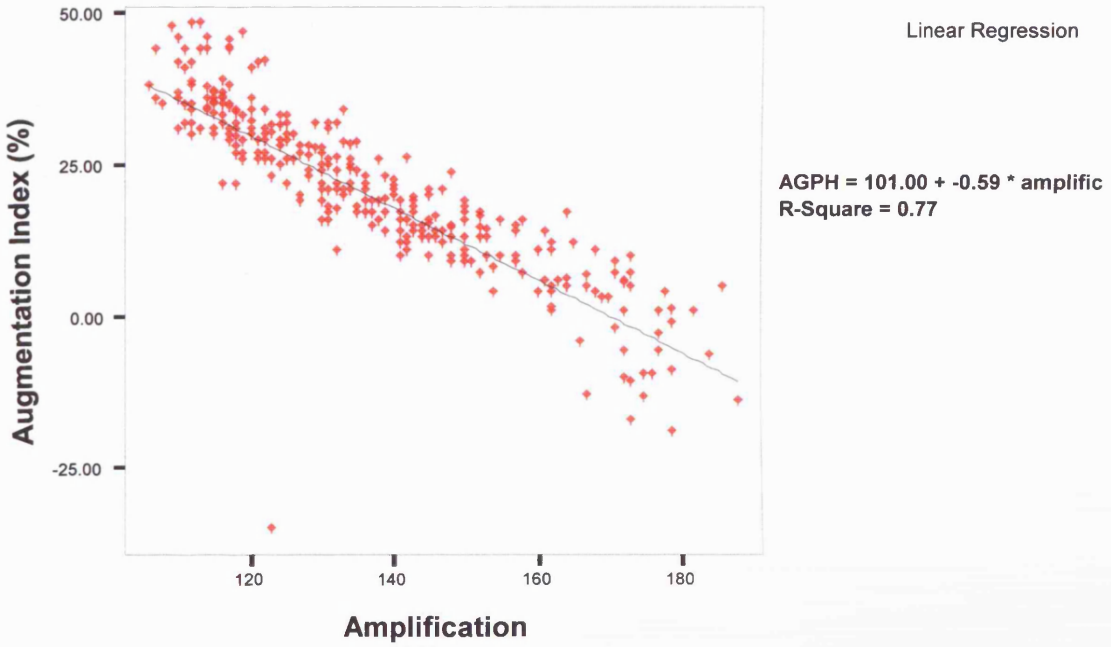
PWA offers more than just a measurement of AI however. As discussed earlier, amplification of the pulse from central to peripheral arteries falls with age, as an increase in augmentation index secondary to faster pulse wave velocity raises central pressures to comparable to those seen in peripheral arteries. Consistent with this, our data shows a clear reduction in pulse wave amplification with age, in our normal control population. (figure 9)

Figure 9: Age vs. central to peripheral amplification



Since amplification is thought to be due largely to the effects of systolic wave augmentation, it is reassuring to see the anticipated direct inverse correlation of augmentation Index to amplification in these normal subjects. (figure 10)

Figure 10: Augmentation Index vs. Amplification

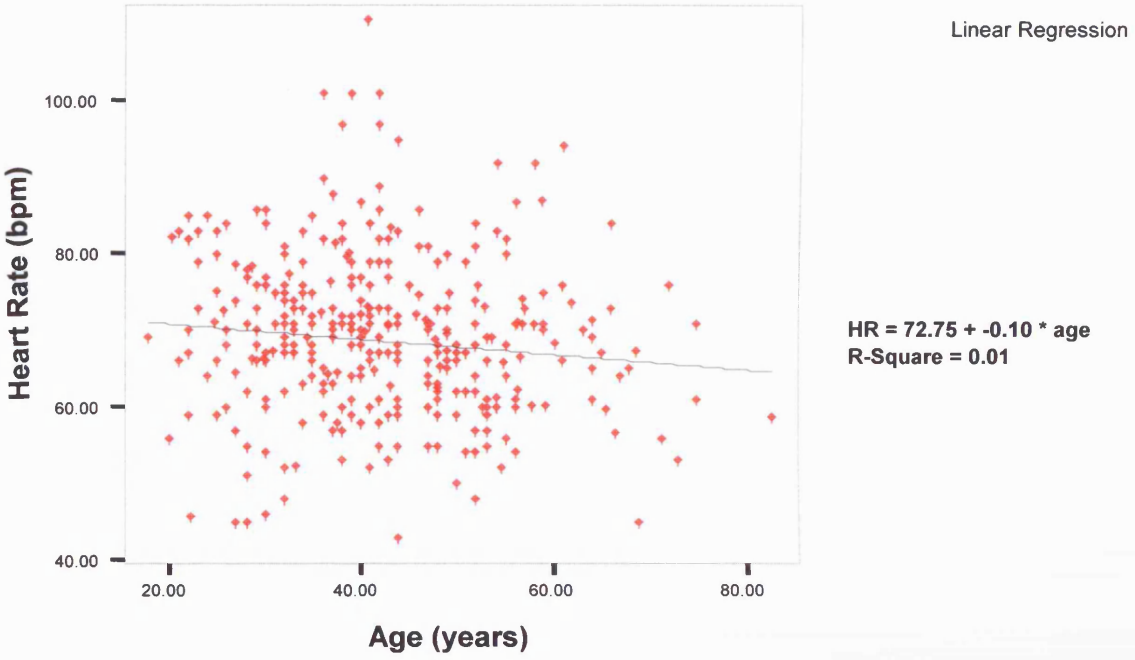


3.3.3 Results (Ageing and Cardiac parameters including Heart rate.)

Given the aforementioned composite nature of AI, obviously cardiac parameters will exert significant influence on indirect measurements of vascular stiffness using pulse wave analysis.

There was a weak but statistically significant negative correlation between ageing and heart rate, which, as mentioned previously, is well described (figure 11).

Figure 11: Age vs. Heart rate



When broken down into systolic and diastolic components, no statistically significant change was seen with age. There was an observed trend to reduction in systolic duration (figure 12) and increase in diastolic duration (figure 13) with age. This could reflect two factors, 1) older, stiffer ventricles requiring greater filling times in diastole or 2) alternatively the changes in systolic and diastolic time intervals may just reflect the fall in heart rate associated with ageing. Specifically selecting out hypertensive individuals from our population may have potentially lessened the effect of age on

cardiac duration by reducing the number of individuals with coexisting left ventricular hypertrophy associated with hypertension.

Figure 12 Systolic (ejection) duration vs. age

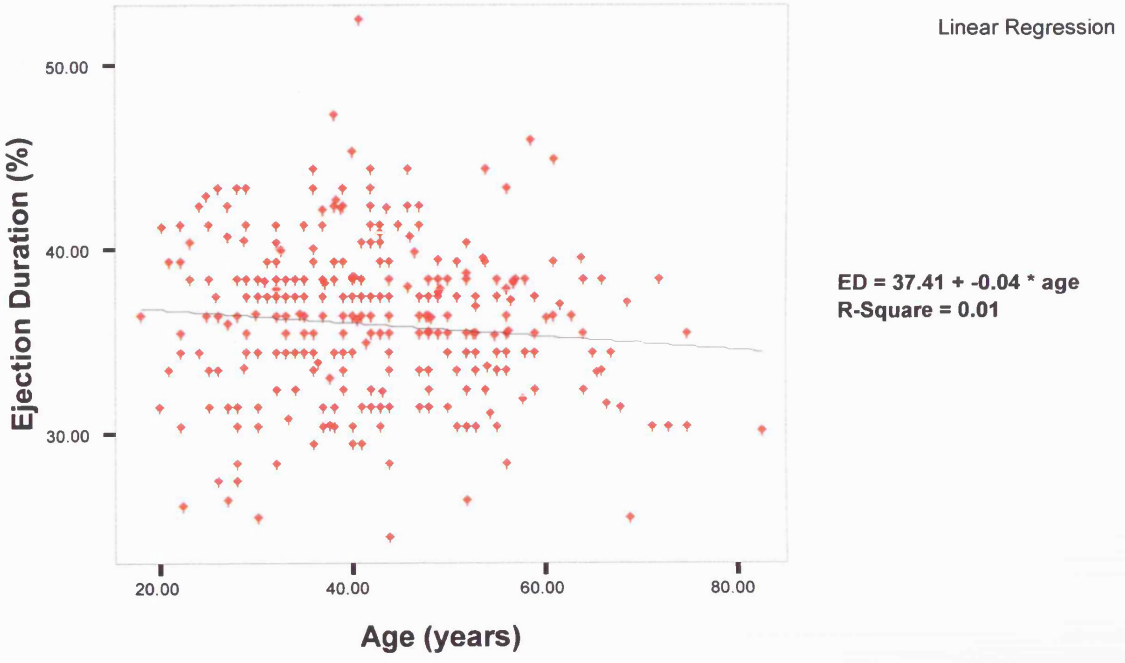
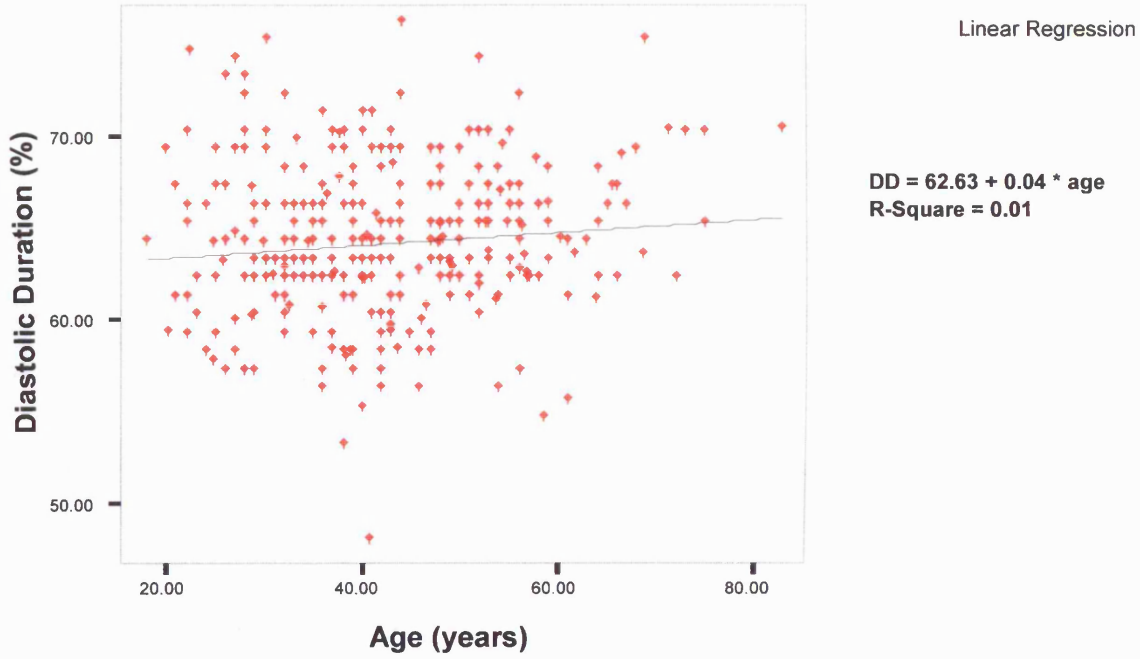
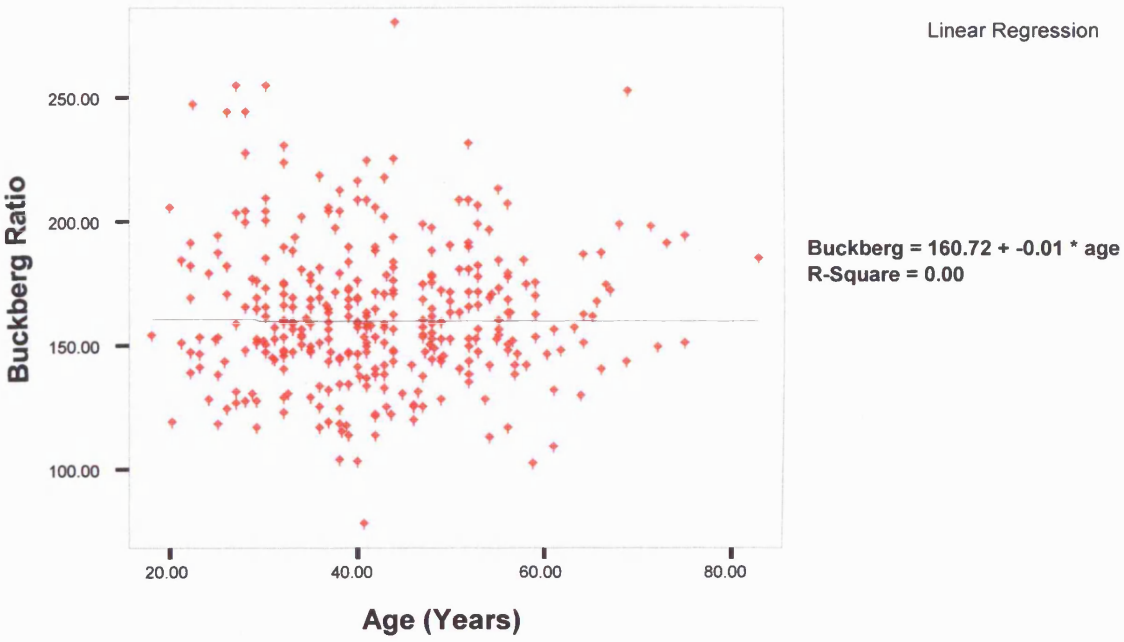


Figure 13 Diastolic duration vs. age



In support of these findings, evidence of reduced sub-endocardial viability was not apparent with ageing (figure 14) when measured by the Buckberg ratio, which is discussed in detail later in relation to AI.

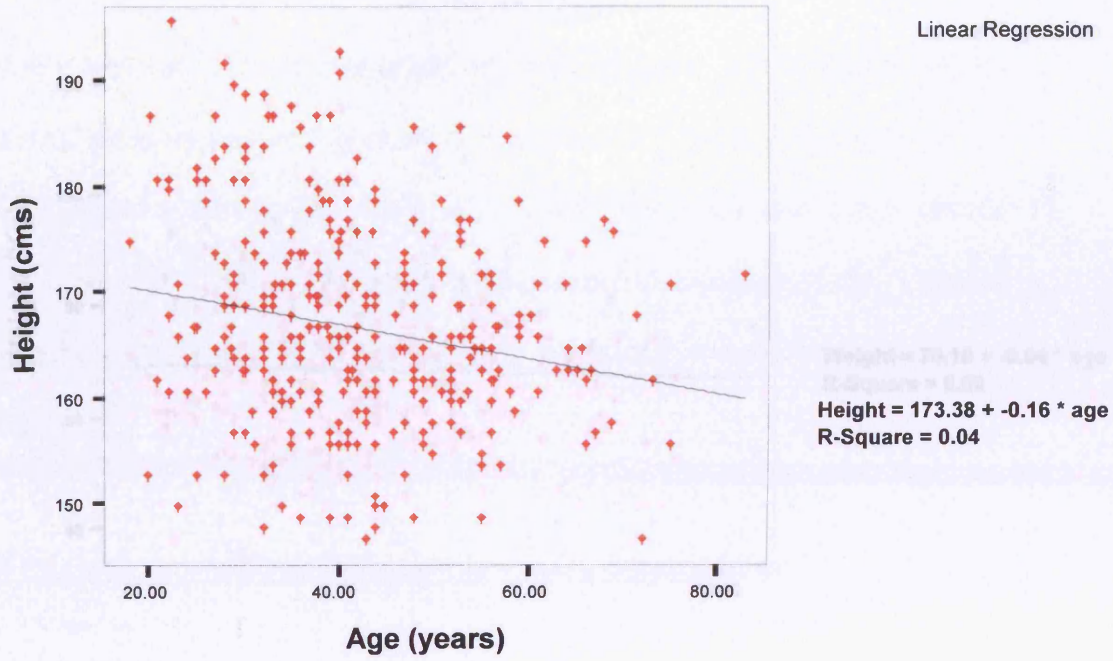
Figure 14. Age vs. Buckberg Ratio



3.3.4 Results (Ageing and Anthropometric parameters)

Although the elderly control subjects in our population were significantly shorter than the younger subjects (figure 15), this trend was not large and is unlikely to account fully for the elevation seen in AI with age. Augmentation index is however strongly and inversely related to height, and will be discussed later.

Figure 15: Age vs. height



Weight and BMI showed no statistically significant correlation with age in our population (figures 16 and 17).

Figure 16: Weight vs. age

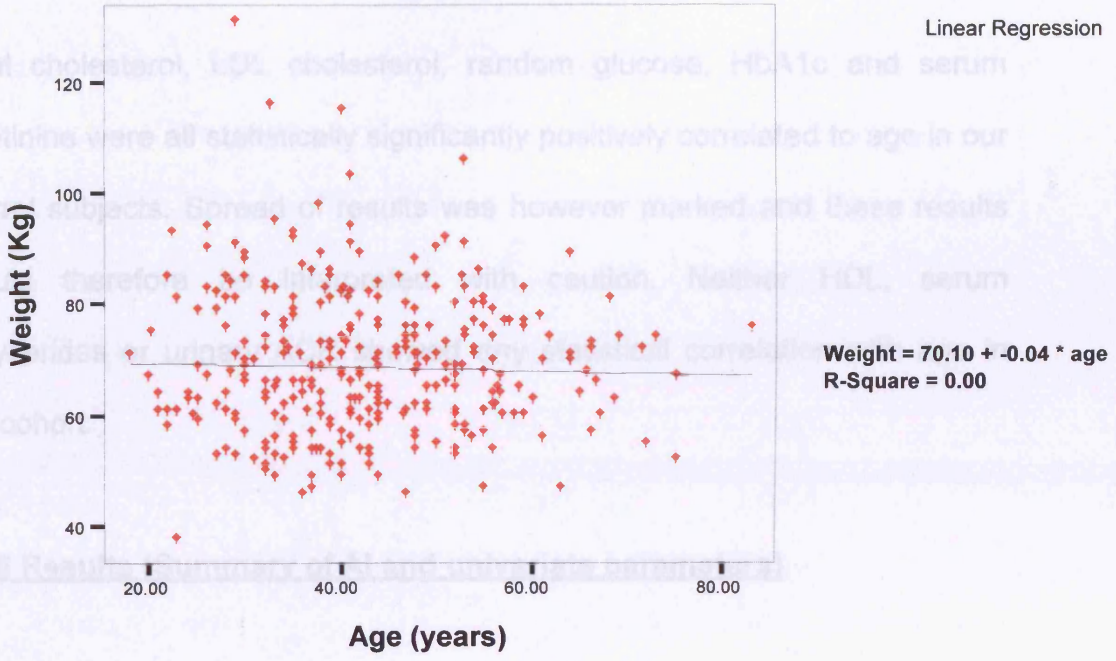
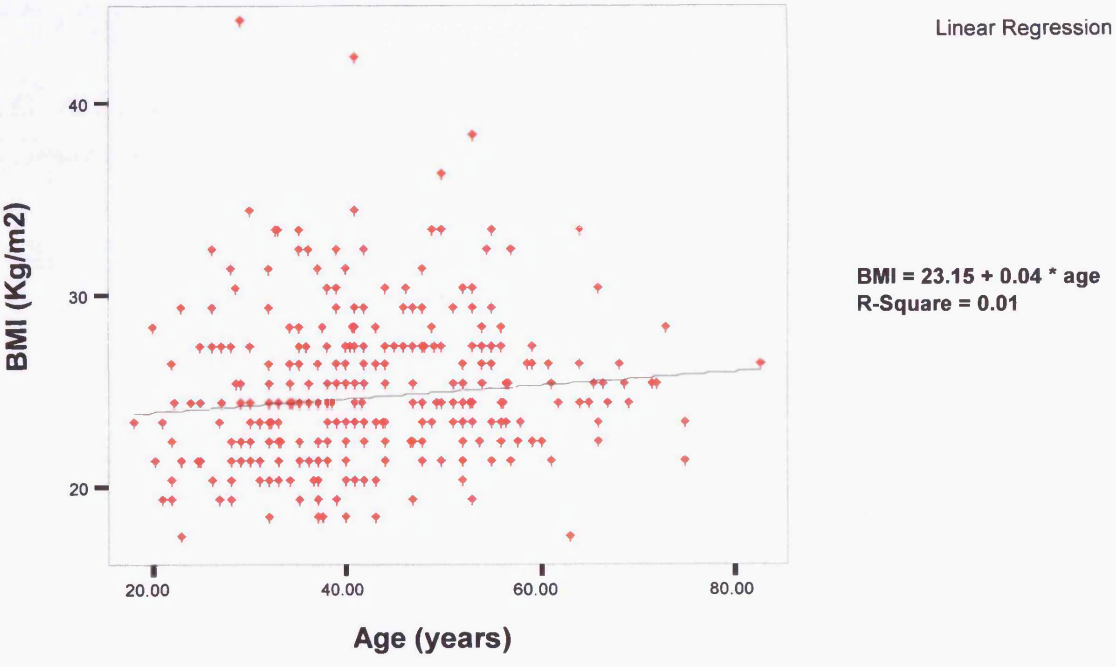


Figure 17. BMI vs. age



3.3.5 Results (Ageing and Biochemical parameters including Serum Lipids and urine ACR)

Total cholesterol, LDL cholesterol, random glucose, HbA1c and serum creatinine were all statistically significantly positively correlated to age in our control subjects. Spread of results was however marked and these results should therefore be interpreted with caution. Neither HDL, serum triglycerides or urinary ACR showed any statistical correlation with age in this cohort.

3.3.6 Results (Summary of AI and univariate parameters)

Table 5 below summarises the univariate parameters which appear statistically significantly correlated to Augmentation Index and will be discussed further.

Table 5. Significant univariate correlates with AI

Variable	n	Correlation Coefficient	Two-tailed significance
Age (years)	342	0.173	0.01
SBP (mmHg)	342	-0.119	0.05
MAP (mmHg)	342	0.126	0.01
PPP (mmHg)	342	-0.209	0.01
Height (cm)	313	-0.456	0.01
Weight (Kg)	312	-0.259	0.01
Gender	338	0.339	0.01
Heart Rate (BPM)	342	-0.364	0.01
Cholesterol (mmol/l)	308	0.189	0.01
HDL (mmol/l)	306	0.173	0.01
LDL (mmol/l)	302	0.203	0.01
Creatinine (umol/l)	305	-0.147	0.05

3.3.7 Results (Augmentation Index and Blood pressure parameters)

Although AI is generally considered a blood pressure independent parameter (augmentation is expressed as a percentage of total pulse height), AI is often seen to be increased in association with an elevated distending blood pressure increasing PWV due to *functional* vascular stiffening. Blood pressure indicators of stiffer vessels (such as increased

pulse pressure) could also be expected to be related to an increased AI for the same reasons i.e. assumed increased PWV.

In this population, SBP and PP were negatively correlated to AI, whilst MAP was positively correlated (Figures 18 to 20) and DBP not significantly correlated (figure 21).

Figure 18. Augmentation Index vs. SBP

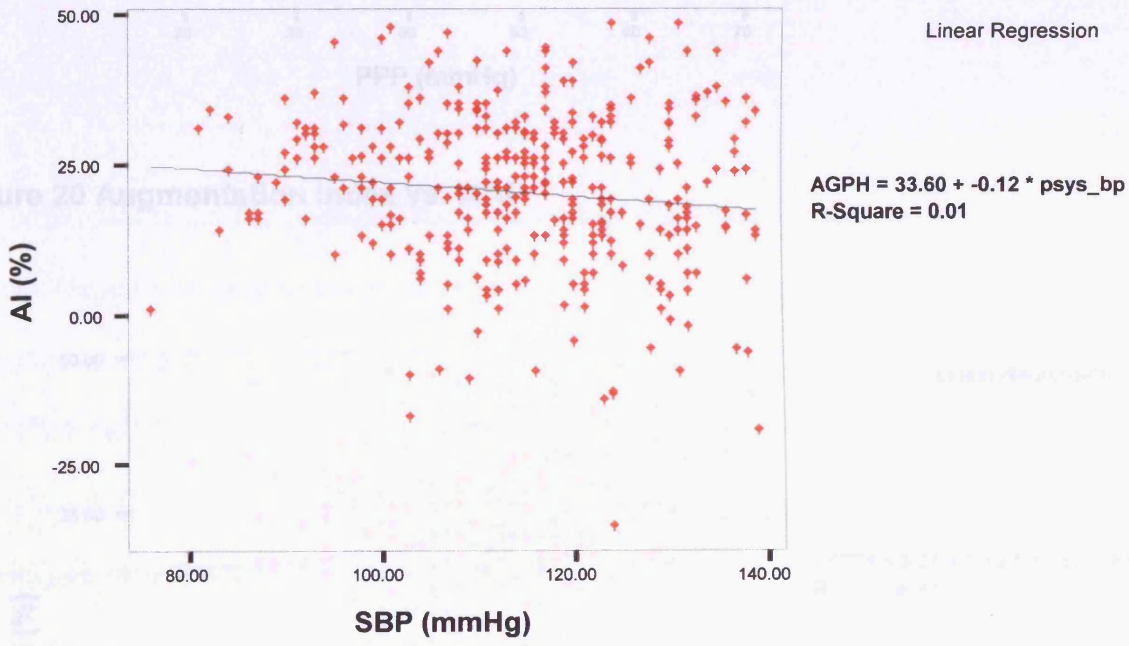


Figure 19. Augmentation Index vs. Peripheral PP

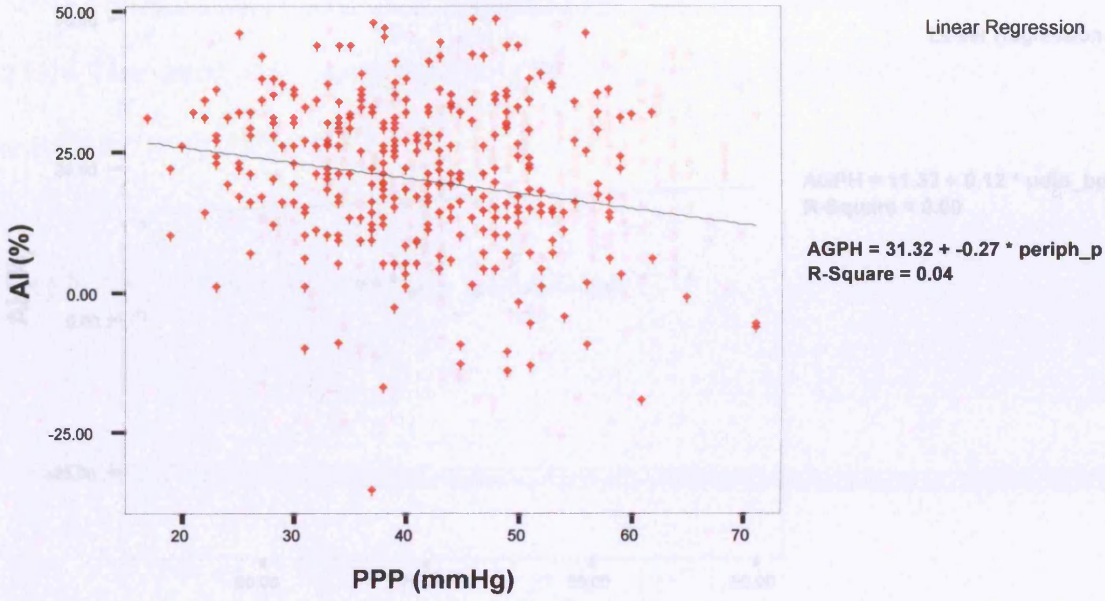


Figure 20 Augmentation Index vs. MAP

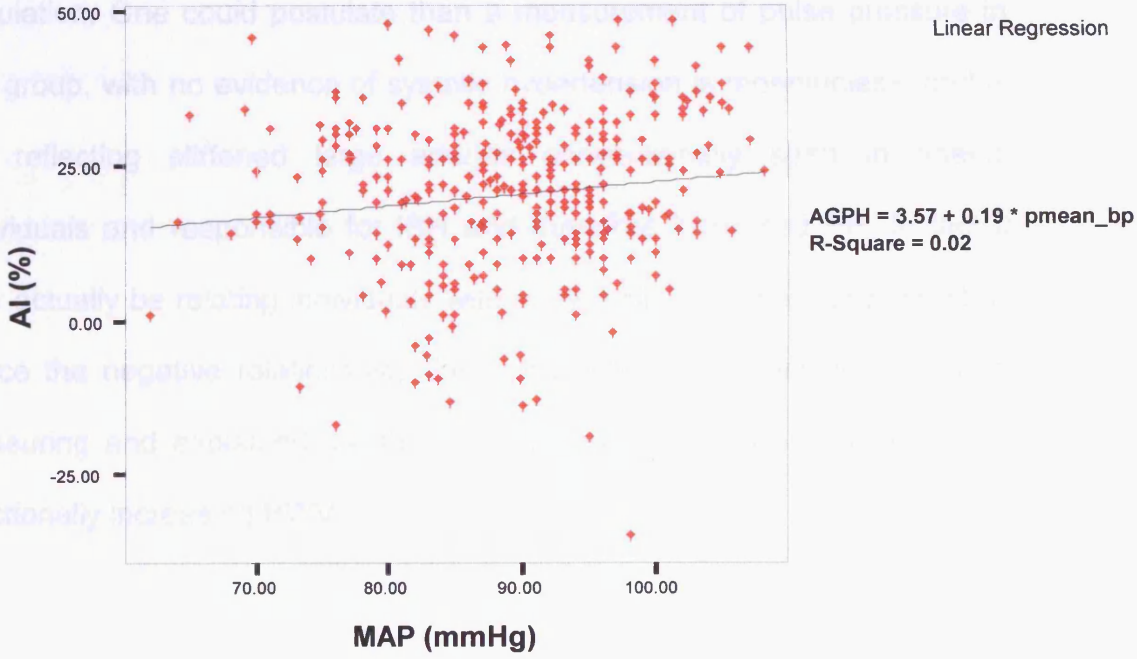
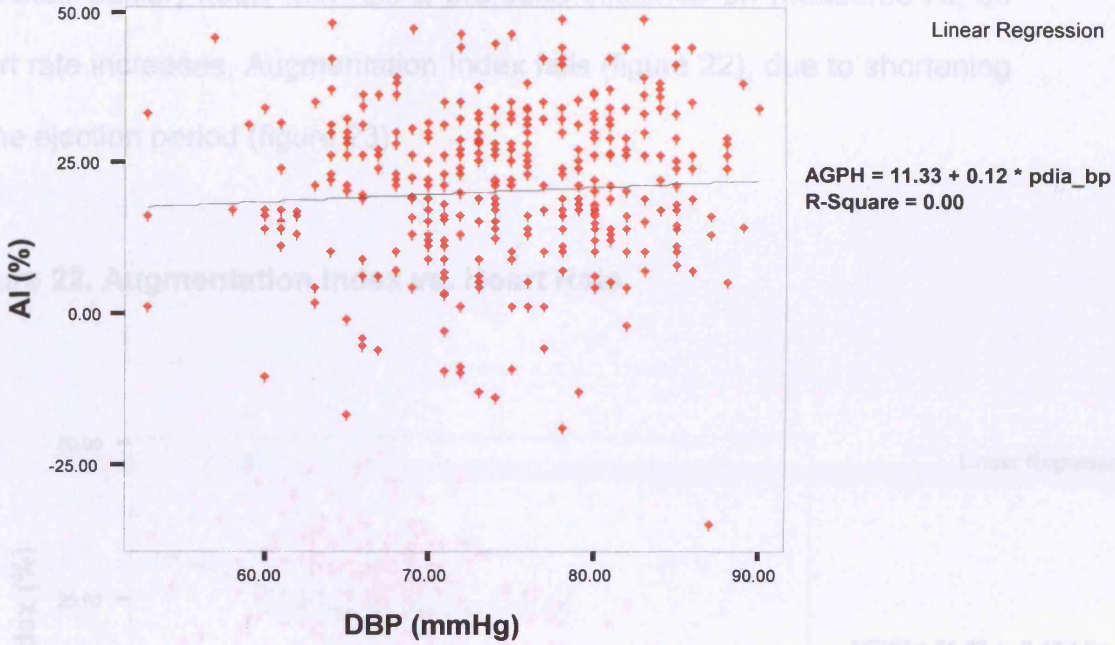


Figure 21 Augmentation Index vs. DBP



The explanation for the negative associations of SBP and PP are not clear but are likely to be due to the artificial cut off in blood pressure used for this population. One could postulate than a measurement of pulse pressure in this group, with no evidence of systolic hypertension is meaningless, and is not reflecting stiffened large arteries conventionally seen in ageing individuals and responsible for ISH and therefore increased PP. In fact it may actually be relating individuals with a low DBP, and therefore low MAP hence the negative relationship. The positive MAP relationship with AI is reassuring and explained as above by increases in distending pressures functionally increasing PWV.

3.3.8 Results (Augmentation Index and Cardiac parameters)

As noted earlier, heart rate has a profound influence on measured AI, as heart rate increases, Augmentation Index falls (figure 22), due to shortening of the ejection period (figure 23).

Figure 22. Augmentation Index vs. Heart Rate

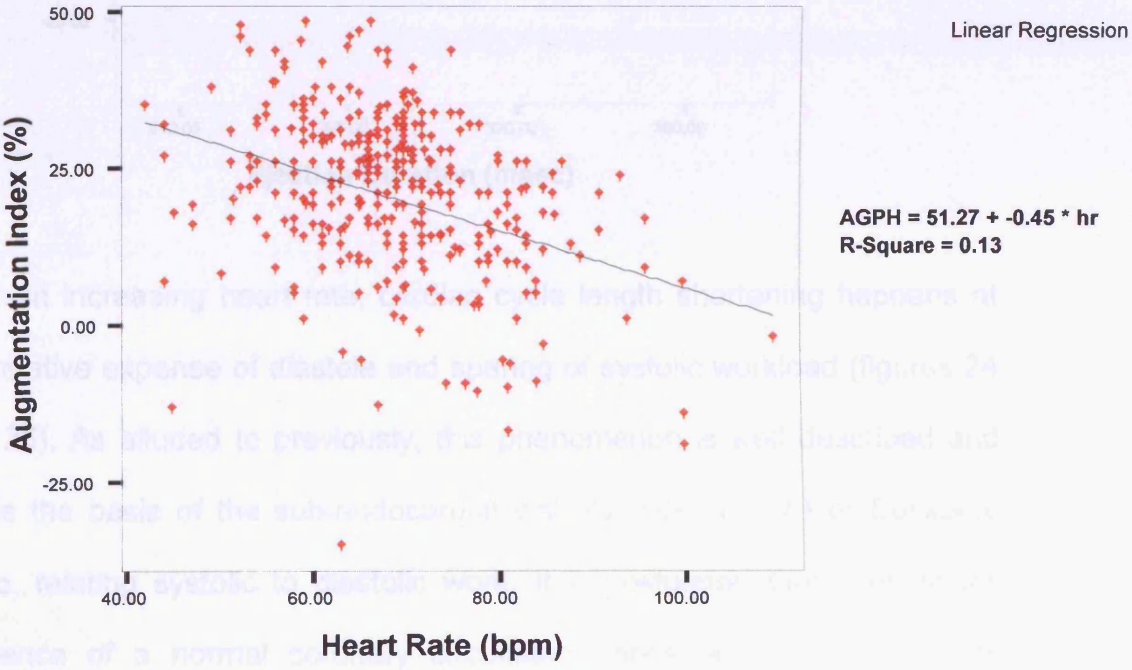
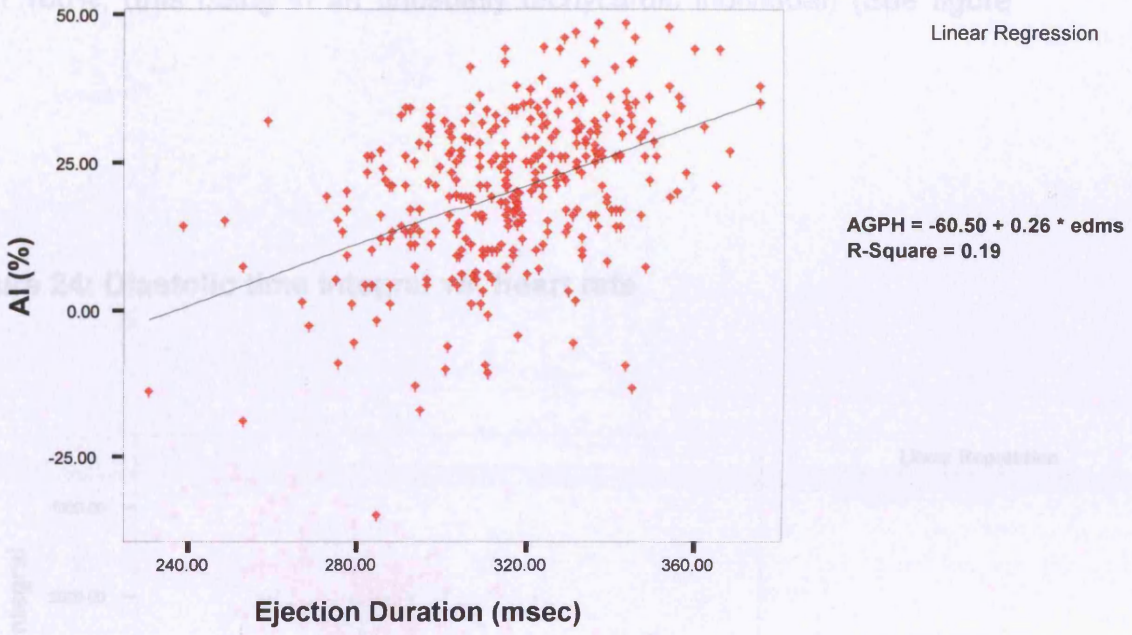


Figure 23: Augmentation Index vs. cardiac ejection duration



With an increasing heart rate, cardiac cycle length shortening happens at the relative expense of diastole and sparing of systolic workload (figures 24 and 25). As alluded to previously, this phenomenon is well described and forms the basis of the sub-endocardial viability Index (SEVI) or Buckberg Ratio, relating systolic to diastolic work. It is postulated that even in the presence of a normal coronary circulation, ratios less than 100% are associated with under perfusion of sub-endocardial muscle. This goes some way to explain why chronic stable angina is largely exertional (and hence aggravated by increased heart rate, reduced Buckberg and diastolic coronary blood flow reduction in the face of an increasing myocardial oxygen demand), It also helps explain why rate limiting drugs such as beta blockers are so useful in this setting.

In our normal population, a classical inverse relationship between heart rate and SEVI was observed, and reassuringly it was rare to see SEVI of less than 100%, (this being in an unusually tachycardic individual) (See figure 26).

