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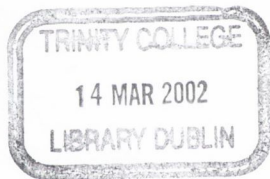
ARTERIAL PULSE IN HEALTH AND DISEASE

Azra Mahmud

A thesis submitted for the degree of doctor of philosophy
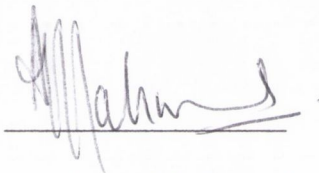
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DECLARATION

I declare that, except where otherwise acknowledged this thesis is entirely my own work and that it has not been submitted previously for a Higher Degree at this or any other university.

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Azra Mahmud

ACKNOWLEDGEMENTS

Embarking on 'Arterial Pulse Wave Analysis' in Ireland was sheer adventure but transformed into great passion and a sense of challenge with time. Only those who have undertaken a project such as this can truly appreciate the magnitude of the commitment of time and energy. After all, tracing the footsteps of giants like Marey, Mahomed and McKenzie was by no means an easy task. But my faith in Almighty Allah kept me going. Many thanks, therefore, go first and foremost to him. The journey to this final moment of submission of my thesis would have been far tougher were it not for the love and support of my family and friends who were always there in my moments of despair.

All authors face a daunting task when thanking valued friends and supporters for inspiration and dedication. Recognizing that I will surely fail to acknowledge all those whose valued guidance sustained this effort and turned a dream into reality, the following individuals merit special mention:

First and foremost, all my thanks go to Professor John Feely, for giving me the wonderful opportunity to undertake this research for a PhD and certainly, the journey has been a most exciting one. I have lost count of instances when I found a photocopy of a relevant paper on arterial stiffness on my desk with "*for Azra*" written on it in a handwriting so familiar and the endless emails of Medline searches I would get from him every day – such was his level of motivation and enthusiasm for this work. The challenging and admirable style of his supervision and leadership are matched only by their exemplary nature. I wish to thank him for his friendship, understanding and kindness, all of which helped me cope with many obstacles and unpleasant situations during the course of this research.

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SUMMARY

Arterial stiffness exerts a major influence on cardiac afterload and coronary perfusion. Indices of arterial stiffness have therefore been proposed as surrogate markers of cardiovascular risk and as a potential therapeutic target. A description of arterial stiffness in both healthy and diseased populations and an understanding of the variables that affect it is an essential prelude to further work in this area. Therefore, I applied the techniques of pulse wave analysis to measure augmentation index (AI%), an index of wave reflection in the ascending aorta with measurement of pulse wave velocity (PWV) in 25% of cases, to 416 individuals (aged 16-86 years, 228 healthy, 188 untreated hypertensive patients). AI% and PWV were correlated (r = 0.62, n=130, p<0.001). In the older hypertensive subjects, heart rate and height and diastolic blood pressure (DBP) and gender in the younger normotensive population were the major determinants. For PWV, age and systolic blood pressure were the principal determinants in both normotensive and hypertensive subjects. Also, PWV was significantly higher in males, while AI% was higher in females. The aortic systolic blood pressure (SBP) was significantly higher in the young males compared to females but was the same in both sexes in the hypertensive group. Pulse pressure (PP) amplification, which was higher in males than females had a stronger inverse relationship to AI% than PWV and was significantly reduced in the hypertensive compared to the healthy subjects. I identified a group of healthy young individuals who had pseudo-systolic hypertension, a state of elastic arteries with markedly increased PP amplification, giving rise to a normal aortic but elevated brachial SBP. These individuals were all male, non-smokers and active sportsmen.

In a double blind randomised crossover study, caffeinated but not decaffeinated coffee increased blood pressure and arterial stiffness and the effects on arterial stiffness were independent of blood pressure (BP) changes. No change in heart rate was noted. The preferential increase in aortic SBP may be of relevance in the light of a recent meta-analysis showing that chronic caffeine may increase SBP and suggests that arterial stiffness is a potential mechanism. I also compared red wine containing alcohol (0.8g/kg) to de-alcoholised wine and observed decreased arterial stiffness only with red wine containing alcohol. This is in contrast to my observation in the population database analysis showing that males with a history of alcohol excess (> 21 units/week)

have evidence of increased arterial stiffness. These findings are analogous to the effects of alcohol on BP, acutely causing a reduction but in the long term increasing it.

My studies on smoking may have important public health ramifications. Healthy young adults compared with non-smoking peers shows evidence of arterial stiffness, but in males only. Exposure to passive smoking in an atmosphere equivalent to a smoky pub was sufficient to cause acute stiffening of the arteries in healthy males but of interest, no such effect was seen in females. I also observed that acutely smoking a cigarette increases BP, heart rate and arterial stiffness. The effect appears to be attenuated in chronic smokers.

Earlier studies and my present work demonstrated that acutely, Glyceryl trinitrate reduces arterial stiffness by decreasing the amount of wave reflection in the ascending aorta. In a group of treated hypertensive men with chronic impotence, I demonstrated that sildenafil citrate, a phosphodiesterase type-5 inhibitor that enhances nitric oxide activity, is associated with a fall in BP and decreased wave reflection in the ascending aorta indicating decreased arterial stiffness. This suggests that the effects of sildenafil extend beyond the penile vasculature.

Finally, I explored the effect of angiotensin II receptor antagonists on arterial stiffness. I compared losartan to hydrochlorthiazide in essential hypertension, both of which provided an equal reduction in BP but only losartan decreased arterial stiffness. In a comparative study of an angiotensin II receptor antagonist, valsartan and the angiotensin-converting enzyme inhibitor, captopril, both significantly reduced BP and arterial stiffness to a similar extent. By combining both therapies, it was possible to show an enhanced degree of reduction in arterial stiffness that was independent of BP reduction suggesting a rationale for using the two agents in combination. To demonstrate the practical usefulness of such a combination in 18 previously poorly controlled hypertensive patients adding an angiotensin II receptor antagonist to their drug regimen including an angiotensin-converting enzyme inhibitor, markedly reduced BP, arterial stiffness and increased PP amplification. These studies clearly show the usefulness of measures of arterial stiffness, which will be employed increasingly as a therapeutic target, for risk stratification, and the choice of the appropriate drug in hypertensive patients and to monitor the response to therapy.

TABLE OF CONTENTS

METHODOLOGICAL CONSIDERATIONS FOR MEASURING ARTERIAL STIFFNESS

THE DETERMINANTS OF ARTERIAL STIFFNESS

Determinants of Arterial Stiffness in the Normotensive and Hypertensive Populations

Pseudo-Systolic Hypertension in Young Men-Elastic Not Stiff Arteries 127

EFFECT OF CAFFEINE AND ALCOHOL ON ARTERIAL STIFFNESS

Acute Effect of Caffeine on Arterial Stiffness and Aortic Pressure Waveform

1. Subjects
2. Blood Pressure Measurements
3. Derivation of Aortic Pressure Waveform
4. Pulse Wave Velocity Measurement
5. Statistical Analysis

1. Acute Effect of Caffeine on Blood Pressure and Heart Rate
2. Acute Effect of Caffeine on Pulse Wave Velocity
3. Acute Effect of Caffeine on Arterial Wave Reflection

MODULATION OF NITRIC OXIDE AND ARTERIAL STIFFNESS-EFFECT OF GLYCERYL TRINITRATE AND SILDENAFIL CITRATE

EFFECT ON ARTERIAL STIFFNESS OF DRUGS THAT MODULATE THE RENIN-ANGIOTENSIN SYSTEM

Favourable Effect on Arterial Wave Reflection and Pulse Pressure Amplification of Adding Angiotensin II
Receptor Blockade in Resistant Hypertension

TABLE OF FIGURES

in the normotensive and hypertensive subjects.

in healthy subjects.

TABLE OF TABLES

Page

PUBLICATIONS FROM THIS THESIS

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1. The Young Investigator Award, 9th European Society of Hypertension Meeting 1999, Milan, Italy.

2. The Young Investigator Award, 10th European Society of Hypertension Meeting 2000, Goteborg, Sweden.

3. Best Poster Presentation " Pseudosystolic Hypertension in young men-elastic not stiff arteries". 3rd European Pulse Wave Analysis Meeting 2001, London, UK.

4. Best Poster Presentation" Smoking stiffens Arteries-as young as 22 years", National Scientific Meeting 2001, RCPI, Dublin, Ireland.

5. The Young Investigator Award, 11th European Society of Hypertension Meeting 2001, Milan, Italy.

LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
AI%	Augmentation index
ARIC	Atherosclerosis risk in communities study
ATII	Angiotensin II
BP	Blood Pressure
CHD	Coronary heart disease
CO	Cardiac output
DBP	Diastolic blood pressure
DVP	Digital volume pulse
e-NOS	Nitric oxide-synthase
GTN	Glyceryl trinitrate
HR	Heart rate
IDDM	Insulin-dependent diabetes mellitus
IP (DVP)	Percent maximal digital volume pulse amplitude
ISH	Isolated systolic hypertension
LVED	Left ventricular ejection duration
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
MI	Myocardial infarction
NIDDM	Non-insulin dependent diabetes mellitus
NO	Nitric oxide
Pd	Minimum diastolic pressure

PDE5	Phosphodiesterase type 5
Pi	Inflection point
PP	Pulse pressure
PR	Peripheral resistance
Ps	Peak systolic pressure
PWV	Pulse Wave Velocity
RAS	Renin-Angiotensin System
SAVE	Survival and Ventricular enlargement study
SBP	Systolic blood pressure
SHEP	Systolic hypertension in the Elderly programme
SOLVD	Studies of Left Ventricular dysfunction
SYS-EUR	Systolic hypertension in Europe study
Tf	Transfer function
ΔTr	Transit time

CHAPTER ONE

INTRODUCTION

1.1. Rediscovery of the Pulse

"Still our old ally, the pulse, ranks the first amongst our guides; no surgeon can despise its counsel, no physician shut his ears to its appeal. Since, then, the information which the pulse holds is of so great importance, and so often consulted, surely it must be to our advantage to appreciate fully all it tells us, and to learn from it every detail that it is capable of imparting ". (Mahomed 1872)

This is how Frederick Akbar Mahomed, a 23-year old student at Guys Hospital in London introduced his first scientific paper on the sphygmograph in 1872. In the absence of a clinically reliable sphygmomanometer, which was not introduced until 1896 by Riva Rocci, he showed that high arterial pressure could be gauged from the examination of the arterial pulse. More precisely, he described how the arterial pressure could be determined from the contour of the palpated radial pulse and from the pressure required to eliminate it. Mahomed correctly recognised the pressure pulse waveform in the hypertensive subject as one with a late systolic peak and with little or no diastolic fluctuation (Mahomed 1874). The most recent development in the study of the arterial pulse is a refinement of sphygmography. Using applanation tonometry, together with computerised methods of analysis, permits accurate derivation of the aortic pressure waveform from non-invasive recording of the radial or carotid artery pressure waves. Utilization of this new technique of sphygmography *'pulse wave analysis'* opens an exciting era to the oldest of all clinical examination that of the arterial pulse.

Cardiovascular disease is the leading cause of mortality in most industrialized populations and over 40% of middle aged and older Irish people have hypertension (Cardiovascular Health Strategy, 1999). Large artery pathology is a major contributor to cardiovascular disease morbidity and mortality. Given the insidious nature of most of the cardiovascular risk factors and the atherosclerotic process, early detection of both functional and structural arterial changes may help identify patients who are at high risk of clinical complications. The public health benefit of recognizing such early arterial damage and possibly preventing progression to established cardiovascular disease is considerable.