Figure 24: Diastolic time integral vs. heart rate

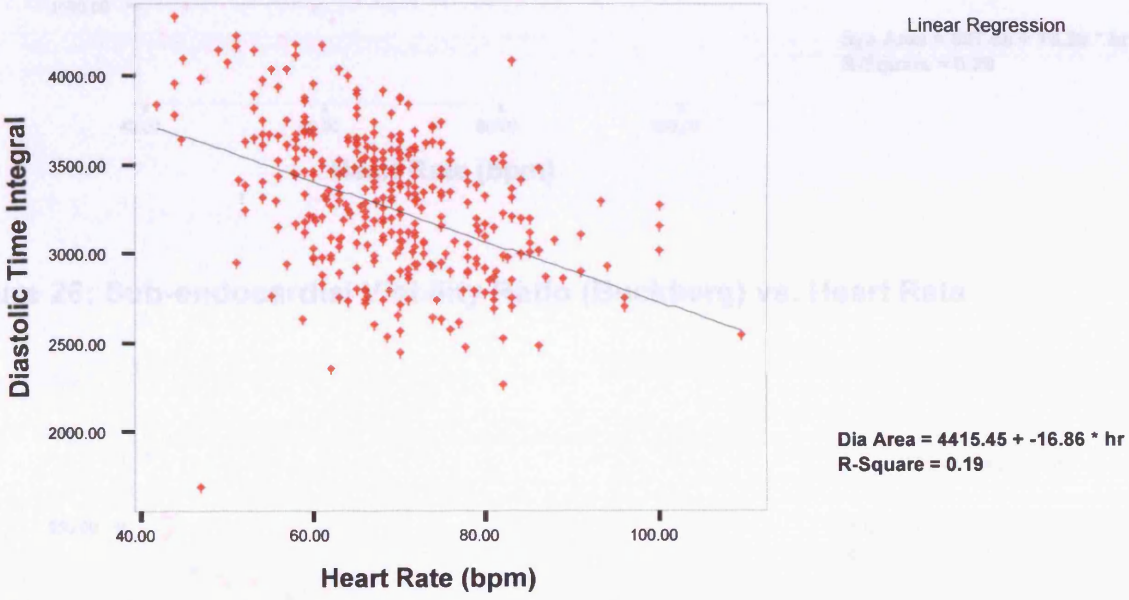


Figure 25: Systolic work vs. heart rate

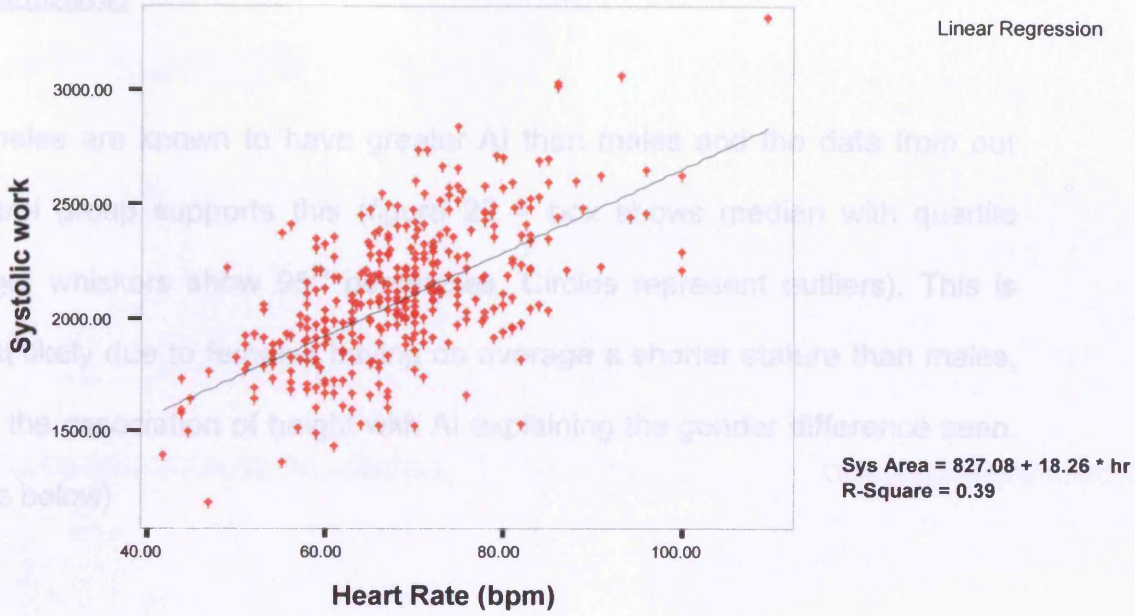
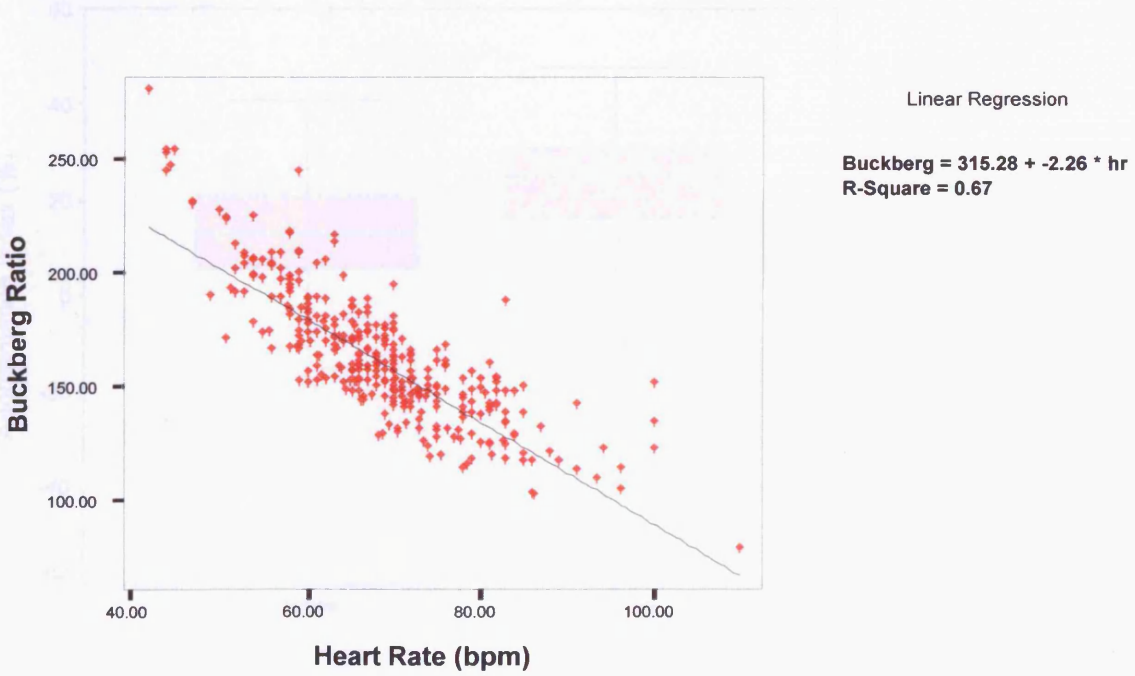


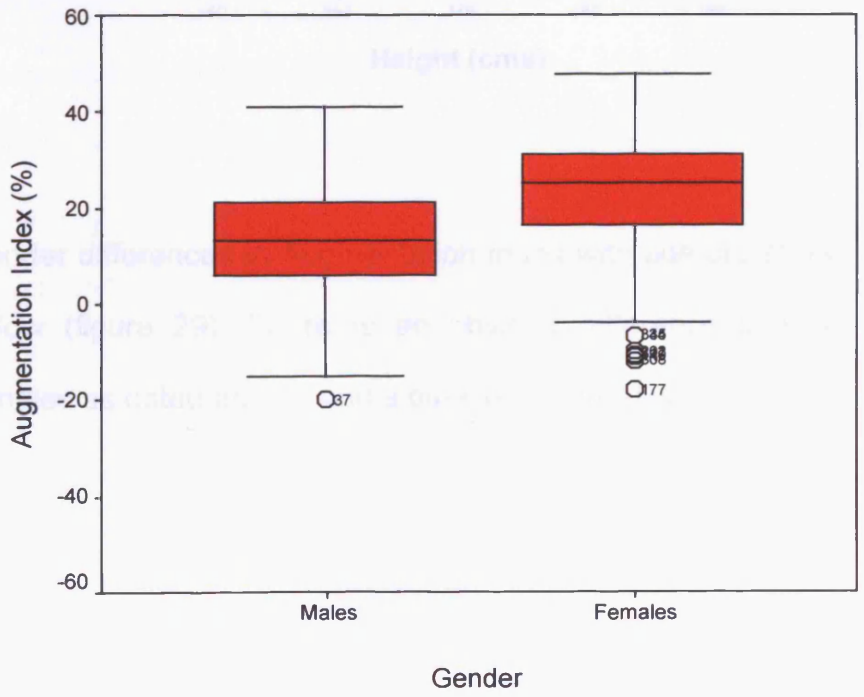
Figure 26: Sub-endocardial Viability Ratio (Buckberg) vs. Heart Rate



3.3.9 Results (Augmentation Index, gender and anthropometric parameters)

Females are known to have greater AI than males and the data from our control group supports this (figure 27 – box shows median with quartile range, whiskers show 95th percentiles. Circles represent outliers). This is most likely due to females having on average a shorter stature than males, and the association of height with AI explaining the gender difference seen. (See below)

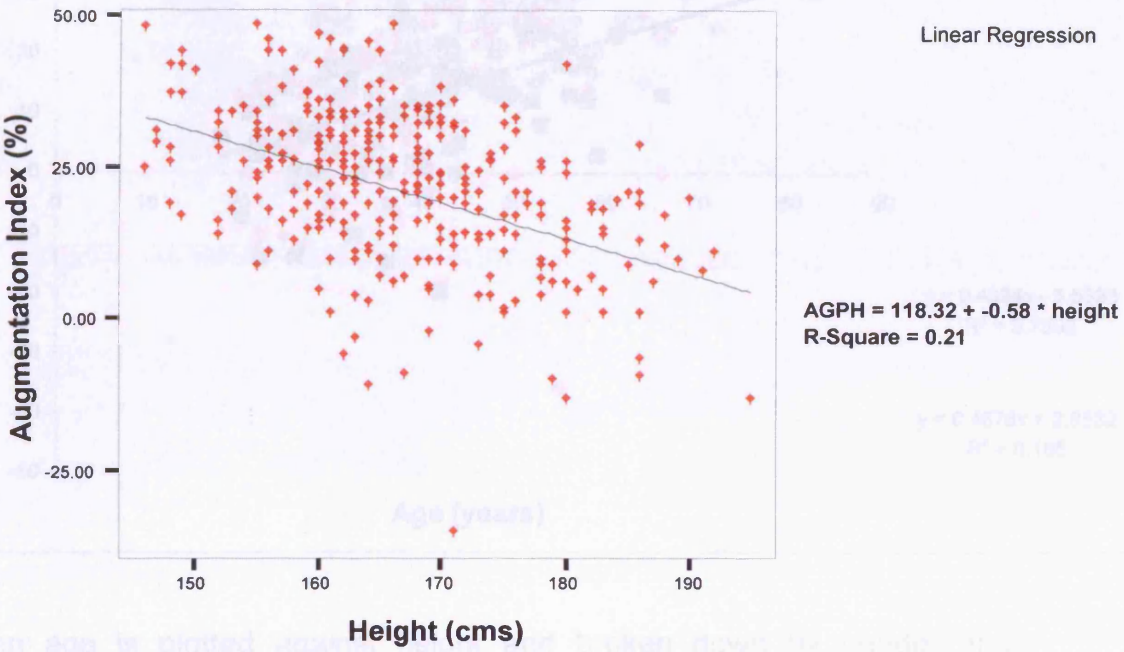
Figure 27. AI and gender



Height is well recognised as a significant factor in influencing measured AI. It is inversely related and shown here in figure 28. A reasonable adjustment

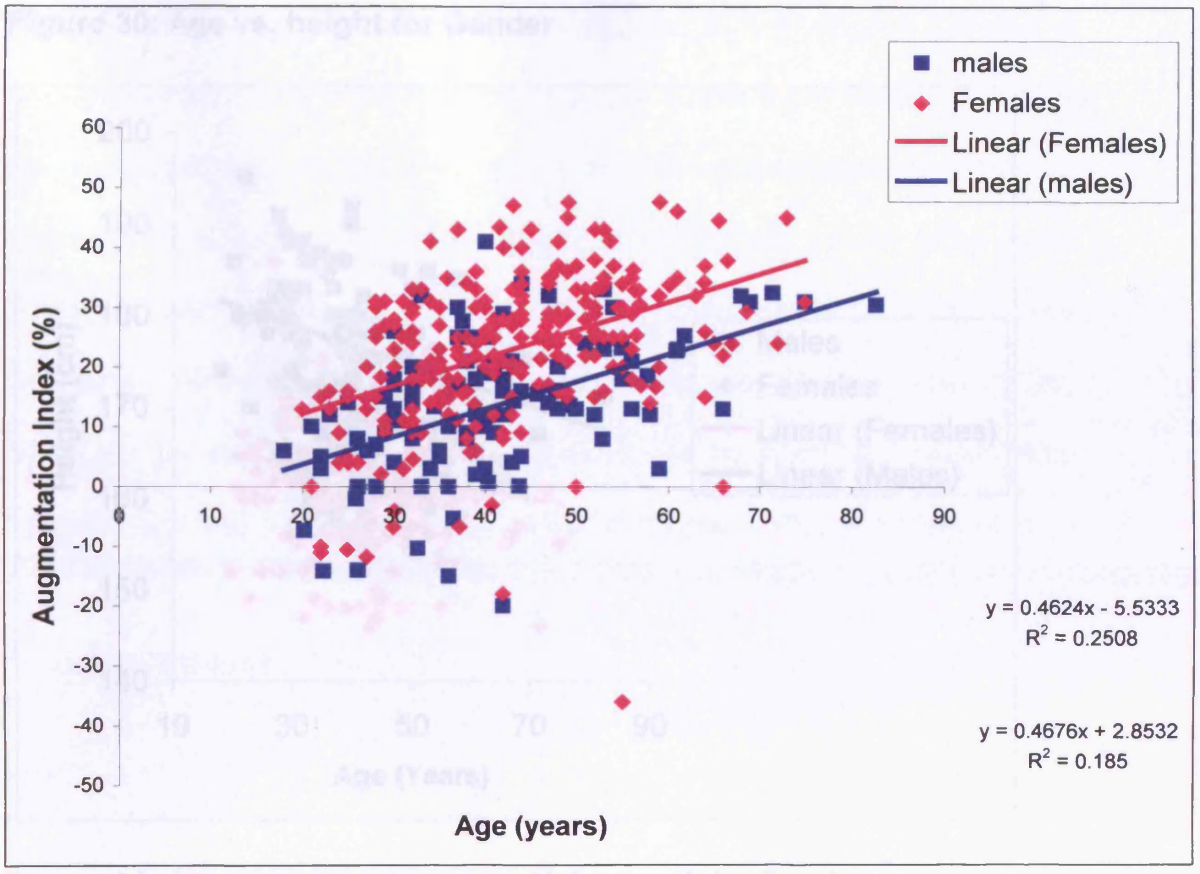
for the effects of body height, based on our control data, is a reduction in measured AI of 0.39% for every centimetre increase in body height.

Figure 28. AI and height



Gender differences in Augmentation Index with age are shown in the graph below (figure 29). There is an obvious difference between males and females as noted above, and a parallel rise in AI with age for both sexes.

Figure 29: Augmentation index vs. age by Gender



When age is plotted against height and broken down by gender, the difference between males and females is very apparent (figure 30). If our original graph of Augmentation Index versus height shown previously is colour coded for gender then the impact of Gender on this variable is obvious (figure 31) and is largely reflecting height differences between the sexes, the slope of the regression line being virtually the same for both genders.

Figure 30: Age vs. height for Gender

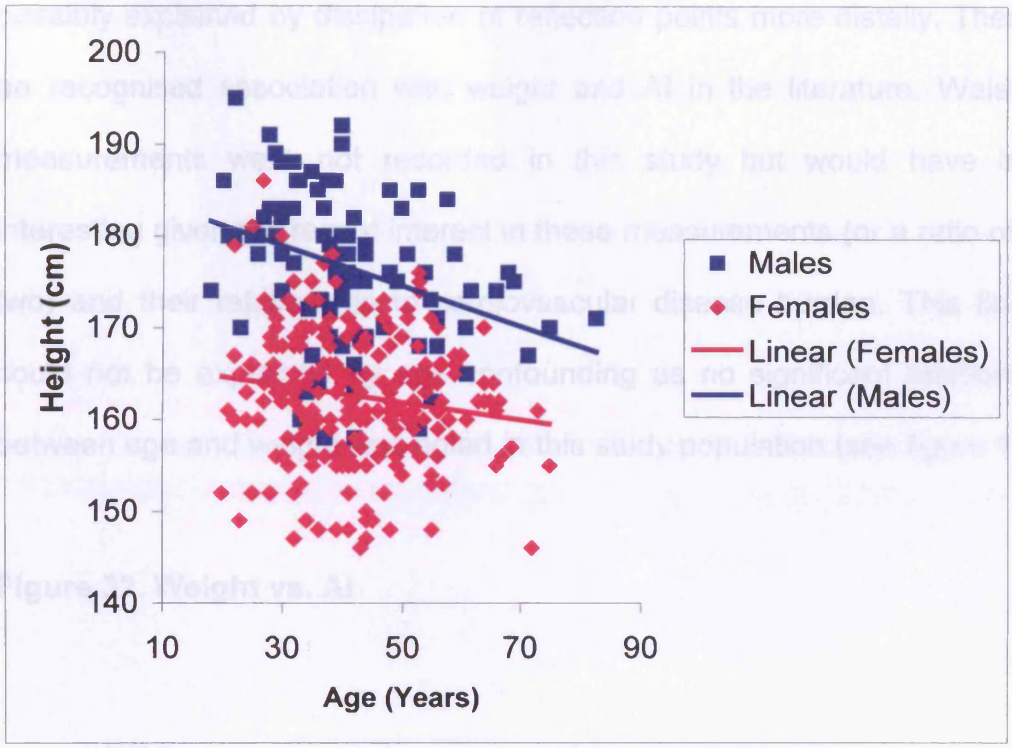
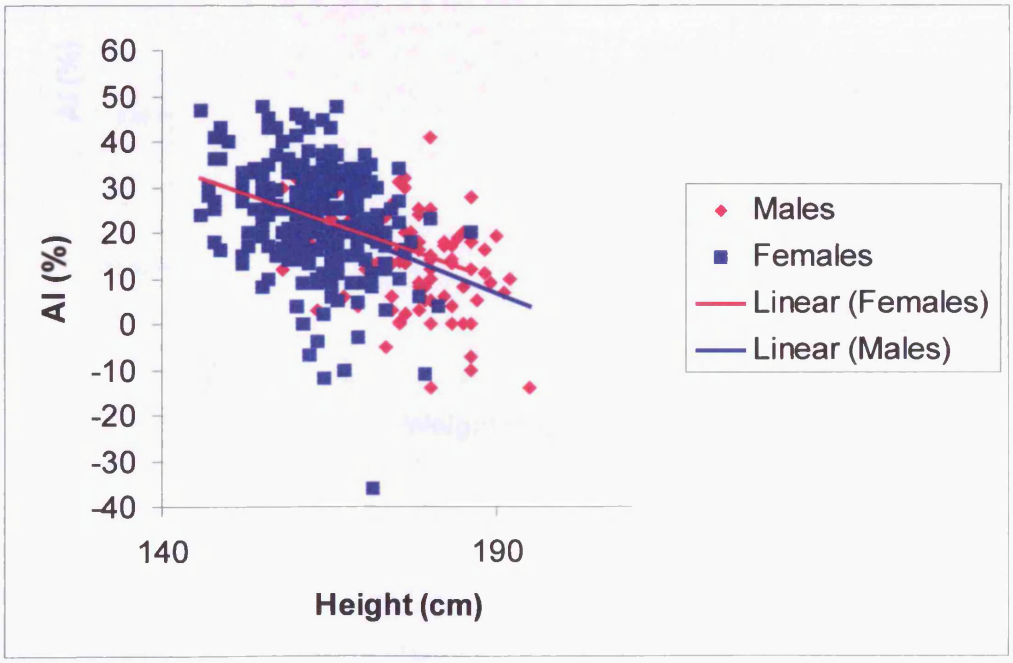
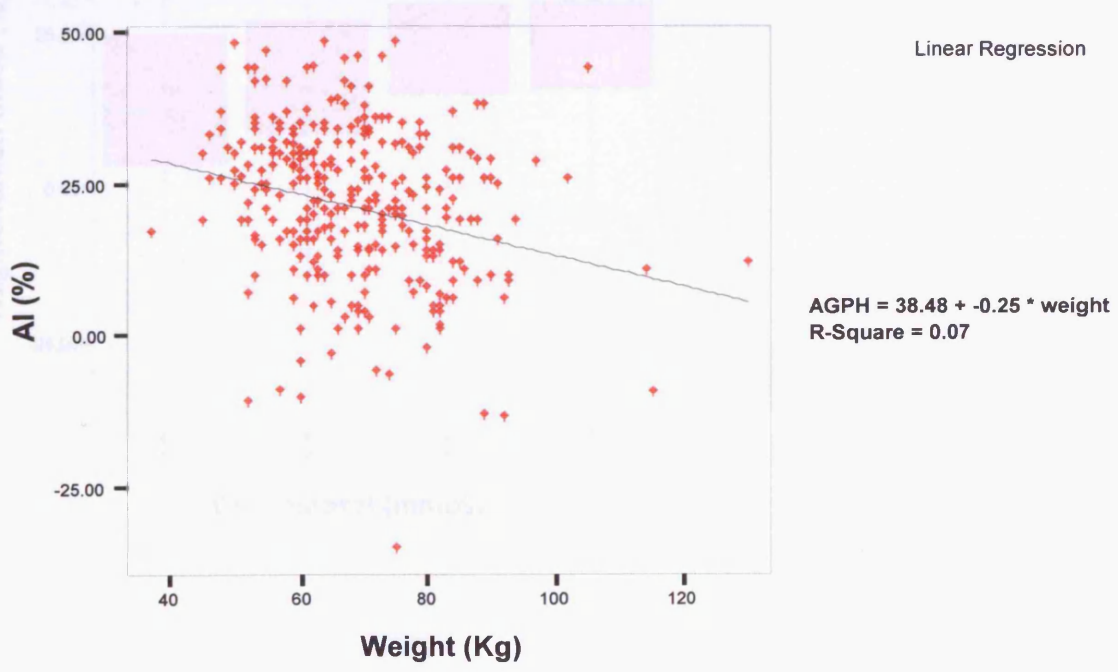


Figure 31: Augmentation Index vs. Height split by Gender



Weight showed a significant inverse correlation with AI (Figure 32) and is possibly explained by dissipation of reflection points more distally. There is no recognised association with weight and AI in the literature. Waist/hip measurements were not recorded in this study but would have been interesting given the recent interest in these measurements (or a ratio of the two) and their relationship to cardiovascular disease burden. This finding could not be explained by age confounding as no significant relationship between age and weight was noted in this study population (see figure 16).

Figure 32. Weight vs. AI



3.3.10 Results (AI, Biochemical parameters and smoking status)

Total cholesterol and LDL cholesterol were statistically correlated to AI (figures 33 and 34), although the scatter of results throughout this control population was again large. The relationship of HDL cholesterol (figure 35) appeared essentially neutral.

Figure 33: Augmentation Index vs. Total serum Cholesterol

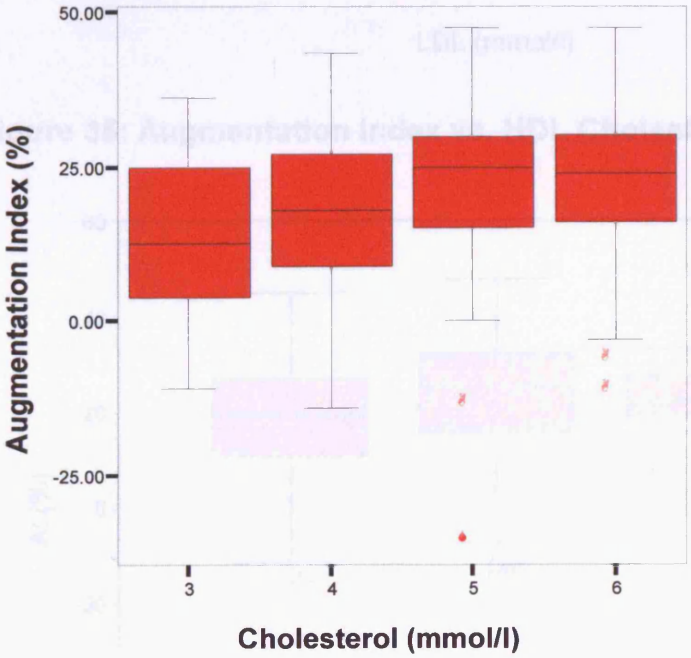


Figure 34: Augmentation Index vs. LDL Cholesterol

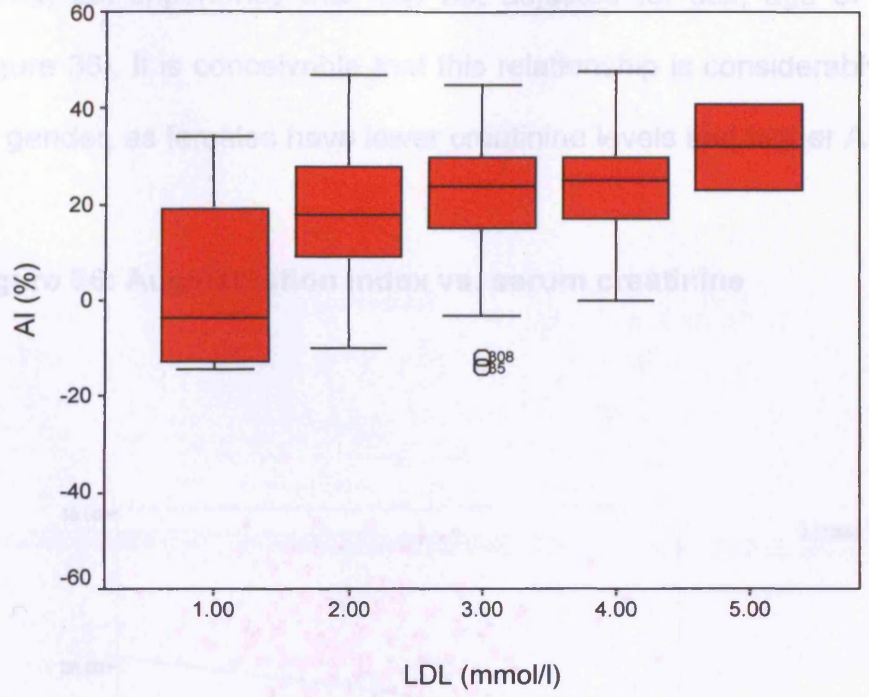
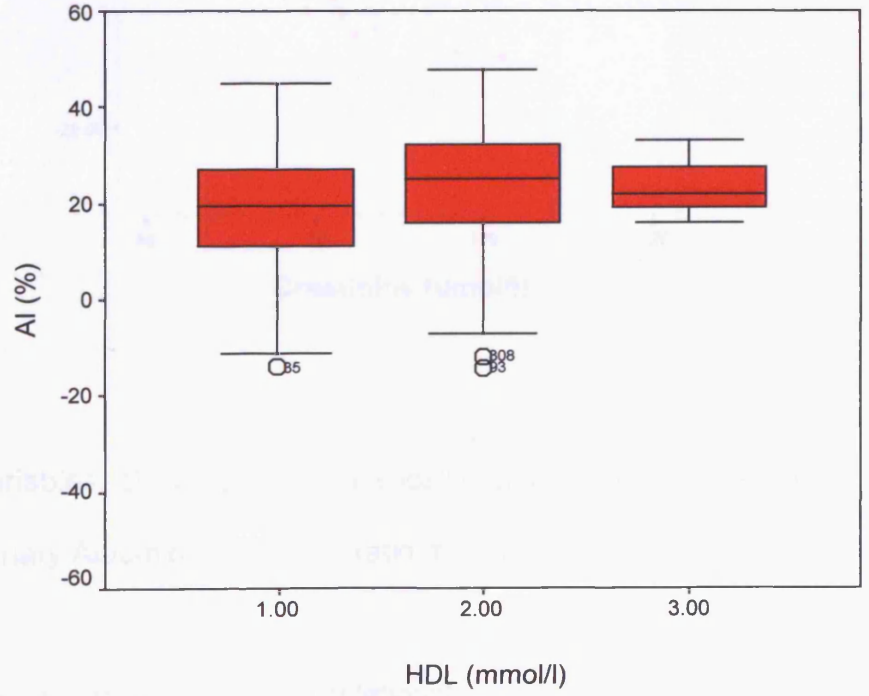
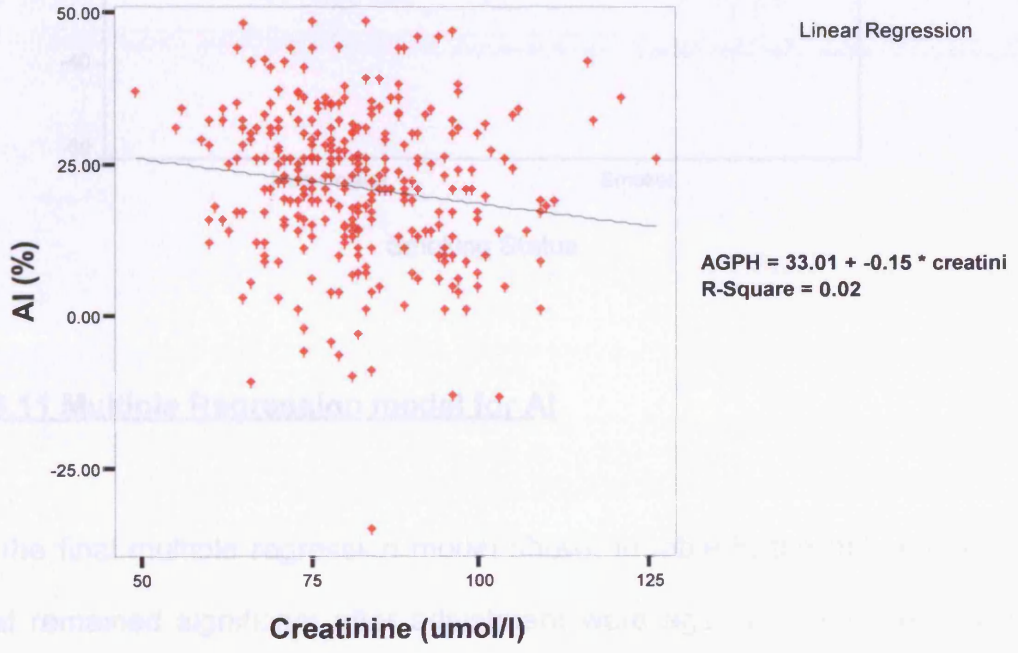


Figure 35: Augmentation Index vs. HDL Cholesterol



A negative but significant relationship of AI to serum creatinine was also noted, but importantly this was not adjusted for sex, age or body mass (figure 36). It is conceivable that this relationship is considerably influenced by gender, as females have lower creatinine levels and higher AI.

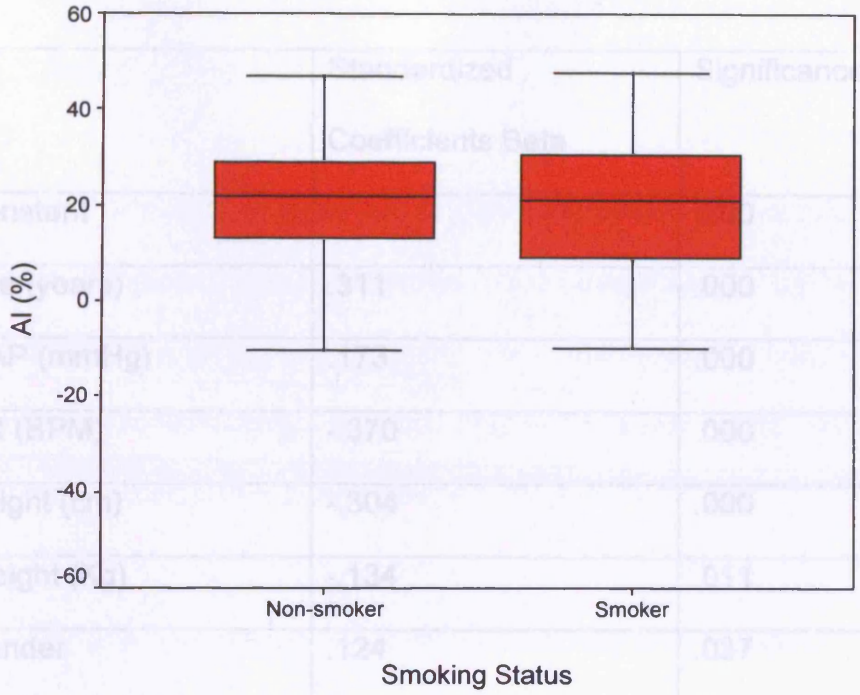
Figure 36: Augmentation Index vs. serum creatinine



Variables showing no statistically significant correlation to AI included urinary Albumin: creatinine ratio, fasting glucose, triglycerides and HbA1c.

There was no significant relationship between AI and smoking status in this population (figure 37).

Figure 37. Smoking status and AI



3.3.11 Multiple Regression model for AI

In the final multiple regression model shown in table 6, the only predictors that remained significant after adjustment were age, MAP (chosen as BP parameter), heart rate, height, weight and gender.

Neither serum total cholesterol, LDL or HDL cholesterol remained as significant predictors of AI in our final model. Possibly this can be explained by the fact that lipid levels in the majority of our group were within normal reference range as defined by our local laboratory and quite probably due because of the relatively small sample size of the group.

Table 6. Final multiple regression model

	Standardized Coefficients Beta	Significance
Constant		.000
Age (years)	.311	.000
MAP (mmHg)	.173	.000
HR (BPM)	-.370	.000
Height (cm)	-.304	.000
Weight (Kg)	-.134	.011
Gender	.124	.037
HDL (mmol/l)	.033	.524
LDL (mmol/l)	.101	.161
Cholesterol (mmol/l)	-.041	.570
Creatinine (umol/l)	-.065	.204

3.4 Chapter Summary

3.4.1 Age

As alluded to above, age has a profound effect on vascular stiffness in human subjects. This observation has been made by a number of investigators using a variety of techniques, as simple as sphygmomanometrically derived peripheral pulse pressure to Doppler studies of aortic pulse wave velocity[28-30;50;65;67;72;73;138-141].

Whether this age related stiffening is interlinked with an age related increase in blood pressure is still a contentious issue. Westernisation appears to be a stimulus for both increasing blood pressure and arterial stiffness seen with aging in many populations studied.

Despite little increase in peripheral blood pressure in this population with age, the remarkably linear and tight relationship of Augmentation Index with age adds weight to the concept of age related arterial stiffness possibly independent of blood pressure. AI is seen to increase by 0.3% per year of ageing. This relationship will be reviewed in the following chapter.

Preliminary findings from this data would suggest that Augmentation Index may provided a reasonable marker of ageing and is certainly deserving of further study.

3.4.2 Heart Rate

The negative relationship of AI and HR is not a novel finding[132;135;142-144]. Very simplistically, as heart rate increases, there is less time in the cardiac cycle for the reflected peripheral wave to augment the outgoing systolic wave. As ejection duration falls, so to does Augmentation Index. As heart rate increases, the relative time spent in systole and diastole alter, in an attempt to both preserve systole and therefore stroke volume, but also diastole to allow adequate ventricular filling and coronary blood flow.

In the normal population studied, although the trends above were noted, no significant change in the percentage of time spent in systole and diastole was noted with age. It is likely therefore that the relatively minor age related changes in heart rate have little effect on the more significant age related increase in AI seen within the group.

The fact that AI is very dependent on heart rate however, has profound implications when studying the impact of various pharmacological agents on measured vascular stiffness using this technique. In addition to a fall in mean arterial pressure (and therefore arterial distending pressure), rate altering agents such as beta-blockers and certain calcium channel blockers will result in an increased AI as heart rate falls (converse to the mechanism described above, greater cardiac cycle time allows greater time for the reflected wave to augment the outgoing systolic wave).

To further complicate matters, the unopposed alpha effects seen in beta blocked patients may result in greater peripheral vasoconstriction and may therefore alter peripheral reflection sites, making them functionally more proximal. As discussed previously, It has been suggested that the resultant increase in AI seen with rate limiting agents may go some way to explain the disappointing results in terms of left ventricular hypertrophy regression seen with these agents despite respectable falls in peripheral (brachial) BP. The beneficial effect on vascular compliance and wave reflection seen with other anti hypertensive drugs e.g. ACE inhibitors may explain why the reverse of this is true with these agents.

Whether potentially adverse effects arterial wave properties remain significant after adjusting for the beneficial effects of BP reduction on cardiovascular outcome remains to be seen in large, randomised clinical trials e.g. ASCOT[121]. The impact of these therapies on PWA derived parameters is currently being specifically assessed in a major sub-study within ASCOT, the conduit artery functional end-point study (CAFÉ)[80].

It is therefore important to recognize the impact of HR on AI when comparing heterogeneous groups. Certain disease states e.g. DM and indeed hypertension can result in significant changes in resting HR as a result of the underlying disease process on autonomic regulation and sympathetic drive. It is vital not to over-interpret these differences in

measured AI as alterations in vascular stiffness as they may be fully explained after adjustment for HR differences.

Using our control population, a reasonable adjustment factor for heart rate is a decrease in AI of 3.7% for an increase in HR of 10 beats per minute. Although caution is advised when applying adjustment factors derived from data from normal control populations to patients with various disease states, data from hypertensive and diabetic populations we have studied yields a similar relationship between AI and heart rate. This adjustment factor also compares favourably to that provided by an independent pacing study performed to specifically assess the impact of alteration in HR on measured AI[143].

3.4.3 Height

Body height predictably has a significant impact on AI that is most likely and simply explained by a longer aorta in taller people, and thus a greater distance to distal reflecting sites, delaying return of the reflected wave. This phenomenon has been documented by other investigators[90;132;145-147] and our data above suggests that differential height is the simple explanation for gender differences in AI.

Females have a greater AI for any given age than their male counterparts (figure 29). This is likely to be predominantly due to their shorter stature (figure 31). This observation refutes alternative more speculative

explanations suggested by others, i.e. that the differences in AI may be due to the vasoactive properties of oestrogen[148;149]. It also addresses one of the conundrums as to why women seem to have a higher age adjusted AI than men and yet less cardiovascular risk at any given age.

Whether or not the increased AI in women is detrimental in real terms remains unknown.

The observed differences in central haemodynamic parameters, in particular Augmentation Index, between the sexes have been linked by some researchers to the greater age related increase in left ventricular mass seen in females. Higher age-adjusted AI values in women have also been suggested as a reason why females appear more likely to develop symptomatic left ventricular failure following myocardial infarction than males despite higher ejection fractions[150].

These adverse alterations in central haemodynamic parameters may go some way to explain the elevation in cardiovascular disease that has previously been described in association with short stature[151], although supportive data for this association in large study populations is lacking[152;153].

3.4.4 Blood pressure

Although, as noted above, a trend for increasing peripheral systolic blood pressure was seen with age, this trend did not seem as tightly related as that between age and AI.

Peripheral diastolic blood pressure is obviously more closely related to central diastolic blood pressure than peripheral systolic blood pressure is to central systolic blood pressure, due to the phenomenon of systolic wave augmentation discussed previously. This and the fact that AI is largely adjusted for BP may explain why DBP does not remain a statistical predictor of AI.

MAP is most likely to produce 'steady state' distending pressure for the large arteries and given the interaction of blood pressure variables it was taken as the blood pressure parameter of choice, and remained a significant predictor of AI in the final model.

From our data, an increase in MAP of 1 mmHg is associated with an increase in AI of 0.17%.

Caution must be applied when extrapolating this finding to more diverse population groups as our group is somewhat artificially cut off at a systolic blood pressure of 140 and diastolic blood pressure of 90 mmHg. Although this cut off may have effects on subsequent statistical analysis we feel

justified in demarcating our normal group in this way, as these 'artificial' parameters are those we apply in routine clinical practice when we define someone as 'normotensive'.

3.4.5 Weight

Weight remained a predictor of AI in the final regression model. This is difficult explain and shows an inverse relationship with AI. As noted earlier, possible mechanistic explanations may be alteration in peripheral reflection sites associated with increased tissue requirements for blood and a degree of peripheral vasodilatation. Increased adipose tissue is associated with greater oestrogen production which may also act to vasodilate vascular beds. These thoughts are purely speculative and require further study.

3.4.6 Biochemical parameters including serum lipids and urinary ACR

As mentioned earlier, there were no statistically significant correlations between parameters in this group and arterial stiffness as measured by Augmentation Index in our final model.

Serum lipid levels were of particular interest due to the well known association between elevation of these molecules and cardiovascular disease.

Despite this we were unable to confirm a relationship between lipid levels essentially in the normal population range and augmentation index. This is interesting because studies using a more direct method of measuring arterial stiffness i.e. pulse wave velocity, have also been unable to show a significant relationship with lipid levels throughout a "normal range"[29;67;141]. The situation may be different in people with hypercholesterolaemia, one small study having shown a relationship between hypercholesterolaemia and increased arterial stiffness[130].

3.5 Discussion

This control population provides us with a unique opportunity to assess the impact of 'normal' ageing on central haemodynamic parameters once the effects of various co-morbidities such as hypertension; dyslipidaemia, diabetes and renal dysfunction etc had been accounted for.

As already mentioned, despite possible pitfalls in 'selecting out' a group of 'normal' individuals, we felt this was necessary to remove some of the confounding factors e.g. hypertension when assessing the effects of ageing on arterial stiffness healthy volunteers. In this way, the effects of a number of demographic and physiological variables and their impact on various measured haemodynamic parameters can be assessed prior to the application of this technique in future clinical trials.

It is possible that in selecting out a "normal" control group we artificially select those elderly patients who do not display predictable rises in BP seen commonly in a "westernised" society with ageing. It may be therefore that these specific individuals are protected to a degree by some mechanism and to some extent relatively 'abnormal' compared to the general population as a whole.

As mentioned on several occasions previously, ageing is not inevitably linked to hypertension and we feel happy therefore that this artificial demarcation was justified.

This group will act as a control group for any further studies. A large control population such as this gives invaluable insight to predict the magnitude of measured change in vascular indices that can be expected by alteration in simple variables such as heart rate and body height.

The results of this control dataset show that despite little clinically significant change in blood pressure with age, there is a remarkably linear increase in augmentation index (in part a surrogate marker of PWV and therefore indirectly vascular stiffness).

This increased vascular stiffening with age is supported by previous observations of blood pressure and peripheral pulse pressure seen with

ageing in other much larger cohorts with less restriction on blood pressure required for inclusion [17] and population studies measuring PWV[67].

Whether this increase in vascular stiffness is related to factors associated with westernisation independent of blood pressure e.g. increased sodium intake, reduced exercise, smoking and high levels of cholesterol cannot be determined from this study but these factors are likely to play a major role. Interestingly variation in lipid levels and blood glucose within a "normal" reference range appeared to have little impact on Augmentation index in this normal cohort.

Never the less this study adds weight to the growing body of evidence suggesting a link between ageing and arterial stiffness, even in seemingly healthy people.

As discussed previously, age related changes to the cardiovascular system may be structural or functional or more likely a combination of both. It is likely that whatever the underlying mechanism, increased vascular stiffness is inextricably linked to the development of conventional target organ damage (LVH, renal disease and retinopathy) and cardiovascular events (MI and CVA).

A more complete understanding of 'normal' ageing effects on the vasculature may lead to more specific targeting of therapy to help reverse or

at least prevent continuing damage to this important 'target organ' in its own right.

Early use of pharmacotherapy to reduce distending mean arterial pressure in large conduit arteries, and therefore reduce "functional" stiffness may slow pulse wave velocity and limit subsequent early wave reflection from the periphery. This may ultimately go some way to reducing potentially irreversible 'reactive' structural cardiovascular alterations and consequent cardiovascular and cerebrovascular events.

Since normal background vascular ageing appears to be independent of coexistent disease, it may be that greater attention to western lifestyle factors e.g. high salt and fat diet, low levels of exercise and smoking may be the only option for preventative intervention. Although often successful in clinical trial settings, risk factor modification is notoriously difficult to achieve in clinical practice and requires considerable effort and enthusiasm on the part of the treating physician and patient alike.

It is likely that a population based approach rather than an individual one, in addition to a culture shift in western lifestyle may be necessary to affect significant change. Such an approach now underpins the governments new National Service Framework for management of coronary heart disease and we await the results of success of application and subsequent outcome over the coming years.

To follow on from our control group study, we sought to investigate whether the technique of pulse wave analysis to measure augmentation index as a surrogate of vascular ageing would prove a more sensitive method of detecting occult vascular disease in patients with clinically uncomplicated hypertension

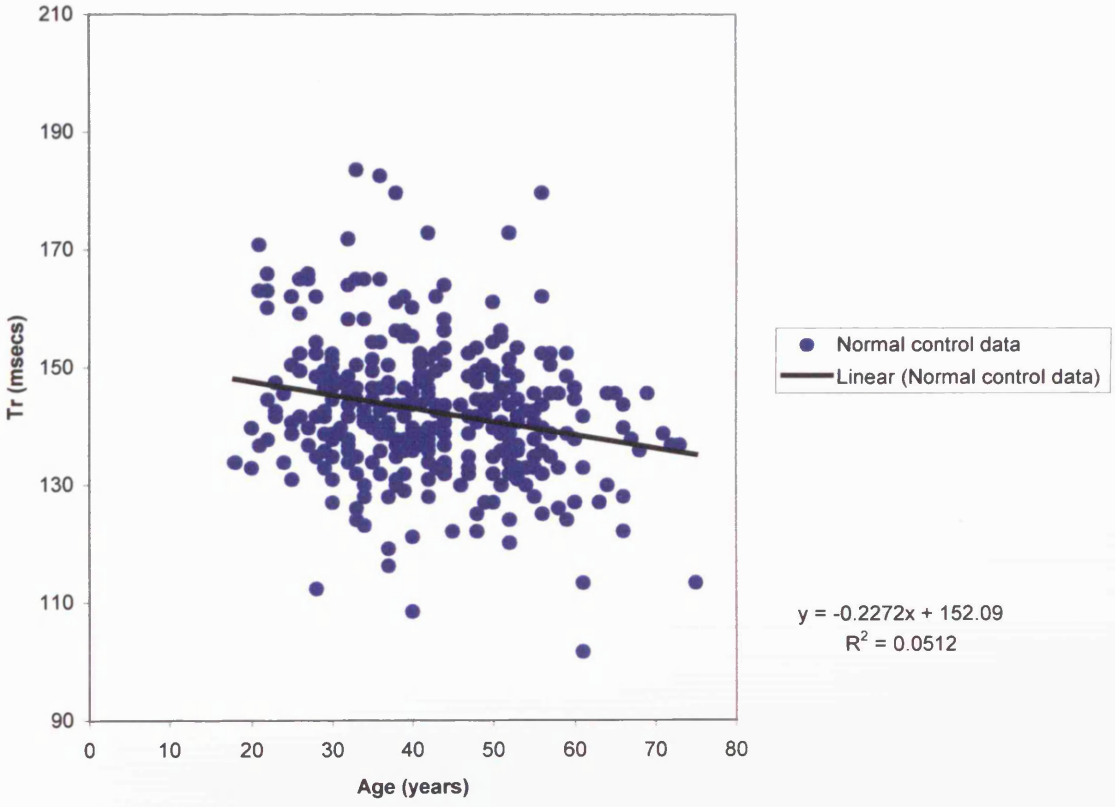
3.6 Addendum

The relationship of AI to vascular stiffness is thought to lie mainly in its relationship to timing of the reflected wave (and therefore indirectly pulse transit time or pulse wave velocity). The timing of the reflected wave on the incident wave (Noted as Tr previously) is often quoted as another indirect marker of pulse wave velocity. It has some advantage over AI in being largely perceived as heart rate independent.

Unfortunately at the outset of this thesis, the available software was not able to extract Tr data and hence this parameter was not discussed in the above chapter. As the commercial software developed it became possible not only to extract Tr data from new recordings but also to re analyse stored data to calculate Tr on previously acquired waveforms.