1

Historically, large arteries were considered simply as passive conduit vessels. More recently, several studies have shown that large arteries constitute a complex and fully functional organ, serving not only as conduits, performing endocrine and paracrine functions but much more importantly, acting as cushions, buffering the pulsatile flow of blood. The latter mechanical properties of large arteries are an important determinant of circulatory physiology both in health and disease. Elastic large arteries absorb energy during the systolic complement of pulsatile flow and thereby reduce the cardiac work for a given amount of cardiac output. The continuous pulsatile stresses to which large arteries are exposed may break down the elastic element in the vessel wall producing dilatation and stiffening of the wall. According to the law of Laplace, dilatation worsens the pulsatile stress on the arterial wall even if pressure is unchanged. This may lead to vascular smooth muscle cell hypertrophy; increased collagen production and elastic tissue breakdown and so further stiffening of the normally compliant arterial wall. Many risk factors can accelerate this vicious cycle of conduit vessel stiffening and importantly pharmacological interventions vary in their ability to terminate, attenuate or in some cases exacerbate this cycle. Despite advances in other areas, modern clinical practice has not done justice to the arterial pulse and the new methods, which have been developed to measure, analyse and interpret the pulse, have not been widely used. The aim of this chapter is to trace the developments in this field and to consider how clinically important information can be gleaned from studies of the arterial pulse.

The history of the arterial pulse is the history of medicine, of its art and of its science. Scientific understanding of the pulse and its application to medicine has at all times been dependent on the knowledge of theory and of physiological mechanisms and the availability of methods for its measurement. In ancient times the examination of the arterial pulse was an essential part of Chinese, Indian and Greek medicine. The art of medicine, however, dominated the science.

O'Rourke et al. (1992b) in their text on the 'The arterial pulse' and Naqvi and Blaufox (1998) in 'Blood Pressure Measurement – An Illustrated History', have reviewed the history of the pulse and blood pressure measurement extensively and only a brief synopsis is given here. Most of the Figures for the Introduction and Methods Section have been

taken from *Theoretical experimental and clinical principles by* Nichols & O'Rourke (1998).

1.1.1 Chinese Medicine: The Chinese medical literature contains numerous references to the practice of feeling the pulse as an important part of clinical examination. One of the earliest and best-known Chinese medical texts is *Huang Ti Nei Ching Su Wen*, the Yellow Emperor's Classic of Internal Medicine.

' Too much salt hardens the pulse'

The Yellow Emperor may have in as early as 2697 BC recognized the role of excess salt in hypertension and suggested the ability to recognize these changes by examining the pulse *(Figure 1.1).*

1.1.2 The Egyptians: In 2000 BC, the priests of Sekhmet, physicians to the ruling Pharaoh, appeared to have special knowledge of the examination of the pulse. An inscription on one of the stones in a priest's tomb reads:

' *Powerful and clever in his art, who lays his hand on the sick and knows'*

The Egyptian physicians undoubtedly recognized that the heart beats, and the pulse was accelerated in physical exertion, fear and fever and they could easily determine whether it was slow or fast'.

1.1.3 Indian Civilization: Dominant personalities such as the Hindu physician Susruta, gained a huge following and substantial wealth from the pronouncements made after palpation of the pulse.   Examination of the pulse took the form of a mystic rite.   The physician offered the diagnosis and prognosis without any examination at all.   It was generally believed that a good physician could diagnose a disease and its severity, could detect pregnancy and even determine the sex of an unborn child from the study of the arterial pulse.

Figure 1.1. *' Too much salt hardens the pulse'* from **Huang Ti Nei Ching Su Wen, the Yellow Emperor's Classic of Internal Medicine who may have discovered the role of excess salt in causing stiffness of arteries more than 2000 years ago.**

Figure 1.2. *"An anatomical essay on the movement of the heart and blood in animals"* **in which William Harvey proved the circulation of blood.**

1.1.4 Greek Civilization: Hippocrates and other ancient Greek physicians introduced some order to the 'mythology' of the pulse with scientific ideas. Their approach was essentially practical and clinical and in the 4th century BC, they recognised and named various pulses and their features. In 460 BC Hippocrates devoted a whole book '*De Corde*' to the clinical aspects of the heart. He thought that since the arteries were empty at death, they contained air and not blood. He believed that the arteries contracted intrinsically and furthermore, did not describe any relationship between the arteries and the heart. The arterial pulse itself, however, is not mentioned in his writings. In 400 BC, Praxagoras, a contemporary of Hippocrates, introduced the art of *sphygmopalpation* – (Greek sphygmos ' pulse'; Latin palpare ' feel') and may well be the first Western physician to write on this subject. One of his students was the Alexandrian Hirophilus (335-280 BC) who classified variations in the pulse and assigned them different names. There is persuasive historical evidence that he was the first person to count the pulse using the primitive water clock known as *'calypsidera'*.

Another famous Alexandrian, deservedly called the 'father of physiology' is Erasistratus. He observed that when the heart contracted the arteries dilated, and the opposite occurred during the next cycle. This led him to conclude that the arteries did not contract intrinsically which was the standard teaching up to that time. He is also the first physiologist to describe the idea of a finite pulse wave velocity. Unfortunately, in his view, the arteries contained air and veins blood.

Galen, born in 131 AD, was the most influential of the ancient Greeks who wrote on the pulse. His voluminous writings dominated the practice of medicine for almost 15 centuries right up to the time that William Harvey in 1628 described the circulation of blood. Galen noticed a relationship between the heart, arteries and veins and pointed out that the arteries during life-contained blood, not air. However, he still believed that the arteries dilated actively and therefore generated a pulse through their own innate action. In contrast to other great teachers Galen had no important protégés. His influence came from his written work rather than from his oral teaching. One suspects that his contemporaries could not understand him. It is not necessary to follow Galen in the enumeration of names of a variety of pulses. His account of them is characterised by

extreme verbal subtlety and one cannot but wonder that his terms furnished matter for inexhaustible discussions by his followers who were so entangled for over 14 centuries. Much of the Galenic approach still remains when we use terms as "dicrotic", "*anacrotic*", "*bisferiens*" to mystify our students and ourselves.

1.1.5 The Middle Ages: Avicenna, an Arab physician who travelled widely across Europe (980-1037 AD) is considered by many to be the successor of Galen and his teachings were influential for over 500 years. Like Galen he devoted a large portion of his work to the study of the pulse. In *A Treatise on the Canon of Avicenna* he writes:

'The pulse is a movement in the heart and arteries... which takes the form of alternate expansion and contraction'.

He gives two good reasons why the wrist was an ideal place to examine the arterial pulse: it is easily available and the patient need not be distressed at the exposure of the body. Avicenna comprehensively covers the subject of the pulse and records the effects of environment, food, drink, age and exercise. The other physicians at that time who contributed to the study of the pulse are Yuhanna Bin Masawayh (777-857), Rahazes (865-923/932) and Maimonides (1135-1208).

1.1.6 The Renaissance: By the time of the Arab decline in the middle of the thirteenth century many Arabic texts had already been translated into Latin and other European languages and this process laid the foundation of work done during the Renaissance, particularly in the emerging Universities of Cordoba and Palermo. Arab teachings on the pulse became the standard reference, many of which have survived into the present day. In 1553 Miguel Serato was the first to describe the pulmonary circulation. Joseph Struthius in his most important work, *Art Sphygmica*, records what is considered to be the earliest graphic presentation of the pulse, published in 1555 in Basel:

.........if you place adjacent to the arteries upon the outside of the skin some leaf, or membrane, or linen or small scarp of cloth or some other similar object. You will see whatever you have placed on top, moved and be raised together with the artery below it.

By using this rudimentary but revolutionary method, Struthius laid the foundation of a remarkable diagnostic tool, the sphygmograph, which was destined to achieve practically universal application.

1.1.7 Move Away From Galen - The Modern Cardiovascular Era:

The modern era in cardiovascular medicine began with the publication in 1628, of William Harvey's classic monograph *"An anatomical essay on the movement of the heart and blood in animals"* in which he described the circulation of the blood *(Figure 1.2)*. Harvey described how cardiac systole caused passive distension of the systemic arteries whence blood was 'continuously, evenly and uninterruptedly driven by the beat of the heart into every chamber and part'. Harvey likened the arterial system to a distended bladder, which attenuated the pulsations while distributing blood to the peripheral organs through vascular conduits. Harvey, while Anatomist to the Royal College of Physicians of London, addressed his book to the President Dr. Argent and to Fellows. The crest of the Royal College of Physicians of London and also that of Ireland features palpation of the arterial pulse *(Figure 1.3)*. Harvey's work established the pulse as a manifestation of cardiac ejection and modified by vascular properties, and the pulse in disease as a consequence of abnormalities in the function of the heart and blood vessels. He also described the effects of wave reflection on the arterial pulse. Harvey set the scene for the improvement in the physiological knowledge of the mechanism of the arterial pulse and for its clinical application.

The Reverend Stephen Hales (1677-1761) recorded arterial pressure in a horse from the height of the blood column *(Figure 1.4)*, determined the response of arterial pressure to blood loss, formulated the concept of peripheral resistance and showed that the greatest resistance to flow resides in the tiny blood vessels that are not visible to the naked eye. Hales likened the elastic arterial system to the air filled chamber of the contemporary fire engine, which converted pulsatile flow at its input to a steady stream from the fire hose nozzle. German writers subsequently translated this as *"Windkessel"* a term, which endures till today to describe the cushioning function of arteries *(Figure 1.5)*. Hales believed that the pulse was a consequence of cardiac ejection into conduit vessels upstream from the major resistance to flow. However, Hales conceptual model of windkessel and peripheral resistance is now considered unduly simplistic because it does not consider the effects of wave travel and reflection.

Figure 1.3. Earliest crest of the Royal College of Physicians of Ireland
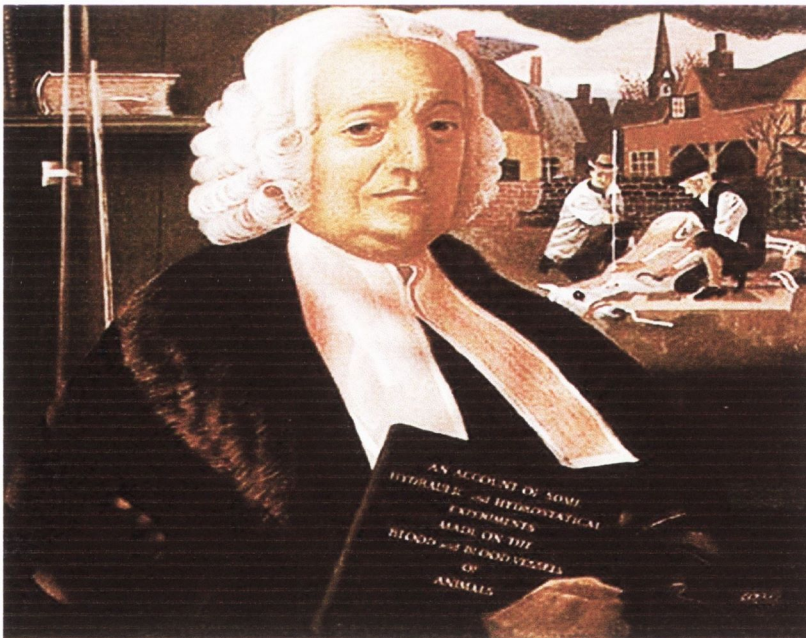
Figure 1.4. The first measurement of arterial pressure from the height of the blood column in the horse by Reverend Stephen Hales in 1769.