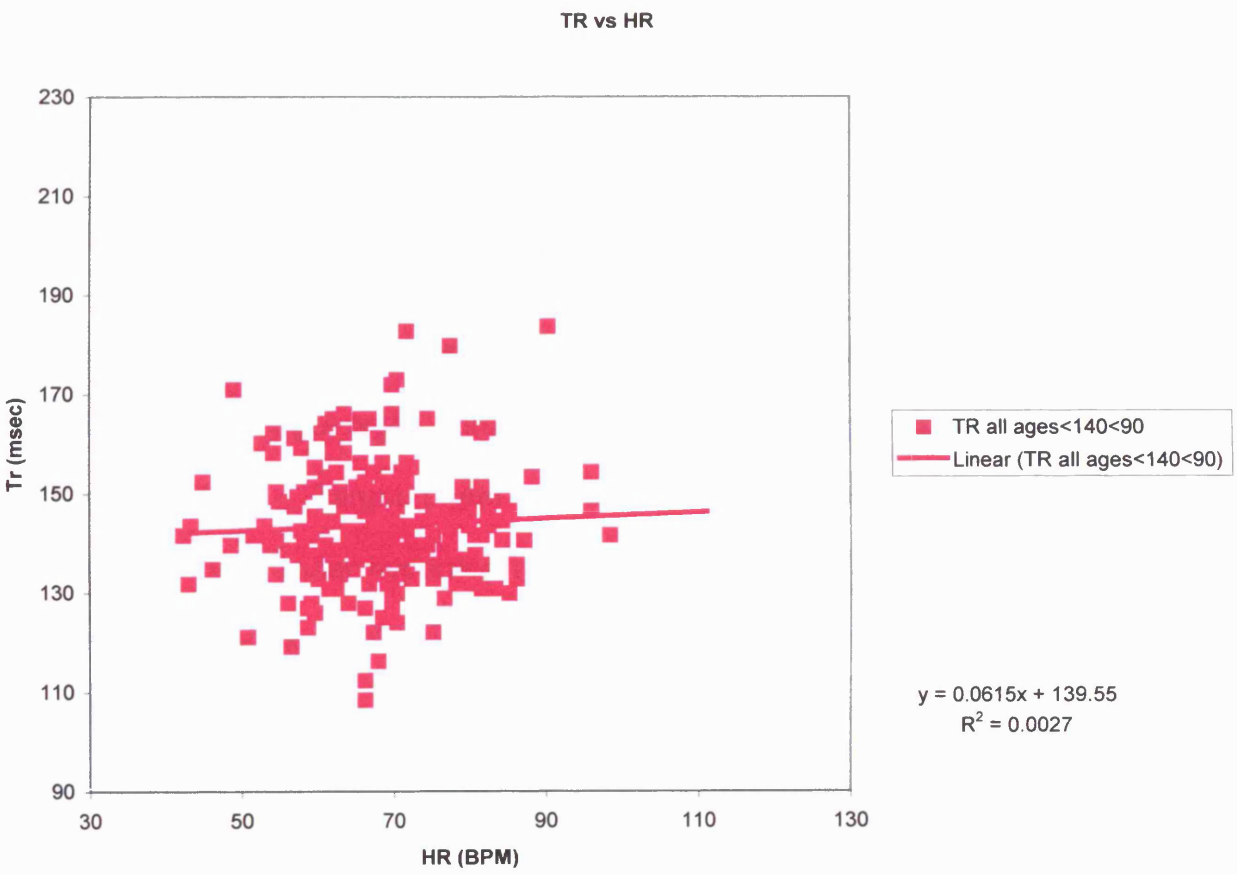
As Tr would prove a useful measure in further studies, the normal control data above was re-analysed for Tr and showed a reducing Tr with age consistent with increasing pulse wave velocity and increasing vessel stiffness (figure A).

Figure A



In accordance with the published literature, the relationship between HR and Tr was neutral for our control group, artificially cut off at SBP<140 and DBP<90. (See figure B)

Figure B


TR vs HR
230
210
190
170
150
130
110
90
Tr (msec)
30
50
70
90
110
130
HR (BPM)
TR all ages<140<90
Linear (TR all ages<140<90)
y = 0.0615x + 139.55
R2 = 0.0027

4.0 The use of Pulse Wave Analysis in the assessment of hypertensive patients. (Results 2)

4.1 Introduction

Current guidelines now advocate the treatment of borderline (Stage 1) hypertension only if target organ damage is present or the patient is at high cardiovascular risk[64] . Target organ damage is usually defined as the presence of left ventricular hypertrophy by ECG or echocardiography, the presence of renal impairment or proteinuria / microalbuminuria or evidence of hypertensive retinopathy. High risk for the purpose of this study was defined as per the BHS guidelines for treatment of hypertension i.e. a CVD risk of >20% over 10 years[64]. Remarkably, to date, no method of assessing occult injury or dysfunction of the vasculature has been applied to the routine assessment of target organ injury in hypertensive patients. This is despite the fact that experimental evidence suggests the presence of ongoing vascular injury in people with hypertension. For example, it is postulated that people with hypertension exhibit disturbance in vascular endothelial function[154-156], although whether cause or effect is still much debated. It is also well established that the haemodynamic performance of the vasculature declines with age. In this latter regard, vascular stiffness has been shown to increase with age resulting in a doubling of pulse wave velocity between the ages of 20yrs and 80yrs[30;67]. This increase in vascular stiffness may in part relate to declining endothelial function with age but is

also due to age-related changes in vascular structure culminating in a progressive loss of vascular compliance [45;50].

Recent studies have suggested that the development of increased vascular stiffness can be impacted upon by factors other than age, notably co-existing disease, i.e. hypercholesterolaemia, diabetes mellitus and chronic renal disease [69;123;125;130;157-167]. Thus, the determination of vascular stiffness may provide an insight into early functional and structural changes in large blood vessels that could ultimately be used to assess whether or not the vasculature as a "target organ" exhibits evidence of dysfunction or injury.

Such a finding would not be without importance because the vasculature is chronically and directly exposed to increased mechanical stress in people with hypertension and thus might be expected to be the most sensitive marker of cumulative injury.

Moreover, the consequences of increased vascular stiffness are important from a pathological perspective. Increased vascular stiffness increases pulse wave velocity and leads to systolic pulse wave augmentation. This increases systolic work and the systolic: pressure time interval and thus by necessity decreases the diastolic pressure time interval, predisposing to left ventricular hypertrophy and myocardial ischaemia[45]. Furthermore, increased vascular stiffness has now been shown to be an independent risk factor for cardiovascular disease[19;69;70;72;157].

Increased vascular stiffness ultimately leads to an increase in systolic blood pressure and a widening of pulse pressure, both of which independently predict an increase in cardiovascular risk[19;21;70;168] .

Since systolic wave augmentation is indirectly related to aortic pulse wave velocity, it has been suggested that AI in fact acts as a surrogate marker for vascular stiffness. Although augmentation itself is related to blood pressure, AI, expressed as a proportion of total pulse height, and is therefore considered to be largely blood pressure independent.

Therefore, the purpose of the present study was to use a simple non-invasive technique to determine whether young people with either uncomplicated, borderline (stage 1) hypertension or overt hypertension show any evidence of increased vascular stiffness as suggested by Augmentation Index when compared with a normotensive population.

4.2 Experimental Design and Methods

See general methods previously described in chapter 2.

4.21 Study Population Characteristics

Never treated hypertensive patients (n=78) (systolic BP>160mmHg and/or diastolic BP>100 mmHg) were invited to take part at the time of being assessed at the Leicester Hypertension Clinic. Patients were selected for

inclusion if they were aged less than 50 years and had uncomplicated essential hypertension. Uncomplicated hypertension was defined as the absence of target organ damage as determined by routine clinical examination and investigation. The absence of left ventricular hypertrophy was confirmed by the Sokolov voltage criteria on a routine 12-lead ECG, and by echocardiography. The presence of hypertensive retinopathy was determined by clinical fundoscopy and proteinuria was excluded by routine urine dip-stick testing.

Normotensive controls (n=216) (systolic BP<140 mmHg and diastolic BP<90 mmHg) were recruited as part of a volunteer cardiovascular screening programme of "healthy volunteers" within hospital employees and their families. The "borderline hypertension" group was taken from patients either screened in the hypertension clinic or referred up by their local GP and who were thought not to require treatment.(n=53)(systolic BP 140-159mmHg or diastolic BP 90-99mmHg).

All participants in the study gave signed informed consent and the study was approved by the Leicestershire Health Local Research Ethics Committee (see appendix).

4.22 Blood Pressure measurement

As previously described in chapter 2, peripheral blood pressure was measured over the brachial artery of the right arm after a five minute seated

rest using an Omron 705CP automated oscillometric sphygmomanometer. Three readings were taken five minutes apart and the mean of the last two readings were used as the measure of their peripheral blood pressure. Immediately after blood pressure was measured, applanation tonometry was performed over the right radial artery with the patient remaining seated and relaxed.

4.23 Applanation Tonometry

As previously described, pulse wave analysis using applanation tonometry was performed at the right radial artery site. We adhered to a previously described predetermined criteria of pulse height and variability to ensure quality control. (Pulse height >100, pulse height variability <10% and diastolic variability<10%). Any recordings not satisfying these criteria were discarded and the measurement repeated.

4.24 Statistical methods

One-way analysis of variance (ANOVA) was used to assess differences between the three groups for the continuous demographic and clinical variables that appeared normally distributed, and Chi-squared tests for differences between categorical variables. Kruskal – Wallis analysis was performed for comparison of non-parametric data.

Multiple linear regression was used to further examine AI as a surrogate of vascular stiffness controlling for the influence of age, heart rate, height, gender, diastolic and systolic blood pressure. The influence of these variables on augmentation index was examined and SPSS (v.11.5) was used for all statistical analyses.

4.3 Results

4.31 Demographics of the study population

Tables 1 and 2 show descriptives and haemodynamic parameters for the three groups respectively. The study entry criteria excluded patients with evidence of target organ damage (i.e. ECG Sokolov criteria for left ventricular hypertrophy or an LV mass index by echocardiography of >125g/m^2 (males), >110g/m^2 (females), proteinuria or retinopathy.)

Table 1 Demographic details for the three study groups

Group (n)	Age (yrs)	Height (cm)	Weight (Kg)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	Heart Rate (bpm)
Normotensive (216)	36.9 (7.8)	166.5 (10.3)	70.1 (14.0)	114.4 (12.9)	75.7 (7.4)	88.6 (8.6)	69.3 (10.9)
Borderline Hypertensive (53)	37.8 (8.8)	170.2 (10.7)	80.8 (14.8)	143.3 (9.7)	91.5 (4.6)	108.7 (4.3)	72.5 (13.6)
Hypertensive (78)	37.4 (7.6)	168.9 (10.6)	80.7 (7.1)	166.2 (20.5)	106.4 (9.7)	126.3 (11.2)	75.1 (14.1)
	P=0.62	P=0.33	P=0.58	P=<0.001	P=<0.001	P=<0.001	P=<0.001

As defined by the protocol, statistically significant differences existed between the three groups for both systolic ($p<0.001$) and diastolic ($p=<0.001$) blood pressure. In addition, there was also a statistically significant difference between groups for MAP and peripheral pulse pressure (PPP) ($p=<0.001$)

There was no statistically significant difference in age ($p=0.62$), height ($p=0.33$) or weight ($p=0.58$) between the groups.

Of interest, the hypertensive group had a significantly higher mean heart rate than the normotensive controls (75.1bpm vs. 69.3bpm). This is a well-recognised phenomenon attributed to the increased adrenergic drive in hypertensive patients.[169] More surprisingly, however, was the finding that the borderline hypertensive patients in this young age group without evidence of target organ damage also showed a significantly elevated mean heart rate when compared to normotensive controls (72.5bpm vs. 69.3bpm), probably reflecting early evidence of disturbance in autonomic cardiovascular regulation.

A significant difference in gender distribution between groups (p=<0.001) was also noted

Table 2 Demographic details for the three study groups

Group (n)	PPP (mmHg)	CPP (mmHg)	CAI (%)	PAI (%)	Amp.	Tr (msecs)	Smokers (%)	Gender split (%) (m:f)
Norm. (216)	38.7 (9.0)	28.3 (7.2)	18.9 (11.9)	70.6 (18.8)	1.38 (0.19)	143.0	18	32 (69:147)
Borderline Hypertens (53)	51.8 (10.9)	38.2 (9.0)	19.7 (12.6)	72.1 (17.5)	1.38 (0.21)	140.0	20	49 (26:27)
Hypertens (78)	59.8 (18.4)	44.8 (15.4)	22.1 (10.3)	78.6 (19.6)	1.36 (0.19)	135.2	16	59 (46:32)
	P<0.001	P<0.001	P=0.88	P=0.12	P=0.63	P=0.005	P=0.83	P<0.001

4.32 Augmentation Index and Amplification

Augmentation Index for the three groups is shown in figure 1. Despite clear differences in MAP and PPP between groups (figures 2 and 3), there was no difference in unadjusted central Augmentation Index between the three groups. Augmentation Indices were in the same order of magnitude as described in the literature in people of the same age, and in other control populations previously studied by our group[112;127].

Figure 1. Augmentation index between groups

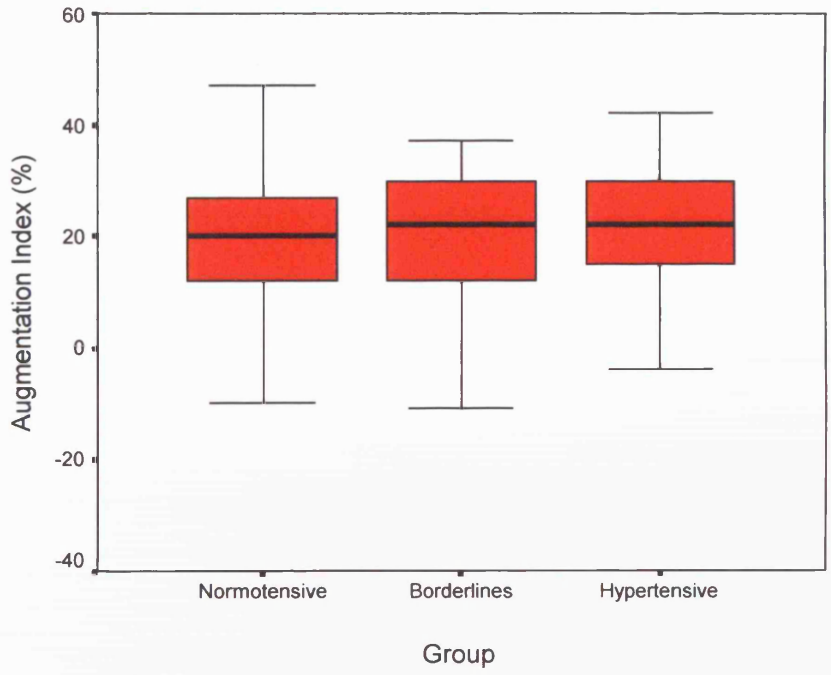


Figure 2. MAP between groups

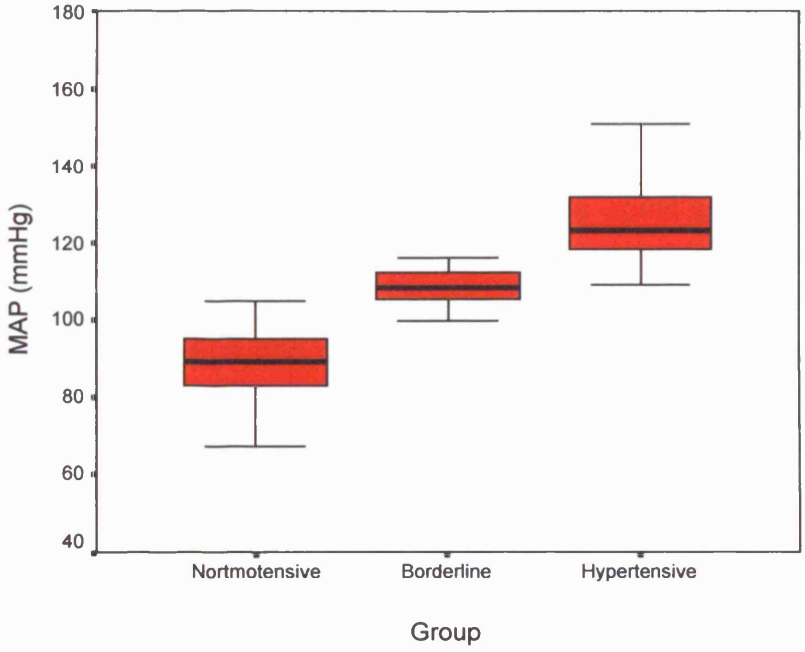
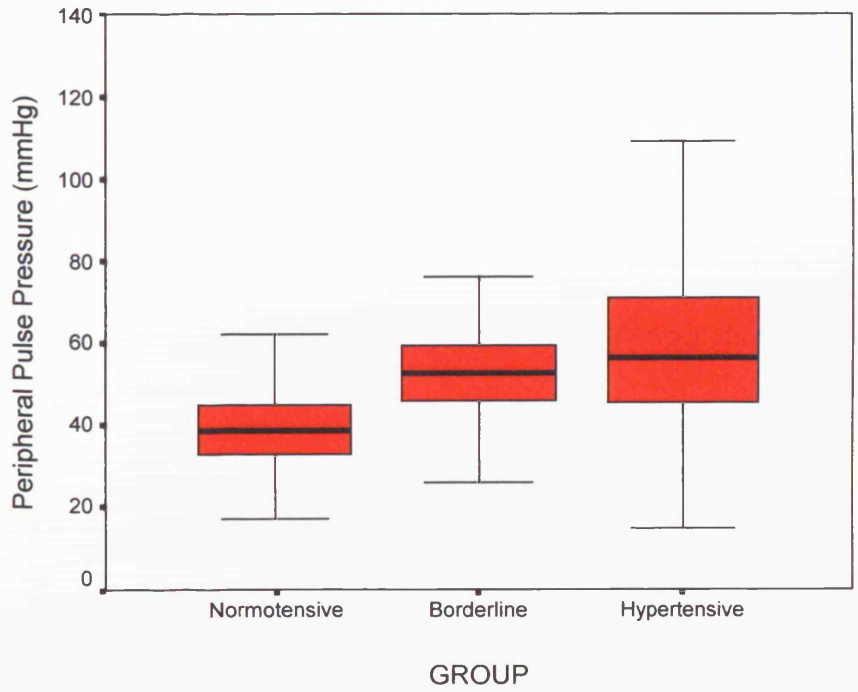


Figure 3. Peripheral Pulse Pressure between groups



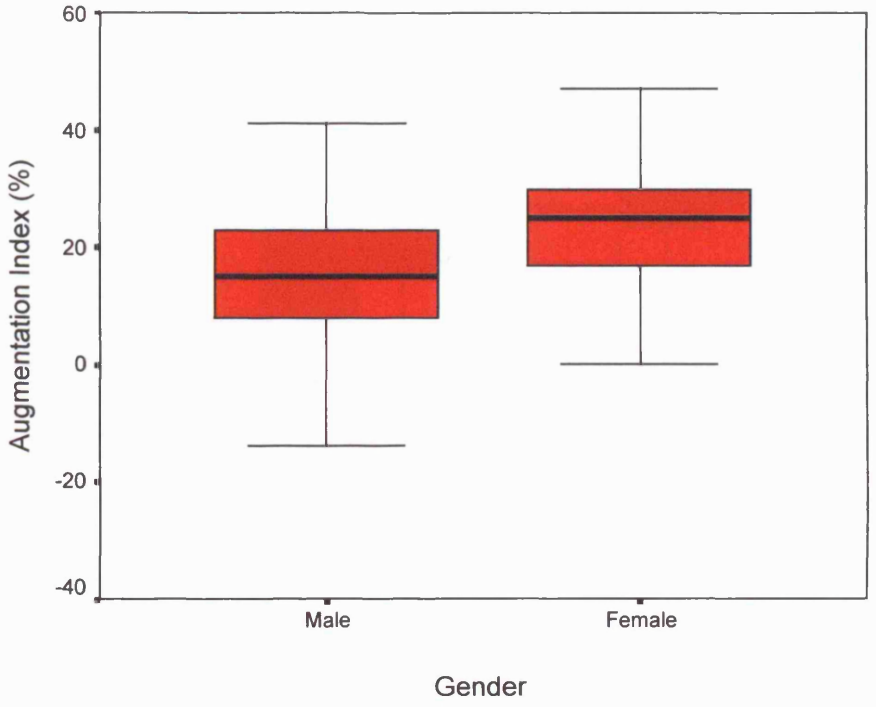
As discussed previously, the influence of heart rate on Augmentation Index is well documented in the literature. Increased heart rate is associated with diminished AI. Since heart rates were significantly different between the groups, a significant impact on AI can be expected. The higher heart rate in the borderline and overt hypertensive groups would act to reduce their AI.

As heart rate is just one of the many potential confounding variables in the estimation of AI we did not 'adjust' AI for heart rate differences but instead analysed heart rate as a component of a multiple regression model to further study the differences between groups.

Gender differences could also profoundly influence AI, and this is largely thought to be due to differences in height. Although significant differences in gender were reported between groups there was no significant difference in height between the groups, making the likely impact of sex distribution small.

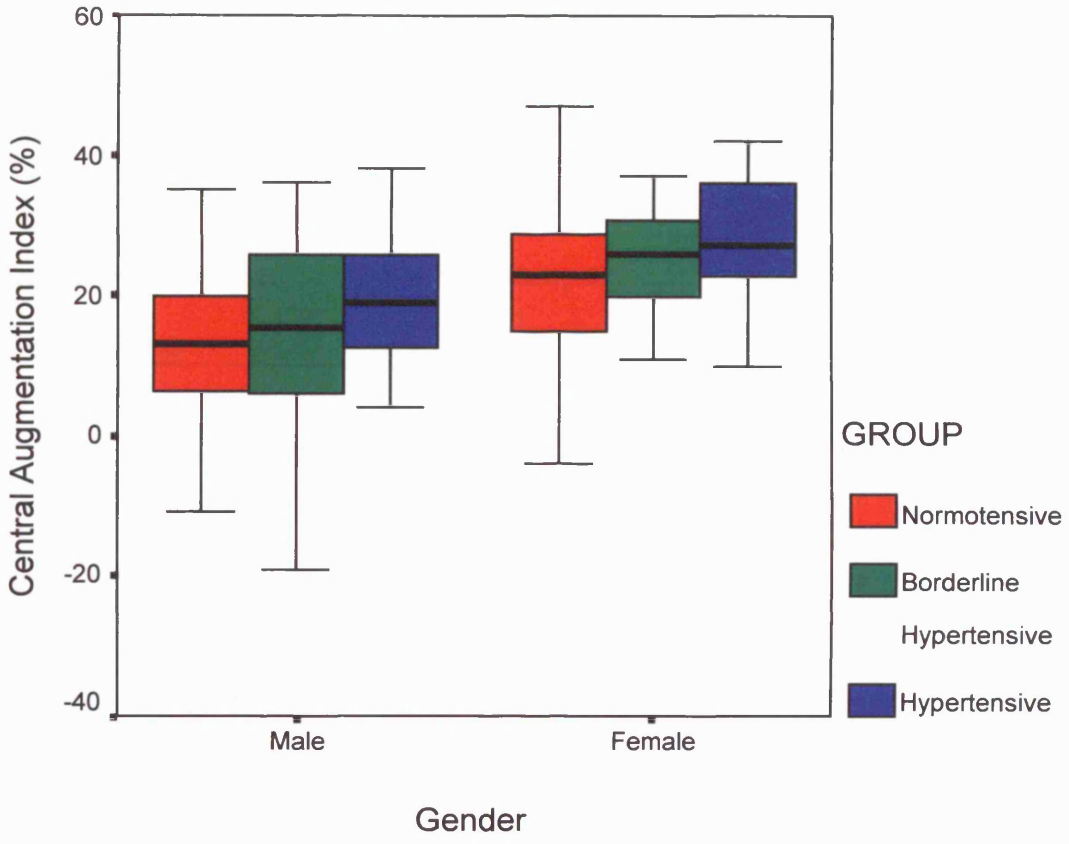
The effects of gender on AI for the whole study population is shown below in figure 4.

Figure 4. Effects of Gender on AI for whole of study population



Although AI was not statistically significant between groups as a whole, given the influence of gender, further analysis of the data subdivided for gender was undertaken. Although no statistical difference in mean AI between groups remained for females, differences in mean AI between normotensive and hypertensive males became significant ($p<0.005$) (See figure 5)

Figure 5 AI between groups split for gender



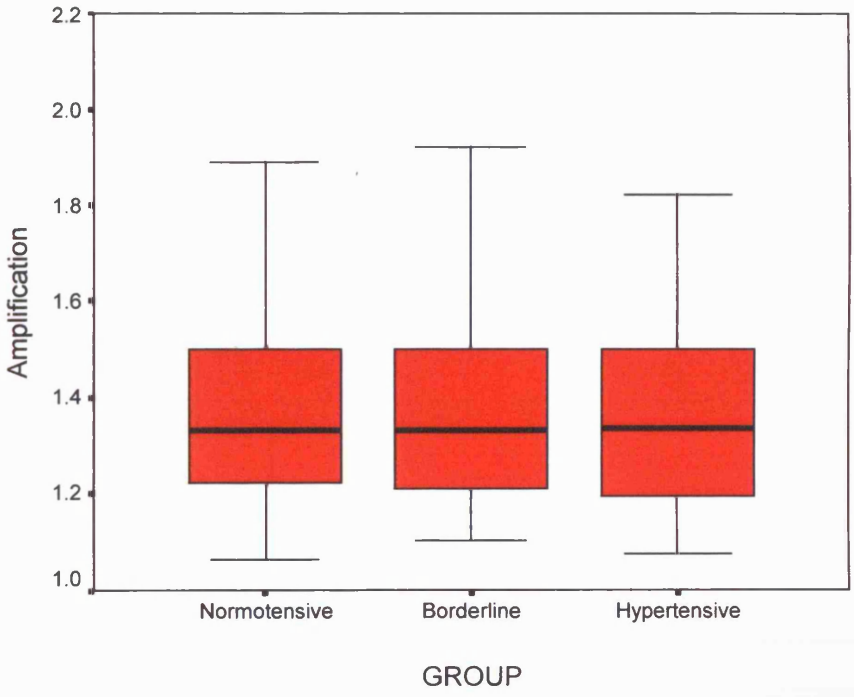
A key finding is that there was no significant difference in AI between the groups, despite the fact that peripheral blood pressures, central systolic, central diastolic and central pulse pressures were significantly different between the three groups

As discussed previously, changes in the amplification of pressure from central arteries to the peripheral circulation (PPP: CPP) is another derived index that is often quoted to be a surrogate for changes in arterial stiffness.

A youthful compliant aorta normally results in a measurable pulse pressure gradient from the central vessels to the periphery. As central vessels

become stiffer and augmentation of central pressure occurs, this gradient falls. We found no difference in pulse pressure amplification between groups (see figure 6). This finding suggests that in addition to AI, pulse wave amplification was also a poor discriminator between groups in our study.

Figure 6. Amplification for the three groups

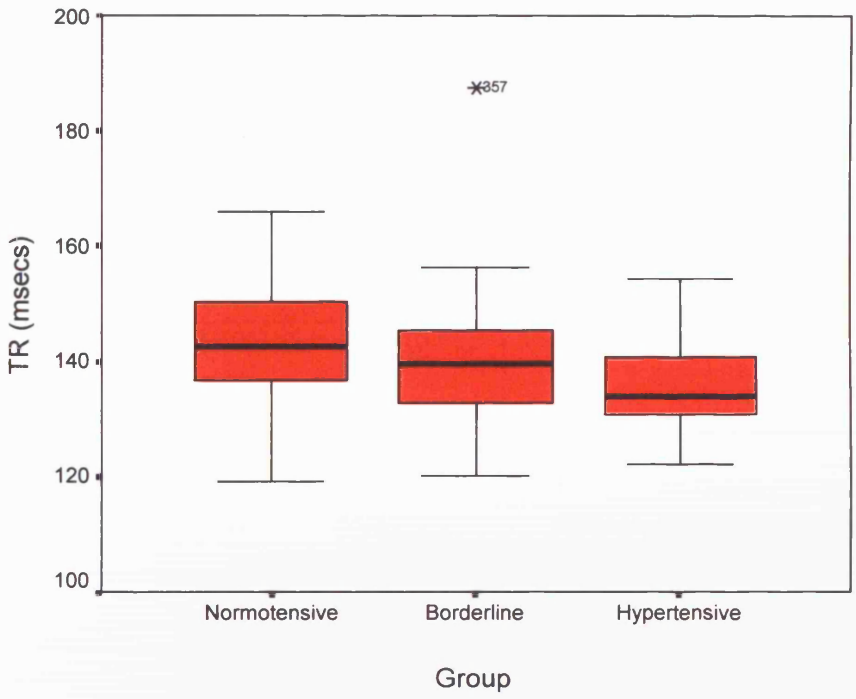


4.33 Time to wave reflection (Tr)

When this study was undertaken the pulse wave velocity software was not sufficiently well developed to measure Tr. However, we were able to re-examine the data with upgraded software to measure Tr. As discussed earlier, Tr is reported to be another surrogate for arterial stiffness. The rationale being that stiffer arteries will lead to increased pulse wave velocity, earlier wave reflection from the periphery and thus shorter Tr.

We found a significant difference in mean Tr between the three groups, (figure 7). Mean Tr was significantly shorter in both borderline hypertensives and overt hypertensives when compared to controls. This finding is consistent with the hypothesis that patients with borderline or overt hypertension have stiffer central arteries, leading to shorter pulse wave transit times and earlier wave reflection.

Figure 7 Tr data for the three groups.



4.34 Other variables

The other measured variables are shown in table 3. There was a significant trend for higher triglyceride levels in the borderline and overtly hypertensive patients (figure 8). This is consistent with the observations of the Tecumseh Blood Pressure study and is consistent with the well recognised presence of

a metabolic syndrome in people with hypertension[170]. Of interest, there was also a significant trend to higher urinary albumin-creatinine ratios in people with hypertension (figure 9), consistent with the association between higher blood pressures and albuminuria[171-173]. The impact of these parameters, if any, on pulse wave characteristics is examined in our multiple regression model below.

Table 3. Other measured variables for the three groups

Group	HDL (mmol/l)	LDL (mmol/l)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Glucose (mmol/l)	HbA1c (%)	ACR	Creatinine (umol/l)
Normotensive	1.47(0.24)	2.95(0.58)	4.94(0.59)	1.16(0.46)	4.9(0.2)	5.5(0.3)	1.0(0.1)	81.7(0.9)
Borderline hypertensive	1.38(0.65)	3.08(0.17)	4.99(0.12)	1.59(0.89) *	4.9(1.0)	5.7(0.9)	1.2(0.3)	84.6(2.0)
Hypertensive	1.28(0.66)	3.60(0.19)*	5.37(0.10)*	1.94(0.12) *	4.8(0.9)	5.60.1)	2.1(0.8)	85.0(2.2)
p-value	0.066	0.01	0.001	<0.001	0.958	0.118	0.105	0.158

We acknowledge that the study lacks power to examine all of the potential variables with confidence, in addition to the relative weighting of data towards the control group.

Figure 8 Triglyceride levels for the three groups

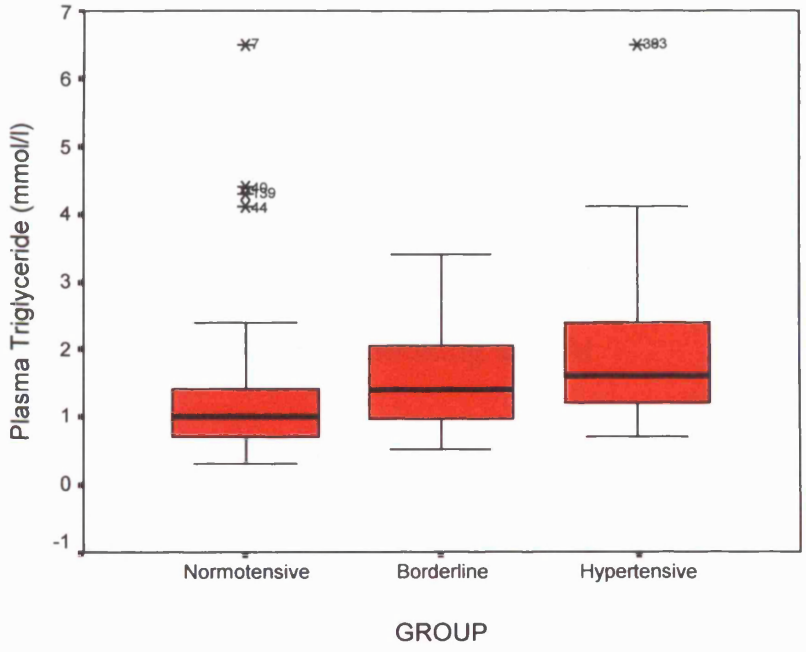
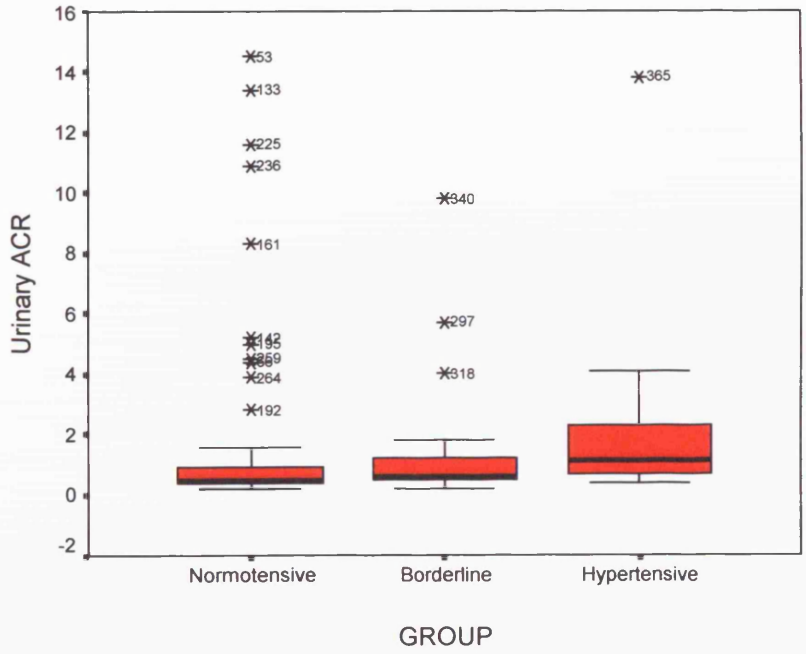


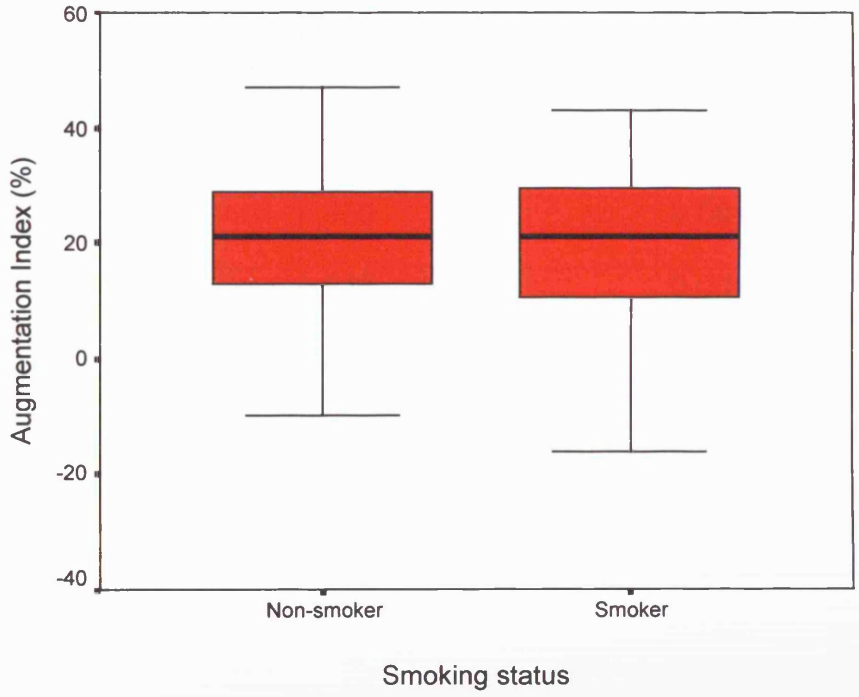
Figure 9 ACR for the three groups



As noted in table 3, total cholesterol, LDL and HDL cholesterol, glucose, creatinine and HbA1C were not significantly different between groups.

Despite cigarette smoking being strongly associated with cardiovascular disease, there was no significantly significant relationship between smoking status and AI in any group. Smoking status and AI for all subjects also showed no relationship (figure 10).

Figure 10. Relationship of smoking and AI



4.33 Multiple Regression

As alluded to above, many factors influence pulse wave analysis by applanation tonometry i.e., age, gender, heart rate, height and blood pressure itself and thus it was important to define how this reported measure of "vascular stiffness" was influenced by these factors.

A Multiple linear regression model was fitted, specifying augmentation index as the outcome. The parameter estimates for the final models (as defined by criteria in methods) fitted to these outcomes are presented in Table 4

Table 4 Results, including parameter estimates, for the multiple regression model fitted to augmentation index

	Standardized Coefficients Beta	Significance
(Constant)		0.001
Group	.159	0.001
Age (years)	.315	0.001
Heart rate (BPM)	-.373	0.001
Height (cm)	-.373	0.001
Weight (Kg)	-.091	0.103
Female Gender	.178	0.004
Cholesterol (mmol/l)	.078	0.128
Smoking status	-.006	0.897
ACR	.016	0.722
Triglycerides (mmol/l)	.091	0.091

This model explained a considerable proportion of the between subject variation, both having adjusted R-squared values of 0.51 and $p<0.001$

Analysis confirmed that the group to which a patient was allocated to, based on brachial blood pressure, was predictive of central AI. When "group" as the dependent variable is substituted with any other brachially derived blood pressure parameter, such as MAP, SBP, or DBP, predictably, all produce similar regression models and equally predict AI. This suggests that "group" is acting as nothing more than a surrogate for the pre-defined sub-division of our population by brachially derived blood pressures.

Reassuringly for the population as a whole, the effects of age, height and heart rate on AI are predictable and significant as expected. These factors, in addition to blood pressure group remain as powerful predictors of AI in our final regression model.

Interestingly, despite the adjustment for difference in height, gender remains a significant predictor of AI in the final model. This intriguing association is repeatedly found by researchers in this field[130;132;149] and has led to much speculation as to the cause of this phenomenon. The impact of gender is discussed in detail in the previous chapter. Height is the key determinant of the impact of gender on pulse wave analysis. The finding that gender is an independent predictor of higher AI in this much smaller number of patients (when compared to our much larger control population described in chapter 3) is probably a chance finding. It may also be that our population bias to female subjects, especially in the control subjects is influencing this relationship.

Lipid status, ACR, serum creatinine and smoking status appear to have no significantly predictive value for AI in the final model in our study population.

4.4 Discussion

It would be clinically useful to have a simple marker of vascular stiffness that can be measured at the bedside, especially if that marker could discriminate between those with and without occult vascular damage, early in the evolution of hypertensive injury. Pulse wave analysis has been promoted as having such characteristics. Our study suggests that this is not the case.

This study explored the possibility that Pulse wave analysis could provide evidence of functional or early structural change in the blood vessels of relatively young patients with no conventional evidence of target organ damage. One interpretation of our study findings is that these patients with borderline or overt hypertension had perfectly healthy large arteries and consequently we were unable to discern any differences in AI. Indeed previous studies have also failed to show an alteration in radial PWA parameters in patients with systo-diastolic hypertension compared with those with isolated systolic hypertension[164;174-176] (and therefore evidence already of stiffened vessels). Whilst possible, this explanation is unlikely.

An increase in MAP between groups would be expected to produce functional stiffening of larger arteries secondary to increased vessel

distension. This would result in an increased PWV. Our finding of a reduced Tr in people with borderline or overt hypertension is consistent with this assumption.

Although our multiple regression model suggests that AI is different between groups after adjustment for all confounding variables, herein lies the problem. What clinical value does the measurement of AI have, if it has to be adjusted for many confounding variables. Moreover, it would seem that this parameter offers little extra in terms of discrimination between groups than can be achieved easily and less expensively with a conventional sphygmomanometer.

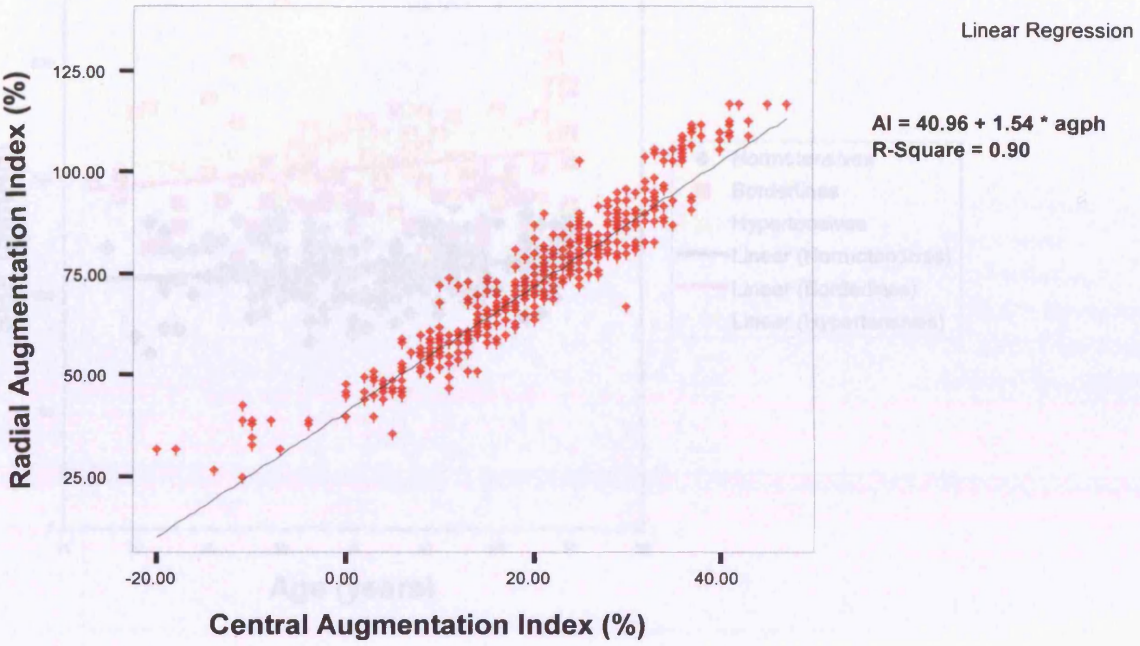
The reason for this finding is most likely to be due to the aforementioned composite nature of the derived measurement of AI. Whilst originally heralded by many as a marker of vascular stiffness, (a view that led to a more critical evaluation in this study), it is plain that AI is principally an index of peripheral wave reflection, and critically dependent on cardiac cycle times, distance to refection sites and the behaviour of the peripheral vascular beds.

The Tr data for the groups is more interesting and is suggestive of an elevated pulse wave velocity in people with borderline and overt hypertension, when compared to those with normal blood pressures .Tr has been regarded as a surrogate for PWV and the basis for this assumption seems sound. Unfortunately, at the time we undertook this study we did not

have the technology to simultaneously measure PWV in our patients, so we cannot comment on the relationship between Tr and PWV in this patient population. Nevertheless Tr has been used by many groups as a surrogate for PWV[130;177]. We remain cautious about this approach and we believe that Tr needs to be independently subjected to critical evaluation before it can be adopted as a surrogate for PWV. For example it is claimed that Tr is more robust than AI and less influenced by other variables such as heart rate. However it is conceivable that the differences in Tr noted in our study may be reflective of the differences in heart rate between groups, rather than the differences in PWV due to changes in vascular stiffness. This is discussed in more detail in the next chapter.