WINDKESSEL
[CUSHION]
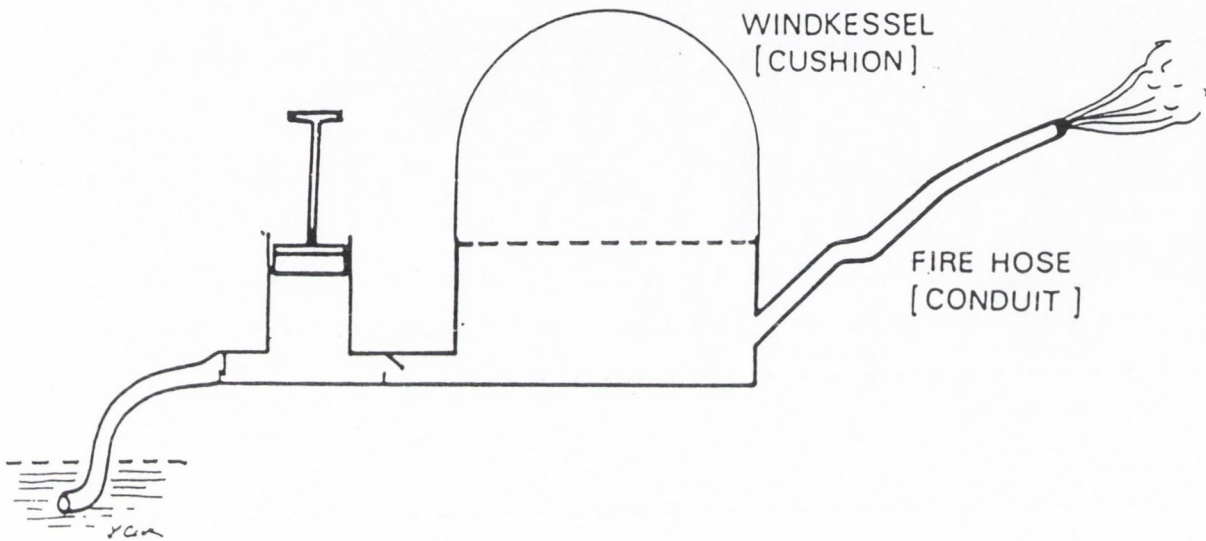
FIRE HOSE
[CONDUIT]

Figure 1.5. The arterial system was likened by Steven Hales (1769) to the contemporary fire engine whose air-filled dome or '*Windkessel*' acted as cushion, and whose fire hose acted as conduit. The Windkessel smoothed out the intermittent spurts from the pump so that water was delivered through the hose in a steady stream. The Windkessel represents arterial distensibility, the fire hose the distributing arteries and the nozzle the peripheral resistance.

During the 18th and 19th centuries the theories of fluid and blood flow and of wave transmission and reflection were established. This was principally done by physician/scientist such as J Poiseuille, EH Weber and Thomas Young. Therefore the application of physical principles to physiological phenomena was more readily made. In 1828 Poiseuille measured arterial pressure by substituting a mercury column for one of blood or saline and established that there is no significant difference in mean arterial pressure between central and peripheral arteries. Poiseuille noted fluctuations in pressure with each beat of the heart but his equipment had such high inertia that pulse contour could not be determined with any accuracy at all.

1.1.8 Dawn of Spygmography: Étienne Jules Marey (1830-1904) was the first to record accurately the arterial pulse in man. Marey developed a series of sphygomagraphs for this purpose and applied these to physiological as well as to clinical studies. His technique for pulse wave recording in the study of human disease were improved and extended principally in England by Mahomed, Broadbent, McKenzie and others. The sphygmograph was introduced into clinical medicine at an opportune time. Richard Bright (1827) had drawn attention to the effects of kidney disease on arterial pressure, cardiac hypertrophy and the risk of stroke. In the absence of a clinically reliable sphygmomanometer, which was not introduced until 1896 by Riva Rocci and Korotkov in 1905, high arterial pressure was gauged from examination of the arterial pulse but lacked reliable quantification. Mahomed *(Figure 1.6)* described how the arterial pressure could be determined from the pressure required to eliminate the radial pulse and also from its contour. Mahomed correctly recognised the pressure pulse in the hypertensive subject as one with a late systolic peak and with little or no diastolic fluctuation *(Figure 1.7)* by using a sphygmograph *(Figure 1.8)*. He was able to detect this by palpation and confirmed his observations by using his first sphygmographic records of typical radial pulse contours in hypertension and ageing, which were confirmed over 100 years later. Mahomed described essential hypertension and chartered its clinical course with uncanny accuracy on the basis of his diagnosis from the arterial pulse in 1874:

Figure 1.7. Frederick Akber Mahomed (1874)
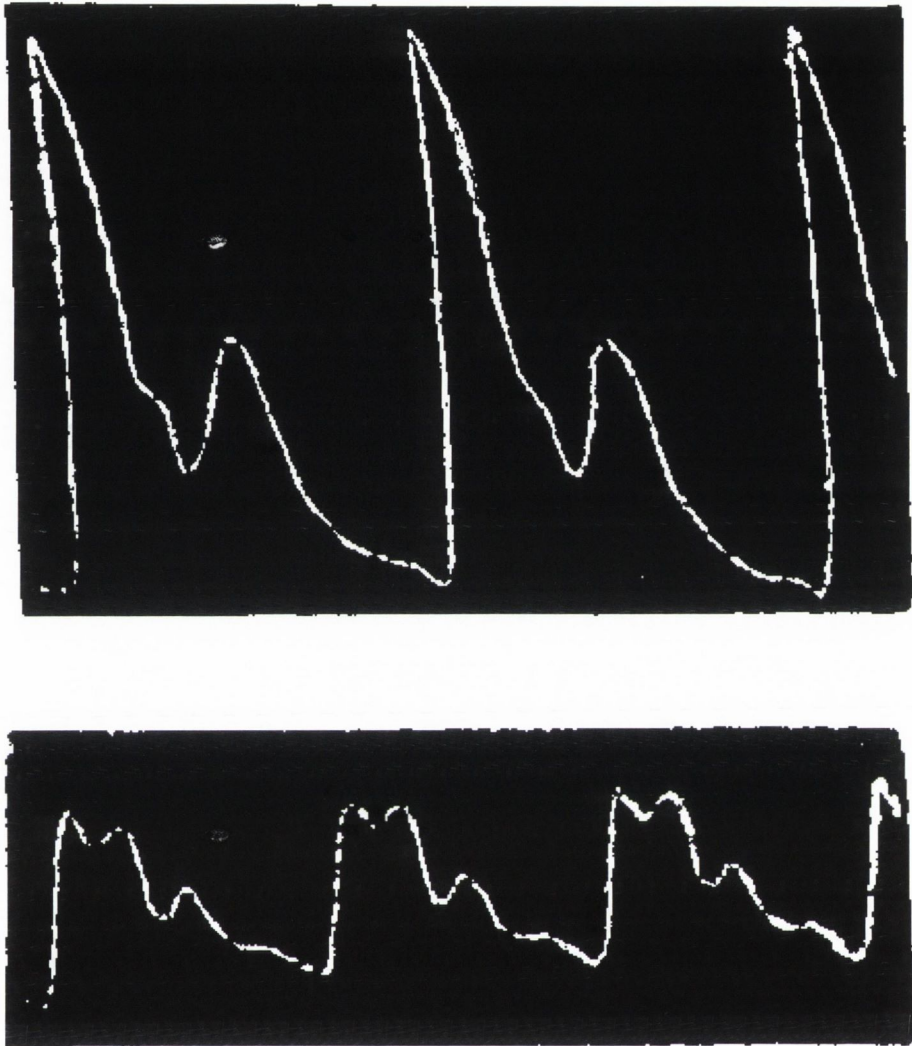
Figure 1.7. Radial artery tracings from a norma
subject (top) and a hypertensive subject (bottom)
Mahomed (1874) correctly recognized the hypertensiv
subject as one with a late systolic peak and little or n
diastolic fluctuation and clearly distinct form a tracin
taken from a normal subject.

"These persons appear to pass through life pretty much as others do and generally do not suffer from their high blood pressure except in their petty ailments upon which it imprints itself. As age advances, the enemy gains accession of strength. The individual has now passed 40 years perhaps 50 years of age. His lungs begin to degenerate. He has a cough in the wintertime but by his pulse you will know him. Alternatively, headache, vertigo, epileptic seizure, passing paralysis and more severe epileptic seizure and then the final blow".

This work clearly described a deterioration of asymptomatic individuals over years with the development of left ventricular failure complicated later by transient ischaemic episodes and then by a fully developed cerebrovascular accident and death from either this or heart failure.

Referring to the aetiology Mahomed writes:

"What has been the cause in one case may be the result in another; thus general disorder may cause high arterial pressure and this in turn kidney changes; While on the other hand kidney changes may be primary and acute, and they may in their turn produce impurity of blood and this general pressure. But whether we read the tale backward or forward, it is the same tale in the end".

Here he describes essential hypertension as distinct from chronic glomerulonephritis but when it has progressed to renal failure, no clear distinction could be made as to the cause.

By the end of the 19th century examination of the pulse had clearly reached a high level of refinement and different types of pulses were recorded with a variety of sphygomographs. Three major books were written during this period, by Marey (1881), Broadbent (1890) and by Sir James McKenzie (1902). These books were all written when the sphygmograph was the only aid to the examination of the cardiovascular system available. There was no x-ray, no electrocardiogram, and no sphygmomanometer available, even in hospitals. Although modern practice has doubtlessly been improved by the introduction of these new aids, it seems certain that the average physician's ability to feel and to interpret the pulse has declined.

1.1.9 Decline of Sphygmography: The introduction and acceptance of the sphygmomanometer following the work of Riva Rocci in 1896 and Korotkov in 1905 led to the disappearance of sphygmograph from clinical practice. It also led to a simplistic approach to arterial function regarding systolic and diastolic blood pressure as a sole guide to cardiac action and vascular response. In retrospect, we can now appreciate with regret that the clinical acceptance of the sphygmomanometer in the early 20th century marked the demise of the sphygmograph at least for another 80 years.

In the first half of the 20th century, the major advance in arterial haemodynamics was the development of accurate monometers. Optical monometers were introduced by Frank in Germany and later refined by Wiggers and then by Hamilton in the United States. These monometers and introduction of catheters that could be placed into the heart chambers allowed pressure waves to be recorded with great accuracy. The introduction of diagnostic cardiac catheterisation in man by Cournanad and Rangers in 1944 has subsequently led to the extensive use of invasive monitoring of the arterial pressure waves in operating theatres and critical care areas of hospitals. However even here it seems that the new technology has not been exploited adequately. Heart rates are being recorded with considerable accuracy with emphasis on systolic, diastolic and the mean pressures but scant attention is being paid to the arterial pressure wave contour. *'High tech' recording has become linked to 'low tech' interpretation.*

The interpretation of pulsatile phenomena in arteries has been greatly aided by the steady state approach proposed and popularised by McDonald in 1960 and later by his followers.
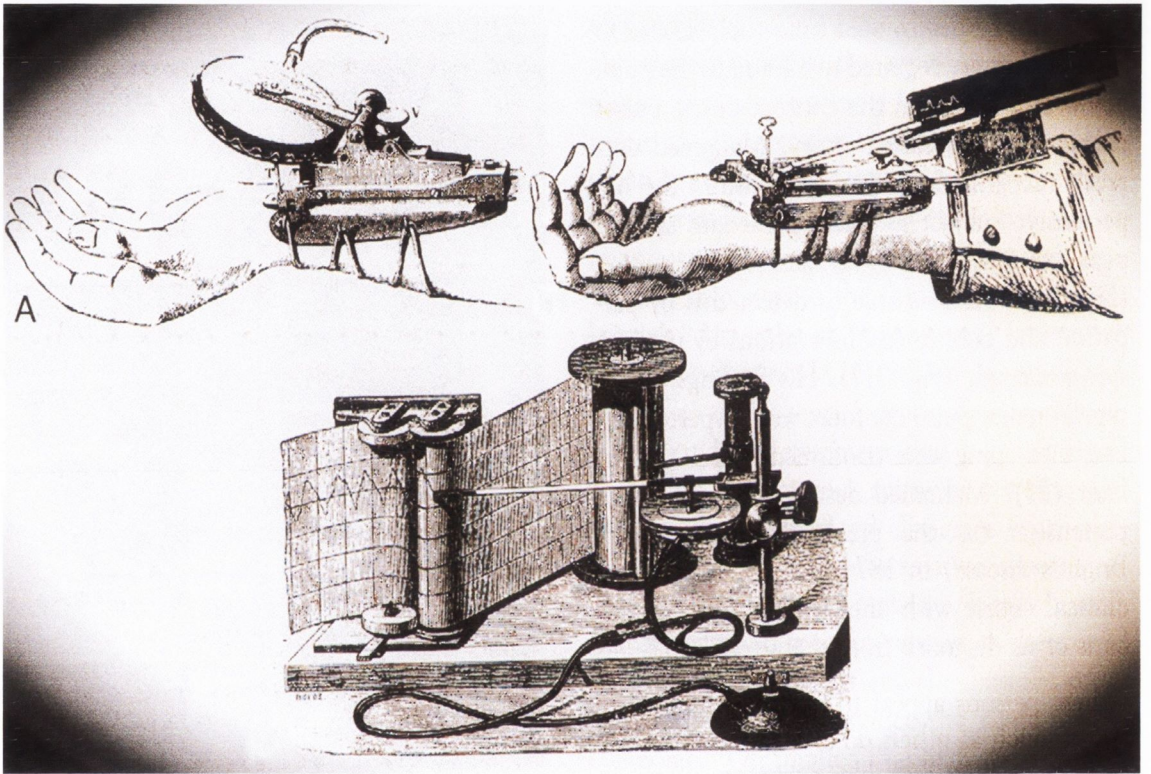
Figure 1.8. Sphygomographs developed by E. Marey. A) Air-filled system with base-plate overlying artery connected to transducer. B) Mechanical sphygmograph without air-filled tubing, which writes directly onto smoked paper. C) Recording system with lever directly over smoked paper passing over a drum that is driven by a clockwork mechanism.

McDonald and Taylor and others showed how harmonic analysis of arterial waves could be used to describe pulsatile pressure-flow relationships by vascular impedance and how impedance plots can be interpreted in terms of the properties of the vascular bed downstream. Extension of this work enabled the compound pressure and flow wave in an artery to be decomposed into forward and backward travelling waves. This allowed the sites of wave reflection, the timing of reflected waves and the intensity of the reflection to be determined with some accuracy. The recent development in the study of the pulse is the refinement of non-invasive methods, for example, Doppler ultrasonic techniques for measuring arterial flow, Phase logged echo tracking systems for measurement of arterial diameter and introduction of applanation tonometry for registration of the arterial pressure wave permitting the accurate registration of pressure flow and diameter pulses from transducers applied gently over the skin. Utilisation of these new techniques of measurement together with the application of the sophisticated principles of analysis opens endless possibilities in the oldest of all clinical examination, that of the arterial pulse. The history of the pulse like history in general should teach the lesson that new information must add to the old and not demean it.

' Science like life feeds on its own decay. New facts burn old rules; then developed concepts bind old and new together into a reconciling law' (James 1896).

1.2   Principles of Arterial Haemodynamics

Like a good friend the arterial system is usually taken for granted. Fault is only found when an organ and tissues suffers as a consequence of narrowing of one or more of its peripheral branches. Little attention is paid as to how changes in arterial properties may alter the function of the heart itself. Indeed, most studies of left ventricular load and its alteration by disease or by drugs focus on the tiny peripheral resistance vessels - the arterioles - and pay no attention at all to the large arteries that distribute blood to the arterioles and which cushion the pulsatile flow from the heart.

The function of the circulation is to supply nutrients to, and to extract waste product from the tissue of the body. This is achieved by slow, steady and non-pulsatile flow through a vast capillary network in the organs and tissues of the body. The force of such flow is

16

generated by the left ventricle of the heart, which contracts and relaxes rhythmically dispersing blood at high velocity into the ascending aorta. However, the arteries have another important function, which is to convert pulsatile flow of blood that results from intermittent ventricular ejection, so that capillary flow may be continuous and therefore most efficient.

The form of the arterial pulse is dependent upon the pattern of ventricular contraction and on the properties of the arterial system and arterioles. In order to understand the arterial pulse it is necessary to consider some of the fundamentals of arterial haemodynamics including the concepts of wave travel and reflection. Wave reflection is important because it is responsible for secondary fluctuations in the pulse, as the heart beats but once with each cycle, secondary waves must have such an explanation (Snellen 1980).

The arterial pulse as a diagnostic tool has attracted people for several thousand years. It is generally agreed that a high percentage of cardiovascular disorders is associated with increased rigidity of the arterial wall due to arterial sclerosis. Mahomed as far back as 1874 (Mahomed 1874) described the changes in arterial pressure pulse in a hypertensive subject as distinct from the arterial pressure pulse of a healthy person; but he could not measure it in numbers. The recent developments of non-invasive methods such as Doppler ultrasound techniques, pressure transducers and applanation tonometry to record arterial flow and pressure waves respectively, together with new techniques of analysis and automatic and computerised calculations opens up a new dimension to the clinical application of the analysis of the contour, amplitude and velocity of the pressure pulse.

1.3   The Arterial Pulse

Arterial pulse wave is any periodic fluctuation caused by the heart and occurs at the same frequency as the heart beat. Ejection of blood with every cardiac contraction is converted to flow pulsations, pressure pulsations and diameter pulsations in arteries throughout the body (Remington and Wood 1956; Snellen 1980). All of these can be described as the pulse, however clinicians refer to the pulse only as the arterial pressure pulse, which can be palpitated in large accessible arteries.   To understand arterial haemodynamics, flow and diameter pulses are described briefly and pressure pulsations, which have been used to measure pulse wave velocity (PWV) and arterial wave reflection in this thesis, explained in greater detail.

1.3.1 Diameter Waves: The contours of the pressure and diameter pulses appear almost identical (Nichols and O'Rourke 1998b; O'Rourke et al. 1992b; McDonald 1960) however; the fine differences can be demonstrated when pressure and diameter are plotted against each other. Then the plotted line is not a straight dichotomous line, but assumes the form of a curvilinear clockwise hysteretic loop *(Figure 1.9).* The *curvilinearity* is attributed to the non-linear elastic properties of the arterial wall, whereas the hysteresis (caused by lagging of diameter) is attributed to the viscosity of the arterial wall. The *hysteresis* loop provides important information regarding the mechanisms involved in alteration of elastic properties of the arterial wall (Stefanadis et al. 1995a; Stefanadis et al. 1997a; Stefanadis et al. 1998a). For the same modification of distension pressure the large elastic arteries present a greater modification of diameter than the smaller muscular arteries (Snellen 1980).   The amplitude of the diameter fluctuation is quite small relative to the mean diameter of the artery and sophisticated techniques are required to measure it (Stefanadis et al. 1995a; Stefanadis et al. 1997a; Stefanadis et al. 1998a; Hoeks et al. 1990).

1.3.2 Flow Waves: The flow pulsation in an artery is the longitudinal movement of blood that occurs with each beat of the heart. Flow in an artery cannot be appreciated by inspection but can be measured by electromagnetic flow meters applied around the artery or inserted into the artery incorporated on the tip of a catheter; it can be measured non-invasively by a Doppler ultrasonic probe (Remington and Wood 1956; Snellen 1980). The contour of the flow wave in the ascending aorta has a triangular shape during systole with

no flow in diastole. In other arteries, secondary fluctuations are superimposed on the flow wave as a result of wave reflection from the upper and lower part of the body. The amplitude of the flow fluctuation in greatest in the large arteries close to the heart (300% or more of mean flow) but attenuated in small peripheral arteries due to the cushioning effect on arterial pulsation and a progressive increase in cross-sectional area of the small arteries in relation to the proximal aorta *(Figure 1.10)*.

1.3.3 Pressure waves: The contraction of the left ventricular myocardium and the ejection of blood into the ascending aorta generate a pressure wave, which will travel along the arterial wall throughout the body. Due to amplification, the pulsatile fluctuation in the peripheral arteries reaches a level of 40% to 80% of the mean arterial pressure (MAP) inside the artery (O'Rourke and Kelly 1993). This is considerably less than the pulsatile fluctuation of 300% or more of mean flow especially in the ascending aorta, but more than the tiny fluctuation in the diameter, (5-10%) in a large artery with each heart beat *(Figure 1.10)*.

1.3.4 Properties of the pressure wave: Pressure waves can be recorded invasively by pressure catheter or a non-invasively by an external transducer. Several non-invasive methods have been used for pulse recording including external volume capsule, piezo-electric transducers, plethysmography, tonometers etc. each having some specific limitations and advantages. The pressure waveform changes both in amplitude and contour as it travels from the aorta to the peripheral arteries *(Figure 1.10)*. The advantage of the non-invasive pressure transducers is their ability to record pressure waves at different arterial sites (Kelly et al. 1989b; Kelly et al. 1989a) and their main disadvantage is the need to validate against intra-arterial recordings. Understanding of the mechanisms that contribute to the genesis of the normal pulse facilitates the understanding of mechanisms involved in the formation of the abnormal pulse. This in turn leads to a keen approach into the pathophysiology of disease states.
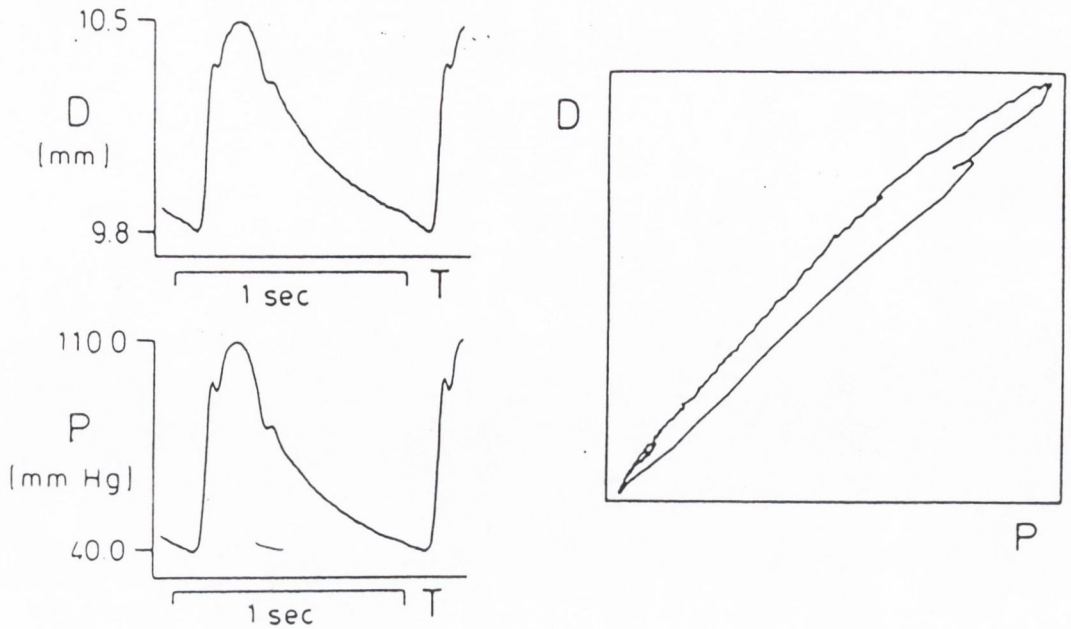
Figure 1.9. Diameter (above) and pressure (below) waves recorded simultaneously from the common carotid artery of a human subject. The figure at right shows instantaneous diameter (ordinate) plotted against instantaneous pressure (abscissa), the hysteresis loop.
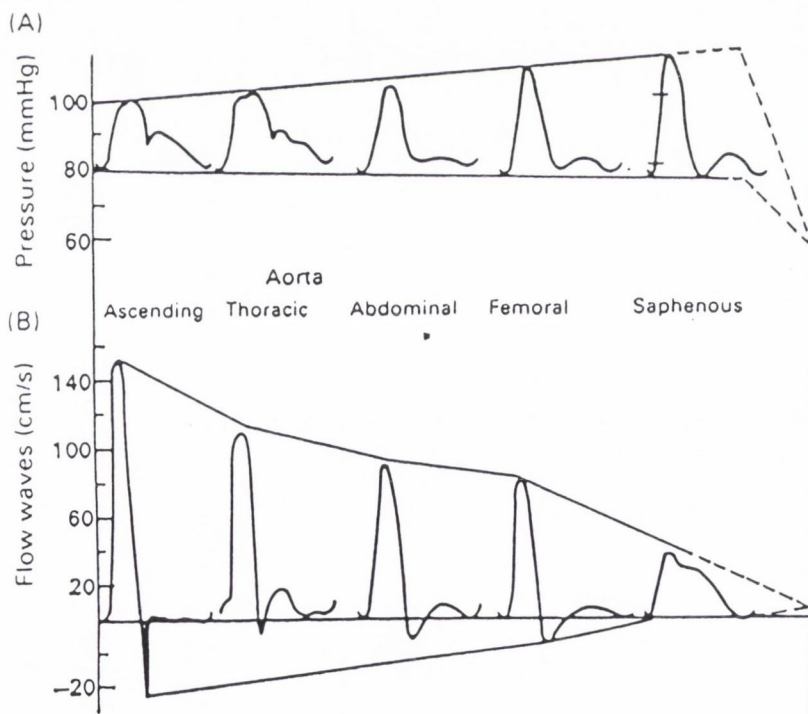
Figure 1.10. Diagrammatic representation of change in (A) pressure and (B) flow waves between the ascending aorta and peripheral arteries. There is a progressive rise in amplitude of the pressure pulse and decrease in the amplitude of the flow pulse.