Pulse wave analysis may have greater use in the assessment of central haemodynamic parameters, rather than the focus on AI. A number of researchers have now looked at the Transfer function and its use in the synthesis of a central pressure wave from a peripheral wave contour. Whilst the carotid waveform is very similar to that of the central aortic waveform, this is not the case for the radial waveform. The use of a transfer function when applied to radial traces has been hotly debated in the literature, with some groups now questioning whether or not it is required at all[99-103;178]. Indeed much of the information is present in the radial pressure wave contour alone, and the relationship between radial AI and central AI is remarkably linear. Indeed this was the case in our study population, see figure 11.

Figure 11. Central vs. peripheral (Radial) AI



Supporters, some would say evangelists for this technique would argue that if all data was present in the peripheral artery without requiring the generation of a central waveform using a transfer function, then the age related changes in AI would be mirrored by changes in brachial blood pressure or perhaps more reliably brachial artery pulse pressure. This is not always the case and was not seen in any of our study populations who showed remarkably little variability in SBP, MAP or PP with age, when studied for individual blood pressure groups. (See figures 12,13 and 14) This finding also lends weight to the concept of AI providing no more information than peripheral blood pressures alone.

Figure 12. Change in brachial SBP with age for the three groups

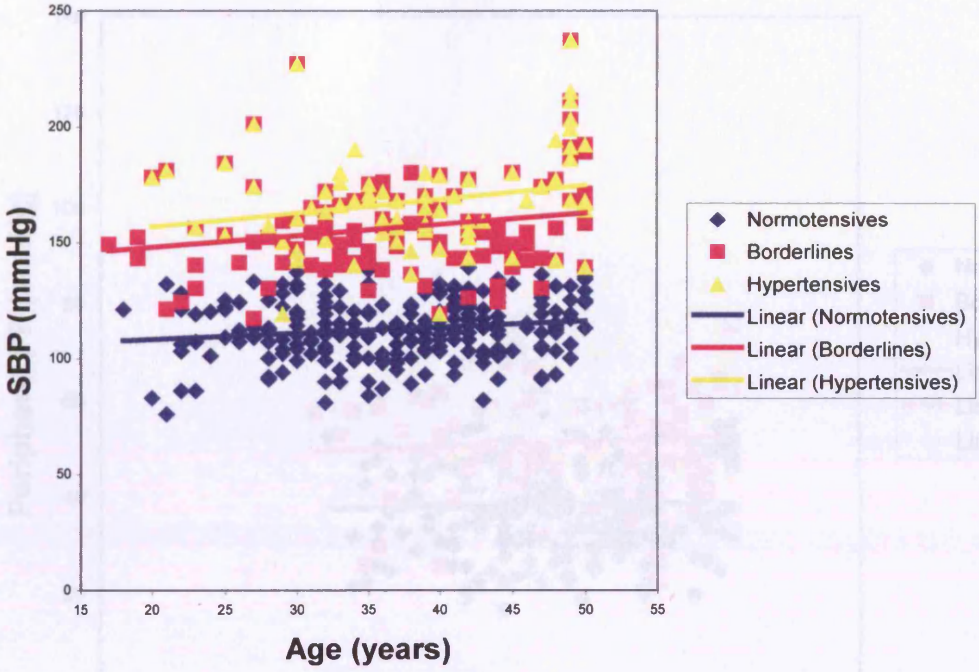


Figure 13 Change in MAP with age for the three groups

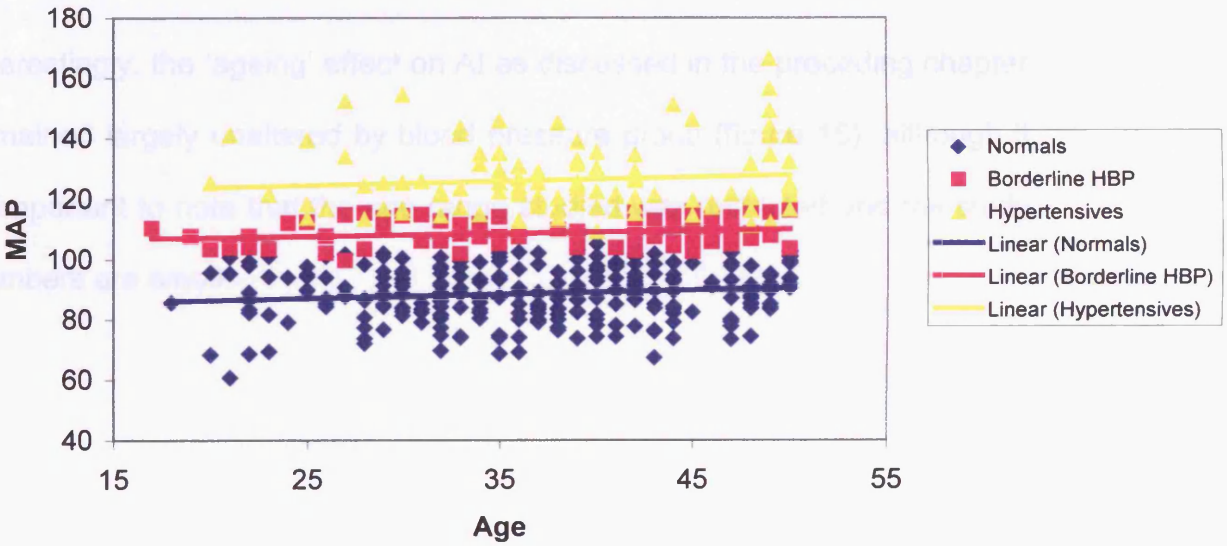
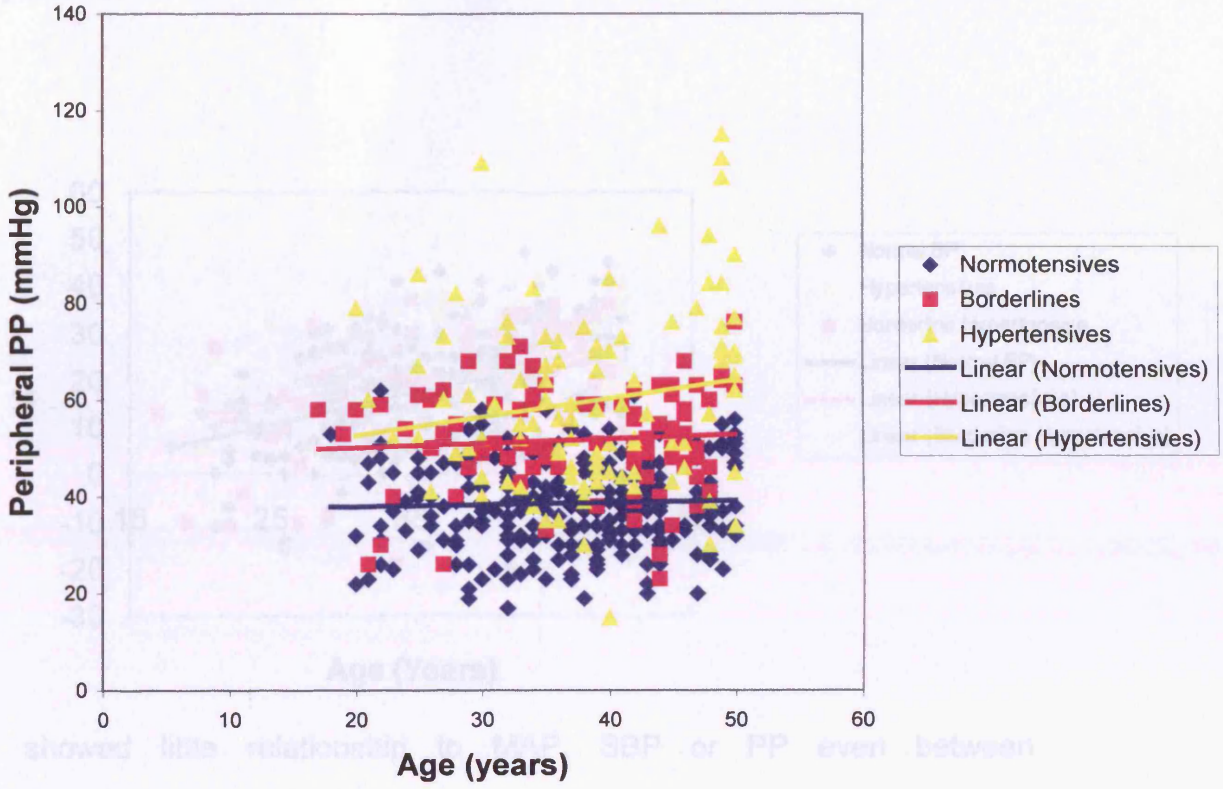
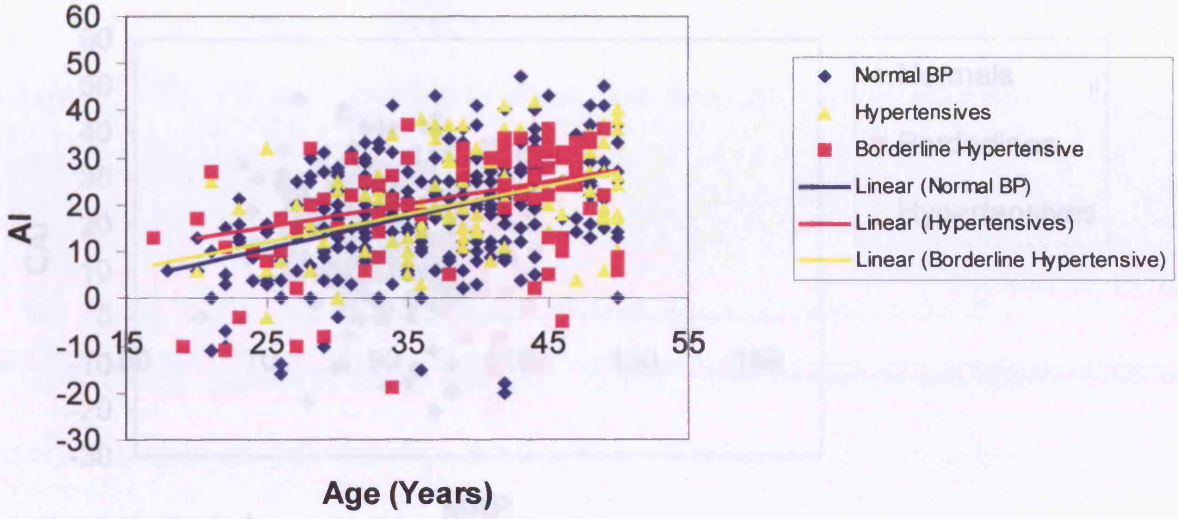


Figure 14. Change in brachial PP with age for the three groups



Interestingly, the 'ageing' effect on AI as discussed in the preceding chapter remained largely unaltered by blood pressure group (figure 15), although it is important to note that the age range studied was restricted and the study numbers are small.

Figure 15 AI in normotensives, borderline hypertensives and overt hypertensives



AI showed little relationship to MAP, SBP or PP even between groups.(figures 16-18)

Figure 16 AI vs. SBP for the three groups

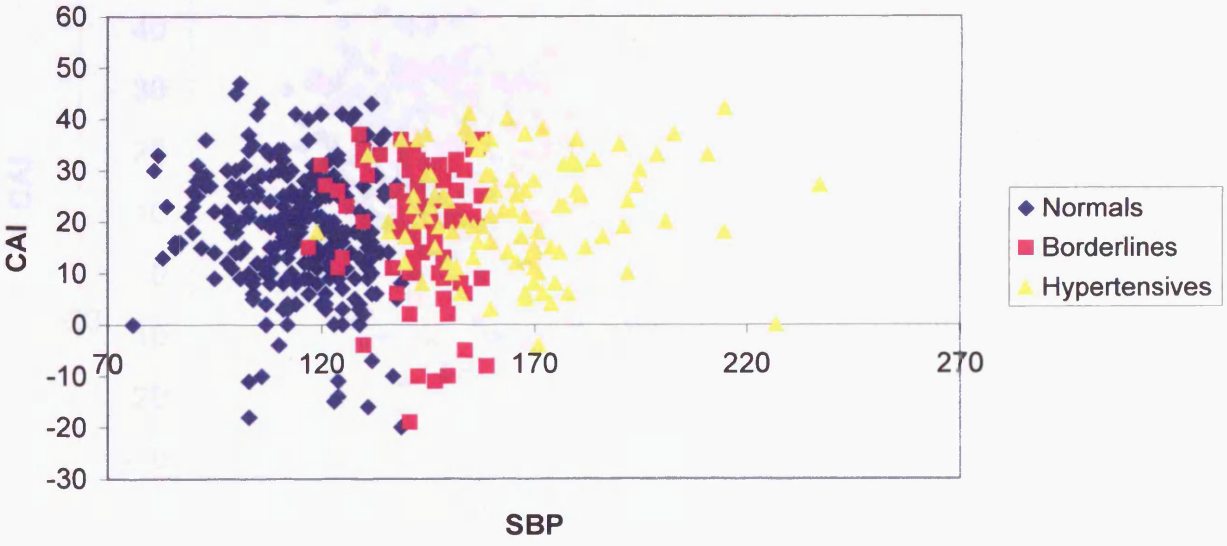


Figure 17 AI vs. MAP for the three groups

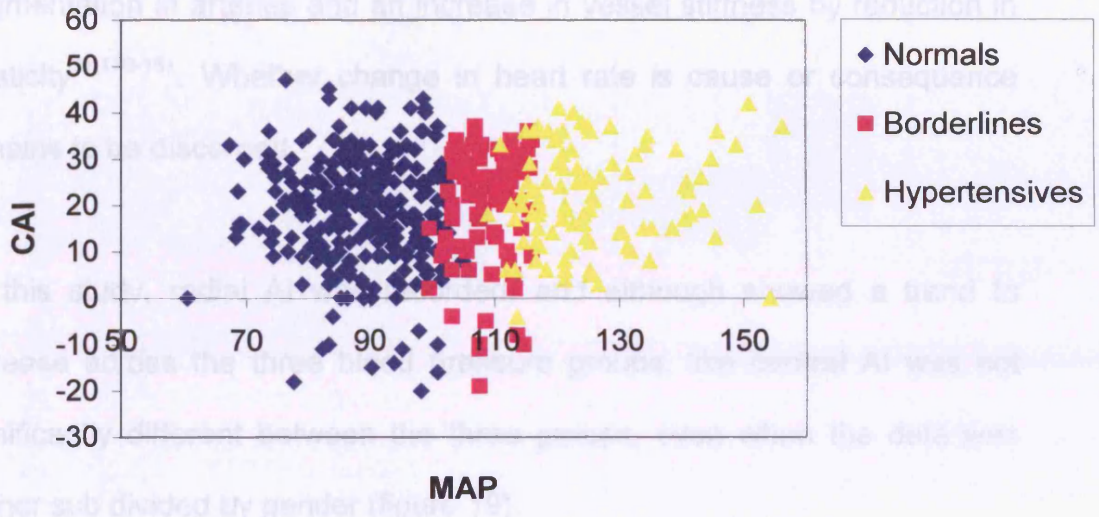
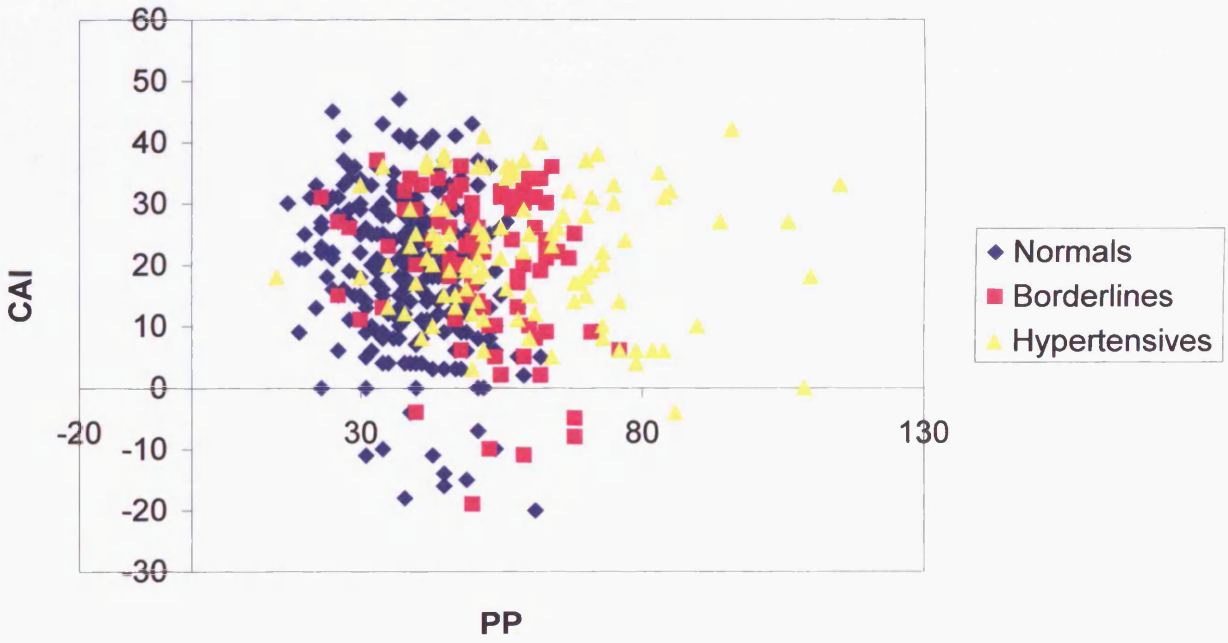


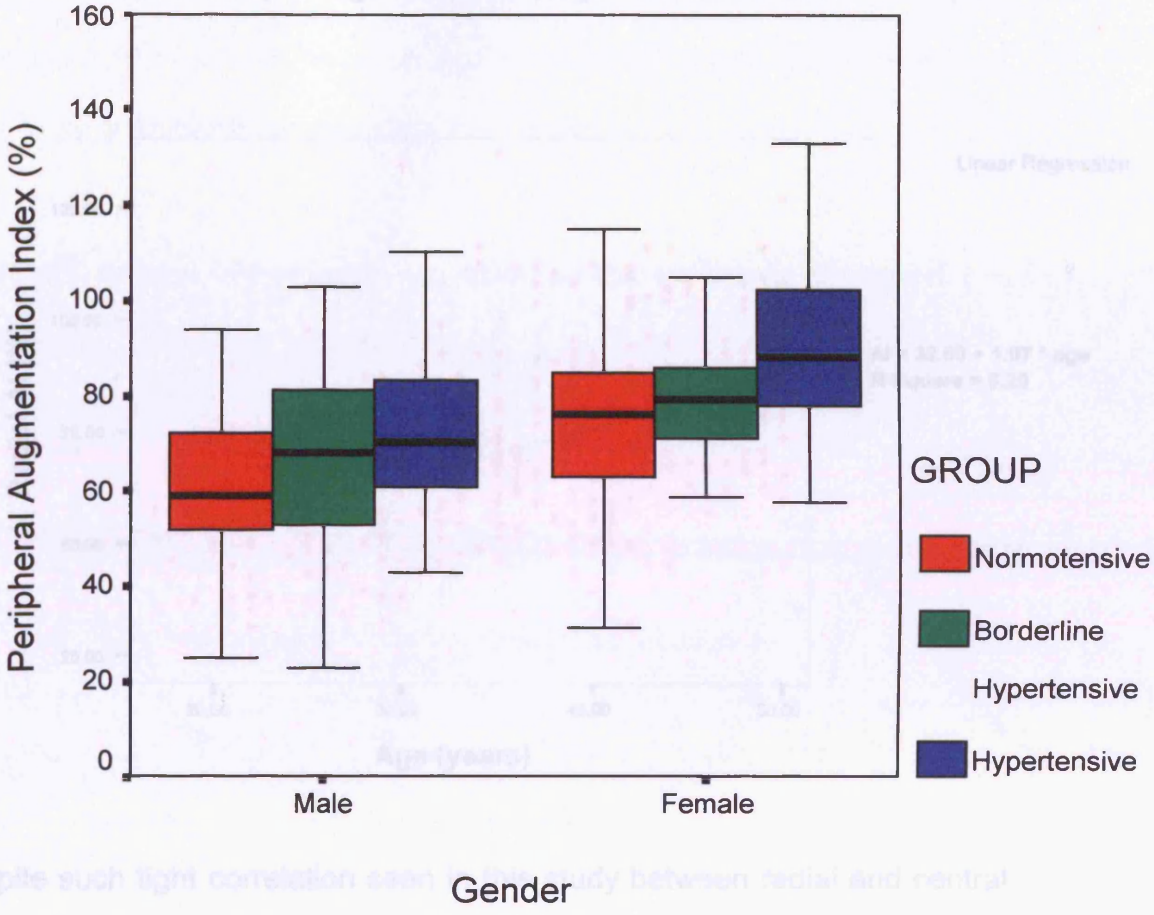
Figure 18 AI vs. Peripheral PP for the three groups



It is possible that the difference in heart rates between groups in some way serves to reduce or 'normalise' AI against the effects of elevated MAP. A chronic elevation in heart rate has been shown to be associated with elastin fragmentation in arteries and an increase in vessel stiffness by reduction in elasticity[51;179-181]. Whether change in heart rate is cause or consequence remains to be discerned.

In this study, radial AI was recorded, and although showed a trend to increase across the three blood pressure groups, like central AI was not significantly different between the three groups, even when the data was further sub divided by gender (figure 19).

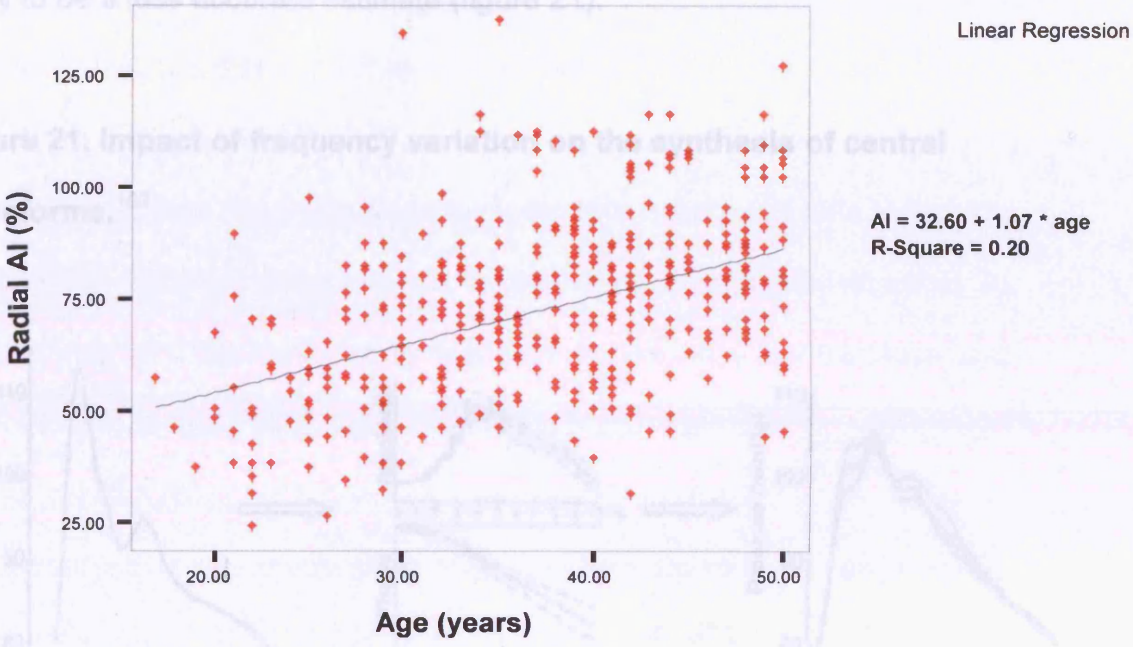
Figure 19. Peripheral AI for group and gender



This implies that the inability to detect a difference in central AI was not necessarily due to a problem with use of a generalized transfer function.

Sceptics of applanation tonometry and the generalized transfer function would claim that nothing can be added to the radial wave contour that isn't already there. Interestingly, with this in mind and the relationship of central to peripheral AI as noted above, we looked at the relationship of age on peripheral AI and confirmed that it largely mirrored that of central AI shown previously (figure 20).

Figure 20 Relationship of age on radial Augmentation Index

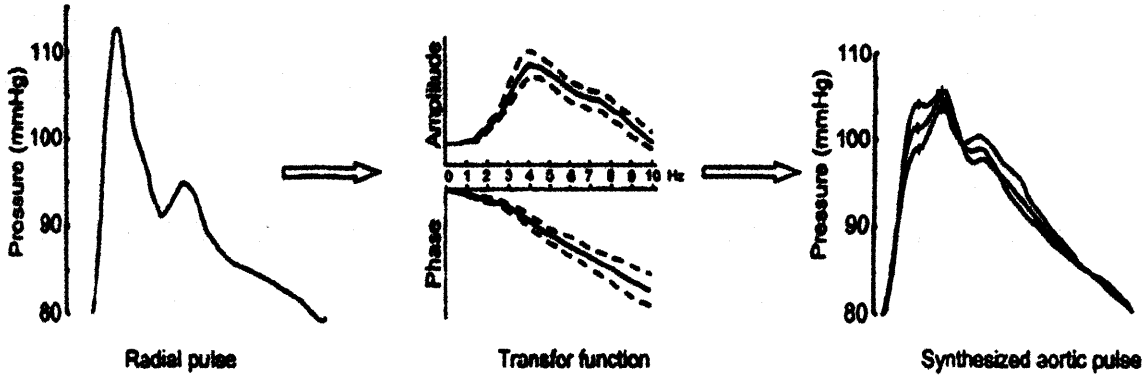


Despite such tight correlation seen in this study between radial and central AI, other investigators have raised significant concerns regarding the derivation of central AI from peripheral waveforms using a generalised transfer function. These concerns do not seem to be as marked in the estimation of central blood pressure parameters from radial arterial traces[103].

Why does this discrepancy between central AI and central haemodynamic estimates exist when using a generalized transfer function when such good correlation exists between radial and central AI? As mentioned previously, It has been proposed that correlations between peripheral and central blood pressures depend largely on low frequency information which shows

relatively little variation. This is in contrast to the relationship between peripheral and central AI, which depends mainly on higher frequency information, shows more variation and is therefore more prone to error and likely to be a less accurate estimate (figure 21).

Figure 21. Impact of frequency variation on the synthesis of central waveforms.[103]



Although peripherally derived central blood pressure parameters are likely to be a reasonable estimate of true central blood pressures their documentation must be seen to provide considerably more than theoretical benefit or academic interest to justify the time and expense recording them over straight forward brachial pressures.

As yet there is little robust data, and certainly no outcome data in hypertensive patients to suggest the use of central pressure measurement in preference to conventional blood pressure. Whether or not all hypertensive patients or just sub groups such as those with stiffer vessels

i.e. isolated systolic hypertension or those with cardiac target organ damage i.e. those with left ventricular hypertrophy would benefit from titration of blood pressure treatment against central blood pressure is not known but would be an interesting concept to test. Studies are currently underway in attempt to address some of these issues (CAFÉ)[80].

As indicated above, we were disappointed not to have been able to include pulse wave velocity measurements in this study and related them to AI measurements. This was due to fact that at the time, the software and equipment to record PWV was not available in our unit.

In summary, in a reasonably sized study, we have failed to be convinced as to the reliability of AI as a surrogate for arterial stiffness. AI measurements were unable to discriminate between patients with varying degrees of hypertension above and beyond conventional brachial blood pressure. It is possible that arterial stiffness did not differ between groups, however this is unlikely as the distending force of increased blood pressure induces a functional increase in conduit arterial stiffness. Moreover, the observation that Tr was reduced in people with borderline and overt hypertension is consistent with the likelihood that arterial stiffness was increased, but that this increase in stiffness was not reflected by any change in AI.

Interestingly previous studies have also shown radial artery distensibility in patients with systo-diastolic hypertension to be unchanged or increased compared with that of the carotid artery[164;176;182]. It is conceivable that our

findings have been influenced by the use of the radial artery as our tonometry site. We did not perform carotid artery tonometry, and therefore cannot address this possibility. However, carotid artery tonometry is technically more difficult and unlikely to be adopted as a bedside technique for the routine assessment of patients.

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Despite the ease of acquisition, the inadequacies of AI to reflect potentially increased PWV as suggested by the Tr data, led us to turn our attention to the incorporation of non-invasively derived directly recorded carotid to femoral pulse wave velocity into future studies. Again prior to the integration of PWV measurements into ongoing clinical trials we first sought to prove reproducibility by our own in house study.

5.0 Reproducibility of Non-Invasive measurement of Aortic Pulse Wave Velocity utilizing Applanation Tonometry. (Results 3)

5.1 Background

Arterial stiffness, whether structural or functional, is an increasingly recognized "risk factor", fundamental in the progression and adverse cardiovascular outcomes of not only a number of pathological states such as hypertension and diabetes, but also in normal ageing.[19;21;23;69;70;72;123;157;168;183]

This powerful predictor is often assessed by the measurement of the speed of transmission of the arterial pulse as it travels along the aorta. Large arteries become stiffer as elastin fibres fragment and increasing wall tension is transferred to collagen fibres. These stiff vessels are obviously less able to accommodate the volume of blood ejected from the left ventricle due to their lack of compliance, and so more energy is available for forward flow of blood through the arterial tree. The inability of these large blood vessels to smooth out pulsatile blood flow, and the delivery of high velocity blood flow to the smaller blood vessels is likely to result in structural damage to these vessels in addition to other "end organs" and result in the adverse outcomes eluded to above.

Although clinical study evidence is now available to support the finding of an increased aortic pulse wave velocity (PWV) as an independent predictor of

both cardiovascular risk and all-cause mortality, studies have been relatively heterogeneous in the methods used to assess PWV. A Variety of techniques, from ultrasound methods of Doppler flow, to tonometry of applanated arteries using a number of commercially available systems have been used, making comparison of studies difficult[69;123;157;183;184].

5.2 Objective

In any new technique, it is imperative to confirm that measurements are reproducible prior to widespread trial or clinical application. To date reproducibility studies of pulse wave velocity have largely been confined to measurements obtained using Doppler traces of arterial flow[90;129;131;185-187].

Assessment of PWV via applanation tonometry using a commercially available system (Sphygmocor) has been tested in a number of small studies with limited populations. These studies, although small, have never the less suggested that the technique has good inter and intra operator reproducibility at least in normal subjects.

Only limited reassurance has been provided however, as the investigators have included very small numbers of patients both with and without disease[129;131;187]. Results have been confounded by the fact that some researchers have studied brachial rather than aortic PWV[132]. Whilst this may be technically easier for the operator (Femoral pulses are often difficult to appropriately applanate), the results must be interpreted with caution as the

brachial artery is seldom affected by atheromatous disease or ageing to the same degree as the aorta, even in severe disease

The following study was therefore designed to add to the already existing trial date for reproducibility of PWV, as measured by applanation tonometry (Sphygmocor), in a large control group, across a wide spectrum of age and in a number of pathological states.

This study will also serve to reconfirm the reproducibility of single arterial site Pulse Wave Analysis (PWA) via applanation tonometry, as previously reported by our group.

Since this study aimed to reflect the 'real life' clinical use of this technique, 'all-comers' were measured, with the acknowledgement that a percentage of patients would not be measurable!

Previous experience by our group, and familiar to all researchers experienced in this technique, is the potential for significant variability in aortic PWV seen in certain individuals when measured either serially or by different operators. This study therefore affords us the opportunity to review data from such individuals in detail, in an attempt to clarify why such variability exists, and if it is possible to reduce this apparent error.

5.3 Methods

5.3.1 Subject selection

One hundred normal subjects, twenty eight patients with chronic renal disease, seventeen hypertensive and fourteen diabetic patients were recruited for a variety of clinical studies in progress in our unit utilizing the technique of applanation tonometry.

Research participants attended for a single 1-hour visit to the Clinical Research Unit at the Leicester Royal Infirmary. Subjects were asked to refrain from eating, smoking and taking caffeinated products in the two hours prior to investigation. A brief cardiovascular history was taken documenting personal and family medical history of cardiovascular disease, lifestyle factors and details of medications. Anthropometric measurements included measurement of height and weight. Urinalysis was performed by stick test to detect protein, glucose and blood. A further urine sample was collected for calculation of an albumin: creatinine ratio. Blood was collected for determination of biochemical and haematological parameters using routine clinical laboratory procedures.

All subjects gave their written, informed consent and the Leicestershire Local Research Ethics Committee approved the study (see appendix).

5.3.2 Brachial artery blood pressure measurement.

See general methods, chapter 2. Three measurements were taken, five minutes apart in accordance with British hypertension Society guidelines [188]. The mean value of the last two measurements was taken as representative of brachial blood pressure.

5.33 Radial artery pulse wave analysis.

See methods detailed in chapter two. Two independent observers (DOB and PSL) carried out all measurements and results expressed as mean of measurements obtained +/- SEM.

5.34 Measurement of carotid-femoral pulse wave velocity (PWV_{cf})

See methods detailed in chapter two. Two independent observers (DOB and PSL) carried out all measurements and results expressed as mean of measurements obtained +/- SEM.

Confirmation of reproducibility was via the well-recognized method described by Bland and Altman[189].

5.4 Results

Demographic data for the individual groups are presented in tables 1 and 2 below. Mean values for PWV and PWA parameters for the individual operators are shown in table 3. Reproducibility for both pulse wave velocity recordings and pulse wave analysis measurements of Augmentation Index are displayed as Bland- Altman plots and shown in the following figures below.

Tables 1:Demographics of the groups

Group	Age (years)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	PP (mmHg)
Controls (n=100)	48.4 (14.3)	129.0 (17.5)	76.4 (9.8)	93.9 (11.6)	52.6 (11.7)
Hypertensives (n=17)	56.8 (14.1)	161.3 (27.7)	92.0 (11.4)	115.1 (14.3)	69.3 (24.9)
Diabetics (n=14)	53.7 (13.8)	140.1 (21.3)	80.1 (12.0)	100.1 (14.0)	60.0 (15.2)
Renal disease (n=28)	49.5 (11.9)	144.3 (17.4)	81.5 (9.6)	102.4 (11.2)	62.8 (13.0)

Tables 2:Demographics of the groups (Cont.)

Group	Height (m)	Weight (Kg)	BMI (Kg/m2)	Gender (male %)	HR (bpm)
Controls (n=100)	168.1 (8.6)	73.4 (13.7)	25.8 (4.0)	36	69.1 (10.8)
Hypertensives (n=17)	166.1 (8.6)	78.3 (14.0)	28.3 (4.1)	35	67.2 (9.4)
Diabetics (n=14)	168.6 (9.0)	78.3 (17.8)	27.3 (4.6)	50	73.4 (8.9)
Renal disease (n=28)	172.1 (8.3)	82.9 (15.6)	27.8 (3.4)	64	61.4 (10.6)

Table 3: Mean values for PWV and PWA parameters between operators for individual patient groups

Patients	DOB AI	PSL AI	PSL PWV	DOB PWV	n	p
Controls	23.4	23.1	7.47	7.58	100	0.66
Hypertensives	29.6	31.3	10.3	10.56	17	0.83
Diabetics	22.3	22.7	9.81	10.16	14	0.81
Renal	23.3	23.3	8.19	8.44	28	0.65
All Patients	24.0	24.1	8.1	8.3	159	0.544

Bland-Altman plots for the groups are shown below. These show that for all groups the majority of the data falls within 2 SD with no evidence of skew.