1.4   Factors Determining Arterial Pulse Wave Contour

Patterns of flow, pressure and diameter pulsations show a considerable variation, not necessarily to the same degree. There are several rules that apply in the description of these variable forms and several mechanisms that explain when and why this variability occurs.

Rules of description:

- Pattern of flow in the ascending aorta is remarkably similar at different ages, in different physiologic circumstances, and in different animal types (Remington and Wood 1956) (Snellen 1980) *(Figure 1.11)*.

- On the contrary, pressure pulsation in an artery differs considerably with age, under different physiologic conditions and in corresponding arteries of different animals (O'Rourke 1982; Remington and Wood 1956; Snellen 1980) *(Figure 1.11)*.

- Both flow and pressure pulsations show different patterns in different arteries of the same subject (O'Rourke 1982) (Remington and Wood 1956; Snellen 1980) *(Figure 1.10)*.

- Diameter pulsations are almost identical to pressure pulsations as they are related with a simple cause and effect relationship (Stefanadis et al. 1995a; Stefanadis et al. 1997a; Stefanadis et al. 1998a) *(Figure 1.9)*.

- Pulsations arrive progressively later at more peripheral sites with the delay depending on the distance from the heart and on PWV *(Figure 1.12)*.

Mechanisms:

- Flow in the ascending aorta is mainly dependent on the pattern of ventricular contraction. However, pressure in the ascending aorta is dependent on the pattern of ventricular contraction and on the properties of the arterial system that determine wave travel and reflection.

- Different flow patterns in different arteries of the same subject are explained on the basis of wave reflection.

21
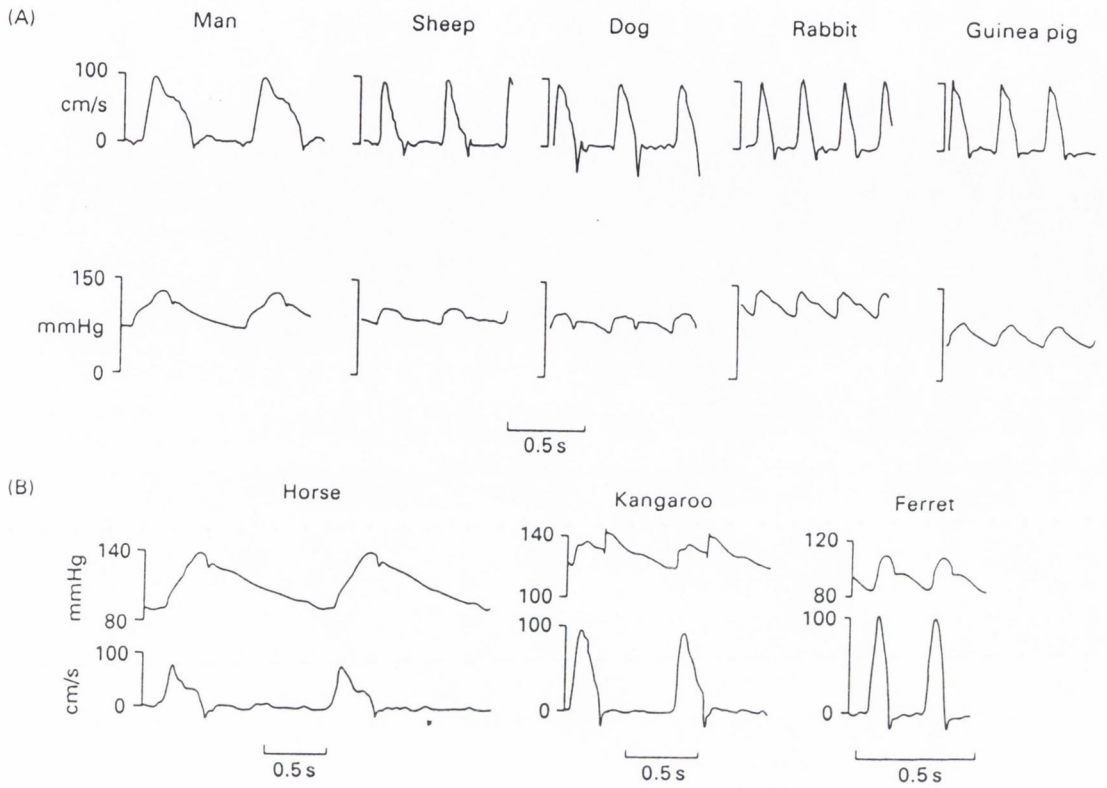
Figure 1.11. Ascending aortic flow and pressure waves in a human being and in a variety of animals. In (A) flow is above and pressure is below; in (B) tracings are inversely arranged.

- PWV is principally determined by the elasticity of arteries; pulse travels faster through stiffer arteries and vice versa.
- Different pressure patterns in different arteries in the same subject result from:
  1. Attenuation of the wave during travel because of the visco-elastic properties of the arterial wall and the viscosity of the blood within.
  2. Dispersion of the wave due to different frequency components travelling at different velocities.
  3. Wave reflection.
  4. Amplification of the pulse in the peripheral arteries as a consequence of their greater stiffness compared with the proximal aorta.
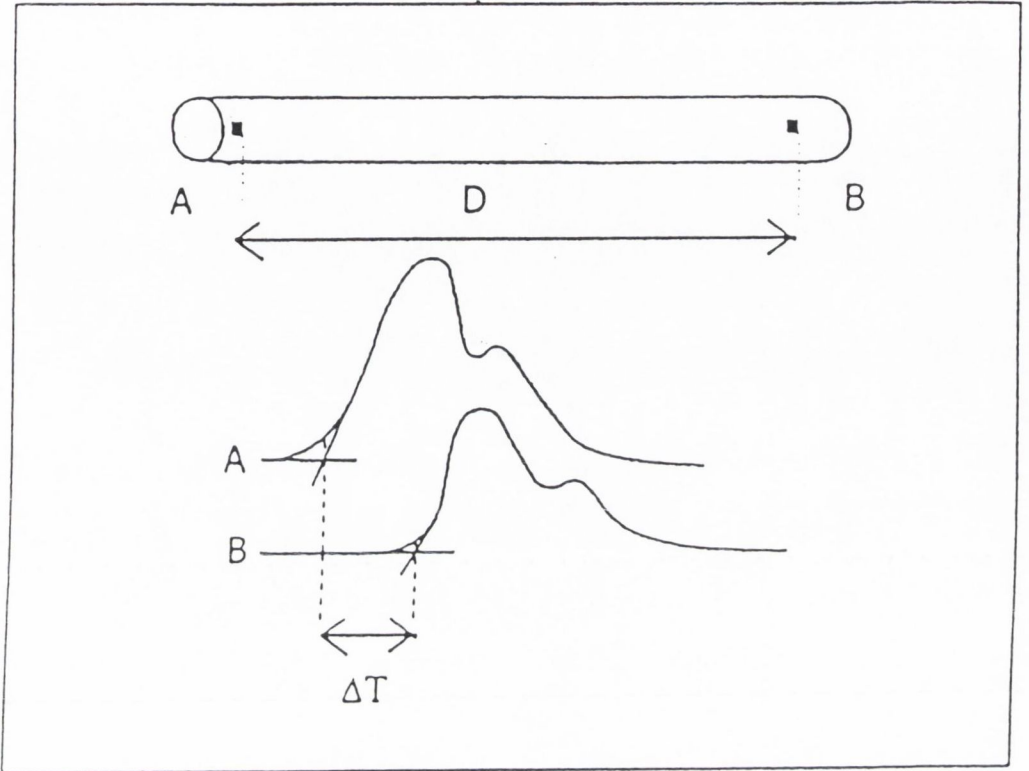
Figure 1.12 The carotid pressure waveform (A) and the femoral artery waveform (B) showing the delay in the transmission of the pulse between the two arterial points shown as ΔT

1.5   Arterial Wave Reflection

Ejection of blood into the aorta dilates the aorta and generates a pressure wave.   In youth, this pressure wave typically has a sharp upward and downward slope and the second wave following the incisura, is quite prominent marking the aortic valve closure *(Figure 1.13a).* This diastolic wave is caused by wave reflection from the periphery, principally from the lower part of the body. In older subjects the aortic pressure wave exhibits a late systolic peak with little or no diastolic fluctuation and mono-exponential fall of blood pressure (BP) in diastole *(Figure 1.13b).*   A combination of these patterns is seen at intermediate ages and may be explained on the basis of peripheral wave reflection.   This characteristic change in pressure waveform shape with age is attributed to an increase in aortic stiffness and PWV, with earlier return of reflected waves from the periphery.   In contrast to the pressure wave, the flow wave shows little or no change with age; only a small decrease in peak velocity attributable both due to decreased cardiac output (CO) and aortic dilatation is noted *(Figure 1.14).*

Wave reflection is responsible for the secondary fluctuations in the arterial pulse (O'Rourke 1982), which are of prognostic importance. Wave reflection is the major cause of increasing systolic blood pressure (SBP) with age (Westerhof and O'Rourke 1995) and is responsible for substantial differences between SBP in the central and peripheral arteries in youth (Snellen 1980). Such differences in SBP depend on the timing and amplitude of wave reflection. However, the common practice was to ignore wave reflection and treat the arterial system as a windkessel.

The *windkessel* is an elastic chamber, which cushions intermittent pulsations from a pump and converts the pulsations into steady flow *(Figure 1.5).* There is no such elastic chamber in the systemic circulation.   There are practical problems with the windkessel model, which make it incomplete and unsatisfactory for explaining arterial phenomenon. In the windkessel pressure changes occur simultaneously in the distensible reservoir and in the distal conduit; this is not the case in life. In the windkessel there is no amplification of the pulse as occurs in peripheral arteries. In the windkessel, pressure falls exponentially during diastole; there is no secondary diastolic wave as usually seen in systemic arteries. The simplistic view of the arterial system as a windkessel lends to

A.
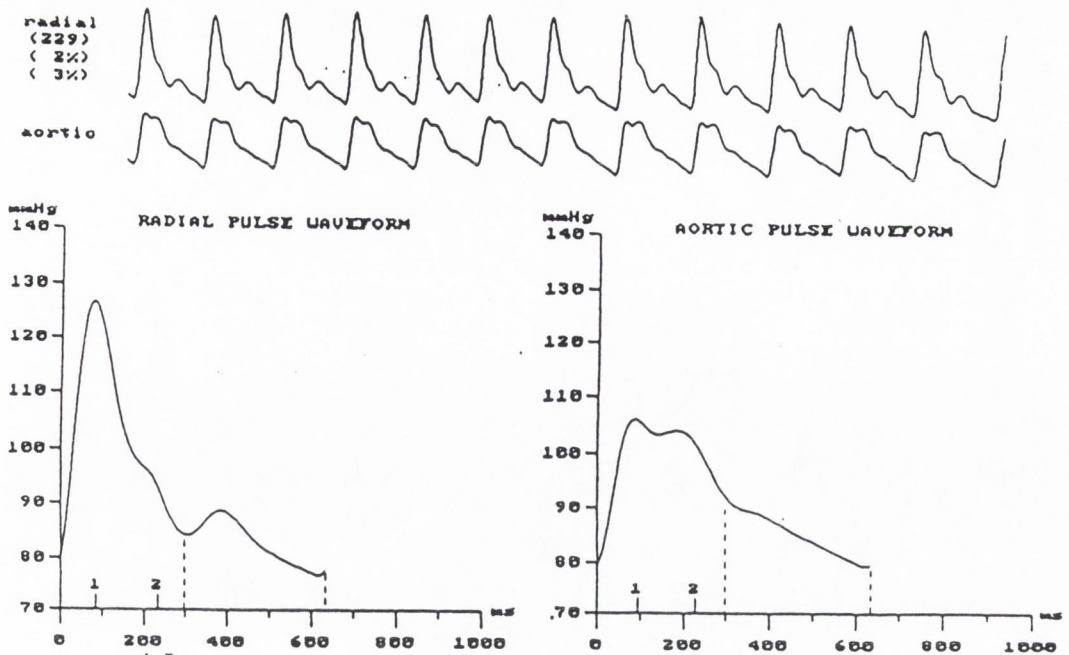
Figure 1.13a. & b.   The radial and aortic pressure waveforms in the young individual (A) and (B) in an older subject.   In the young individual the pressure wave typically has a sharp upward and downward slope and the second wave following the incisura.   This diastolic wave is an echo of the first and is caused by wave reflection from the periphery, principally from the lower part of the body.   In older subjects the aortic pressure wave exhibits a late systolic peak with a very small diastolic wave following the incisura and the fall in pressure during diastole is near exponential.

B.

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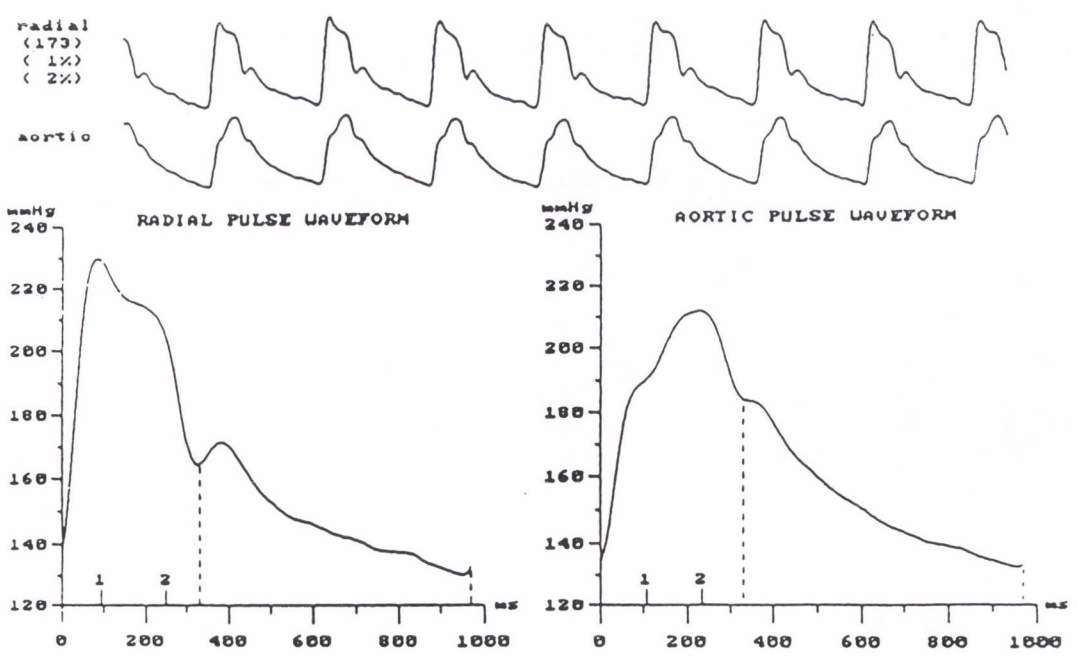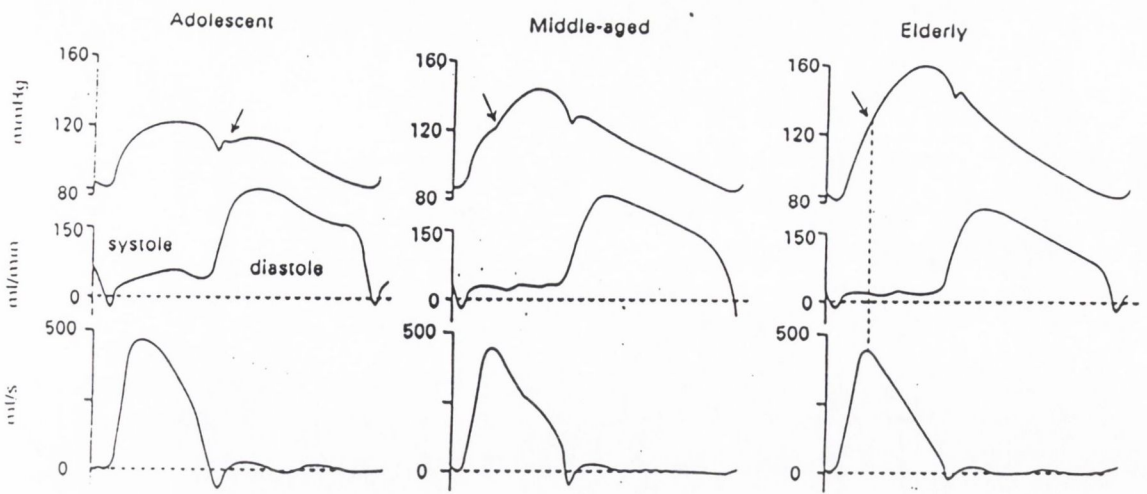
Figure 1.14. Pressure (top) and flow waves recorded in the ascending aorta (bottom) and coronary artery (middle) in normotensive subjects (adolescent, middle-aged, elderly). In adolescents, the reflected pressure wave (beginning at the arrow) occurs in diastole and enhances coronary flow. With advancing age, the reflected wave arrives earlier, occurs in systole during ventricular ejection, and causes a decrease in coronary blood flow. The deceleration phase of the flow wave is convex in adolescents, concave in middle age and linear in the elderly.
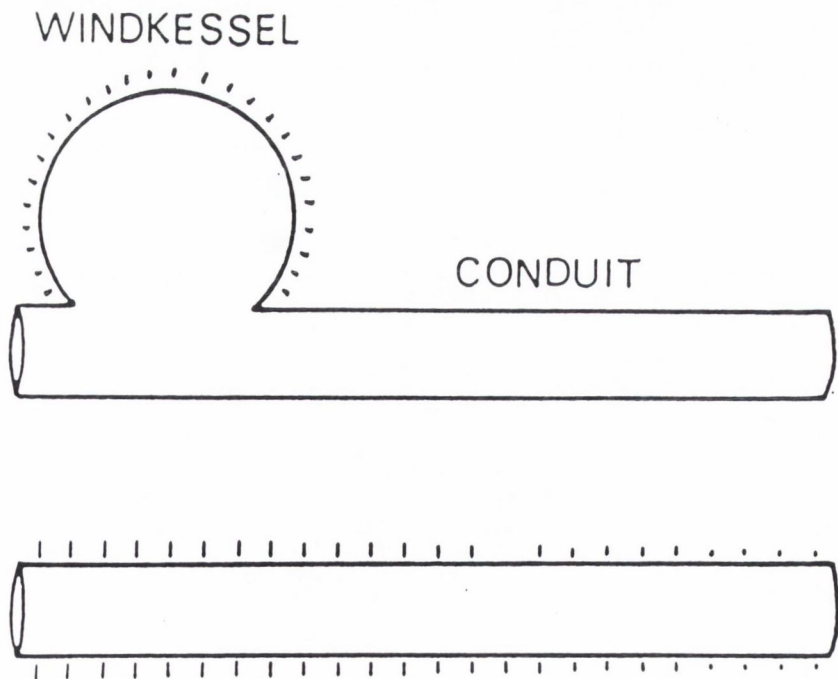
Figure 1.15. The cushioning and conduit functions of the arterial system may be represented separately by a *Windkessel* and distributing tube (above) or by a single distensible tube (below) in which both functions are combined.

27

the concept of a single SBP and diastolic blood pressure (DBP) that is the same in all arteries. The most realistic model of the arterial system is the distensible tube, which joins the heart to the peripheral resistance (PR); in this model, cushioning and conduit functions are combined *(Figure 1.15)*. With this simple model one can consider the pulsatile and steady phenomenon separately, but at the same time, can also account for differences in pressure at different points in the arterial tree, amplification of the pressure wave and secondary oscillations in pressure and flow. This is the simple model proposed by (Hamilton and Dow 1939) and by (McDonald 1960). It can be shown that the simple tube provides a good representation of the whole arterial system. A minor modification (discussed later) is used to account for differences in contour of pressure and flow waves in the upper and lower limbs, and also the eccentric position of the heart in the body, which is closer to arterial terminations in the upper part of the body than to those in the lower part *(Figure 1.16)*.

1.5.1 Wave Travel: The surge of pulse generated by the left ventricle after travelling down the arterial tree is reflected from periphery. Since the velocity of the pulse is fast (of the order of meters/sec), it is apparent that within the same cardiac cycle, the wave generated by left ventricle has adequate time to travel to the periphery and back. Such finite wave travel is evidence against the arterial system acting as a windkessel. When pressure waves are recorded between central and peripheral arteries, finite wave travel is apparent as a delay in the foot of the wave between the two sites *(Figure 1.12)*. PWV can be measured from such waves when the distance between the recording sites is known. The PWV can be measured as an index of arterial stiffness, according to Moens-Korteweg equation as:

$$\textbf{PWV} = \sqrt{\textbf{\textit{E.h}} / \textbf{2} \textbf{\textit{r}} \rho}$$

Where E is the Young's modulus of the vessel wall, h is the wall thickness; r is the internal diameter and ρ is the density of blood.