Figure 1. Bland-Altman plot for PWV for all patients

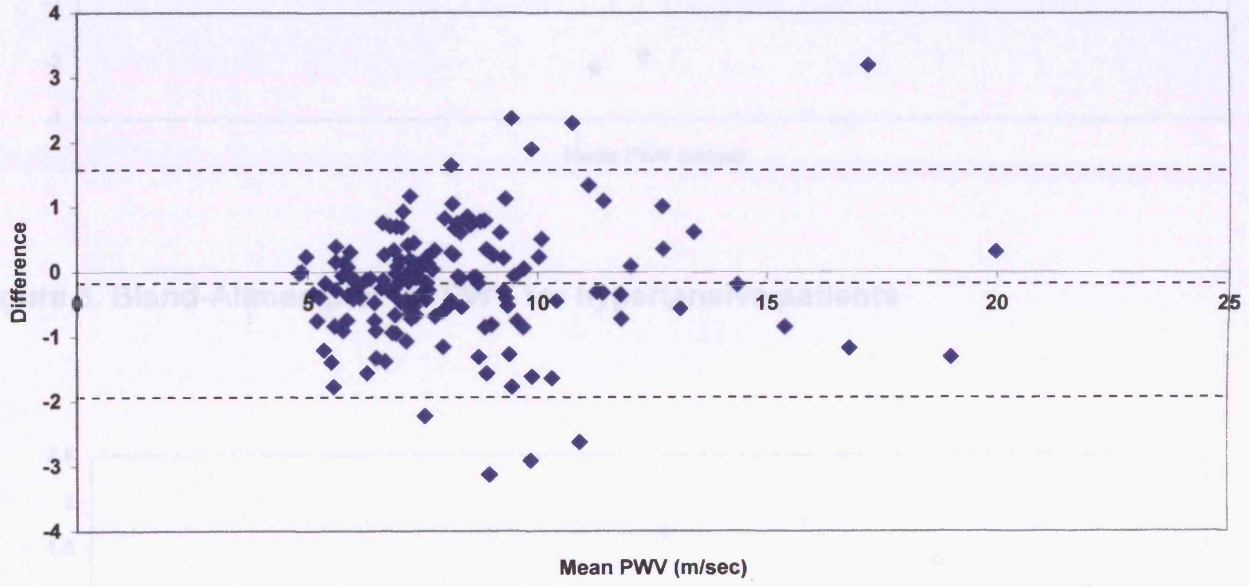


Figure 2. Bland-Altman plot for PWV for control group

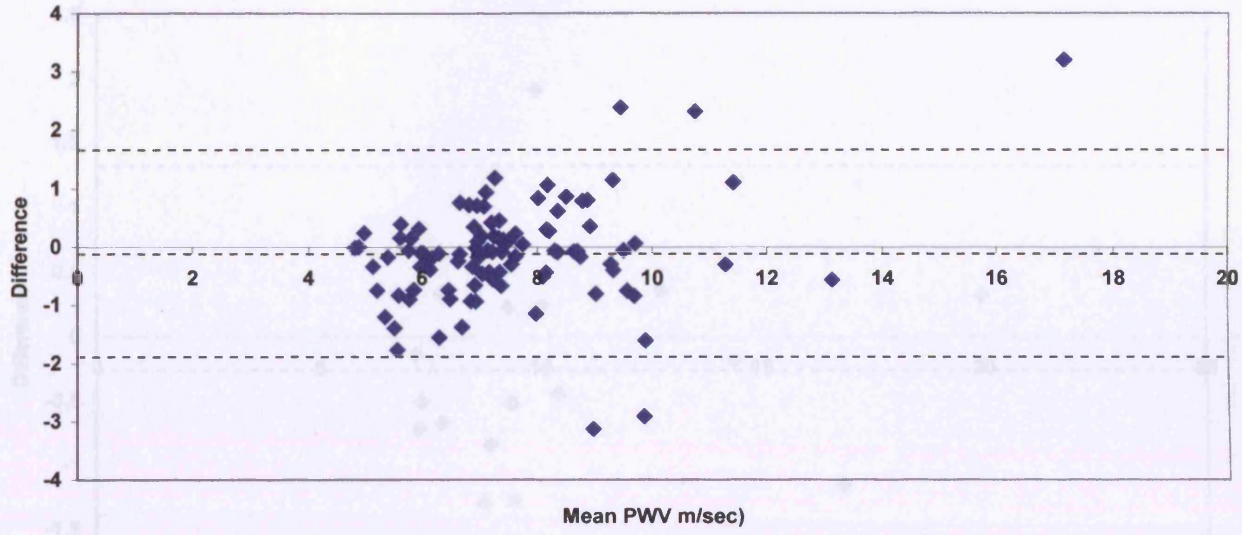


Figure 3. Bland-Altman plot for PWV for hypertensive patients

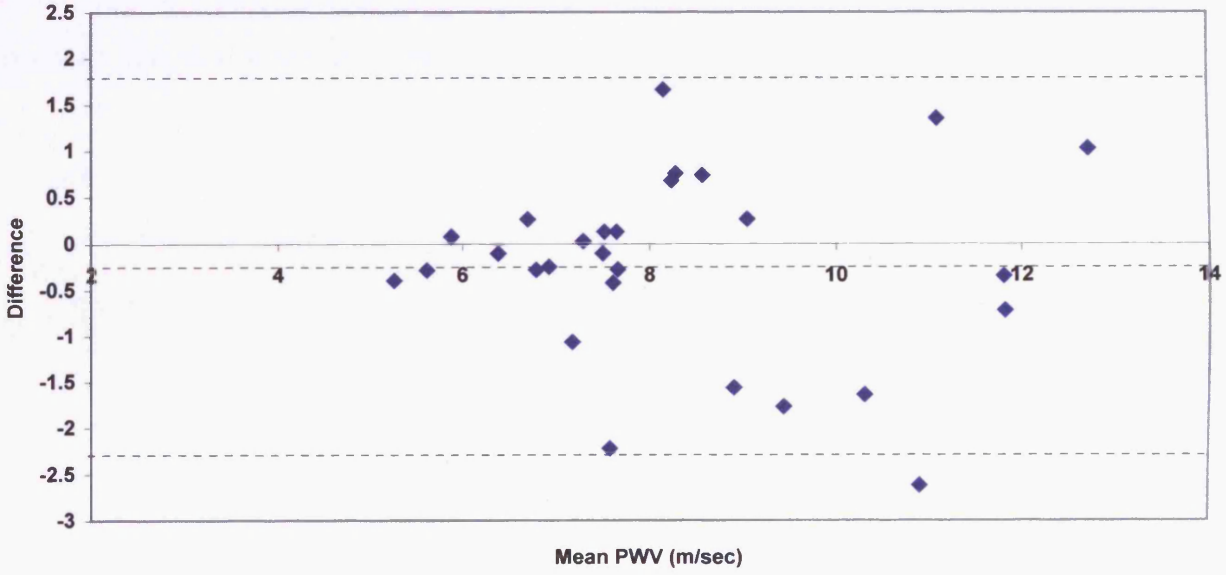


Figure 4. Bland-Altman plot for PWV for diabetic patients

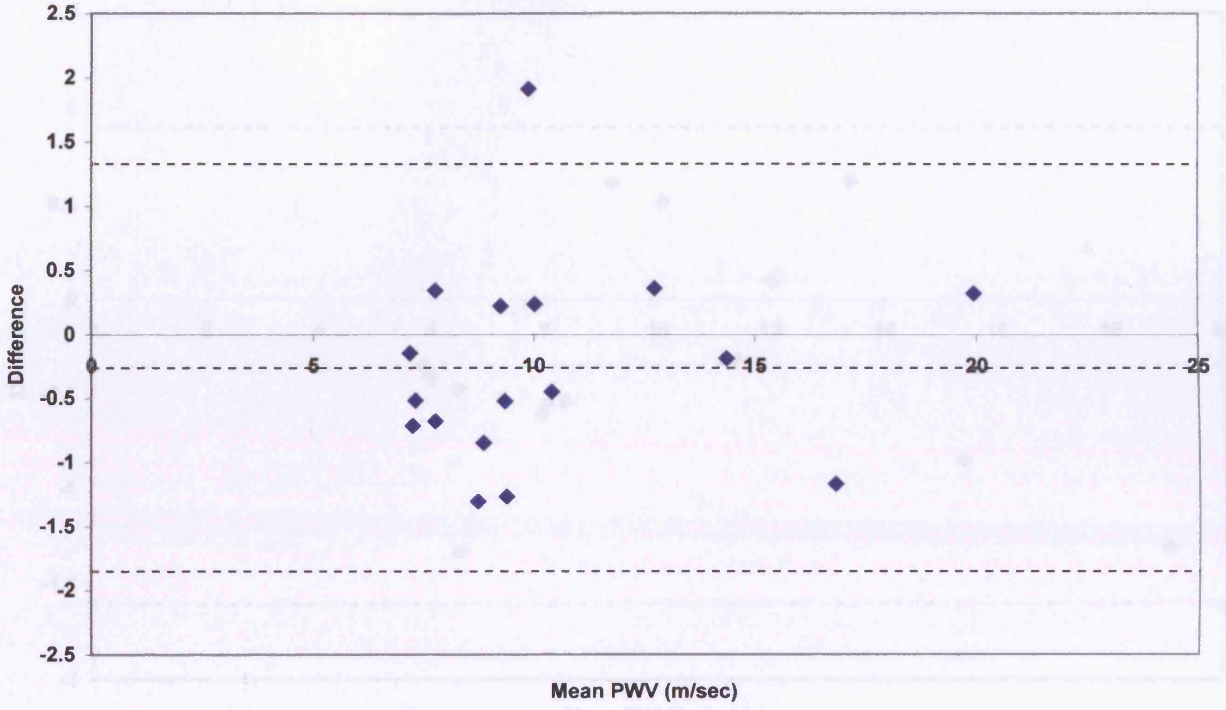


Figure 5. Bland-Altman plot for PWV for renal patients

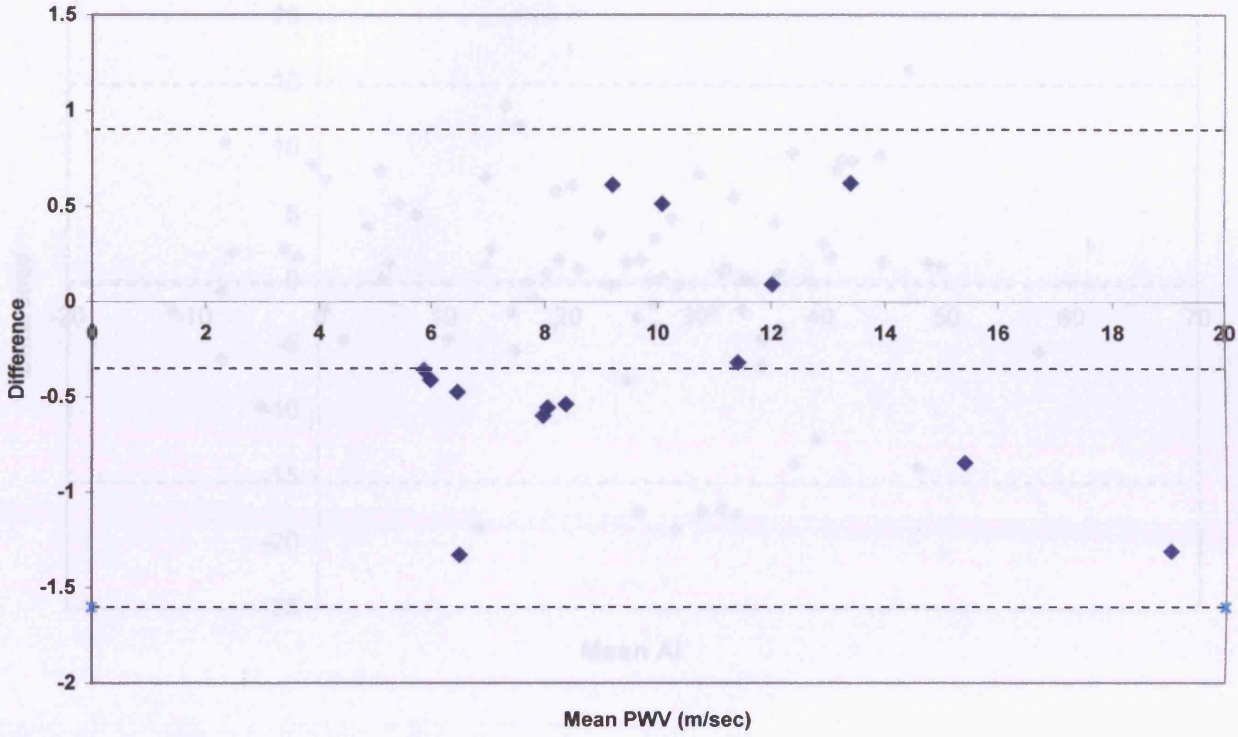


Figure 6. Bland-Altman plot for AI for all patients

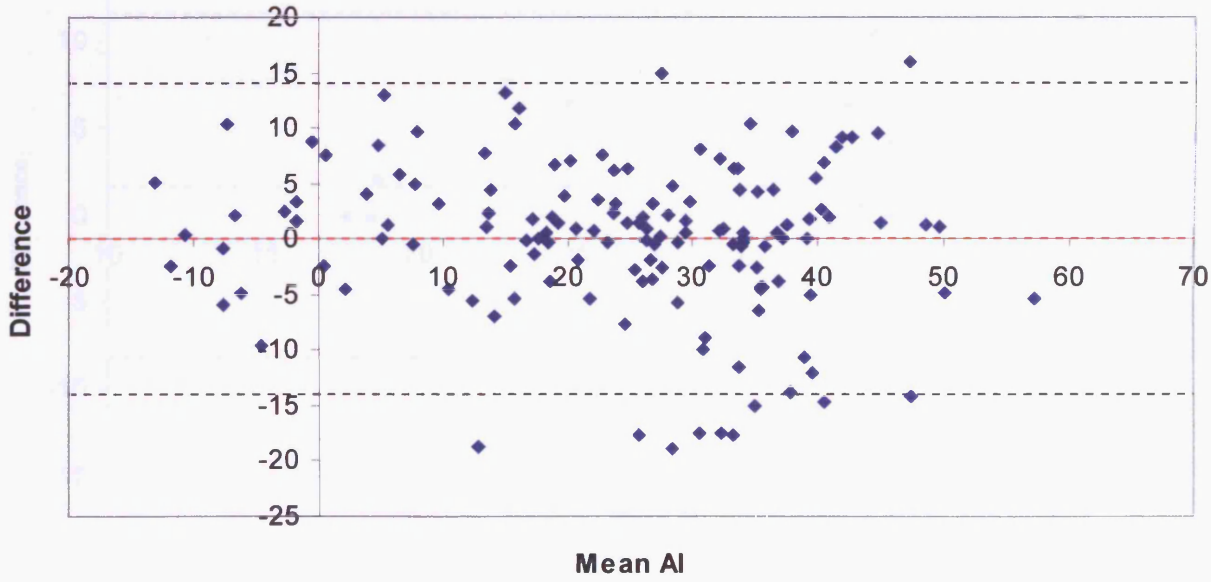


Figure 7. Bland-Altman plot for AI for controls

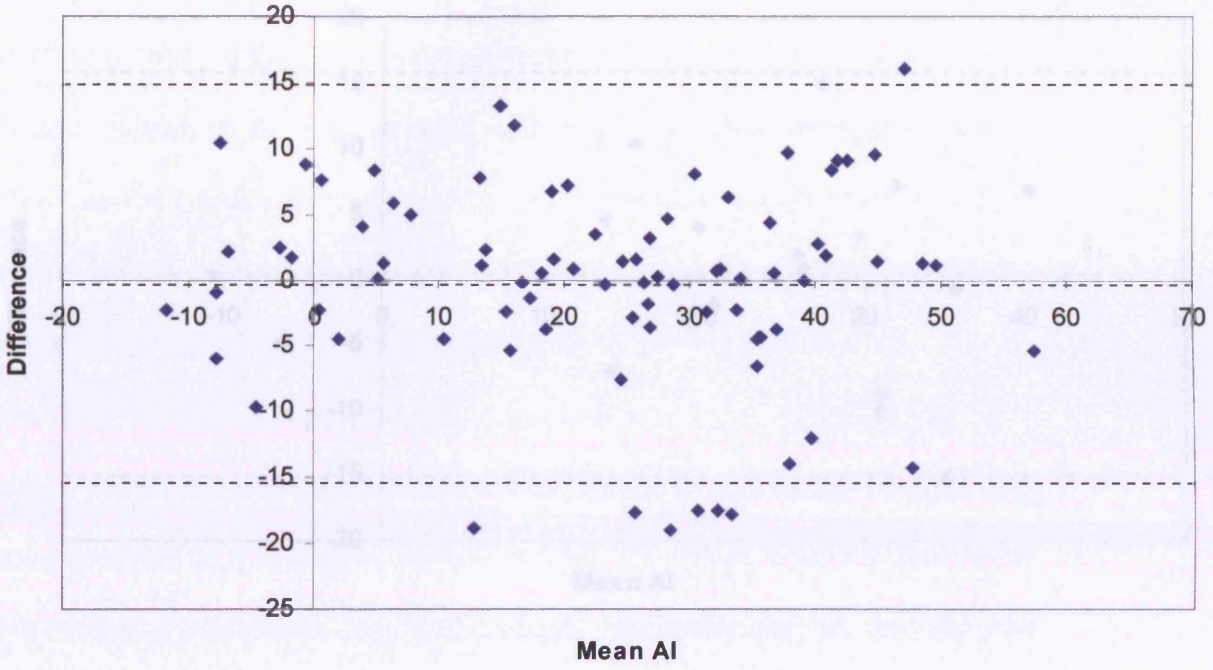


Figure 8. Bland-Altman plot for AI for hypertensive patients

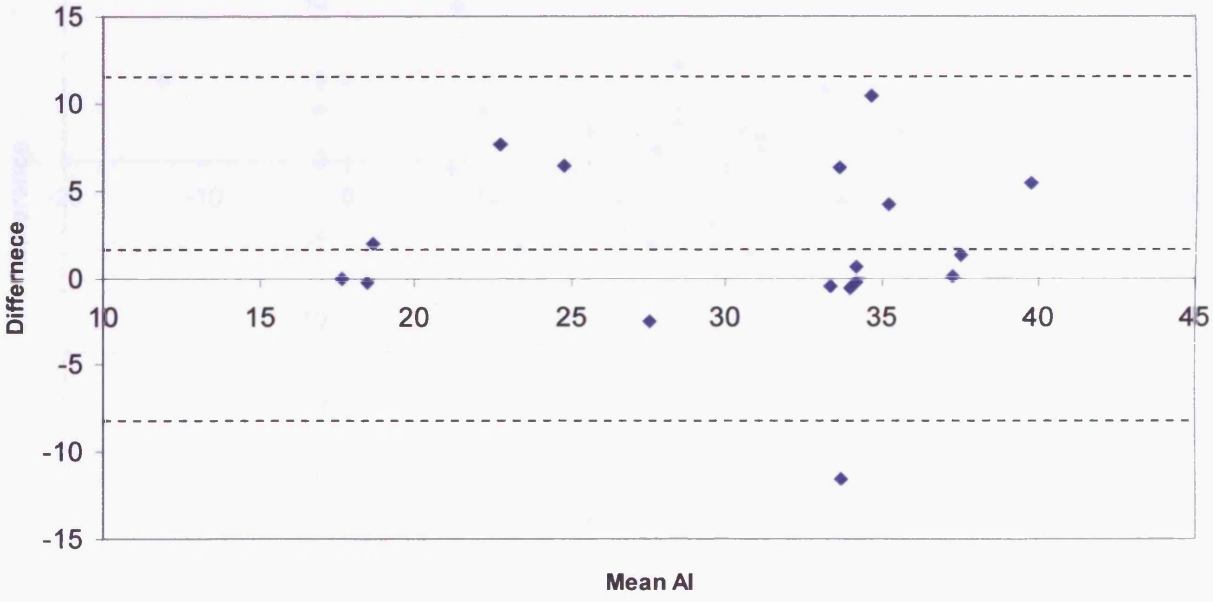


Figure 9. Bland-Altman plot for AI for diabetic patients

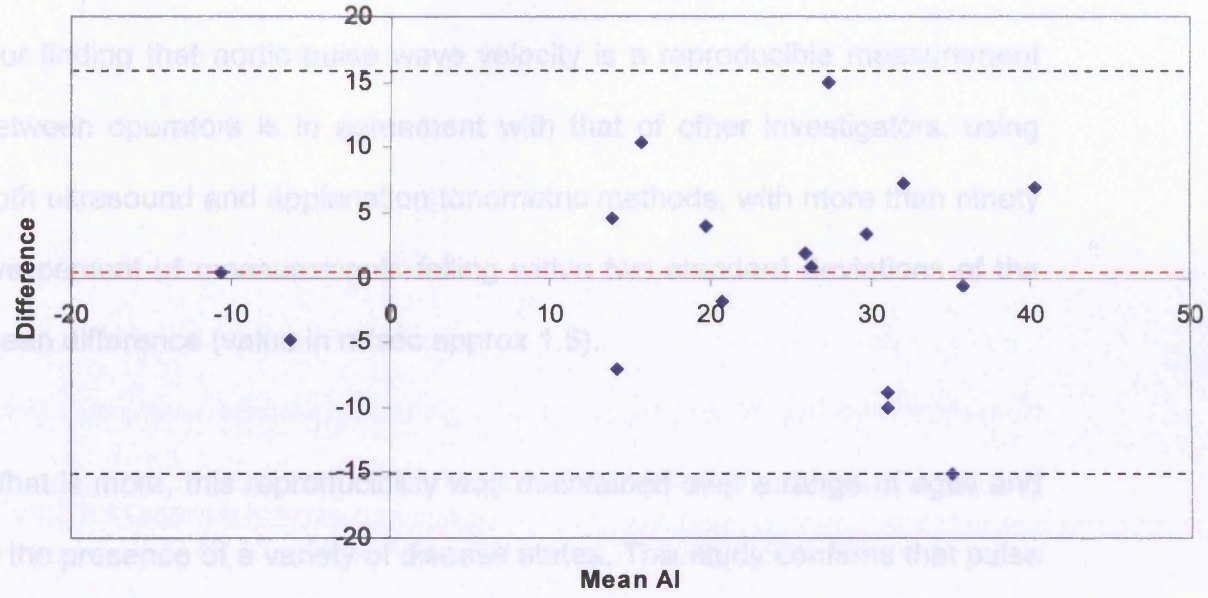
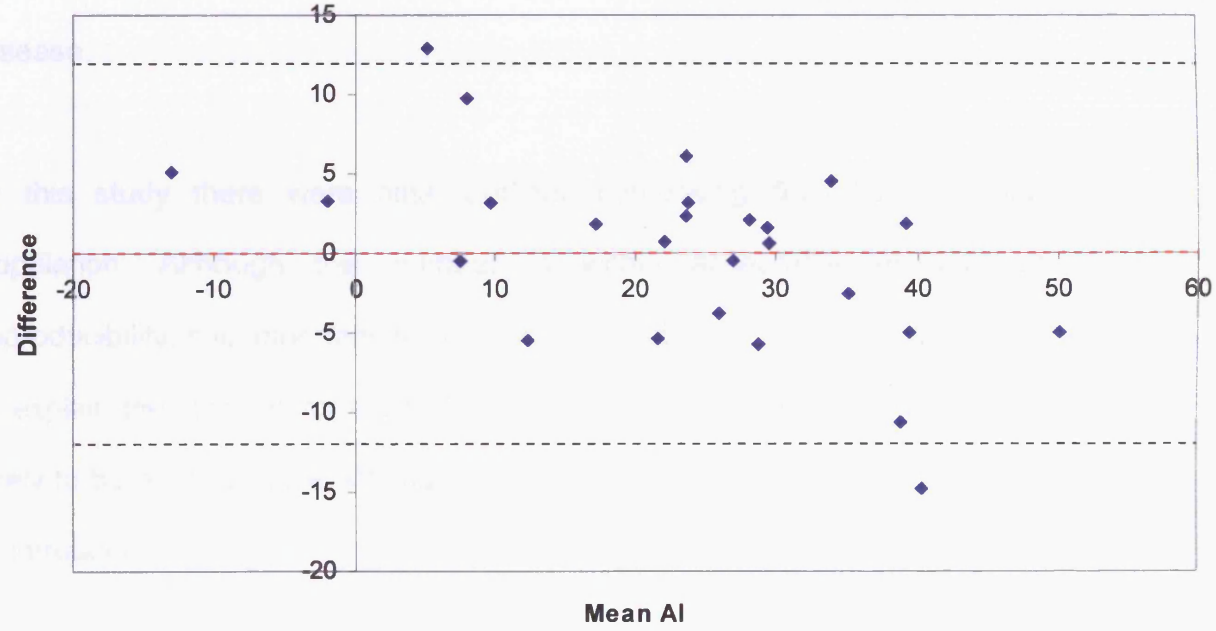


Figure 10. Bland-Altman plot for AI for renal patients



5.5 Discussion

Our finding that aortic pulse wave velocity is a reproducible measurement between operators is in agreement with that of other investigators, using both ultrasound and applanation tonometric methods, with more than ninety five percent of measurements falling within two standard deviations of the mean difference (value in m/sec approx 1.5).

What is more, this reproducibility was maintained over a range of ages and in the presence of a variety of disease states. This study confirms that pulse wave velocity measured by applanation tonometry, is a reproducible measurement for use in clinical trials involving all adult age groups (system not validated in children) and in those patients at greatest risk of vascular disease, namely hypertensive, diabetic patients and those with chronic renal disease.

In this study there were nine 'outliers' comprising 5.7% of our total population. Although this number is within acceptable margins of reproducibility, it is important we look at these individual data points in order to explain the reasons for such poor reproducibility. Potential explanation is likely to be multi-factorial. We have postulated a number of potential factors to introduce error:

- Body Habitus (BMI)
- PWV surface measurement between arterial sites

- Accurate tonometric measurement at both arterial sites (QC)
- Adequate ECG recording (QC)
- Algorithm used for wave detection
- Peripheral blood pressure measurement
- Cardiac rhythm and variation in heart rate
- Underlying disease process

Body habitus is obviously a major potential source in the introduction of measurement error, and is pertinent in the fact that many patients with cardiovascular disease are notable by an increased BMI. Error may be introduced due to the simple fact that a high BMI may significantly influence the surface measurement used to calculate PWV. Whilst in this study this is likely to be controlled for by the fact that the same arterial points were used for both operators and therefore surface measurements were identical, concerns would be in patients attending for serial measurements on separate occasions if identification of arterial points was difficult due to body habitus. Body habitus also profoundly affects arterial applanation. As discussed previously there is scope for potential error if arterial applanation is poor due to interposed tissue between the surface of the skin and the arterial wall. Deviation of the probe from the perpendicular will also introduce error. Whilst often difficult at the carotid site, major difficultly is found at the site of the femoral pulse as this is often a deep and well-covered vessel in patients with especially truncal obesity

As discussed, surface measurement is potentially inaccurate in patients irrespective of body morphology and size. This is a problem with the technique and is difficult to overcome, save by marking arterial sites as described above. It is unlikely that a few millimetres discrepancy in surface distance will influence PWV significantly between individuals.

Quality control at arterial applanation sites is extremely important, and as detailed in the methods, those measurements not satisfying in-built QC were rejected. The QC used were pulse height of 100 as an indicator of a strong arterial signal and a diastolic variability of less that 10 which largely reflects the baseline stability of the trace and therefore indirectly tremor or movement in the hand of the operator or subject movement such as respiratory artefact. Whilst this is important in getting the most accurate pressure trace possible, and for calculation of PWV by foot of the wave method, it says nothing about the determination of inflection points on the waveform and therefore the determination of parameters such as AI by computerised software. Naked eye direct visualisation by the operator is therefore still recommended as even waves satisfying in-built QC may be inaccurate for such PWA indices.

Since timing of the pulse waves is achieved gated to a 3 lead ECG an accurate ECG trace is required and has its own QC incorporated into the equipment. Although important in the calculation of PWV, the ECG clarity required is largely limited to the ability to discern an obvious R wave.

Appropriate attention to good skin preparation and electrode adhesion should be made to maximize ECG clarity and reduce artefact

Several algorithms are available to calculate pulse wave velocity, we have usually favoured the intersecting tangent method for determining the foot of individual waves. Alternative methods include the second derivative method, first derivative method and the point of minimum diastolic pressure method. Intercepting tangent and second derivative methods are considered the most reproducible measurements[190]. Whilst reanalysis of PWV measurements using all four methods yields differing absolute PWV values, reassuringly these remain not significantly different between operators for all the above noted patient groups.

Peripheral blood pressure as noted above may markedly influence many arterial measurements. Since beat to beat variation is the norm, and the acquisition of a number of these measurements can often take several minutes, the potential effect of blood pressure cannot be overlooked. Manipulation of the carotid arterial site may affect blood pressure by carotid body stimulation and femoral arterial tonometry in some individuals may cause anxiety or embarrassment and result in elevation of blood pressure around the time of study. In the same way as peripheral blood pressure measurements can be affected by anxiety, the only way to minimize this variability is a quite and controlled environment with several repeated measurements obtained prior to using an average of the last two obtained results. This method of peripheral blood pressure measurement has been

our standard practice in all studies. To date, no way of incorporating a more beat to beat measure of blood pressure such as that obtained by finnapress into these vascular measurements is available commercially. Whilst parameters such as AI are 'standardised' for BP, PWV measurements are not so and are likely to be influenced significantly. One practical way of reducing this error is to re-measure BP after each measurement. The BP cuff is left deflated in-situ during the PWV measurement and re-inflated immediately prior to the next replicate measurement. If BP has changed markedly (usually a decrease with time) the preceding measurement is discarded and the series of measurements continues until there is little variation in BP between measurements.

In a similar way to blood pressure, since these measurements are ECG gated over several beats and then averaged, cardiac arrhythmias will influence measurements significantly, especially those resulting in marked variability of average heart rate such as atrial fibrillation or frequent ectopic beats. For this reason patients with known or discovered arrhythmia at the time of study were excluded. Whilst PWV seems to be little influenced in our studies by heart rate, AI as noted previously is greatly altered. Although on a beat to beat level the impact is likely to be minimal, in longitudinal studies or studies utilising therapies resulting in heart rate changes could prove difficult to interpret without an artificial heart rate adjustment/standardisation.

We were interested to evaluate if the underlying disease process per se would influence reproducibility of this technique and are pleased to report in

albeit relatively small numbers this did not appear to be the case. Although smaller numbers in the pathological groups may under report the number of 'outliers' in the Bland Altman plots, there appeared to be no greater proportion of poorly reproducible points than seen in the control population.

Review of all outlying patients showed no obvious cause for lack of reproducibility in these individuals related to any of the factors proposed above. It may be that certain measurements were different between operators by a combination of these factors, operator error or merely by chance.

Pulse wave velocity and PWA parameters therefore are reproducible between operators, not only in control subjects but also in those with a number of pathological conditions affecting the cardiovascular system including hypertension, diabetes and chronic renal disease. A major limitation of this study however was in the fact that repeatability over time between operators and in different subjects was not assessed, and therefore the use of this method in longitudinal studies over time would require further study before it was implemented.

6.0 Pulse Wave Velocity in a normal population (Results 4)

6.1 Introduction

Pulse wave velocity (PWV), unlike its relatively poor surrogate Augmentation Index, already has more credibility as a measure of vascular stiffness. As discussed in previous chapters, measurements of predominantly aortic PWV via different methods have already proven themselves as not only predictors of outcome in high-risk subjects such as hypertension and diabetes, but also in unselected ageing populations per se[69;72;123;157].

These previous large scale studies have predominantly used Doppler methods of assessment of PWV by comparing foot to foot methods of Doppler flow waveforms obtained either simultaneously or ECG gated from the aortic arch and femoral arterial sites over a specified surface distance. These methods require relatively specialized equipment and considerable operator expertise. They are reasonably time consuming and do not lend themselves easily to integration into routine clinical assessment of patients. Never the less, if the documentation of this measurement affords us additional information for risk stratification above and beyond conventional measures then it should not be prematurely dismissed.

Using commercially available software and high fidelity tonometers (Sphygmocor TM), as discussed previously, carotid to femoral PWV (and hence PWV across the aorta) can be measured relatively quickly with this

very portable device. Although the problems with carotid artery tonometry have already been mentioned in previous chapters, and although some degree of expertise is required, this technique is relatively straight forward and quickly learned. As noted above, most of the previous work in this area has been performed using Doppler estimates to calculate PWV.

Following our in house study to confirm reproducibility (see chapter 5), we were interested to explore the influence of the effects of ageing in addition to other haemodynamic and demographic parameters on this measurement in the same way we assessed PWA parameters (See chapter 3). Given the fact that this measurement was being performed by PWA at sites including the carotid artery, we would also have the unique opportunity to compare not only peripheral indicators of vascular stiffness, (e.g. peripheral pulse pressure), but also much heralded PWA derived markers of pulse wave velocity (e.g. Tr and AI) with a direct measure of PWV itself.

6.2 Methods

Using a similar control population and methodology as in our previous study of the assessment of reproducibility for PWV, anthropometric and demographic data were added for use in this study. Patients with known or treated hypertension, ischaemic heart disease, diabetes or dyslipidaemia were excluded. All tonometry data was screened for quality control using our now standard criteria of pulse height >100 and Pulse height variability and

diastolic variability < 10. Data not conforming to these criteria was removed.

As previously, all data was assessed statistically using SPSS v 11.5.]

6.3 Results

6.31 Demographic and haemodynamic Data

Demographic and haemodynamic data is presented in the tables below.

Table 1. Demographic and peripheral haemodynamic data

	n	Minimum	Maximum	Mean	SD
Age (years)	107	20.2	82.7	49.7	14.6
PSBP (mmHg)	107	95.0	181.0	129.6	17.2
PDBP (mmHg)	107	57.0	105.0	76.5	9.7
MAP (mmHg)	107	69.7	128.0	94.2	11.5
PPP (mmHg)	107	31.0	88.0	53.1	11.5
HR (bpm)	107	38	110	69	10.6

Table 2. Anthropometric and laboratory data

	n	Minimum	Maximum	Mean	SD
Height (cm)	101	152.0	200.0	168.1	9.0
Weight (Kg)	101	51	115	73.5	13.7
BMI	102	18	39	25.9	4.1
Cholesterol	102	3.8	7.7	5.4	0.85
Triglycerides		0.5	8.0	1.8	1.2
ACR	90	0.2	5.2	1.1	0.87

Table 3. Central haemodynamic data and PWV

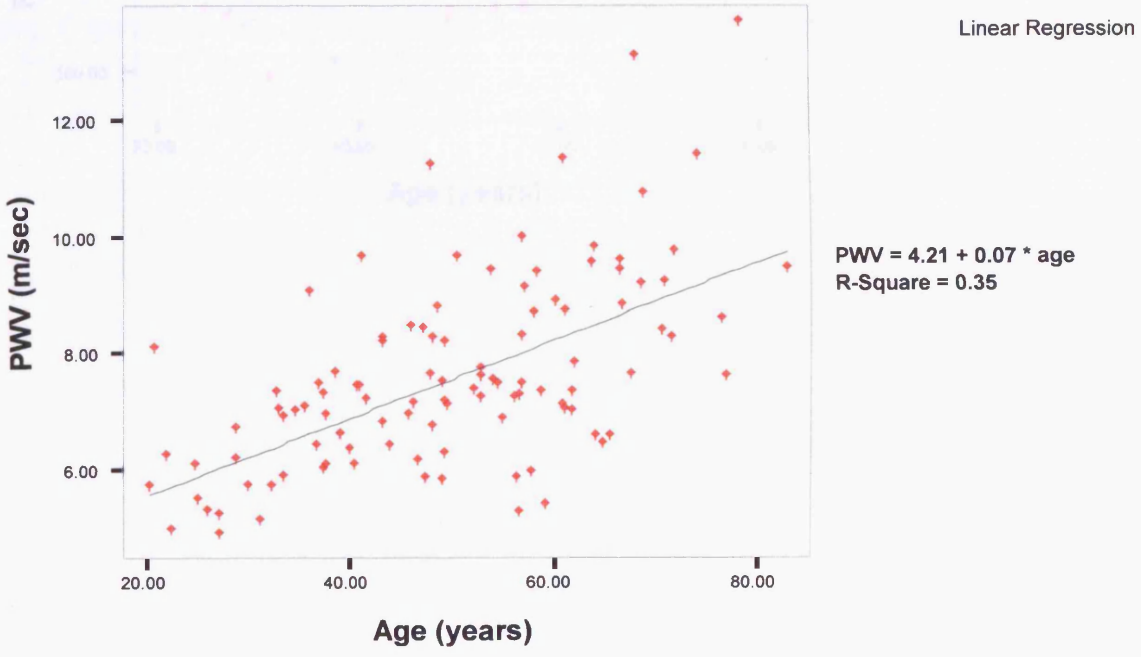
	n	Minimum	Maximum	Mean	SD
PAI (%)	106	31.2	149.1	77.2	22.6
CSBP (mmHg)	107	87.0	176.5	117.6	17.8
CDBP (mmHg)	107	57.0	107.3	77.6	9.9
CPP (mmHg)	107	23.5	78.6	40.0	11.0
Amplification	107	1.05	1.84	1.36	0.22
CT1R (msec)	107	105.0	203.1	140.1	16.1
CAI (%)	107	-14.2	57.4	23.1	16.3
PWV (m/sec)	107	4.82	13.62	7.5	1.66

Gender split was recorded as 36% male and 64% female. Smoking status was documented and as a group overall, smokers comprised 36%.

6.32 Effects of ageing on PWV, haemodynamic parameters, AT indices and demographic variables.

In agreement with many other studies utilizing alternative systems for assessing aortic PWV, there was a remarkably linear relationship between ageing and arterial stiffness in a population free of overt cardiovascular disease[30;67] (see figure1).

Figure 1 Relationship of Age and PWV



In contrast to this, although statistically correlated (DBP and MAP, not SBP) the relationship between age and brachially derived blood pressure indices,

even when parameters more suggestive of vascular stiffness such as Pulse Pressure (PP) were studied, was much less impressive (see figures 2,3,4 and 5 respectively).

Figure 2. Relationship of Age and Systolic Blood Pressure

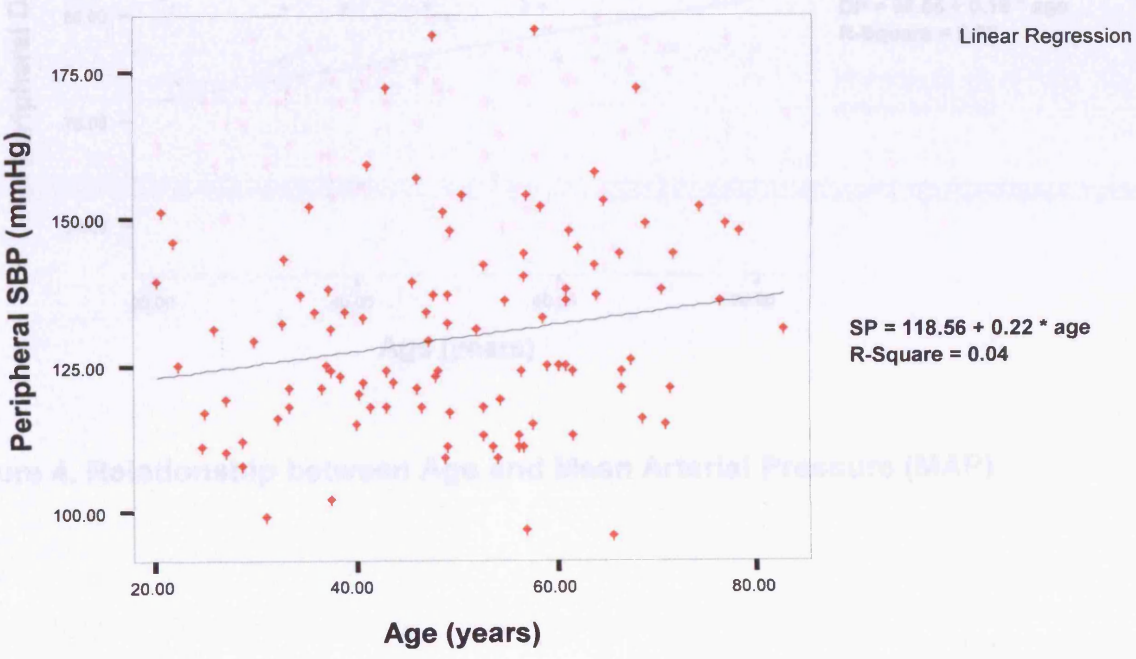


Figure 3. Relationship between age and Diastolic blood pressure

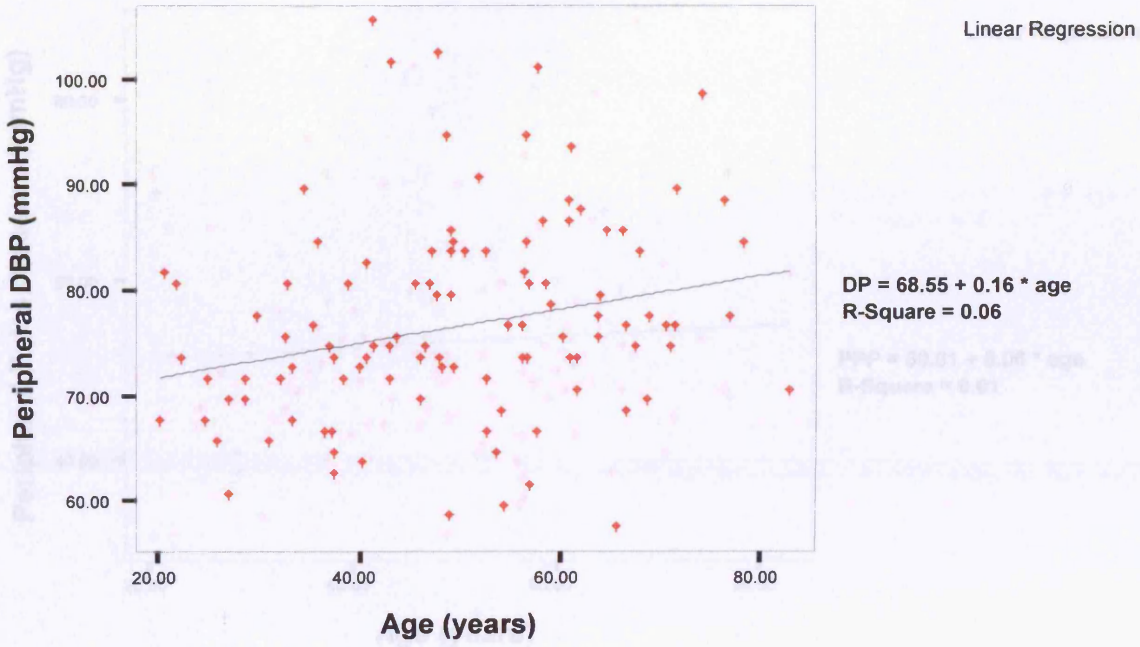


Figure 4. Relationship between Age and Mean Arterial Pressure (MAP).

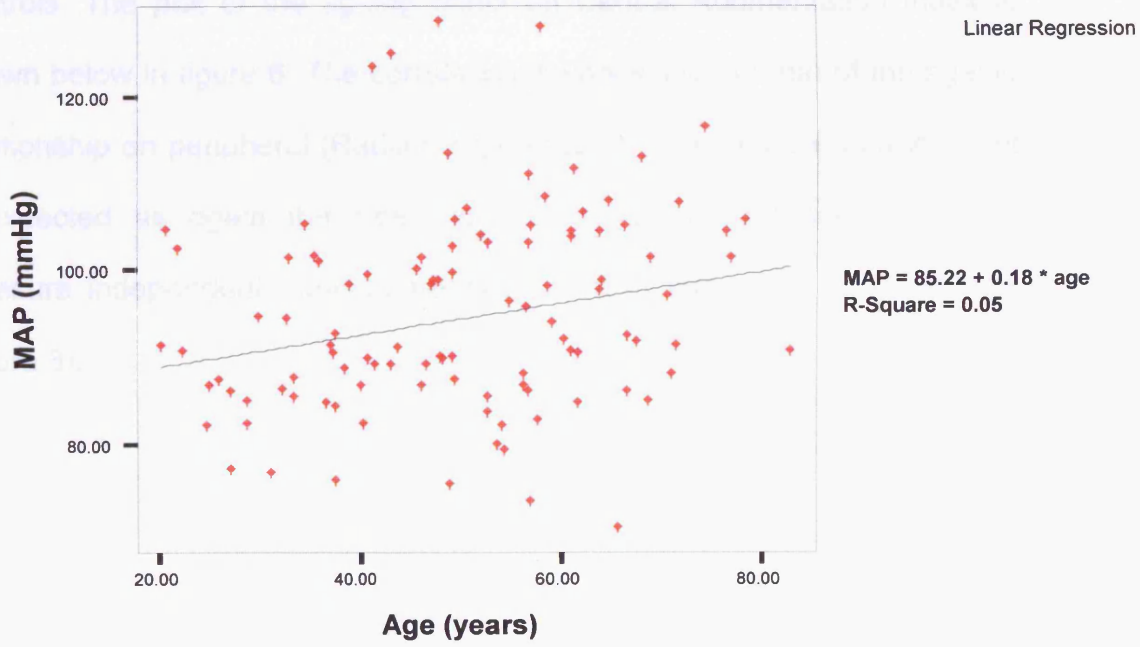
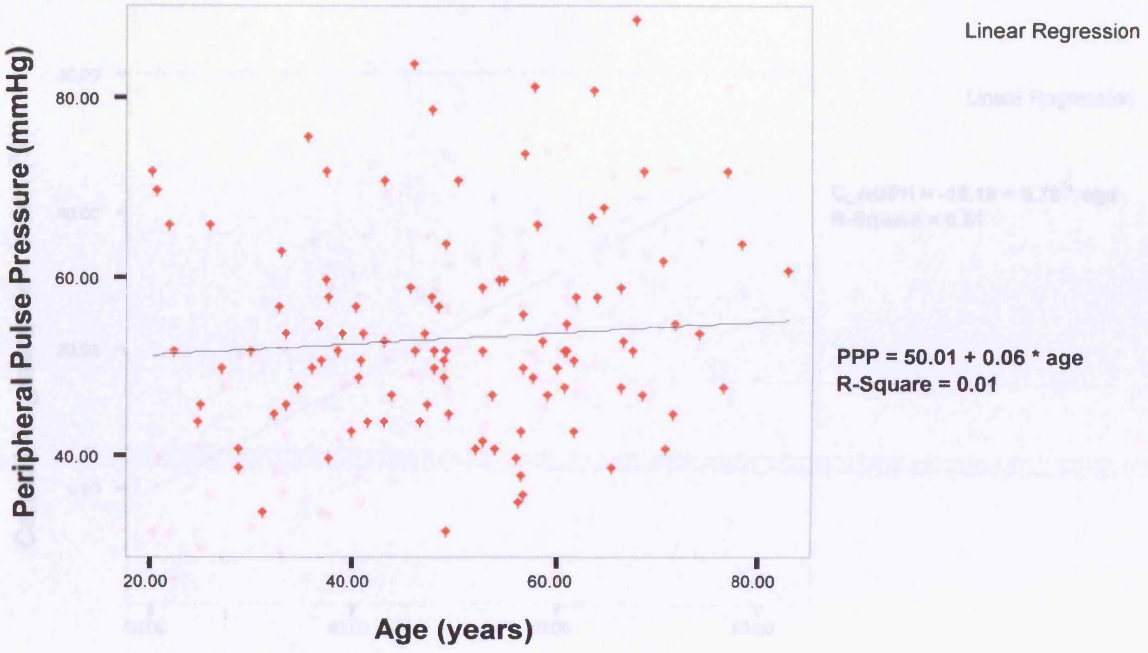


Figure 5. Relationship between Age and peripheral pulse pressure



Parameters obtained by Applanation Tonometry show similar linear relationships to ageing to that seen in our previous data set of normal controls. The plot of the ageing effect on Central Augmentation Index is shown below in figure 6. The correlation is very similar to that of the ageing relationship on peripheral (Radial) Augmentation Index (figure 7) and is not unexpected as again the close relationship between these two blood pressure independent measurements is seen in this group of individuals (figure 8).

Figure 6. Relationship of Age and Central Augmentation Index

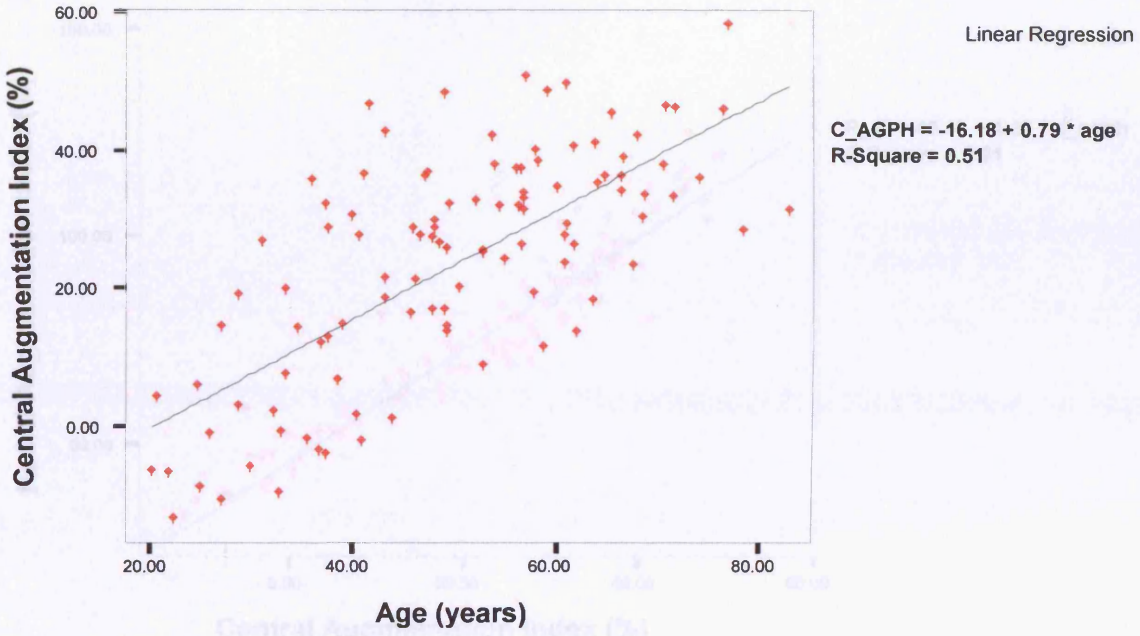


Figure 7. Relationship of Age and Peripheral Augmentation Index

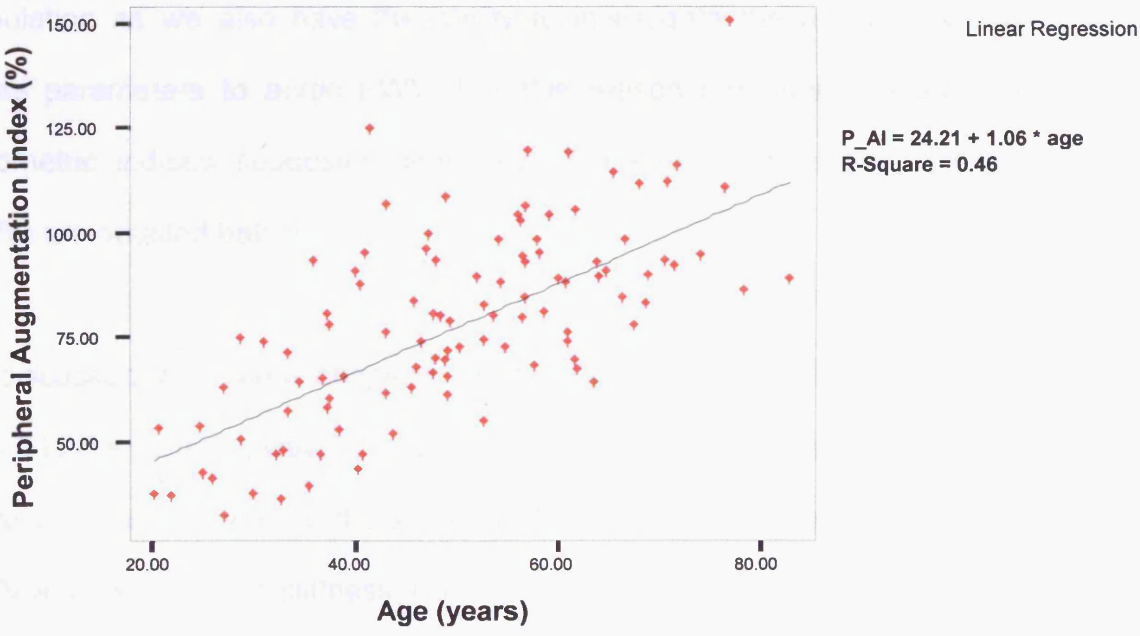
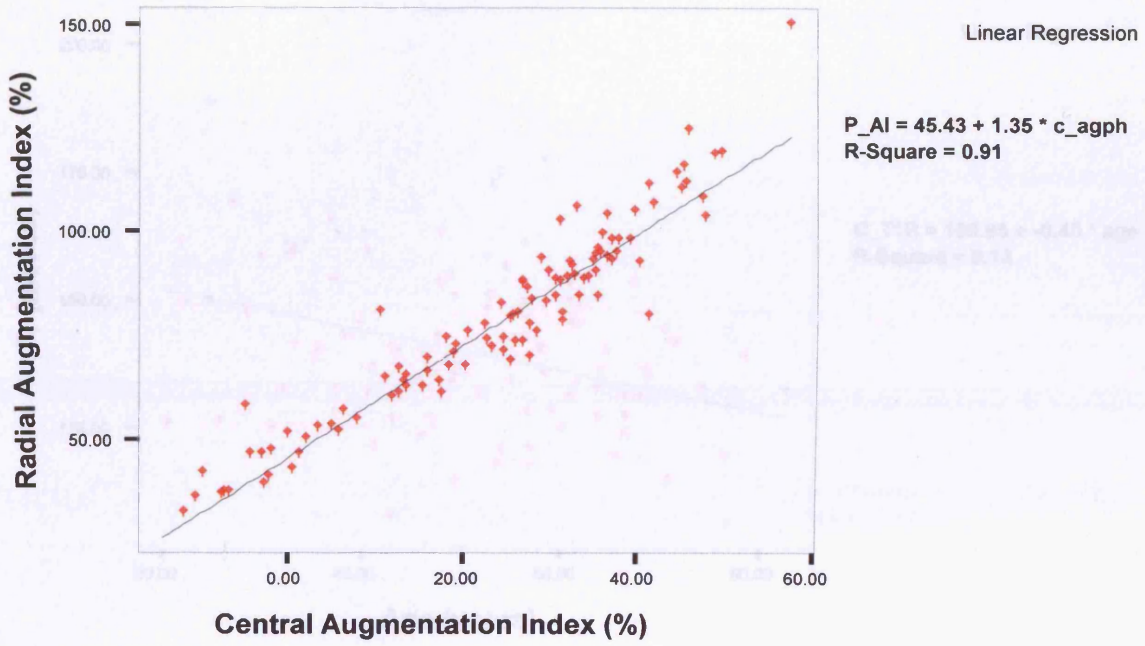


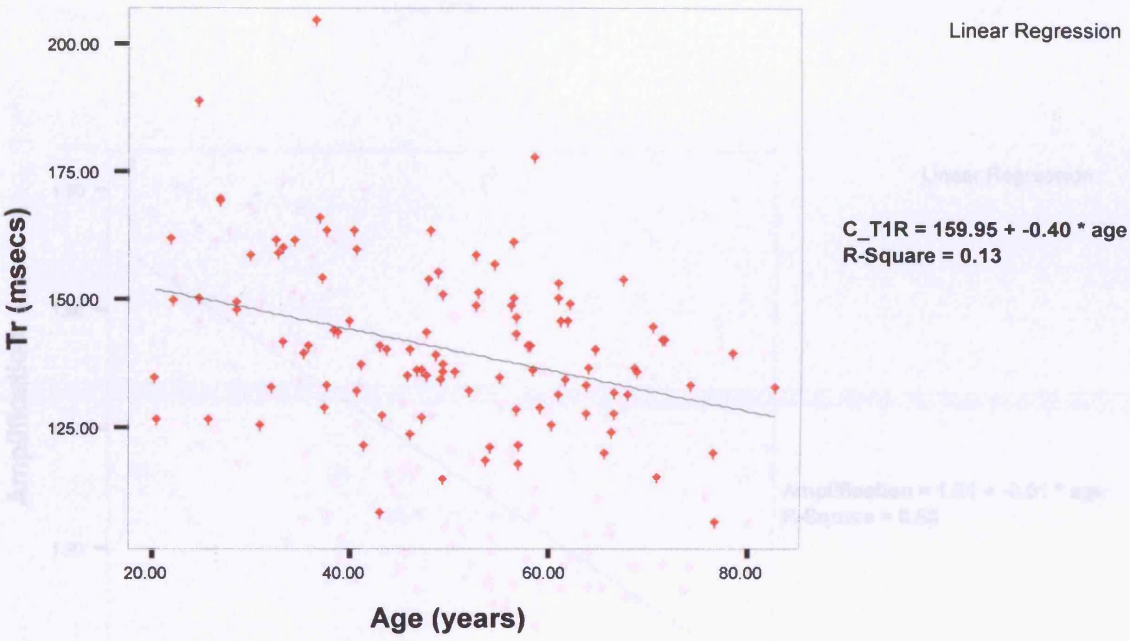
Figure 8. Relationship of Central Augmentation Index and peripheral Augmentation Index



Although not meaning to revisit the effects of age on applanation tonometry parameters in this chapter it is important we study the relationships in this population as we also have the ability to investigate the relationships of these parameters to aortic PWV. For this reason the effects of age on tonometric indices *suggestive* indirectly of arterial stiffness (by inferring PWV) are detailed below.

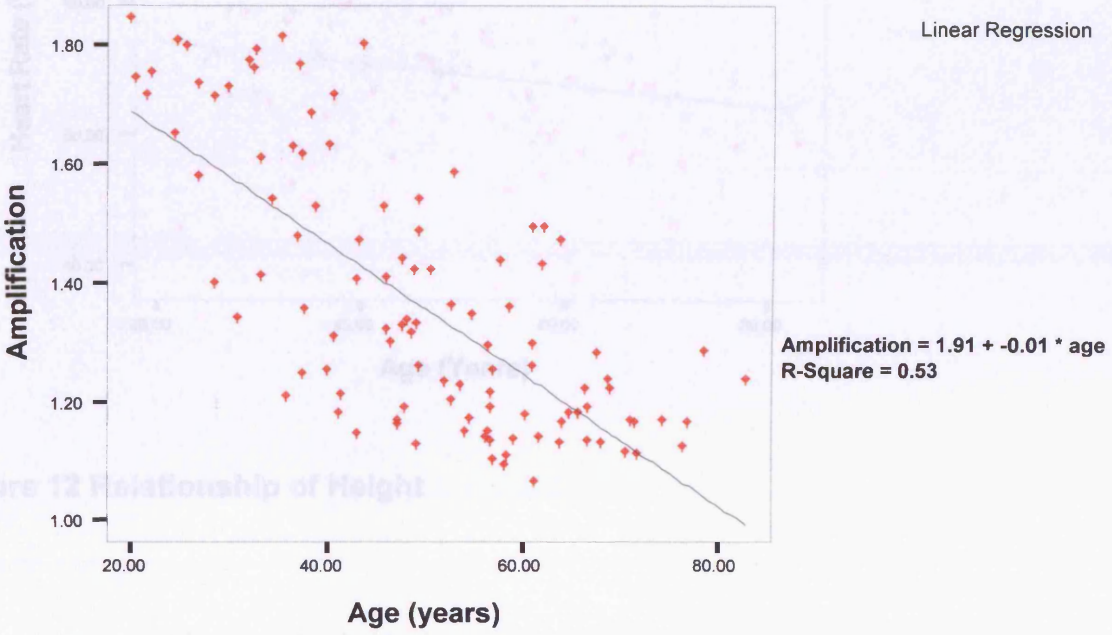
As discussed in previous chapters, the time taken for the reflected wave to return to the outgoing wave (Tr) is an indirect measure of the speed of pulse travel i.e. PWV. Obviously, the shorter the Tr, then the greater the predictive PWV and hence aortic stiffness. An expected significant inverse relationship of Tr and age is shown for our population in figure 9.

Figure 9. Relationship of age and CT1R



Amplification gradient between the central and peripheral vessels falls as increasing vessel stiffness increases central augmentation towards that of the periphery. Fall in amplification gradient or ratio can therefore be used to suggest increasing vascular stiffness and one would expect this to be mirrored by increasing PWV. The expected significant inverse linear relationship between Amplification and age for this population is shown in figure 10.

Figure 10. Relationship of Age and peripheral to central pressure wave amplification



Again, similar ageing relationships for height, heart rate, total and LDL cholesterol were seen in this 'normal' population (figures 11 to 14). Although not presented here in graphical format, there were no significant ageing relationships seen for HDL cholesterol, or triglycerides, fasting glucose, weight, Body Mass Index, serum creatinine or surface distance used for calculation of PWV. An increase in ACR with age was noted (see figure 15) and will be discussed later in relation to PWV.

Figure 11 Relationship of Age and Heart Rate

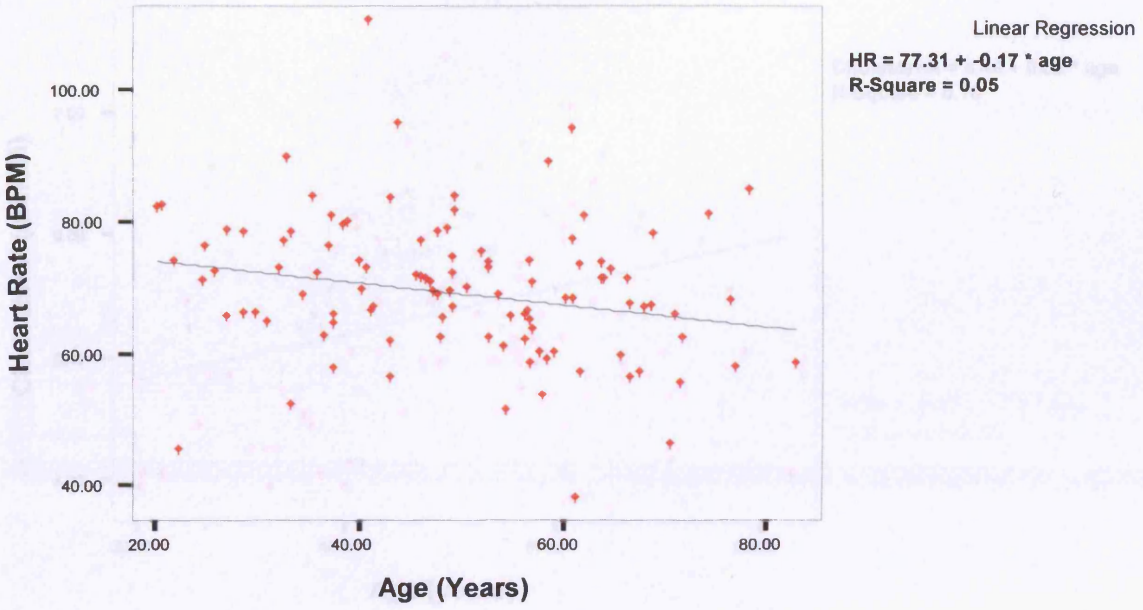


Figure 12 Relationship of Height

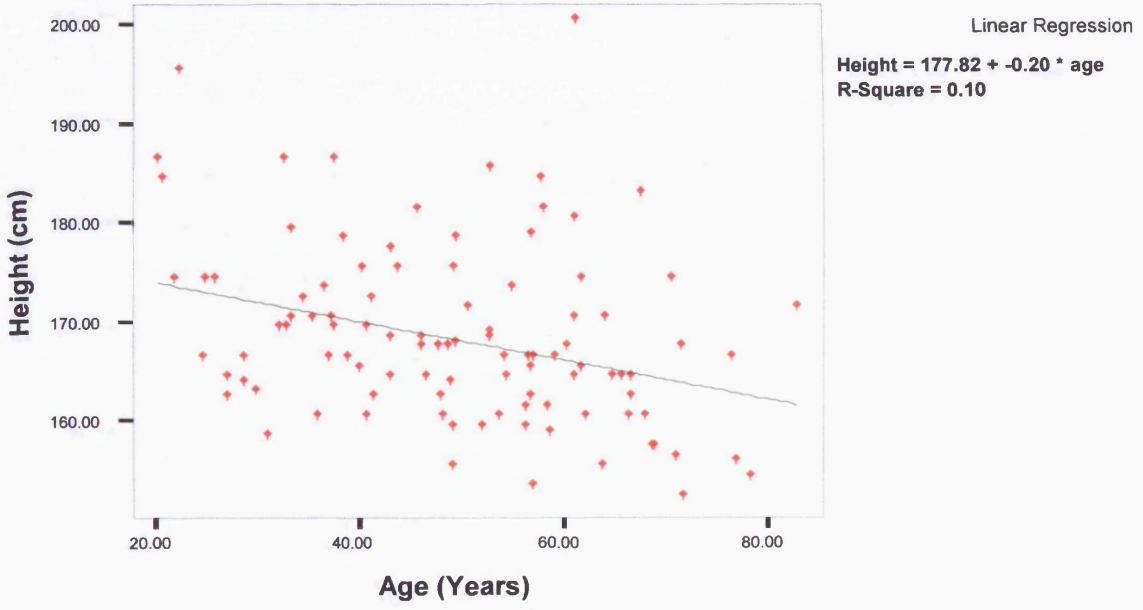


Figure 13 Relationship of Age total cholesterol

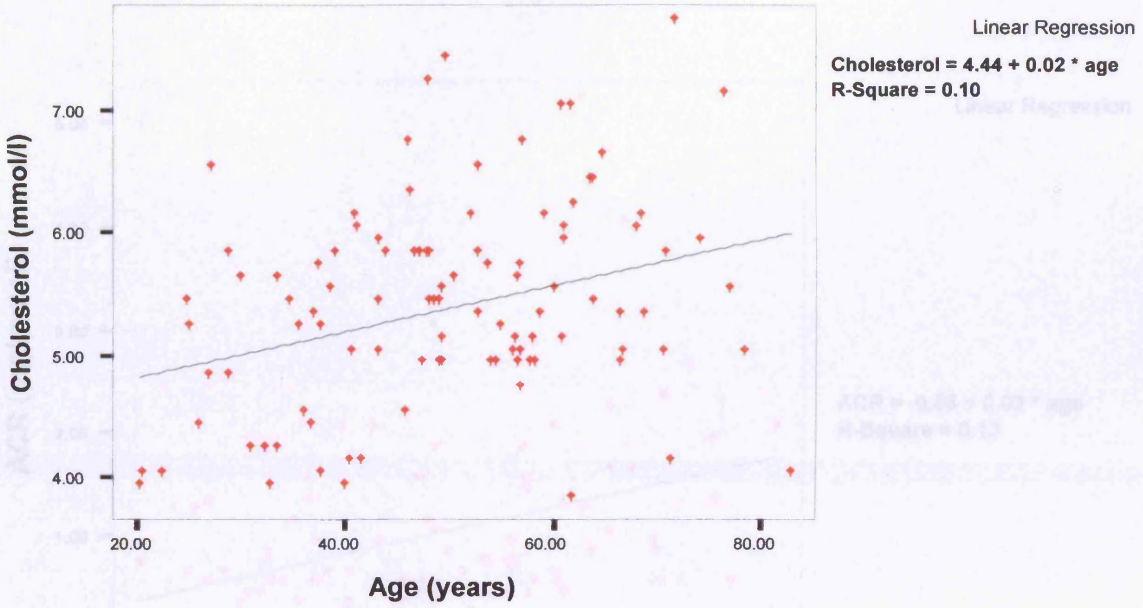


Figure 14 Relationship of Age LDL cholesterol

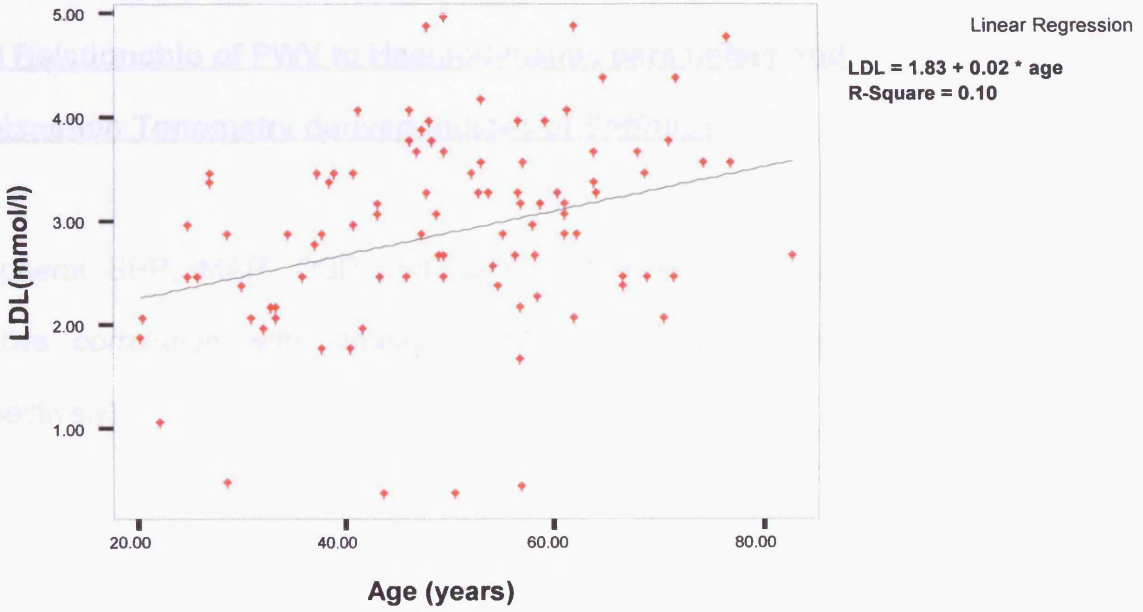
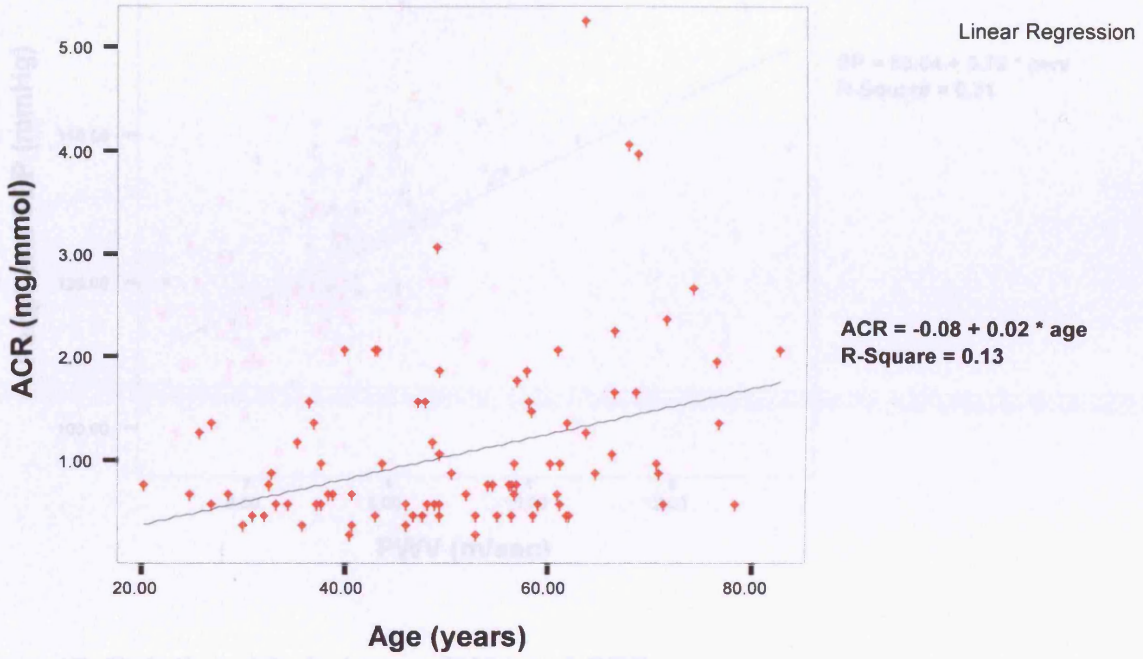


Figure 15 Relationship of Age and ACR



6.33 Relationship of PWV to Haemodynamic parameters and Applanation Tonometry derived Indices of *Stiffness.*

Peripheral SBP, MAP, DBP and peripheral pulse pressure all show a positive correlation with measured PWV (Figures 16, 17,18 and 19 respectively)

Figure 16. Relationship between PWV and peripheral SBP

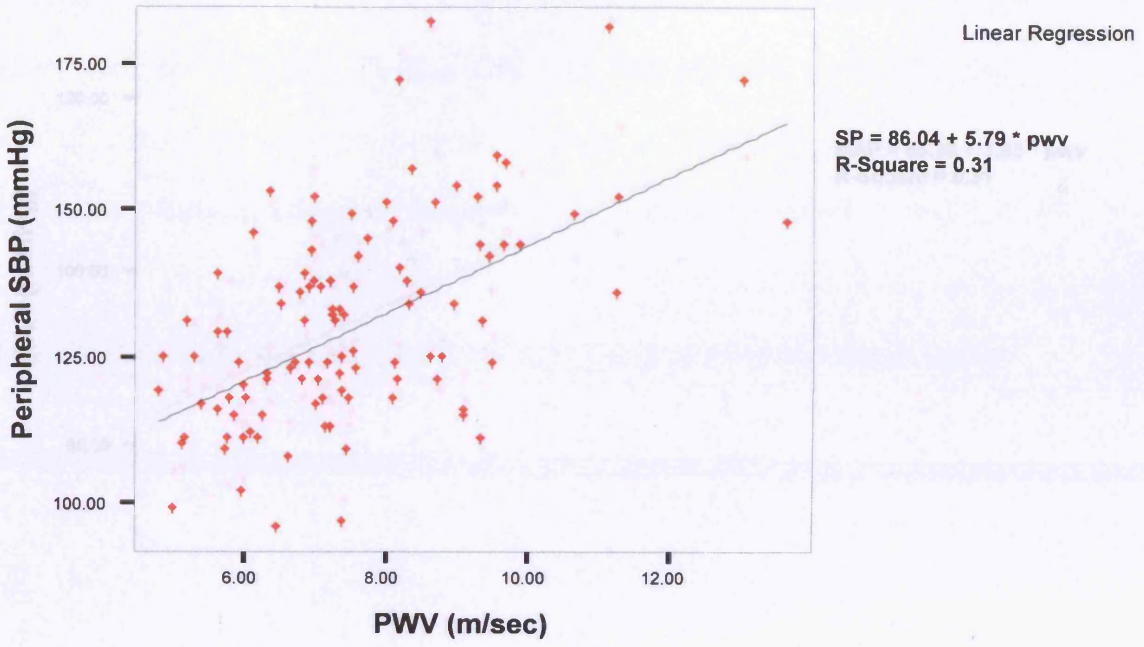


Figure 17. Relationship between PWV and DBP

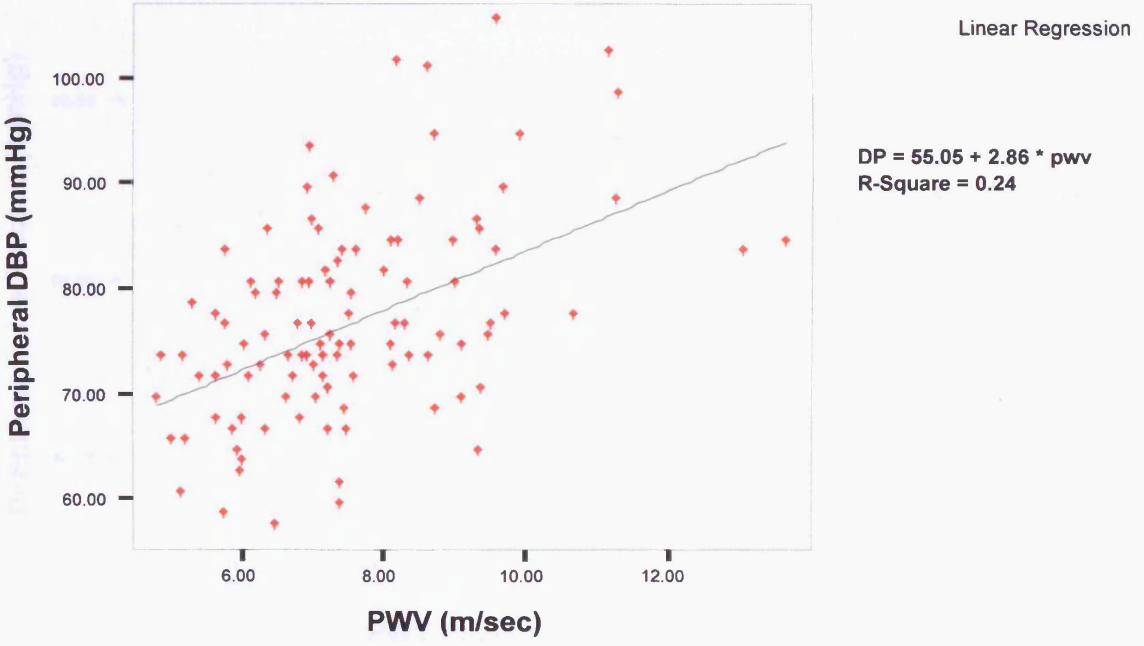


Figure 18. Relationship between PWV and MAP

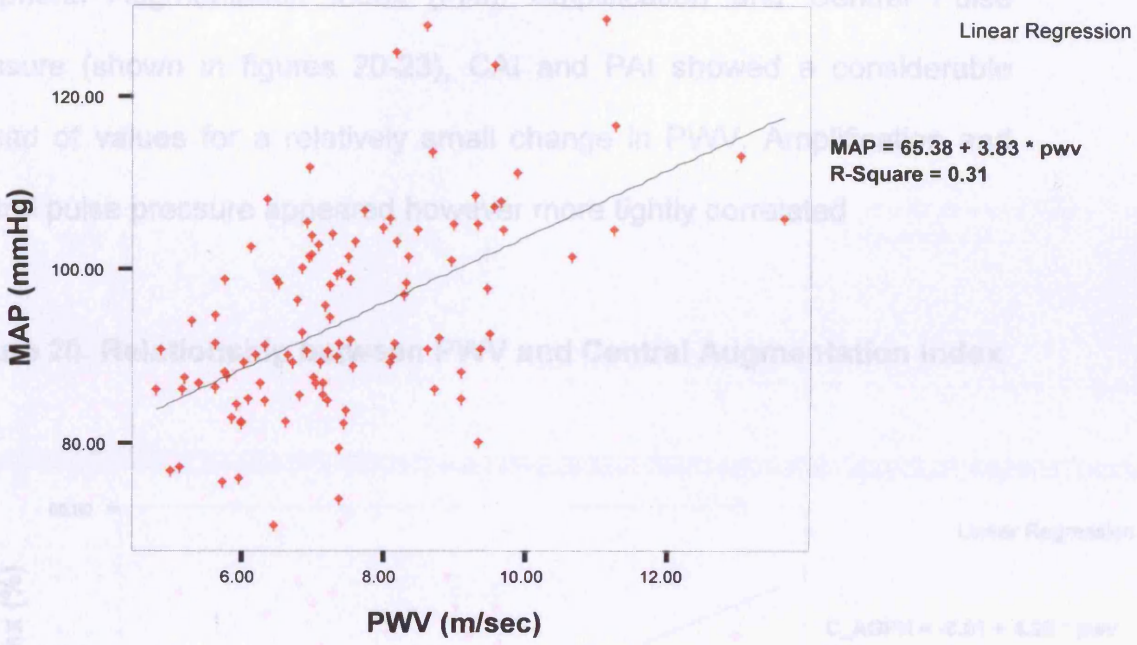
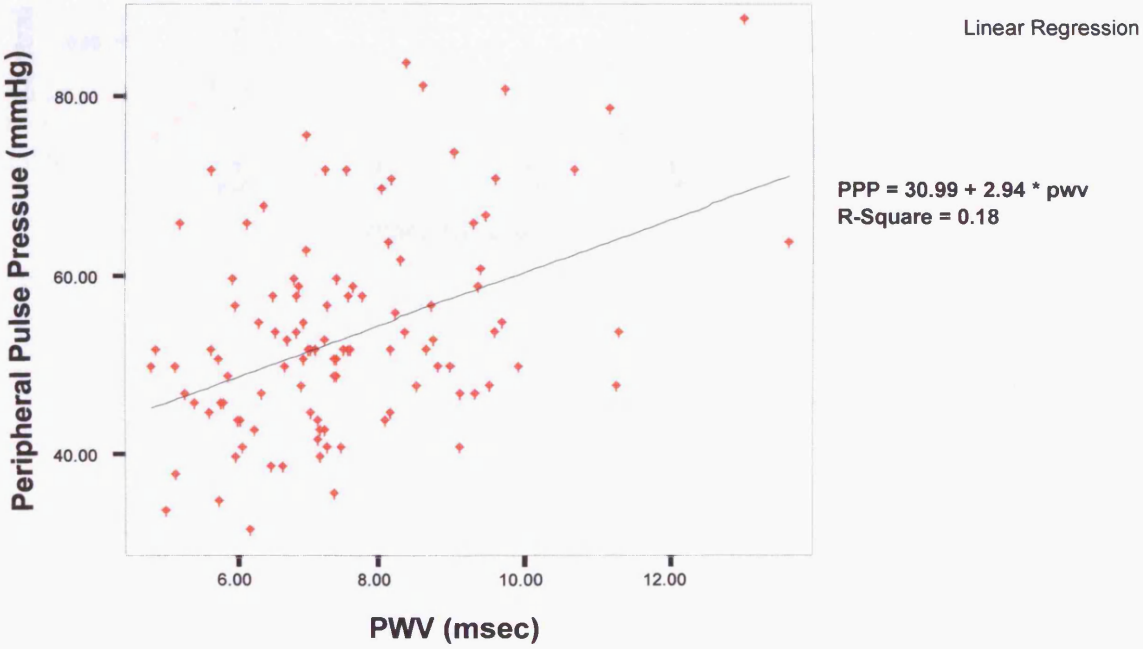


Figure 19. Relationship between PWV and Peripheral Pulse Pressure



Although there was a statistically significant relationship between PWV and the Applanation Tonometry parameters, Central Augmentation Index (CAI), Peripheral Augmentation Index (PAI), Amplification and Central Pulse pressure (shown in figures 20-23), CAI and PAI showed a considerable spread of values for a relatively small change in PWV. Amplification and central pulse pressure appeared however more tightly correlated

Figure 20. Relationship between PWV and Central Augmentation Index

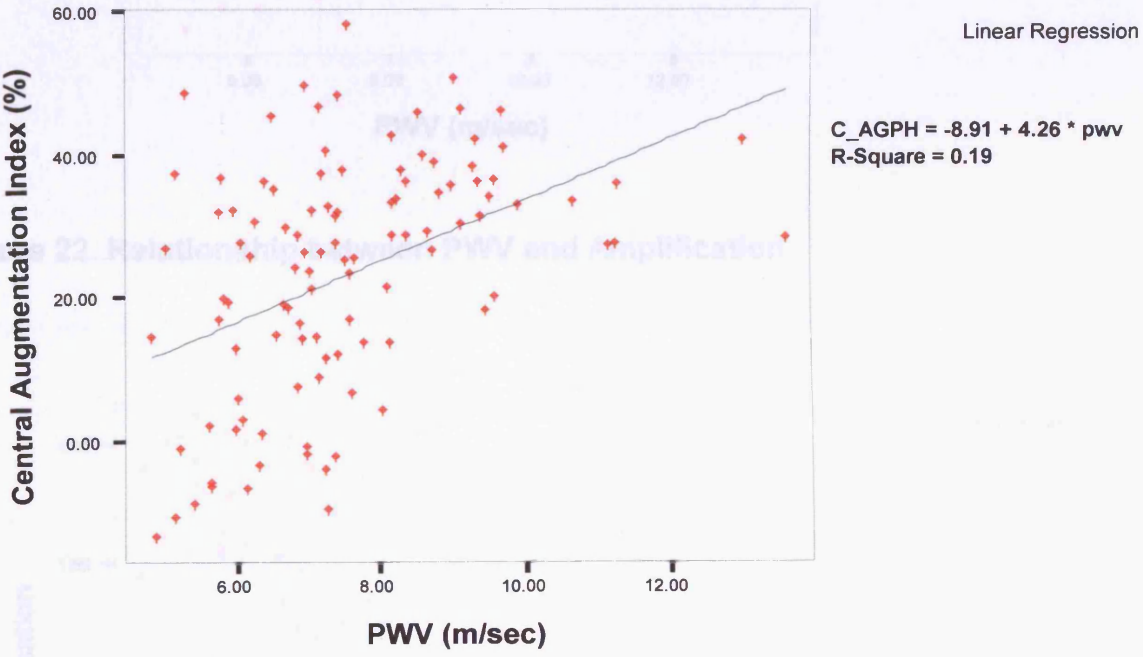


Figure 21. Relationship between PWV and Radial Augmentation Index

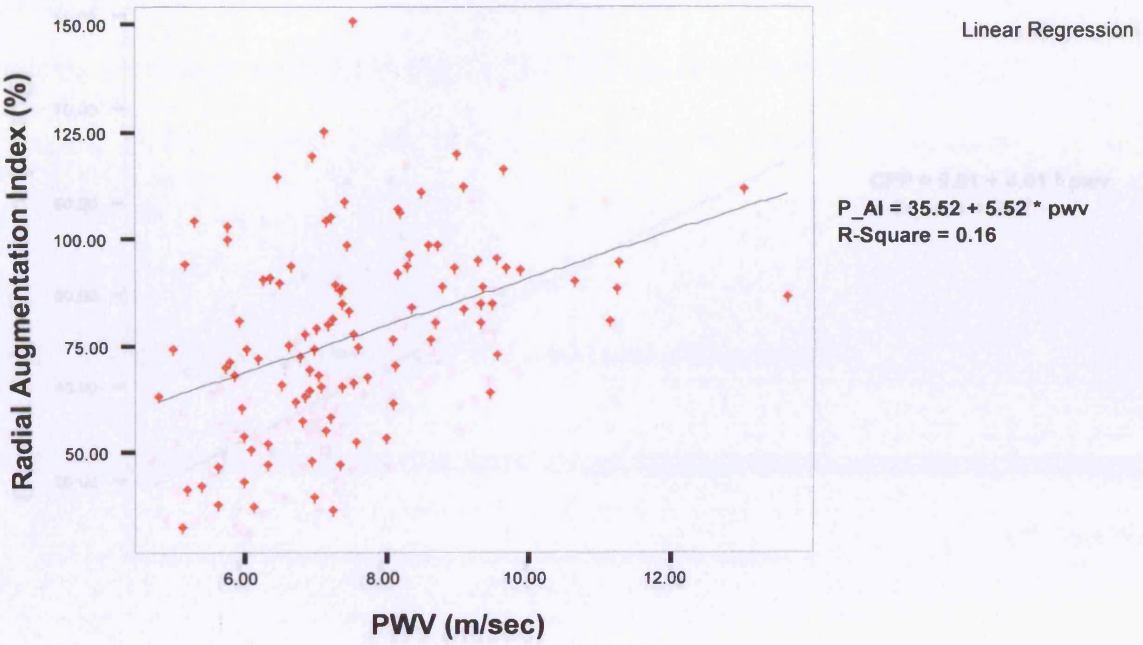


Figure 22. Relationship between PWV and Amplification

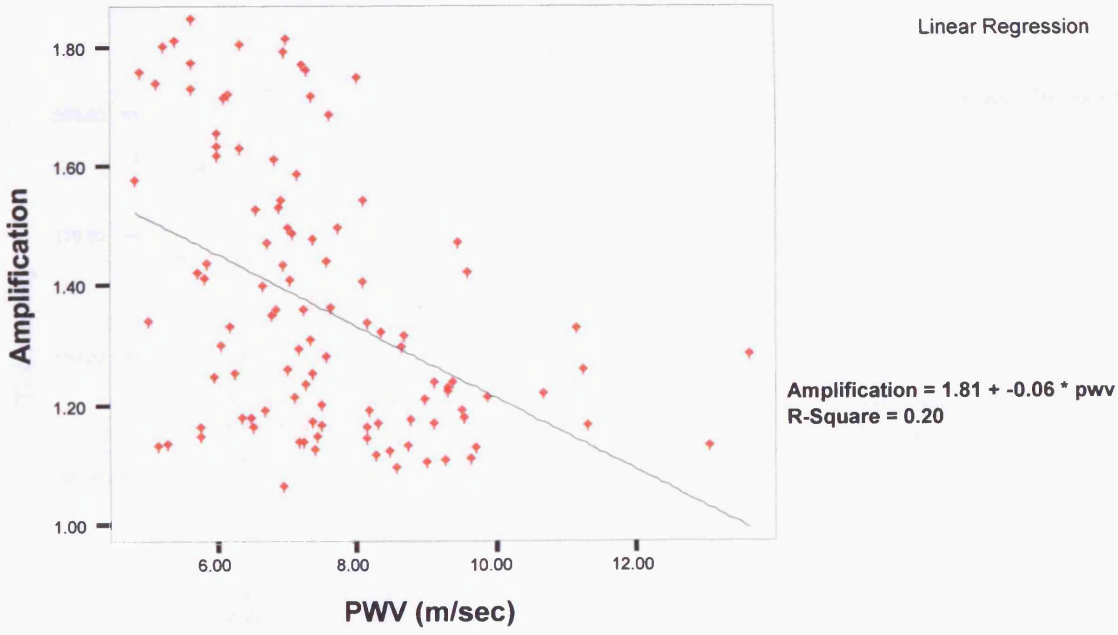
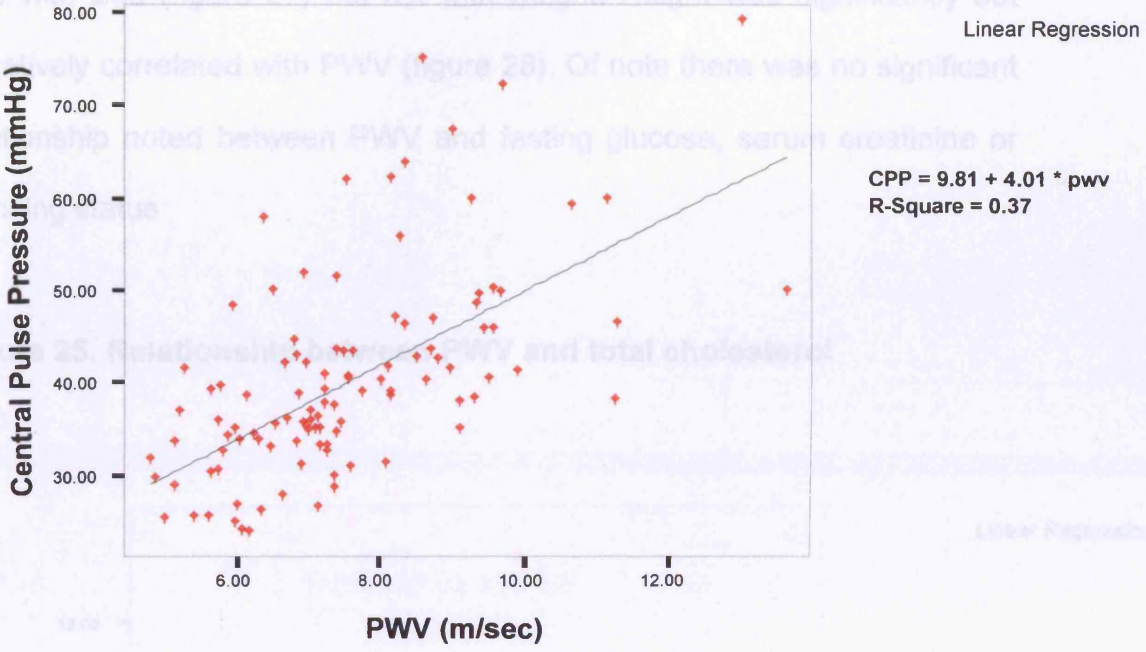
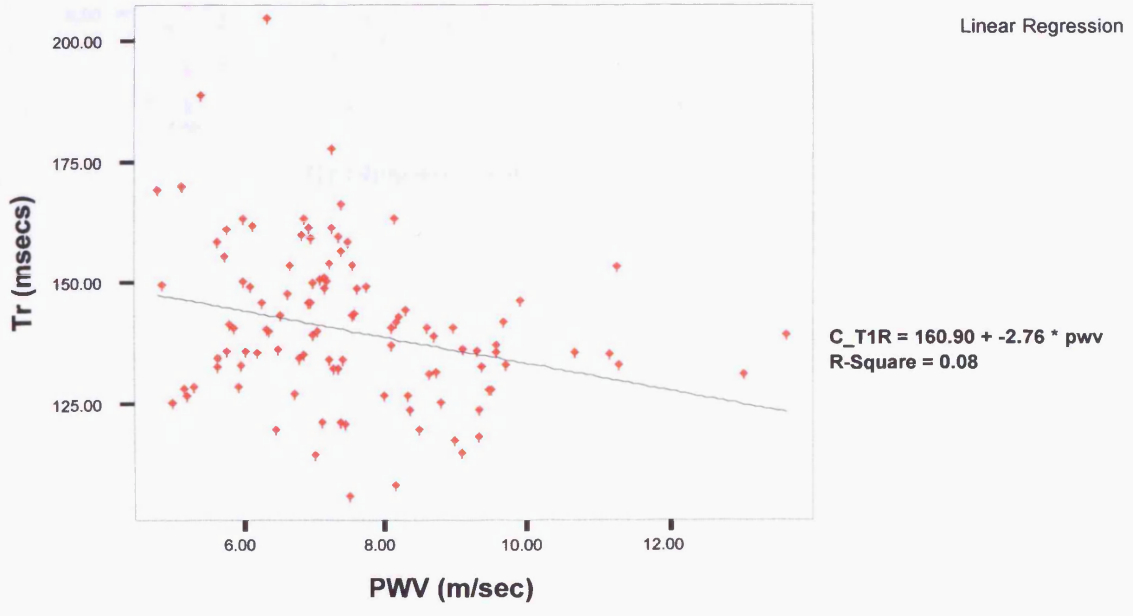


Figure 23. Relationship between PWV and Central Pulse Pressure



Tr was negatively correlated to PWV as shown in figure 24.