PWV is lowest in the highly elastic thoracic aorta and increases progressively in the more muscular peripheral arteries. In experimental animals and young humans it ranges from 5 m/s in the aorta to 8-10 m/s in the iliac and femoral arteries *(Figure 1.17)*. PWV increases progressively with age and hypertension due to the breakdown of the elastic
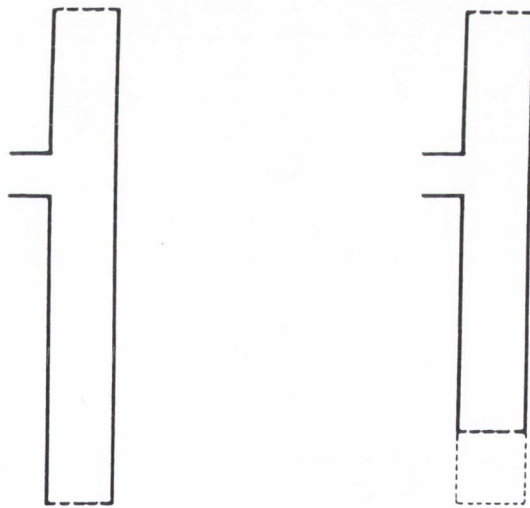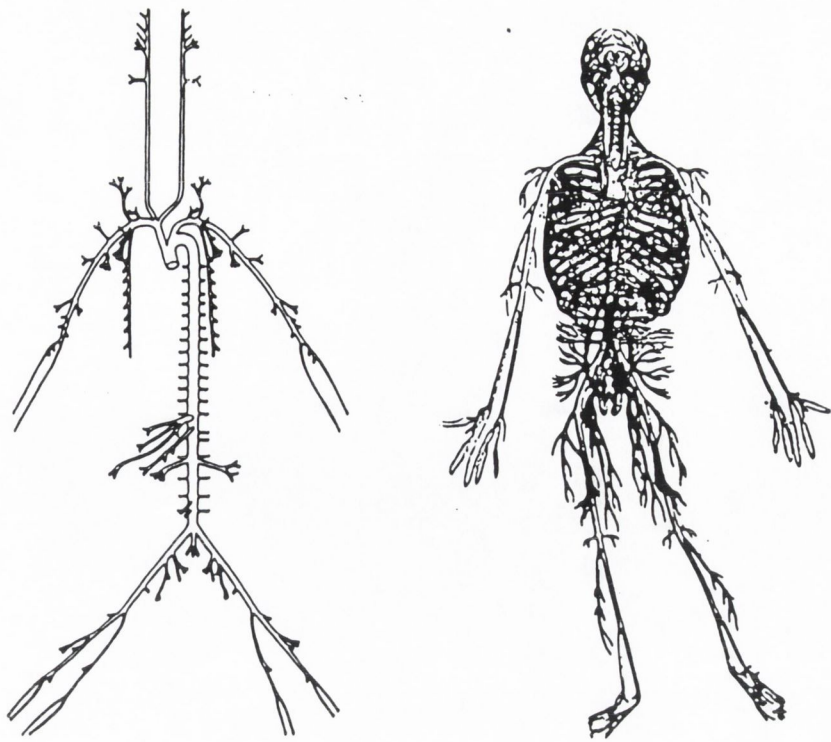
Figure 1.16. Systemic arterial tree of dog (top left) drawn to scale from dissection and human system arterial tree (top right) as drawn by Vesalius with equivalent T-tube models . In adult humans the lower limb of the T appears foreshortened because of increased pulse wave velocity due to arterial degeneration.
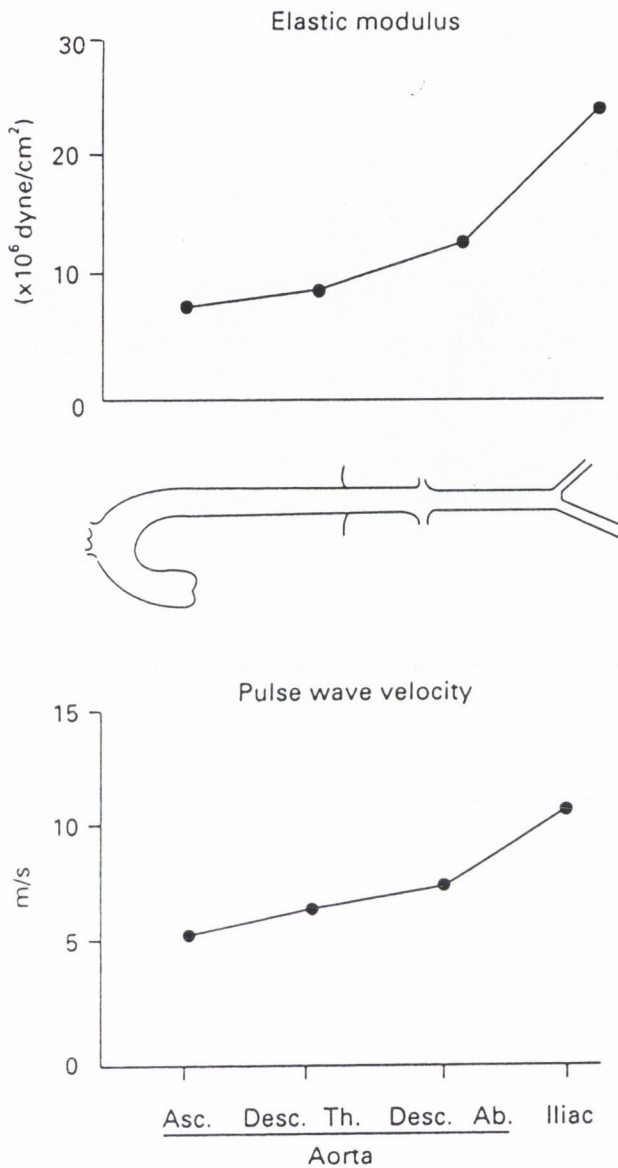
Figure 1.17. Changes in chemical content and in physical properties between central and peripheral arteries. Top: Young's modulus of segments of proximal thoracic aorta, abdominal aorta, iliac artery and femoral artery of young human subjects . Bottom: pulse wave velocity over the thoracic aorta, abdominal aorta, iliac artery of young human subjects.

structures in the arterial wall, which is replaced by the less distensible collagen leading to arterial stiffening. Due to a finite PWV, the pressure waveform recorded at any site of the arterial tree is the sum of a forward and a backward travelling waveform, which is the 'echo' of the incident wave reflected at peripheral sites *(Figure 1.18)* (O'Rourke 1982; Snellen 1980; Yaginuma and Cohn 1992).

1.5.2 Cause of Wave Reflection: The explanation for the existence of wave reflection comes from consideration of the dimensions and branching patterns of the major arteries and subsequent arterioles and from the changes in MAP and the resistance in these vessels (O'Rourke 1982; Snellen 1980; Hamilton and Dow 1939). Major arteries are excellent conduits providing a low resistance for blood over long distances between the heart and the capillaries. Even at arterial branches and bifurcations, there is little fall in MAP. Close to the capillaries, however, MAP falls significantly over short distances within the higher resistance arterioles. It is at this junction between a high conductance large artery and high resistance arterioles that wave reflection occurs *(Figure 1.19).* Under normal circumstances some 80% of the incident wave is reflected from arterioles in the periphery of the body.

There are a myriad of pathways between the heart and periphery within the body. These arise from the ascending aorta and appear to be so grouped that all can be represented by a single, functionally discreet site in the upper body and by a second, functionally discreet site in the lower body. Thus schematically, the whole arterial system can be represented by an asymmetric T-tube; the base of the tube represents the ascending aorta, the shorter limb represents major arteries to the upper body, and the lower limb represents the descending aorta and the major arteries to the lower body *(Figure 1.16)* (O'Rourke 1982; Snellen 1980; O'Rourke and Taylor 1966; O'Rourke 1967; Sipkema et al. 1980). There is no single reflected wave from a single reflecting site, but rather that the wave reflection seen is the resultant of multiple reflections at many sites in the periphery of the body. In the major arteries, only one single discrete reflected wave usually appears, suggesting an origin from a single site (O'Rourke and Kelly 1993). In the past, attempts were made to localize such a site as the terminal aorta or aortic bifurcation (Murgo et al. 1980a; Latham et al. 1985). However this site is only an apparent site, representing the average distance from which reflection appears to arise, as
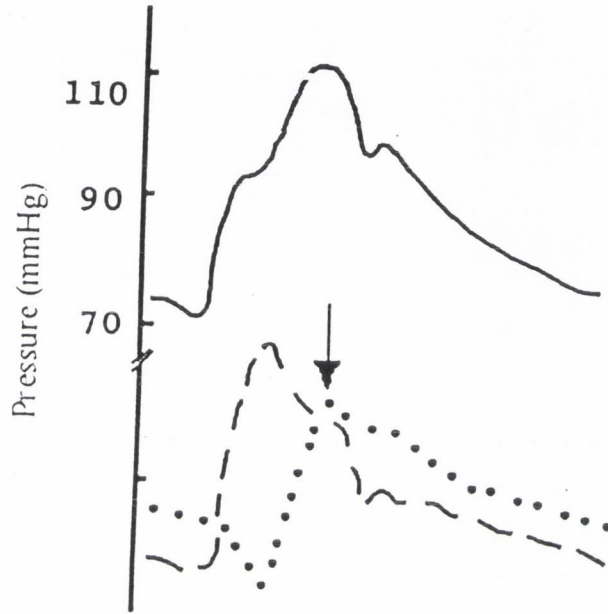
Figure 1.18. Breakdown of the measured arterial pressure waveform(—)
into a forward incident wave (-----) and a backward reflected wave (.....).
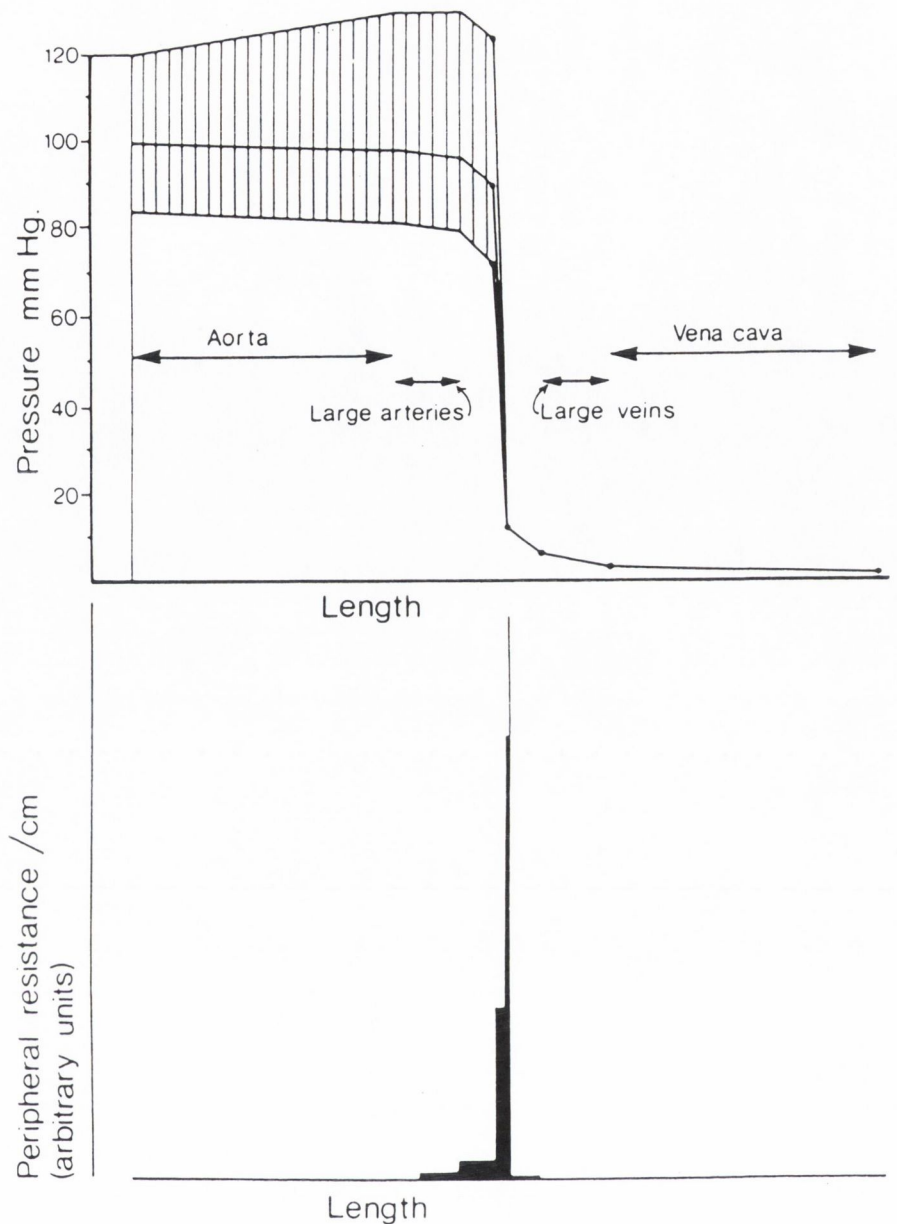
Figure 1.19. Top: changes in mean pressure and in pulsatile pressure generated by the left ventricle between the ascending aorta and the vena cava. Mean pressure falls only slightly in the aorta and large distributing arteries whereas pulsatile pressure is augmented as a result of wave reflection. Mean pressure and pulse pressure fall precipately over a short length in the smallest arteries and in the arterioles as a result of the high resistance to blood flow that these vessels present. Bottom: resistance in corresponding segments of the vascular bed.

visualized from the ascending aorta. Latham et al (Latham et al. 1985) have suggested that there is indeed a strong discreet wave reflection from the abdominal aorta in mature humans. This may be due to relatively greater dilatation of the elastic thoracic aorta than the abdominal collegenous aorta with ageing.