Figure 24. Relationship between PWV and Tr



In univariate analysis, PWV was positively correlated with Total cholesterol (figure 25) and triglycerides (figure 26) but not HDL or LDL cholesterol and also with BMI (figure 27) but not with weight. Height was significantly but negatively correlated with PWV (figure 28). Of note there was no significant relationship noted between PWV and fasting glucose, serum creatinine or smoking status

Figure 25. Relationship between PWV and total cholesterol

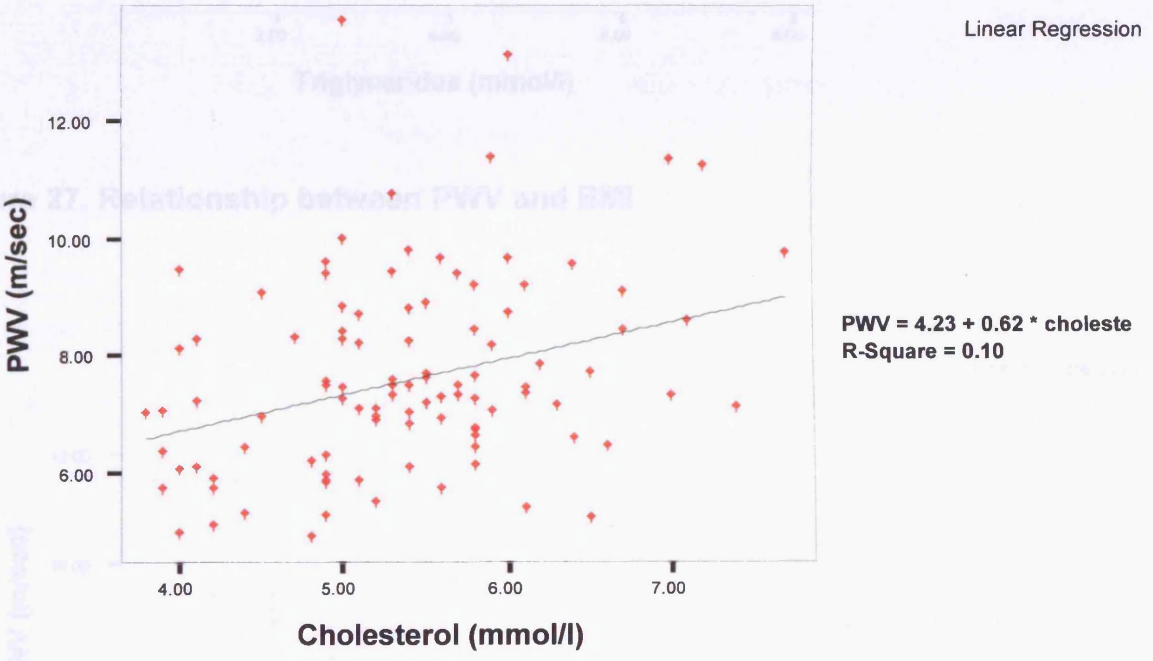


Figure 26. Relationship between PWV and triglycerides

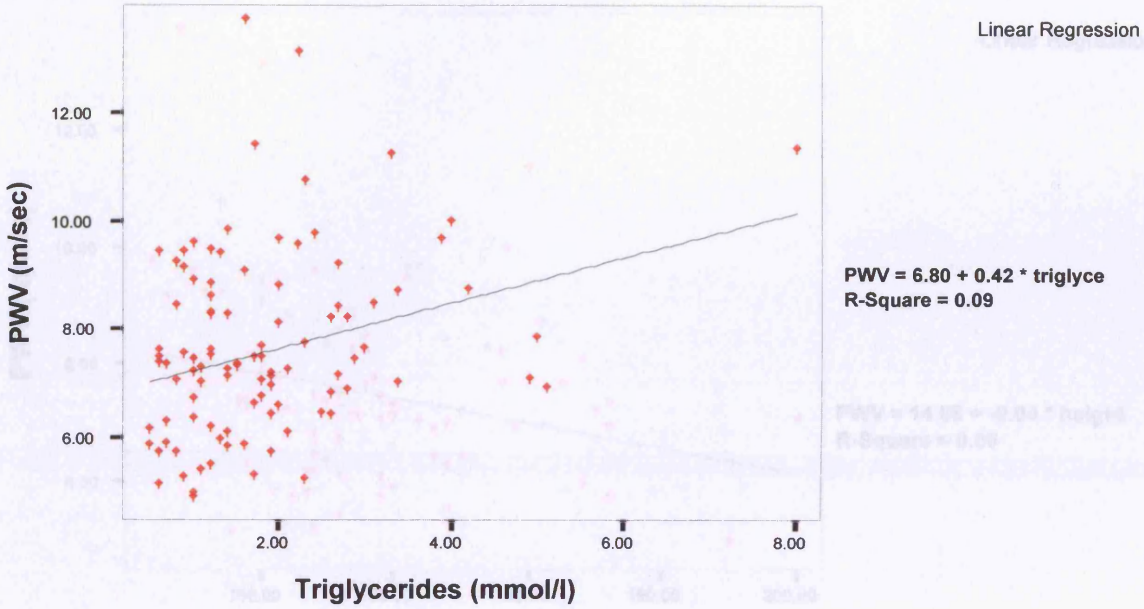


Figure 27. Relationship between PWV and BMI

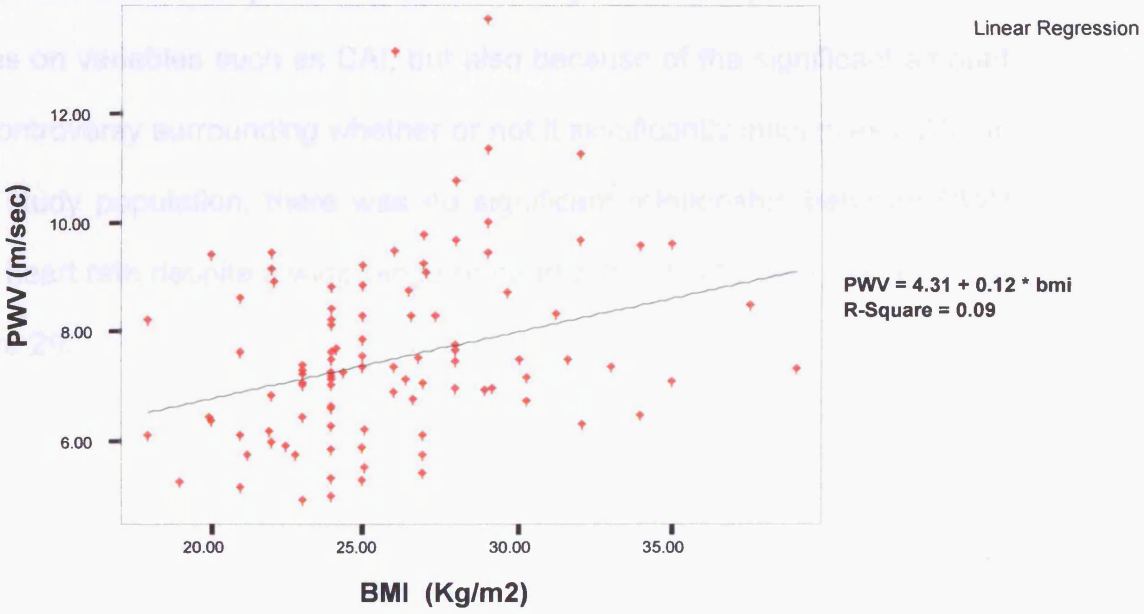
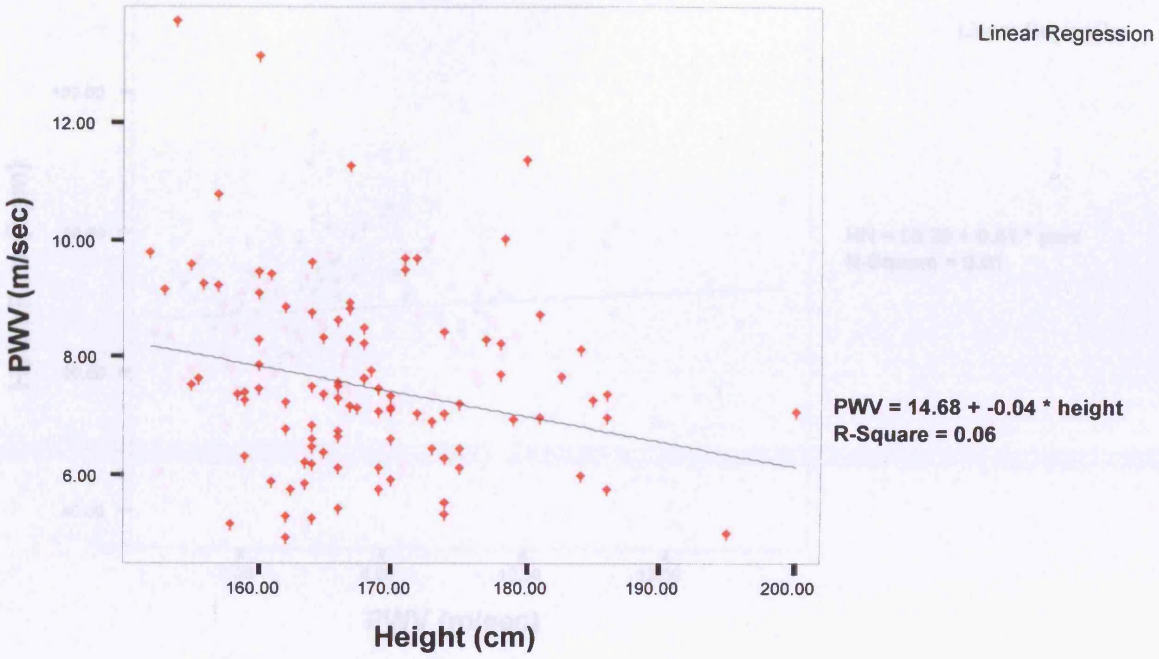
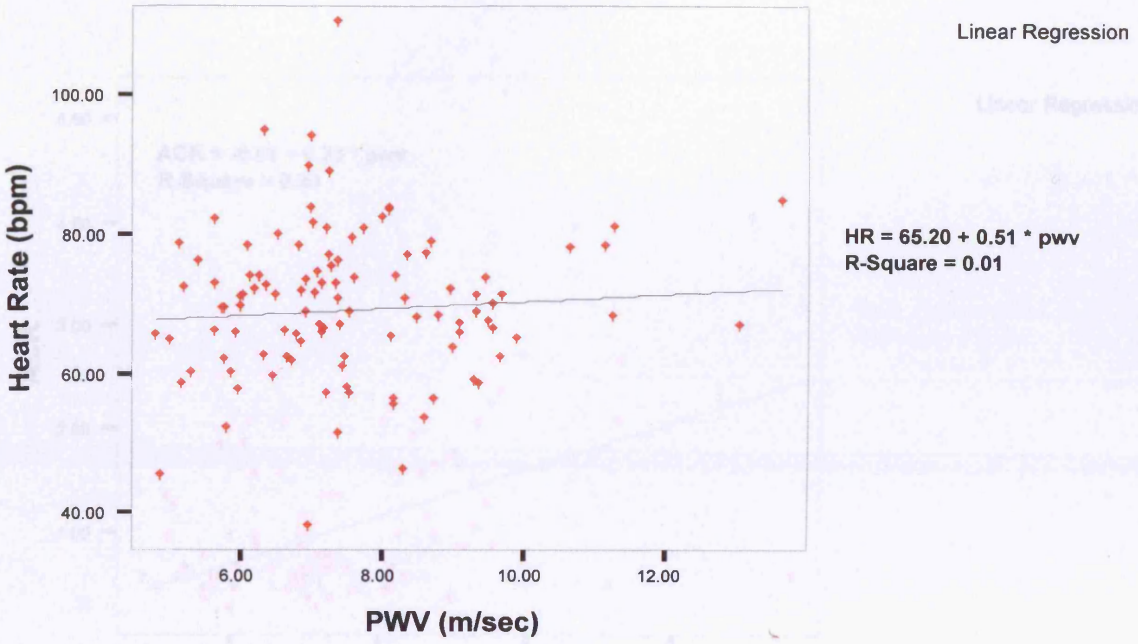


Figure 28. Relationship between PWV and Height



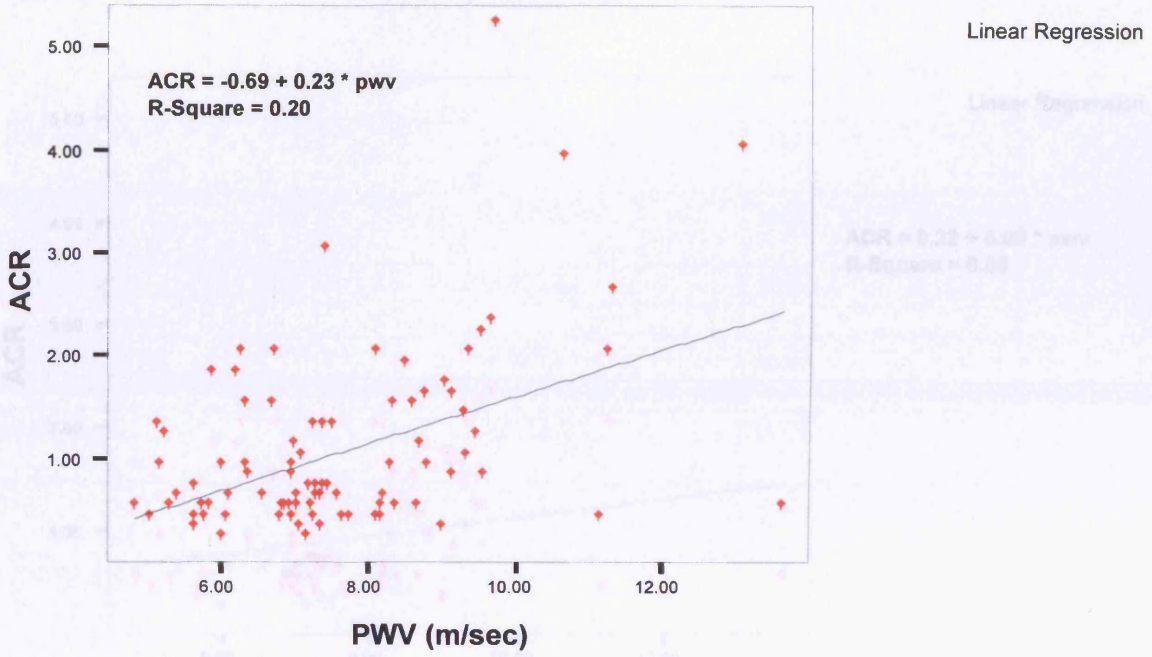
Heart rate deserves special mention, not only due to the profound influence it has on variables such as CAI, but also because of the significant amount of controversy surrounding whether or not it significantly influences PWV. In this study population, there was no significant relationship between PWV and heart rate despite a wide range of heart rate (38-110 bpm SD 10.6) see figure 29.

Figure 29. Relationship of PWV and Heart Rate



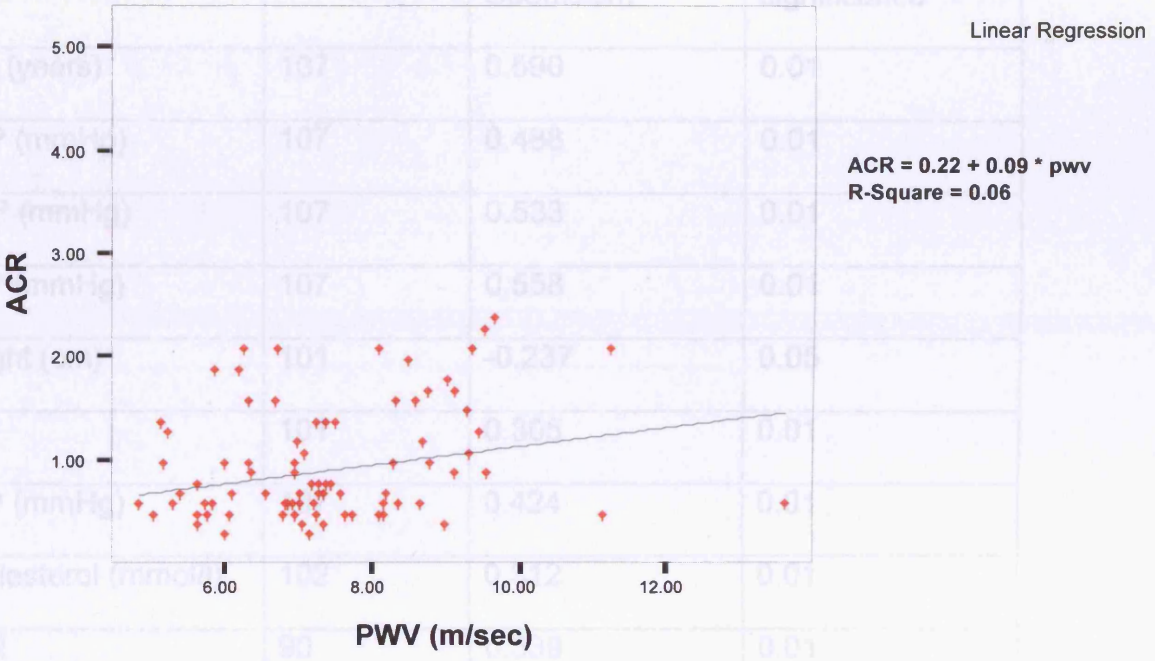
Another variable deserving of specific mention is that of ACR. Proteinuria is not only proving itself as an early marker of, but also a strong prognosticator for, cardiovascular disease. ACR showed a weakly but statistically significant positive relationship associated with increasing PWV in univariate analysis (see figure 30). The limited data for ACR, especially for higher PWV values, in addition to the observed outliers means this data should obviously be interpreted with caution, but never the less is deserving of further study.

Figure 30. Relationship between PWV and ACR



If this relationship is re-analyzed with outliers removed and only those ACR values falling within the 'normal range' studied (less than 2.5 for our local laboratory), then there is still a statistically significant and positive correlation seen($p<0.05$), although the regression slope is obviously much less steep (see figure 31).

Figure 31. Relationship between PWV and 'normal range' ACR



A summary table of the significantly correlated univariate parameters to PWV is presented below.

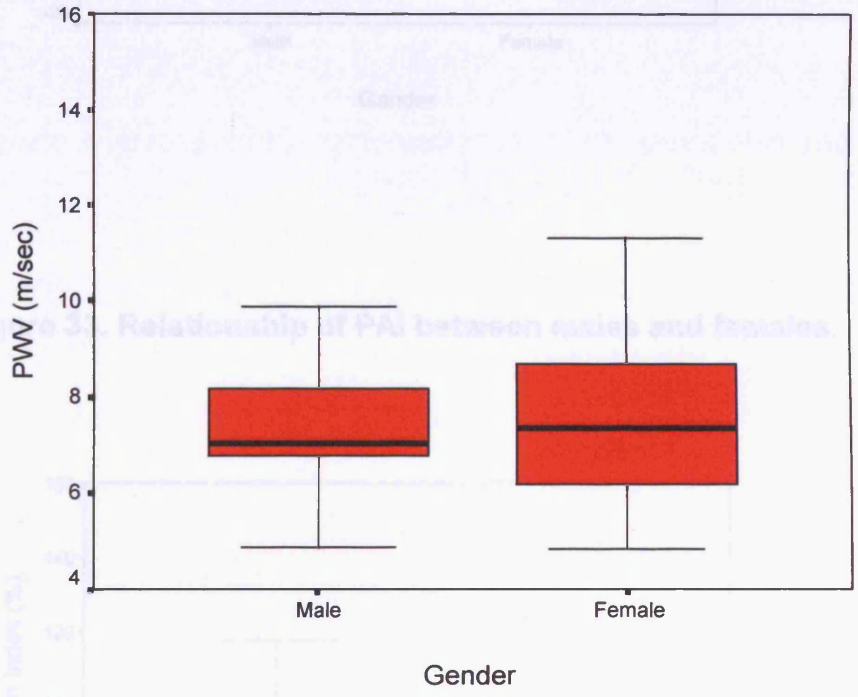
Table 4. Significantly correlated parameters with PWV

Variable	n	Correlation Coefficient	Two-tailed significance
Age (years)	107	0.590	0.01
DBP (mmHg)	107	0.488	0.01
MAP (mmHg)	107	0.533	0.01
SBP (mmHg)	107	0.558	0.01
Height (cm)	101	-0.237	0.05
BMI	101	0.305	0.01
PPP (mmHg)	107	0.424	0.01
Cholesterol (mmol/l)	102	0.312	0.01
ACR	90	0.339	0.01
Triglycerides (mmol/l)	102	0.292	0.01
CAI (%)	107	0.433	0.01
PAI (%)	106	0.401	0.01
Tr (msecs)	107	-0.284	0.01
Amplification	107	-0.445	0.01

6.34 Examination of the data by Gender split

Given the afore-mentioned impact on Gender on a number of tonometrically derived variables the data was then analysed by gender split. Firstly and most importantly there was no significant difference in PWV between males and females (p=0.82) (see figure 32)

Figure 32 PWV and Gender



This finding was seen in the face of females having significantly increased CAI (p<0.001), PAI (p=0.01), faster mean Tr (p=0.04) and lower amplification ratios (p<0.001) all indirectly suggesting increased PWV/vascular stiffness (see figures 32-35)

Figure 32. Relationship of CAI between males and females.

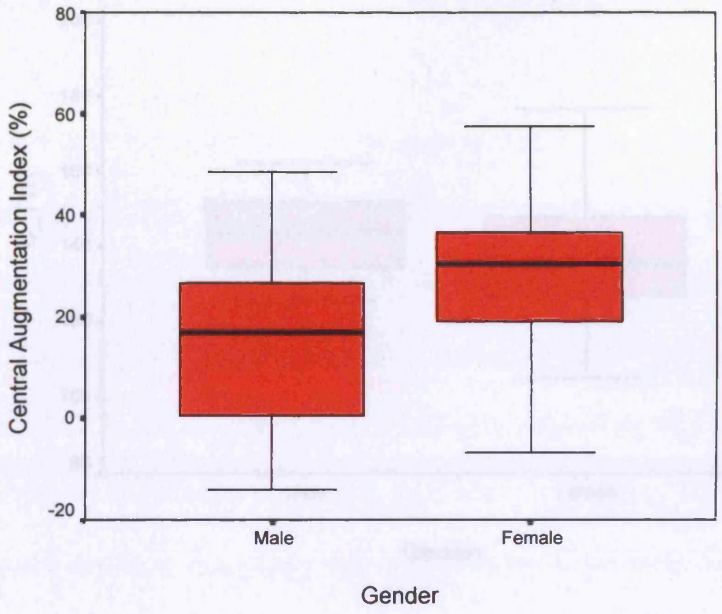


Figure 33. Relationship of PAI between males and females.

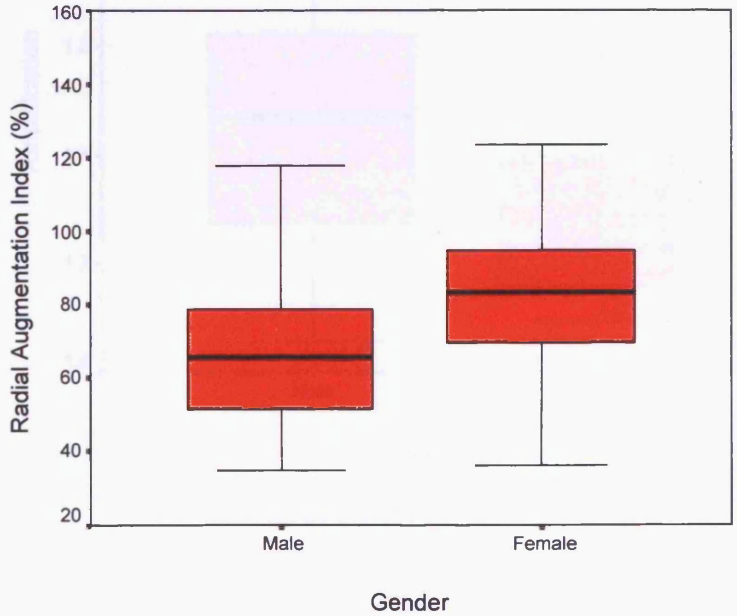


Figure 34. Relationship of Tr between males and females.

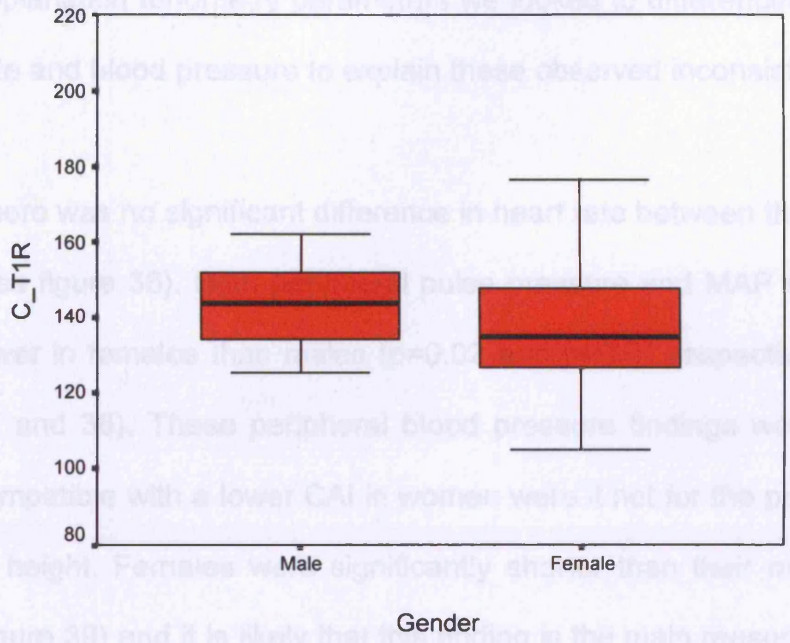
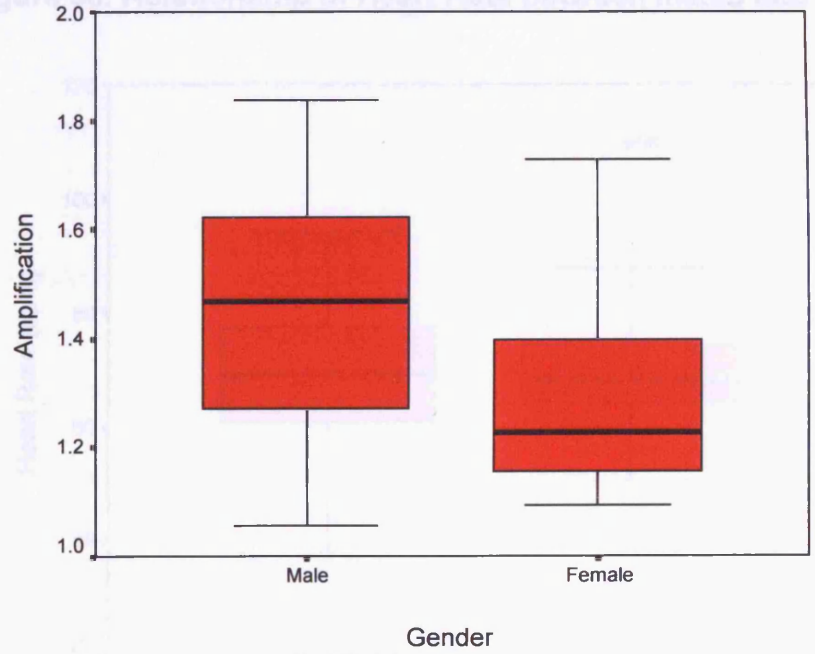


Figure 35. Relationship of Amplification between males and females.



Given our previous knowledge of the impact of certain variables on applanation tonometry parameters we looked to differences in height, heart rate and blood pressure to explain these observed inconsistencies.

There was no significant difference in heart rate between the sexes (p=0.79) (see figure 36). Both peripheral pulse pressure and MAP were significantly lower in females than males (p=0.02 and p=0.01 respectively) (see figures 37 and 38). These peripheral blood pressure findings would obviously be compatible with a lower CAI in women were it not for the profound influence of height. Females were significantly shorter than their male counterparts (figure 39) and it is likely that this finding is the main reason accountable for the discrepancies seen in derived central parameters.

Figure 36. Relationship of Heart Rate between males and females

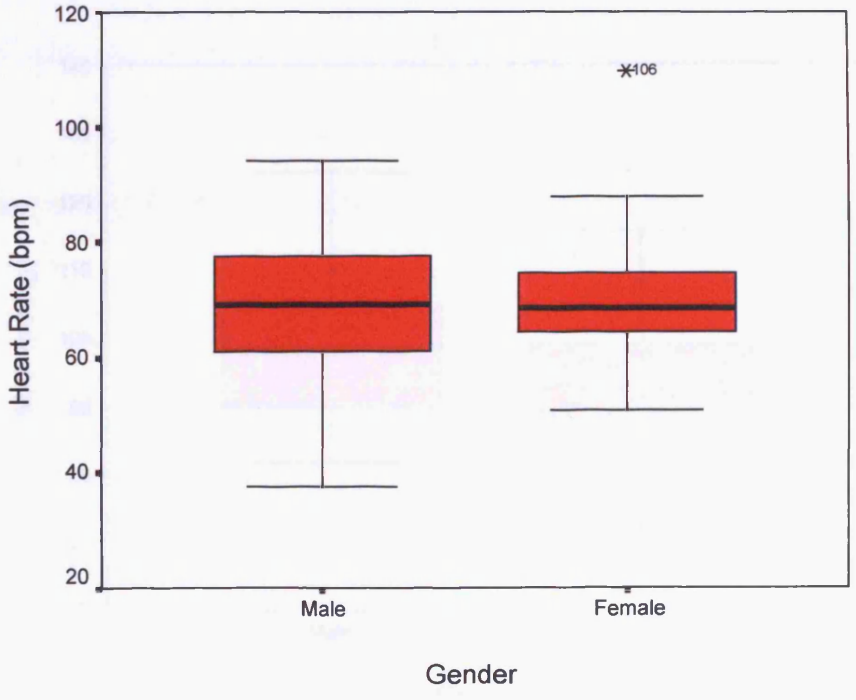


Figure 37. Relationship of Peripheral PP between males and females

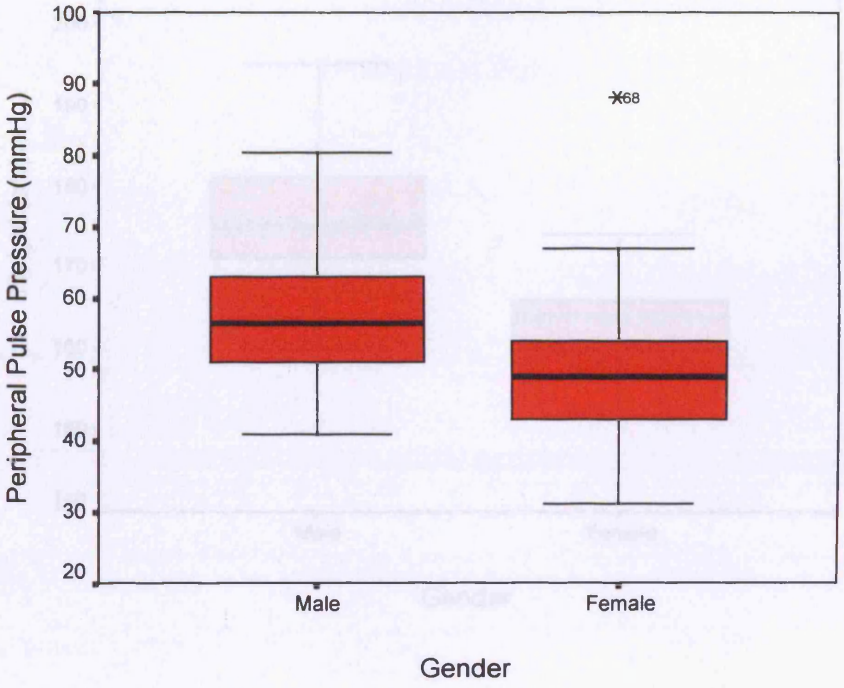


Figure 38. Relationship of MAP between males and females

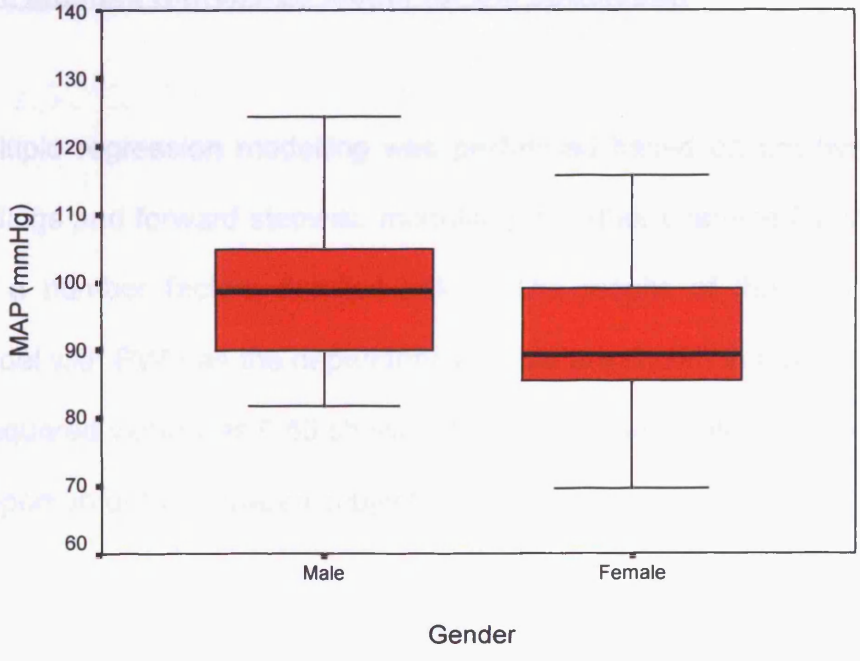
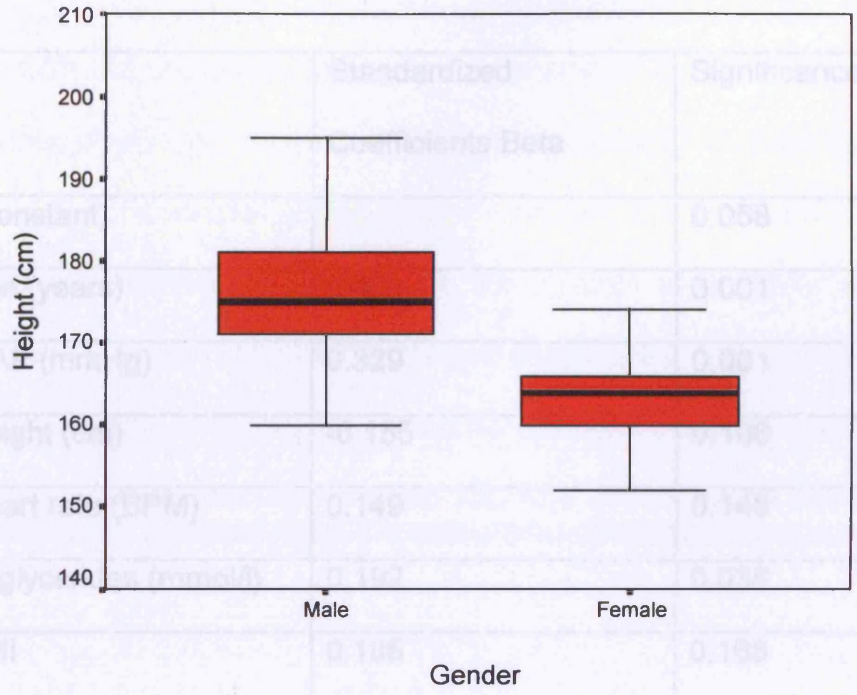


Figure 39. Relationship of Height between males and females



6.35 Multiple regression model for the population

Multiple regression modelling was performed based on positive univariate findings and forward stepwise modelling to further examine PWV controlling for a number factors detailed below. The results of the final regression model with PWV as the dependent variable are shown in table 5 below. The R squared value was 0.66 showing that this model explained a considerable proportion of the between subject variation.

Table 5. Multiple Regression model for PWV

	Standardized Coefficients Beta	Significance
(Constant)		0.058
Age (years)	0.403	0.001
MAP (mmHg)	0.329	0.001
Height (cm)	-0.155	0.106
Heart rate (BPM)	0.149	0.148
Triglycerides (mmol/l)	0.192	0.038
BMI	0.105	0.168
Cholesterol (mmol/l)	-0.075	0.381
ACR	0.205	0.011
Weight (Kg)	0.083	0.326
AI (%)	-0.401	0.188
Tr (msecs)	-0.111	0.255
Amplification	-0.436	0.125
Creatinine (umol/l)	0.032	0.727

Significant factors in predicting PWV in our population were age, MAP, triglyceride level and ACR. Total Cholesterol did not remain a predictor in the final model. Interestingly neither Augmentation Index nor time to reflection of the returning wave (Tr), both heralded as indirect markers of PWV were significant predictors of PWV in the final model.

6.4 Discussion

This control population has provided a unique opportunity to assess the effects of age on measured pulse wave velocity derived non-invasively using the technique of applanation tonometry. It has also enabled the potential influencing variables on this marker of vascular stiffness and indeed outcome to be studied. Alleged alternative indirect indicators of pulse wave velocity and therefore vascular stiffness, also available using this technique have also been studied and their direct relationship to measured PWV assessed.

In support of our theory of vascular ageing, there is a positive linear relationship between age and pulse wave velocity suggesting increased vascular stiffness. This is despite little age related change in conventional blood pressure parameters such as SBP, MAP and PP in our control population. This suggests that PWV may be the most sensitive marker of vascular ageing than others currently available.

Pulse wave velocity correlated positively in univariate analysis with SBP, MAP, DBP and PP. In addition, total cholesterol, triglycerides, BMI and ACR were positively correlated and height was significantly but negatively correlated. Reassuringly all these factors, possibly with the exception of height are well recognised risk factors for vascular disease. As discussed previously, the association of short stature and cardiovascular disease is still debated and the findings of greater pulse wave velocities in shorter

individuals may be influential. The lack of correlation between PWV and individual lipid fractions such as LDL and HDL cholesterol may be due to insufficient power in the study to detect a relationship. This may also hold true for the inability to detect a relationship between PWV and smoking due to the relatively high proportion of non-smokers in this population.

In line with an increasing PWV with age, indirect markers of vascular stiffness such as AI, Tr and amplification were significantly related to age. The relationship between AI and age suggests a positive relationship between AI and PWV if other variables such as heart rate and blood pressure remain relatively constant. Peripheral AI also shows a positive linear correlation with age as expected by its tight linear relationship to central AI. This may suggest that, as proposed by some groups already, peripheral AI be used as a marker of vascular stiffness or ageing without the need for transformation via a generalised transfer function.

Despite significant positive correlations with PWV, both central and peripheral AI showed an alarmingly large spread of values for a relatively small range of recorded PWV values. This obviously has profound implications on the use of this measurement to detect relatively subtle changes in vascular stiffness as considered in the previous chapter relating to the assessment of patients with hypertension.

The relationship between PWV and time to the reflected wave (Tr) although significant appears a weak one with a large spread of values. Those

patients with the highest measured PWV had in fact only slightly reduced Tr, compared with the population mean.

In support of the above concerns, neither AI or Tr proved significant predictors of PWV in the final regression model and add weight to the notion that they should not be used as surrogate markers for PWV.

To further cloud the debate on the relationship between heart rate and PWV, this population showed no significant relationship between the two. It may be that in this particular group, the absence of established vascular disease and the presence of a relatively elastic aorta may have lessened any potential effect of increased heart rate which may have been noted with a stiffer aorta?

What this study does not explain is why, if AI increases with age despite little change in peripheral BP, and if this is not convincingly explained by a close relationship with PWV then what does the change in AI signify? Is it suggesting an age related alteration in peripheral wave reflection due to some alteration in the peripheral circulation or peripheral vascular resistance? If so, and if this is not as obviously recognised by a change in brachial blood pressure parameters, is AI a more sensitive marker of peripheral vascular dysfunction than brachial BP? If so is this clinically relevant given the fact that to date AI has proven a relatively poor marker of outcome in terms of mortality except in a fairly small cohort of the population with end stage renal disease. These questions will deserve further

consideration in larger clinical studies before this technique gains further acceptance into clinical assessment of patients.

Following our inability to integrate measurements of PWV into assessment of hypertensive individuals, and disappointing results by our group to show a difference in AI in the assessment of yet another high risk group, namely those patients with diabetes (unpublished data), we next aimed to compare the results of measured AI and PWV in a diabetic cohort.

7.0 Increased pulse wave velocity is not associated with elevated Augmentation Index in patients with diabetes

7.1 Introduction

Diabetes is an independent risk factor for cardiovascular morbidity and mortality. Cardiovascular death accounts for the majority of deaths seen in patients with diabetes, with mortality rates of up to five times those of non-diabetic individuals [191;192]This excess mortality persists for both males and females, throughout all age ranges and is over and above the effects of other well recognized risk factors for cardiovascular disease such as hypertension, smoking and dyslipidaemia.[192-195] Despite aggressive glycaemic, blood pressure and lipid control, morbidity and mortality rates are not reduced to the level of non-diabetic patients. This has lead to a search for early markers of cardiovascular dysfunction in diabetic patients that may pre-date the development of overt clinical disease, offer a target for early intervention, and delay progression of cardiovascular disease complications.

The assessment of vascular stiffness may prove to be one such early marker. Previous work has documented stiffer arteries in patients with type 1 and type 2 diabetes, with differing degrees of diabetic complications, across various age ranges, at differing arterial sites and using a range of methodologies and measuring techniques[163;196-207]. Moreover, increased arterial stiffness, as assessed by measuring aortic PWV has also been demonstrated in the healthy offspring of patients with type 2 diabetes[208].

However, it should be noted that diabetes is not associated with an increase in measured arterial stiffness in all arteries studied. In some reports arterial stiffness has been found to be increased in only one of a number of arteries studied[137;209;210] whilst other studies demonstrated increased arterial stiffness only in patients with evidence of diabetic complications [211]. Nevertheless, a large body of evidence supports the concept of enhanced arterial stiffness in diabetes.

As previously discussed, PWV is a recognized marker of large artery stiffness. PWV has been shown to be a powerful independent predictor of both cardiovascular and all-cause mortality in many patient groups[69;72;124;157;183;212] and has been shown to be elevated, and predicts premature mortality, in patients with diabetes[123].

To reiterate, PWA uses applanation tonometry to record the peripheral arterial waveform (usually radial) and is well described in the literature[90;107;109;110;213;214].

AI has been heralded by some investigators to be a measure of arterial stiffness and by others as an indirect measurement of pulse wave velocity [129;132;215].

As AI is technically easier to measure than PWV it has been proposed as a convenient and robust alternative indicator of systemic arterial stiffness[216-

[218]. However, the relationship between AI and PWV in people with diabetes is unclear.

In our own clinical studies, despite studying large numbers of people with diabetes, we have been unable to confirm an increase in their AI measurements when compared with non-diabetic controls. We therefore conducted this study to formally define the relationship between AI and PWV in people with diabetes.

7.2 Methods

See general methods, chapter 2.

7.21 Characteristics of Study Participants.

Sixty-six people with diabetes (41 type II, 25 type I) were recruited from an outpatient clinic at the University of Leicester NHS Trust (47 subjects), or from general practice in the Leicester area (19 subjects). Mean duration of diabetes in this group was 14.5±1.29 years. Glycaemic control was maintained through use of insulin (n=44), oral hypoglycaemics (n=20) or dietary control (n=3). Predictably, many patients (n=41) were receiving blood pressure or lipid lowering medications with cardiovascular effects. These included; diuretics (n=14), calcium channel blockers (n=10), angiotensin converting enzyme inhibitors/angiotensin II receptor blockers (n=26), alpha-blockers (n=9), beta-blockers (n=7) and statins (n=15). Some

patients were not receiving concurrent anti-hypertensive or lipid-lowering medications (n=25). Discontinuation of medications for the purposes of this study was not justified. Patients with significant arrhythmias were excluded from the study.

Control subjects were recruited from volunteers attending for a cardiovascular health check at the Clinical Research Unit of the Leicester Royal Infirmary. Three of the control volunteers were taking drug treatments with potential cardiovascular effects (diuretic; n=1, calcium channel blocker; n=1, calcium channel blocker + ACE-inhibitor; n=1).

All subjects provided written, informed consent and the Leicestershire Local Research Ethics Committee approved the study (see appendix).

7.22 Brachial artery blood pressure measurement.

See general methods, chapter 2.

7.23 Radial artery pulse wave analysis.

Pulse wave analysis was used to determine aortic augmentation index (AI), as described in general methods, chapter 2. Augmentation index was defined as the ratio of augmentation to pulse pressure and was expressed

as a percentage (AI=(ΔP/PP) x100, Chapter 1 Fig. 12A). Time to the foot of the reflected wave (Tr) was also identified. Data from the mean of two central aortic pressure waveforms was taken for each subject.

7.24 Measurement of carotid-femoral pulse wave velocity (PWV_{cf}).

PWV_{cf} was determined as described previously in general methods, chapter 2. Data from the mean of two PWV_{cf} measurements was taken for each subject.

All tonometry data was acquired by a single operator. Previous unpublished studies in our laboratory demonstrated an inter-observer variability of 0.12±0.85 $msec^{-1}$ for PWV_{cf} and 0.26±7% for AI. This is consistent with our previously published data [127]

7.26 Analysis of carotid pressure waveforms.

Pressure waveforms acquired at the carotid artery during measurement of PWV_{cf} were extracted for analysis of the arterial waveform. Each series of waveforms was assessed visually to exclude those with significant movement or respiratory artefacts. Pressure waveforms were calibrated with respect to the mean and diastolic blood pressures of the peripheral radial waveform [219]The calibrated peripheral waveforms were analysed using SphygmoCor software and the amplitude of the outgoing and reflected

pressure waves determined. Augmentation index for the peripheral trace was calculated as the ratio of the late systolic peak (P_2) to that of the early systolic peak (P_1), Chapter 1 Fig. 12B). Central aortic pressure waveforms were then generated from the calibrated peripheral waveforms using a generalized carotid transfer function. The resulting derived central aortic pressure waveforms were analyzed by the SphygmoCor software as described for the central waveforms derived from radial pressure waveforms.

7.3 Statistics.

Normality of data was determined by construction of a normal probability plot with the use of the Minitab computer programme (Minitab 13 for Windows, Minitab Inc). Direct comparisons between data from diabetic patients and controls were made using a non-paired student's t-test or a non-parametric test (Mann Whitney) where data distribution did not conform to normality. Multiple regression analysis was used to determine the relationship between augmentation index or PWV_{cf} and diabetes in the whole dataset, controlling for the influence of other variables. A value of $P<0.05$ was taken as significant. Data is presented as mean ±SEM.

7.4 Results.

Demographic, clinical and haemodynamic characteristics of the study participants are shown in table 1. The two groups were well matched for age, gender and height. Predictably, the groups differed with respect to body mass index, haemoglobinA1c, and albumin: creatinine ratio, which were elevated in diabetic patients. Total cholesterol was lower in the diabetic group reflecting the greater number of people receiving lipid-lowering medication. A greater number of diabetic patients were smokers than controls.

Analysis of haemodynamic data showed an elevated brachial blood pressure in the diabetic group and an elevated heart rate (Table 1). Both pulsatile and non-pulsatile components of peripheral blood pressure were elevated in the diabetic group. There was also elevated central pulse pressure and central mean pressure in the group with diabetes. Pulse pressure amplification ratio however, was not significantly different when the control and diabetes groups were compared (control 1.3±0.02, DM 1.34±0.02, P=0.11). PWV_{cf} was markedly elevated in the diabetic patients.

Table 1. Demographic and haemodynamic data for the study populations.

	Control (n=66)	DM (n=66)	P
Age (Yrs)	54.5±1.7	55.4±1.7	NS
Gender M:F	45:21	45:21	NS
Smoking (Y:N)	5:61	15:51	<0.01
Height (cm)	170.6±1.1	170.2±1.1	NS
BMI (kg/m^2)	26.1±0.5	28.3±0.5	<0.01
Glucose (mmol/l)	4.6±0.1	10.8±0.6	<0.01
HbA1c (%)	5.6±0.1	8.4±0.2	<0.01
Total Cholesterol (mmol/l)	5.3±0.1	4.8±0.1	<0.01
Triglycerides (mmol/l)	1.3 (0.4-2.2)	1.4 (0.5-2.3)	NS
Creatinine (mmol/l)	86.3±1.3	91.3±2.6	NS
ACR (mg/mmol)	0.6 (0.2-1)	1.1 (0.5-1.7)	<0.01
Brachial SBP (mmHg)	129.2±1.8	139.2±1.9	<0.01
Brachial DBP (mmHg)	77.3±1.1	80.1±1.3	NS
Brachial MAP (mmHg)	94.6±1.2	99.8±1.4	<0.01
Brachial PP (mmHg)	51.9±1.1	59±1.5	<0.01
Aortic PP (mmHg)	40.5±1.1	45.1±1.4	<0.05
HR (min^{-1})	63.8±1.4	71±1.2	<0.01
PWV_{CF} ($msec^{-1}$)	7.7±0.2	9.3±0.4	<0.01

Figure 1 (A) shows AI derived from transformation of the peripheral radial pressure pulse. AI was little changed between the control and diabetic groups. By contrast, diabetes was associated with a significantly shorter time to the foot of the reflected wave. This decrease in Tr was consistent with the increase in PWV_{cf} observed in diabetic patients. Further analysis of central aortic pressure waveform revealed no difference in amplitude of the reflected wave occurring during systole (augmentation), whilst diabetes was associated with an increase in height of the outgoing pressure wave (P1 height, Fig. 1, (B)) – the latter is predictable on the basis of the higher blood pressure of the group with diabetes. Diabetes was also associated with shorter ejection duration together with an elevated rate of rise of pressure (dP/dT) for the peripheral waveform (Table 2). Measurement of systolic stress from the derived central aortic pressure waveform revealed an elevated tension time integral (TTI) with little difference in the diastolic time integral. Taken together this data suggests a shorter, more forceful systolic ejection in diabetic patients. These changes however, were not associated with increased early pressure wave reflections (AI).

An increase in heart rate is known to reduce AI [143;220;221]. We therefore adjusted the data to account for the higher heart rate of the group with diabetes. After adjusting for the heart rate differences, there were still no significant differences in AI when comparing the two groups (Fig. 1, (A)). This adjustment was made based on the relationship between heart rate and AI from a large database of normotensive controls, acquired in our

clinical laboratory (see chapter 3). Similarly, no difference in AI between diabetics and controls was found when AI for both groups was adjusted to a heart rate of 75bpm using the SphgmoCor software (control AI 21.1±1.5%, DM AI 23±1.4%, n=64, p=0.4). This observation of no difference in AI between the diabetic and control groups was consistent even when the diabetics were sub-divided by type. Tr was significantly reduced for both type II and type I diabetics compared with age and sex matched controls (Table. 3).

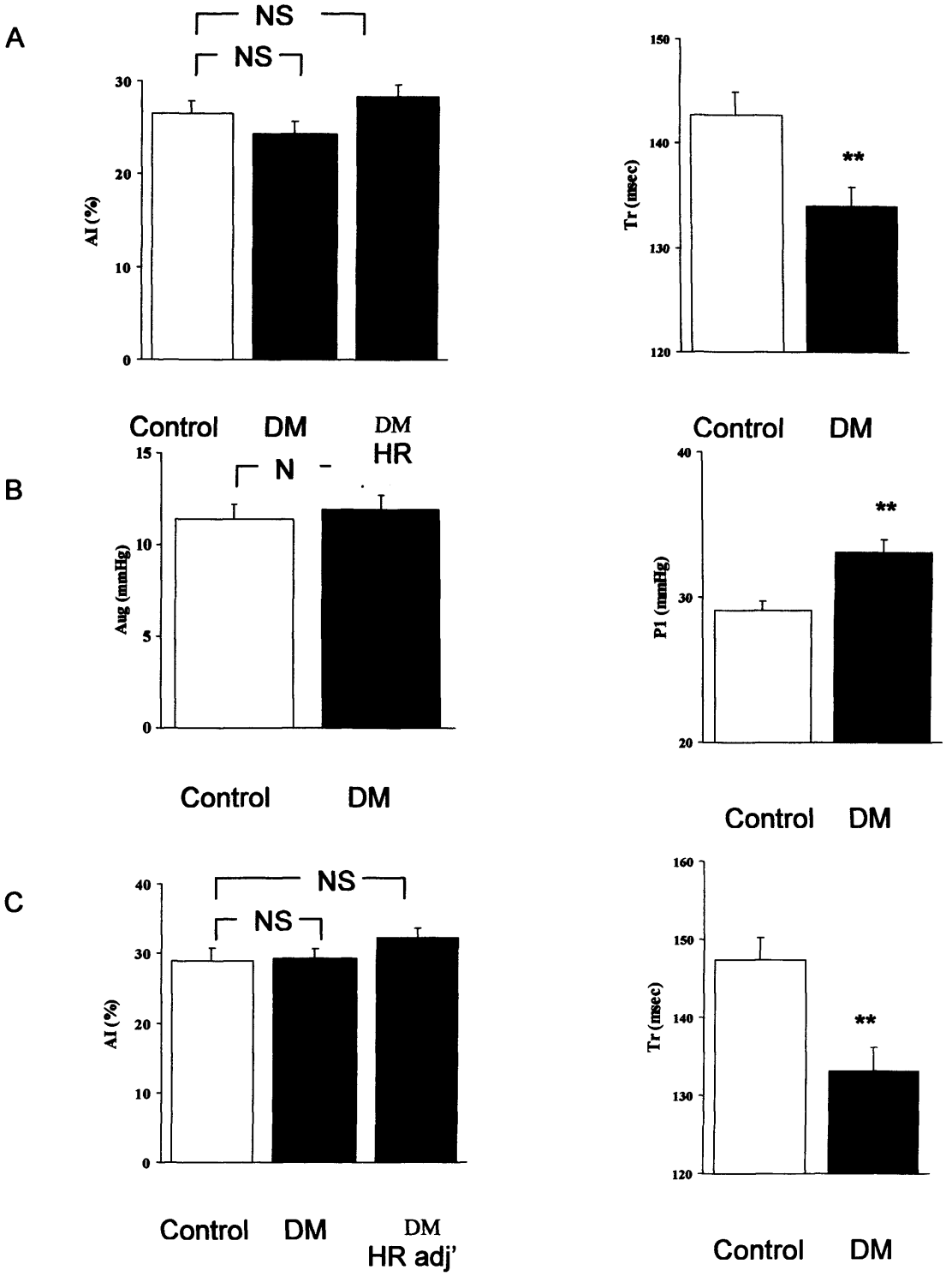


Figure 1. Derived parameters from the transformed central aortic and non-transformed peripheral carotid pressure waveforms in control and diabetic populations

Figure 1 legend: A & B, augmentation index, time to reflected wave (Tr), augmentation and outgoing pressure wave height (P1 height) for the central aortic pressure waveform derived from radial applanation tonometry (n=64). C, augmentation index and time to reflected wave (Tr), for the central aortic pressure waveform derived from carotid applanation tonometry (n=50). D, augmentation index calculated from the peripheral, non-transformed carotid pressure waveform ((P_2/P_1)x100). **P<0.01, NS not significant DM vs. control.

Table 2. Central aortic timing parameters and time integrals derived from radial tonometry for control and diabetic populations.

	Control (n=64)	DM (n=64)	P
T_1 (msec)	106.7±1.2	101±1.5	<0.01
T_2 (msec)	229.9±3	217.1±2.4	<0.01
ED (msec)	328.5±2.6	314±2.9	<0.01
ED (%)	34.4±0.6	36.9±0.5	<0.01
dP/dT	771.2±22	966.8±31	<0.01
TTI ($mmHg.s.min^{-1}$)	2206.9±49.2	2521.5±48.8	<0.01
DTI ($mmHg.s.min^{-1}$)	3571.3±60.6	3524.7±65.2	NS
SVI (%)	167±4.6	142.2±3.1	<0.01

Many of the diabetes group were receiving medications which might influence arterial pressure wave reflections (e.g. rate-limiting agents/ vasodilators/ statins). In order to investigate whether concurrent medication masked the

influence of diabetes on AI, sub-analysis in 25 untreated people with diabetes (mean age 48.4±3 years, BP 132.5±3.3/78.6±1.9mmHg) and 25 age and sex matched controls (mean age 47.2±2.6 years, BP 123±2.2/72.1±1.3mmHg) was performed. In this sub-group AI still was not different between controls and diabetic patients whilst Tr was shorter in the patients with diabetes as expected (Table 3).

Table 3. Central aortic augmentation index and time to reflected wave (Tr) derived from radial applanation tonometry in subgroups of type I diabetics with age and sex matched controls (n=24, mean age 44.9 years), type II diabetics with age and sex matched controls (n=41, mean age 61.8 years) and diabetics not treated with vasodilator or statin therapy with age and sex matched controls (n=25, mean age 48.5 years).

	Augmentation Index (%)			Tr (msec)	
Sub-group	Control	DM	DM HR adj'	Control	DM
Type I DM	20.6±2.3	18.2±2.4	21.5±2.4	154.1±3.9	142.7*
Type II DM	30.6±1.6	28.8±1.3	32.4±1.3	142.7±2.2	134±1.8**
No Concurrent Medication	24±2	21.2±2.8	25.2±2.8	145.9±3.5	136.2±2.8*

* $p<0.05$, ** $p<0.01$ DM vs. control.

Recent studies have suggested that AI calculated from analysis of the transformed radial artery pressure wave may show considerable variability [103]Accordingly, we also calculated AI from the carotid arterial pressure pulse. AI was calculated both from the peripheral carotid waveform and from the waveform transformed using a carotid-aortic transfer function. In agreement with data from the radial artery pressure waveform, no difference in augmentation index was seen between diabetic patients and age matched controls in either the peripheral or the transformed central aortic pressure waveforms (Fig. 1 (C & D)). Further analysis of the central arterial pressure waveform revealed that Tr was significantly reduced in the diabetic patients in agreement with data from the transformed radial pressure pulse.

In a multiple regression model for the whole dataset, age, mean arterial pressure, heart rate and diabetes accounted for 73% of the variability in PWV_{cf} (Table. 4). When AI was used as the dependent variable in the same model, diabetes was no longer seen as a significant determinant of variability.

Table 4. Multiple regression analysis for all subjects (n=122). A: PWV as dependent variable (R2 =0.73, p<0.001), B: AI as dependent variable (R2=0.57, p<0.001).

Data entered into model included age, peripheral systolic blood pressure, peripheral diastolic blood pressure, heart rate, diabetes, previous medical history (PMH), family history of cardiovascular disease, sex, smoking status, height, weight, total cholesterol, triglycerides, blood glucose and microalbumin: creatinine ratio.

Variable	Units	β	SE	P
A				
Age	Years	0.07	0.01	<0.001
Heart Rate	Min^{-1}	0.06	0.01	<0.001
SBP	mmHg	0.07	0.01	<0.001
DBP	mmHg	-0.07	0.02	0.003
PMH	Y/N	1.5	0.5	0.005
Diabetes	Y/N	0.9	0.4	0.04
B				
Age	Years	0.4	0.07	<0.001
Heart Rate	Min^{-1}	-0.4	0.08	<0.001
DBP	mmHg	0.4	0.1	0.001
Smoker	Y/N	6.2	2.3	0.009
Sex	M/F	-5.0	2.2	0.02
Diabetes	Y/N	-	-	0.5

7.5 Discussion.

As expected, our study found that PWV_{cf} and pulse pressure were significantly increased in patients with diabetes compared to age-matched non-diabetic controls. Augmentation index however, was little changed between the two groups, in spite of a significant elevation in blood pressure in diabetics and even after adjustment for their increased heart rate. Moreover, this finding was consistent for central pressure waveforms derived from both radial and carotid arterial sites, observed even in the presence and/or absence of antihypertensive therapy and was not dependent on manipulation by a generalized transfer function or diabetes type.

By contrast, analysis of central arterial pressure waveforms from our study demonstrated that time to the foot of the reflected wave (Tr) was significantly reduced in diabetic patients compared to controls, consistent with the observed increase in PWV_{cf}.

An elevated AI in people with diabetes has been reported in previous studies, however our findings do not confirm these observations [158;177;222]. It is of interest, that the AI data derived from our patients with diabetes is very similar to that reported in the aforementioned and in other studies [158;223-226]. Moreover, the published values of AI in people with diabetes differ little from the regression line for age-related AI in non-diabetics, from a large-scale, published study[137]. Thus the major discrepancy between the present study

and some previous studies investigating AI in people with diabetes is not related to differences in the values for AI in the people with diabetes, but rather, to lower values for AI for the control groups when compared to our study. This may reflect the small sample numbers in some of the aforementioned studies. We emphasise that the values for the age related AI in the control population used in this study are similar to those reported in previously published large populations and in our own studies using large control groups (see chapter 3). [133;137;223;227;228].

Other workers have published findings which supports data from the present study. Two separate studies from Hayward's group [224;229] investigated the influence of hormone replacement therapy on AI. One study recruited patients with diabetes and the other an unselected cohort of post-menopausal women recruited from the community. Although not commented on, these studies showed age related AI to be similar or slightly lower in diabetic subjects both at baseline and after treatment with HRT, in spite of higher brachial blood pressures in the diabetic subjects. Interestingly in patients with diabetes, ejection duration was reduced, and dP/dT was increased in agreement with our current findings. In a separate study investigating the time-dependent influence of insulin infusion on aortic AI in type I diabetic men, Westerbacka *et al.* [230]found no difference in AI between diabetics and age-matched controls at baseline, prior to insulin infusion. In this study, differences in AI between controls and diabetics were only apparent during the first 90 minutes following insulin infusion, due to slower responsiveness in the diabetic patients. When the response to insulin was

at its maximum, no difference in AI was seen between controls and diabetics in agreement with data at baseline. Additionally, in a further study [223] comparing diabetic subjects with treated hypertensives, no difference in AI was reported. However, pulse wave velocity was significantly increased between groups.

In a study of 60 type II diabetics, van Dijk *et al.* [231]demonstrated that AI was not independently associated with brachial pulse pressure from 24 hour ambulatory blood pressure monitoring. These workers concluded that brachial artery pulse pressure was determined by proximal aortic stiffness in a way which is not strongly influenced by peripheral pulse reflection. Additionally, Greenfield [228] found little relationship between insulin secretion, insulin resistance, fasting glucose and measured AI in a large study of female twins. In a recent report of the Hoorn study[207], unadjusted AI was not different in people with diabetes when compared to controls, despite clear differences in vascular stiffness, consistent with data from our study. These studies support our finding of a dissociation between PWV and AI in people with diabetes, and question the validity of AI as a useful index of vascular stiffness [94;119;232;233].

In conclusion, arterial stiffness is increased in patients with diabetes as evidenced by increased pulse pressure, decreased Tr and increased pulse wave velocity. However, diabetes was not associated with increased peripheral wave reflections. An interesting question posed by our findings is "why is the AI not increased commensurate with the elevation in arterial

stiffness in people with diabetes?" Our observations suggest that wave reflections must be altered in diabetes because, despite an elevated PWV, there is no increase in AI. We speculate that this might be due to dissipation of the energy of the incident pressure wave in people with diabetes blunting wave reflections. This observation is potentially important and we suggest that further studies to investigate this observation are warranted. Whatever the mechanism, our study demonstrates that diabetes *per se* is another confounding factor in the use of AI as a surrogate of arterial stiffness.

8.0 Discussion

8.1 General overview

Despite the theoretical limitations of conventional brachial blood pressure measurement in the assessment of the cardiovascular system and cardiovascular risk, it has proven itself clinically as a robust and powerful predictor of clinical outcomes. Such is its importance, that the classification of individuals and treatment of hypertension based on brachial artery measurements alone is established, with both national[64] and international guidelines[12] focussed on managing these numerical values.

Much evidence now exists to suggest that this crude assessment of the cardiovascular system may in fact be limited to changes in brachial arterial pressure which can not necessarily be extrapolated to other vessels, and most importantly not to the aorta, the likely culprit vessel responsible for cardiac alterations seen in association with arterial stiffness and hypertension. In fact, many parameters may be under or over estimated when brachial blood pressure measurements are relied on in isolation. As eluded to in earlier chapters, this may go some way to explain why manipulation of brachial pressures alone may not always have the predicted clinical outcome, and why certain anti hypertensive agents may be more efficacious that others in affecting cardiovascular structural change and possibly overall event rates.

In westernised societies at least, there appears to be a level of ageing-associated arterial stiffening that will obviously lead to a deleterious clinical end if sufficient age elapses. Although almost impossible to untangle, whether the association with hypertension is cause or effect, what seems logical is that in either case, continuing functional adaptation to abnormal stress within a vessel will lead to reactive structural change in order to cope with elevation in wall tension and that this may limit normal elastic arterial wall behaviour and beget further structural (mal)adaptive change. The statement of old that "hypertension begets hypertension" still holds true. What we still do not know is whether treatment of functional change will prevent structural abnormalities and whether structural change once established, will prove to be reversible with therapeutic intervention.

An alternative assessment of cardiovascular and specifically arterial function would therefore seem desirable if we are to move treatment forward a level and potentially treat early signs of increased arterial stiffness pre-emptively to prevent subsequent structural change.

Any new technology to assess arterial function must therefore face an arduous challenge. It must be easily applicable in routine clinical assessment, ideally non-invasively, and must provide parameters that are strongly associated with outcome, above and beyond, or at least in addition to conventional brachial blood pressure assessment. It must provide measurements amenable to intervention, or sufficiently robust mechanistic

concepts to allow potential development of novel therapies. Therapeutic intervention must also lead to a favourable outcome! That such a challenger may come in the form of such an old adversary seems somewhat just.

What then has been learned from application of this old but resurgent technology?

Although not quite as simplistic to apply as a sphygmomanometer, pulse wave analysis via applanation tonometry is easy to learn, quick to apply, non-invasive and amenable to use in a wide range of clinical settings. When faced with such a potentially applicable technology, with the promise of much additional data previously only available from invasive techniques, it is easy to be swept away on the wave of evangelism and lose a degree of healthy scepticism. It also becomes tempting to apply this 'new measurement' in a haphazard fashion to many different areas without fully appreciating the nuances of the technique and its limitations. That it should also be applied hurriedly by some, to large ongoing clinical trials in hypertensive patients prior to us gaining personal experience in its use seemed foolhardy.

We decided initially, therefore, to assess the use of PWA on a normal population to further understand the interaction of various demographic and physiological parameters on measured variables generated by the system. This was truly an eye opening experience! Despite a number of groups previously reporting reproducibility and potentially important variables and

interactions, these previous studies were very small and often from investigators also new to the technique. Our assessment of a large cohort of normal controls proved that AI is a composite measurement profoundly influenced by a number of factors, which are difficult to control or adjust for in routine clinical practice. Augmentation of systolic pressure waves results from a complex interaction of force and timing of ventricular ejection, aortic stiffness (whether functional due to distension by elevated blood pressure or structural due to arteriosclerosis) and wave reflections from the periphery at areas of impedance mismatch. This is a far cry from being a robust indirect marker of pulse wave velocity as proposed by some[130;133;134;177].

The direct influence of age itself, blood pressure, heart rate, height, and possibly gender, all make the use of this marker in small, mixed sex studies fraught with difficulty. The fact that the measure also relies on external calibration from brachial blood pressure introduces further scope for significant error[91;95;96;98;102]. When pharmacological therapy is added to the equation and the influence on heart rate (e.g. beta blockade) and the functional alteration in reflection sites by vaso dilatation (e.g. nitrates and ACE inhibition) the concept of this marker assessing arterial stiffness in a meaningful way seems less likely, indeed fanciful.

To add to these problems, there is controversy surrounding the use of a generalised transfer function (GTF) to synthesise an aortic pressure wave from a radially derived pressure trace. Despite forceful claims at validation for the application of a generalised transfer function in providing a

reasonable approximation in all patient groups[86-89;98], much debate has surrounded this area in the literature. Since the validation of this GTF is founded upon a very heterogeneous population referred for diagnostic cardiac catheterisation, application to patients with specific pathological states has been brought into question[95;234]. The potential variation seen in the higher frequencies used in generating a central AI also introduce considerable error to the point of some investigators suggesting that information deduced from peripheral radial traces alone be used as transforming this waveform adds little extra information and a large degree of error[99;102;103]. Although carotid artery applanation yields a waveform more morphologically similar to that from the central aorta due to its proximity, it is still subject to peripheral calibration and transfer function error, unless the untransformed waveform is analysed and assumed similar to the central waveform.

The assessment of central blood pressure parameters seems less open to error, possibly due to it relying on relatively lower frequencies less susceptible to variability[103]. The application of these parameters will be discussed later in this chapter on future clinical trials.

In the assessment of young patients, with both borderline and established uncomplicated hypertension, the use of AI to predict underlying vascular dysfunction proved singularly unrewarding. Despite having markedly differing blood pressures by design, AI was not significantly different between groups. Could this be explained simply be that fact that the young

cohort of patients studied in this thesis did not have stiff arteries? One would postulate that an obvious difference in MAP between the groups would result in greater distension of elastic arteries and at the very least a 'functional' increase in vascular stiffness and therefore PWV. This has in fact been well documented by other groups using alternative techniques. The assumption held by many that AI was a reliable indirect marker of PWV proved to be ill-founded as discussed above, but at this stage we had already incorporated it into a number of pilot studies.

The data emerging from our control dataset, as discussed previously, was showing that this marker was a very composite measure. If not predictive of PWV, was AI going to tell us something therefore about abnormalities in wave reflection in patients with hypertension? Since these patient groups had predominantly systo-diastolic hypertension, an increase in systemic vascular resistance could be inferred and one would therefore expect to see an increase in wave reflection secondary to this. Was this in fact the case and was the resultant increase in heart rate seen in these groups acting to reduce wave reflection and subsequent augmentation? Is an increase in heart rate seen in patients with hypertension an adaptive response? Certainly the decrease in Tr seen in both the borderline and hypertensive groups would be suggestive of both an earlier wave reflection from the periphery and implies a higher PWV in these groups. The possibility of using central blood pressure parameters rather than AI was discussed and this will be revisited later. A weakness of this initial study of hypertensive patients

was failure to incorporate a direct measurement of PWV, for reasons explained earlier.

In terms of reproducibility, then the technique of applanation tonometry to assess both Augmentation Index and pulse wave velocity has proven itself to be reproducible between operators across a wide age range and a spectrum of disease states[127;129;131;132;187]. Despite this finding it is worthy of note that the spread of derived values, especially for AI is extremely large and this must be considered when designing clinical studies relying on this parameter. Although a number of outliers were noted who could not easily be explained, these outliers were few and well within the acceptable limits for a reproducible technique.

In contrast to AI, pulse wave velocity, in addition to proving reproducible appeared reassuringly and linearly related to age. Predictably, it was influenced by distending blood pressure (MAP), but perhaps less so it was seen to be related to body height. Perhaps a longer aorta is able to dissipate more energy than a shorter one and so reduce speed of pressure wave forward flow? Heart rate did not influence PWV in this study. This is intuitively what one would expect across this essentially normal range of heart rates and there is little physiological reason why increasing heart rate should be associated with an increased PWV. Never the less small studies have shown an increase in PWV with increasing heart rates achieved by right ventricular pacing, which is not easily explained. It was postulated that this finding may have been artefactual due to the computer algorhythms

used by certain software to measure PWV. Use of alternative devices and software has however yielded similar results. The effects of heart rate on PWV remain a contentious issue.

Although total cholesterol correlated positively with PWV on univariate analysis, neither it, nor other lipid sub-fractions remained significantly correlated in the final regression model. This is likely to be due to the lack of significantly dyslipidaemic subjects in our 'normal cohort' and may also simply reflect the fact that the study was under powered to assess these parameters. Triglycerides however remained related to PWV in the final model. The spread of Triglyceride values was however quite wide as is often the case with these molecules which are prone to significant dietary variation.

Given the fact that microalbuminuria has been shown to be a potent predictor of vascular disease in diabetic individuals, and also in non-diabetics, the association of PWV and urinary ACR in a normal population free of occult disease is an interesting one. If, as has been postulated, microalbuminuria is the renal manifestation of vascular endothelial damage or even the result of a more generalised common process also responsible for atherosclerotic disease, then the link between PWV as a measure of vascular stiffness and ACR deserves further study.

Our study of patients with diabetes using the technique of applanation tonometry to assess AI and central haemodynamic parameters in addition to

recording carotid to femoral pulse wave velocity was definitive in dismissing the use of AI as surrogate marker of PWV. In this population known to be at high risk for cardiovascular disease and in whom we know clinically to have accelerated vascular stiffness, AI was not significantly increased versus non-diabetic control patients. This was despite both peripheral pulse pressure and measured aortic PWV being significantly increased in the diabetic cohort. This finding was in direct contradiction to previous smaller studies reporting significant increases in AI in diabetic patients cf controls[158;177;222]. Comparison of data from these studies with our own showed remarkably similar AI results for the diabetic patients but results for the control patients were unusually low in the control populations of the studies reporting increased AI in people with diabetes. We have shown excellent reproducibility for measurement of PWA parameters using this technique[127], after studying almost 1,500 patients in our various studies, and we are confident that our data is robust. What remains interesting is that although AI is obviously not reflecting increased pulse wave velocity in these patients, we would expect that in the face of a documented increase in PWV, AI should be significantly increased if wave reflection from the periphery is not fundamentally altered. Since the regulation of the micro circulation is known to be disturbed in diabetic individuals, and this fact may be responsible for many of the micro-vascular complications of diabetes, the findings of an abnormally 'normal' AI hold significant importance. Could the use of AI in diabetic individuals aid in the detection of occult micro-vascular dysfunction?

8.2 General conclusions

The use of Augmentation Index as a marker of vascular stiffness is largely ill founded. This composite measure comprises a number of variables, all of which can independently be manipulated by both physiological and pathophysiological processes. The profound influence of many demographic and physiological factors on this measurement make it usefulness in comparison studies limited without significant adjustment. In contrast to its inability to reflect PWV, it may in fact be demonstrating alterations in peripheral wave reflection and the peripheral circulation that at present are difficult to explain without further study.

It is undoubtedly able to show an ageing relationship along the same lines as PWV without corresponding changes in peripheral blood pressure measurements and by this finding alone may provide some additional information to clinical assessment of patients. Despite being reproducible between operators, it shows a wide spectrum of values across the 'normal' range and this limits somewhat its clinical application. For example, any proposed change in AI for a given intervention would have to be large to detect a significant difference between groups.

Noted finally, but of great importance is the lack of outcome data available for Augmentation Index. In all but a small population of patients with end stage renal failure, AI has not yet been significantly correlated with outcome. Brachial cuff derived blood pressure on the other hand is strongly correlated

with outcome and leads many sceptics of this technique to question its future worth.

For reasons previously discussed, the use of PWA to derive central haemodynamic parameters may prove more reliable. The theoretical benefit of knowing central or aortic blood pressure lies in its potentially closer relationship between LVH and possibly stroke. Alterations in central blood pressure levels despite little change peripherally, and the mechanistic actions of differing anti-hypertensive agents in terms of vasodilatation and peripheral wave reflection make the conceptual benefits of central BP recording great.

For all the above disadvantages of PWA measurements, researchers persist with this method for a number of reasons. Not only is documentation of central blood pressure attractive as outlined above, the ease of application makes this technique highly desirable. Any measurement seeking to replace or add to conventional blood pressure recording in clinical application, and to vast populations with cardiovascular disease, must be easy and quick to perform.

As detailed previously this technique is easy to learn and reproducible between operators with relatively little training. It results in no significant patient distress, often less than that caused by blood pressure measurement used to calibrate the device.

Pulse wave velocity is proving itself to be a reproducible and robust marker of vascular stiffness and is correlated to hard outcome data such as mortality not only in patients with diseases such as hypertension but also in normal ageing. Changes in PWV associated with outcome are small however, and the size of populations studied to enable these changes to reach significance would need to be large. As a measurement, PWV is less influenced by physiological variables than AI although thoughts on the effects of heart rate differ at present and comparisons of groups with significantly different heart rates would need to be interpreted with caution.

Although not as easy to perform as radial artery PWA, PWV is far from difficult to perform, and certainly less cumbersome than some alternative methods of recording this parameter. In normal populations PWV has shown a remarkable linear relationship with age and this has been shown in our study noted above. Interesting associations of PWV with ACR and certain lipid fractions are cause for further large scale clinical studies incorporating this measurement.

What then does the future hold for this new technique and in particular central haemodynamic parameters and PWV? What is clear is that the need for further clinical studies is very apparent. Since these measurements are non-invasive and relatively easy to acquire, incorporation into large clinical trial protocols should not be difficult. If this were to happen then mortality data for indices such as AI would become readily available to enable us to decide if use in clinical practice will ever be justified.

The use of PWV as an independent predictor of outcome in its own right has largely secured this measurement in terms of further study at least.

In terms of central blood pressure measurements then clinical trials are currently in progress to address the issues of both potential use in clinical practice of this technique and also to help provide a mechanistic insight into certain clinical findings regarding the treatment of hypertension.

CAFÉ[80] or the conduit artery endpoint study was designed to assess the use of PWA parameters in patients enrolled to the Anglo Scandinavian Cardiac Outcomes Trial (ASCOT)[121]. This trial aimed to study the effects of both new and old anti hypertensive treatments both singularly and used in combination with the primary outcome being fatal and non-fatal myocardial infarction. Secondary endpoints such as stroke and all-cause mortality were also addressed. This trial has recently been terminated at the recommendation of the Data Safety Monitoring Committee, and we await the results with interest. The CAFÉ study will afford us the unique opportunity to study a predominantly male cohort of at risk hypertensive patients with equivalent peripheral blood pressures between groups. The study also involves a substantial cohort of diabetic individuals which will be of great interest. All cardiovascular risk factors including lipid levels and presence of left ventricular hypertrophy are documented and can be used in correlation. The potential of lowering central aortic blood pressures by various pharmacological agents can therefore be studied in a large population for the first time. The major concern will be of course the

influence of heart rate on wave reflection and systolic wave augmentation, especially in the individual patients treated with Atenolol. Given the fact that beta blockers have proven themselves to reduce outcomes in hypertensive individuals, deleterious effects on central augmentation may prove to be insignificant and the effect of these agents on MAP more important. Whether or not similar reduction in peripheral blood pressure *in addition* to reduced wave reflection and central augmentation with non-rate limiting vasodilators (ACE inhibitors/Calcium channel blockers) will prove especially beneficial, particularly in stroke outcome and reduction of LVH will also be seen.

The use of these techniques and subsequently derived measurements has much to add to the cardiovascular assessment of patients on a conceptual level at least. Whether or not these measurements prove themselves to be clinically useful is a different matter and remains to be seen. However, it is important to stress that although conceptually attractive, no data has been presented in this thesis to justify the inclusion of measurements of PWA or PWV as an adjunct to current standard clinical practice.

In a recent editorial, it was noted, "there is no doubt that this field is now bedevilled by competing forces of evangelism and scepticism. The truth usually lies between the two." In asking the question whether "central pressures derived from pulse wave analysis will provide more useful prognostic information with regard to cardiovascular outcome than conventional blood pressure measurement alone. 'They will' says the

evangelist, 'they will not' says the sceptic". I tend to agree with the author. "I think they might."[233]

9.0 References

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10.0 Appendix

10.1 Leicestershire Research Ethical Committee approval letters

Melanie Sursham
Direct Dial 0116 258 8610

Health for Leicestershire
LEICESTERSHIRE HEALTH
Gwendolen Road, Leicester LE5 4QF
Tel: (0116) 273 1173 Fax: (0116) 258 8577
DX 709470 Leicester 12

16 July 1998

Professor B Williams
Professor of Medicine
Cardiovascular Research Institute
Robert Kilpatrick Clinical Sciences Building
The Leicester Royal Infirmary

Dear Professor Williams

Comparison of Central Arterial Blood Pressure in Diabetic and Non-Diabetic Subjects: Implications for the management of hypertension in Diabetes Mellitus - our ref. no. 5099 1167

Thank you for your letter of 3 July 1998 responding to the questions raised by the Leicestershire Ethics Committee and enclosing an amended patient information sheet in relation to the above study.

You will be pleased to know that the Leicestershire Ethics Committee has now approved your request to undertake the above-mentioned research.

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when ethics committee approval is given.

Yours sincerely

M. Sursham

R F Bing pp.
Chairman
Leicestershire Ethics Committee

(NB All communications relating to Leicestershire Ethics Committee must be sent to the Committee Secretariat at Leicestershire Health)

Melanie Sursham
Direct Dial 0116 2588610

Health for Leicestershire
LEICESTERSHIRE HEALTH
Gwendolen Road, Leicester LE5 4QF
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17 June 1999

Dr S Carr
Consultant Nephrologist
Department of Nephrology
Leicester General Hospital NHS Trust
Gwendolen Road
Leicester
LE5 4PW

Dear Dr Carr

Re: Applanation Tonometry in patients with Chronic Renal Failure
- our Ref no 4994

Thank you for your letter of 23 April 1999.

I have reviewed the information and on behalf of the Leicestershire Research Ethics Committee, give approval to extend the use of the applanation tonometry technique into the group of patients with chronic renal failure and their controls, as an amendment to study reference 4994.

Could you please let me know the number of patients to be recruited for the Committee's information.

Yours sincerely

M. Sursham

R F Bing
Chairman pp.
Leicestershire Ethics Committee
(Signed under delegated authority

(NB All communications relating to Leicestershire Ethics Committee must be sent to the Committee Secretariat at Leicestershire Health)

Melanie Sursham
Direct Dial 0116 258 8610

Health for Leicestershire
LEICESTERSHIRE HEALTH
Gwendolen Road, Leicester LE5 4QF
Tel: (0116) 273 1173 Fax: (0116) 258 8577
DX 709470 Leicester 12

30 October 2000

Please quote ethics ref 5881

Professor B Williams
Professor of Medicine
Clinical Sciences Building
The Leicester Royal Infirmary

Dear Professor Williams

The relationship between accelerated vascular ageing and vascular matrix synthesis in hypertensive patients – our ref no 5881

I have received Dr David O'Brien's letter dated 1 October advising that he wishes to expand the protocol for the above study to include those patients who suffer with diabetes.

On behalf of the Leicestershire Research Ethics Committee, and by Chairman's action, approval is given to this expansion of the Protocol.

Yours sincerely

pp Dwayne Merry

Rev P Harbord
Chairman
Leicestershire Research Ethics Committee
(Signed under delegated authority)

(NB All communications relating to Leicestershire Ethics Committee must be sent to Leicestershire Health)

RFB 418

Leicestershire NHS
Health Authority

Melanie Sursham
Direct Dial 0116 2588610

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DX 709470 Leicester 12

3 January 2002

Please quote ethics ref no 4994

Dr S Carr
Consultant Nephrologist
Leicester General Hospital

Dear Dr Carr

Blood Pressure Variability with Chronic Renal Failure: Is this an important factor in the development of Target Organ Damage – our ref no 4994

I have received a completed and signed Protocol Amendments form dated 17 December 2001 relating to the above study.

I assume this is the follow up to your letter of 16 March 2000 asking to extend this study. (It was originally described as a pilot study).

By Chairman's action and on behalf of the Leicestershire Research Ethics Committee I have reviewed and approved your proposal to recruit 50 patients in addition to the 60 patients originally approved for this pilot study.

Yours sincerely

M Sursham

P G Rabey pp.
Chairman
Leicestershire Research Ethics Committee
(Signed under delegated authority)

(NB All Communications relating to Leicestershire Research Ethics Committee must be sent to the Committee Secretariat at Leicestershire Health Authority. If however, your original application was submitted through a Trust Research & Development Office, then any response or further correspondence must be submitted in the same way)

Leicestershire, Northamptonshire and Rutland Health Authority

Melanie Sursham
Direct Dial: 0116 258 8610

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Tel: 0116 273 1173
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Our ref: ms/ham/219

3May 2002

Please quote ethics reference no. 6567

Dr S Carr
Consultant Nephrologist/Honorary Senior Lecturer
Leicester General Hospital

Dear Dr Carr

Longitudinal follow up of cardiovascular autonomic function in patients with chronic renal failure – our ref no 6567

I am in receipt of your letter dated 19 April 2002 enquiring whether it would be possible to include pulse wave velocity measurements in your longitudinal study as you did in the cross sectional study.

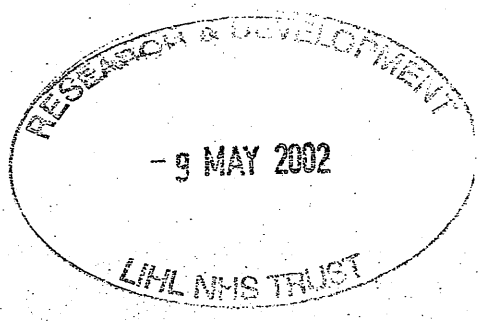
On behalf of the Leicestershire Research Ethics Committee I have reviewed and noted the information and by Chairman's action I approve the inclusion of pulse wave velocity measurements.

Yours sincerely

M Sursham

P G Rabey pp
Chairman
Leicestershire Research Ethics Committee
(Signed under delegated authority)

(NB All Communications relating to Leicestershire Research Ethics Committee must be sent to the Committee Secretariat at Leicestershire, Northamptonshire & Rutland Health Authority. If however, your original application was submitted through a Trust Research & Development Office, then any response or further correspondence must be submitted in the same way.)



10.2 Publications, abstracts and presentations

Publications:

Peter S Lacy*, **David G O'Brien*** et al. Increased pulse wave velocity is not associated with elevated augmentation index in patients with diabetes. *Journal of Hypertension 2004, 22:1937-1944* **(*joint first authors)**

O'Brien DG and Williams B. Assessment of Arterial Compliance in Hypertension in *Current Medical Literature- Nephrology and Hypertension.* Benjamin, Caulfield, Mason and Turner, Eds. 2000

Presentations:

C.Kemp, **D.O'Brien**, B. Williams. Accelerated vascular ageing in young uncomplicated hypertensive subjects. *Hypertension* 2000, Vol 18 (Supp 4). (Poster presentation- International Society of Hypertension, Chicago 2000)

Radial artery pulse wave analysis fails to demonstrate increased vascular stiffness in diabetic patients. **O'Brien DG**, Lacy PS, Stanley AG. & Williams B. Presented at the British Hypertension Society Annual Scientific meeting, Oxford 2001

Renal Function Is A Determinant of Aortic Stiffness in Chronic Kidney Disease (CKD). P.S. Lacy, DG O'Brien, P.P.R. Swales, J. deZoysa, B.A. Fentum, B. Williams and **S.J. Carr**. Presented at the annual meeting of the Renal Association at the Royal College of Physicians on 9th October 2002.

Pulse Wave Velocity but not Radial Applanation Tonometry Demonstrates Arterial Stiffness in Patients with Chronic Kidney Disease. P.S. Lacy, D.G. O'Brien, P.P.R. Swales, B.A. Fentum, B. Williams and **S.J. Carr**. Presented at the annual meeting of the Renal Association at the Royal College of Physicians on 9th October 2002.

Abstracts

Radial artery pulse wave analysis fails to demonstrate increased vascular stiffness in diabetic patients. Lacy PS., O'Brien DG., Stanley AG., Swales PPR., Carr SJ. & Williams B. J *Hypertension* 2002; 20:(suppl 4) s256.

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