Arterial branching sites are of little importance as sites of wave reflection as the total calibre of vessels beyond the point of bifurcation is usually greater than that above and may actually minimize wave reflection.

1.5.3 Evidence for Wave Reflection: The left ventricle expels just a single spurt of blood with each heartbeat, yet the arterial pressure pulse usually shows a secondary wave or sometimes even a tertiary wave. Such pressure waves show different patterns in different arteries ***(Figure 1.20)*** and in the same artery under different circumstances ***(Figure 1.21).*** These secondary waves must be due to wave reflection. They are of lower magnitude than the primary wave and are quite distinct from the high-frequency notch caused by aortic valve closure. They are of greater amplitude in the peripheral arteries due to the proximity of reflecting sites and of lower amplitude in the aorta. The greater amplitude of the peripheral arterial pressure waves results in higher pulse pressure (PP) in the peripheral circulation compared to the central arteries which in turn explains why SBP is higher in peripheral than central arteries ***(Figure 1.22).*** There is no other plausible explanation for these secondary waves. Harvey effectively ruled out intrinsic contractions of the arterial wall by his great work in 1628.

1.5.4 Timing of Wave Reflection: Under normal circumstances, in experimental animals and young humans, wave reflection is timed so as to return to the ascending aorta from the periphery of the body after ventricular ejection has ceased (O'Rourke and Taylor 1967). The foot of the secondary wave thus appears in the ascending aorta to correspond to the high-frequency incisura, which is caused by aortic valve closure. Such timing is desirable since the rise in the ascending aortic pressure caused by wave reflection occurs in diastole rather than in systole. Such a pressure rise augments early DBP and so boosts coronary perfusion pressure without increasing left ventricular afterload (Snellen 1980). Wave reflection appears to be a necessary consequence of vascular design, but nature appears to take advantage of this phenomenon to optimise ventricular – vascular
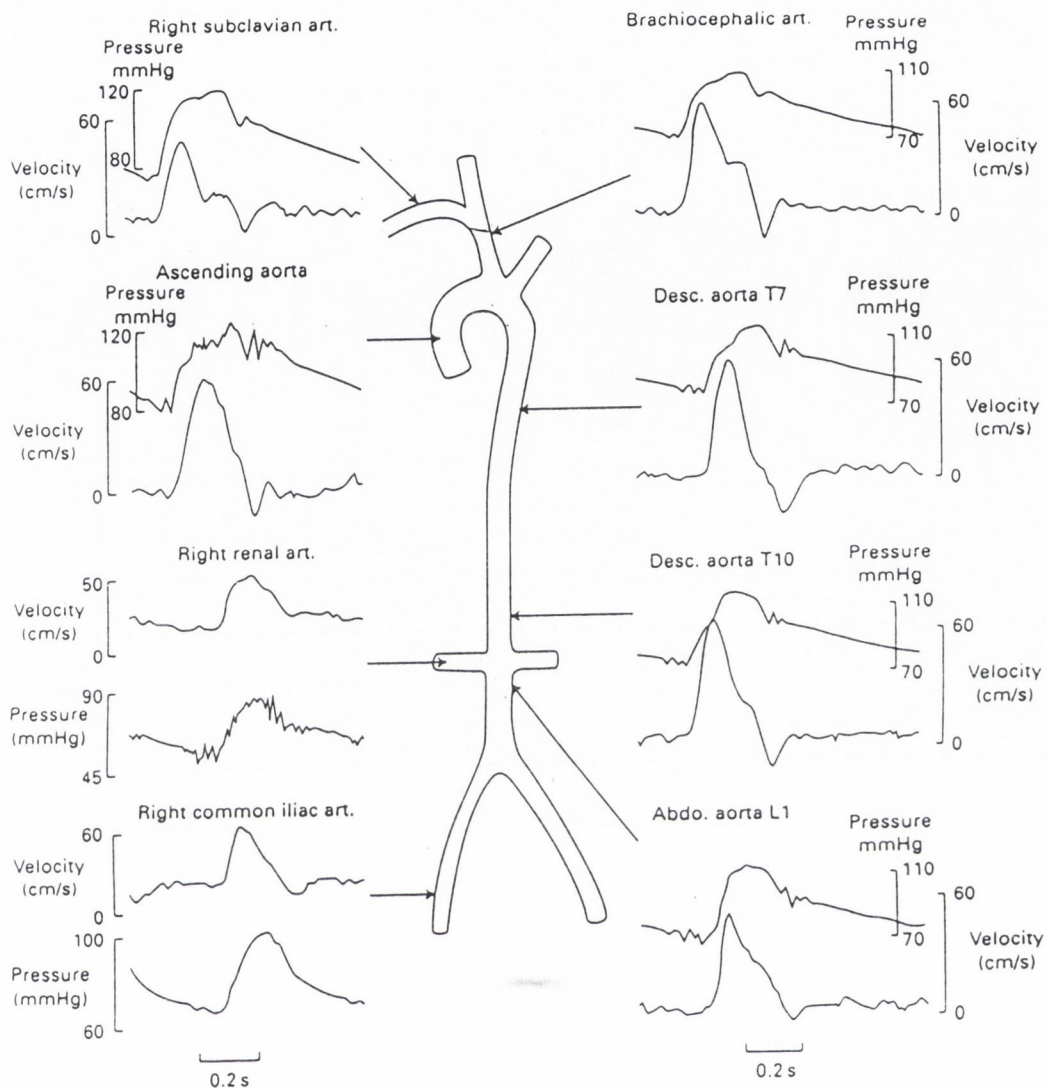
Figure 1.20. Pressure and velocity waveforms in different arteries recorded in a human undergoing diagnostic cardiac catheterization with an electromagnetic catheter transducer.
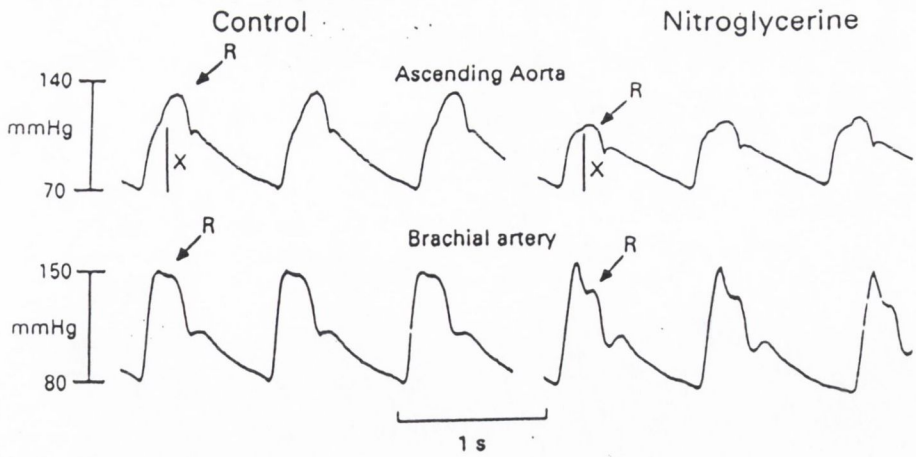
Figure 1.21. Pressure waves recorded with Millar Micromanometer catheters in the ascending aorta above and the brachial artery below of a 54 year old man under controlled conditions (left) and approximately 5 minutes after sublingual administration of nitroglycerin 0.3 mgs (right).
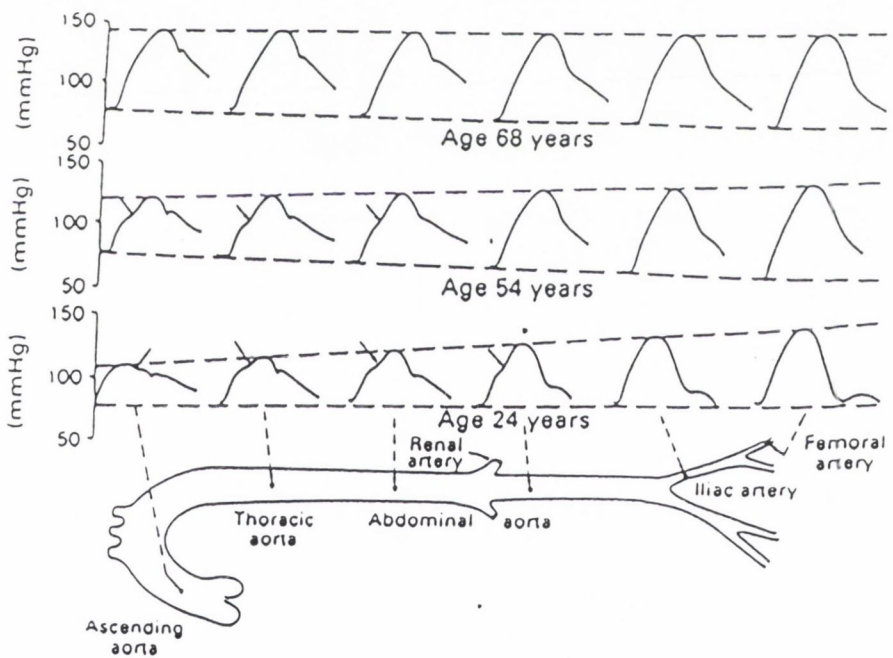
Figure 1.22. Pressure waves recorded along the arterial tree from the proximal ascending aorta through the femoral artery in three adult subjects aged 24, 54 and 68. In the oldest subject there is very little amplification in the pressure wave during transmission; however in the youngest subject the amplitude of the pressure wave increases by approximately 60% during transmission.

36

interaction. In mammals, the average PWV along the arterial wall is of the order of 4-6 m/s. The timing of wave reflection in different animals thus depends upon bodily length, occurring earlier in smaller animals and later in large animals (O'Rourke 1982). However, smaller animals have higher heart rate (HR) and shorter Left ventricular ejection duration (LVED), thus the correspondence between the incisura and the reflected wave foot in the ascending aorta is preserved except in older and hypertensive humans.

Thus the recorded pressure waveform depends upon:

1. The form and duration of ventricular ejection, which determines the incident wave.
2. The intensity of wave reflection from peripheral sites, which determines the size of the reflected wave.
3. The timing of wave reflection, which determines the point of the cardiac cycle at which two waves will meet and merge; the timing itself is dependent on how fast the wave travels towards the periphery and back (PWV) and where the wave is reflected. If the PWV is high, the reflected wave returns early to the ascending aorta and merges with the incident wave at an early point. Thus it adds to pressure and augments the waveform in systole, producing a second (late) systolic peak that is higher than the first peak *(Figure 1.13b)*. In contrast, if the PWV is low, the reflected wave arrives at a later point in the cardiac cycle and thus augmentation is evident in diastole as a convexity rather than a concavity decay of pressure *(Figure 1.13a)*. Intermediate patterns are seen at intermediate ages.

1.5.5 Amplification of Pulse Pressure: In the more distal aorta and the peripheral arteries, the correspondence between the foot of the reflected wave and the aortic incisura is not preserved. The phenomenon that leads to such favourable correspondence in the ascending aorta also leads to amplification of the pressure pulse between central and peripheral arteries and to the incisura preceding the foot of the reflected wave in the lower part of the body *(Figure 1.22)*. Therefore, in young subjects with low or normal PWV and elastic arteries, the SBP in the peripheral arteries is 20 mm Hg or higher than in the ascending aorta and other central arteries. In the elderly with stiff arteries, the pressure wave is transmitted virtually unaltered with SBP increasing by some 8 percent from the aortic root to the femoral artery. In middle-aged subjects, the pressure wave is propagated through the aorta to the femoral artery increasing the PP by about half. In younger

subjects, the amplification of PP is greatest, SBP being 20 mm Hg or more in the brachial artery compared to the ascending aorta and left ventricle of the heart (Kelly et al. 1990; Karamanoglu et al. 1993).

1.6   Types of Arterial Pressure Waveforms

Murgo et al. (1980b) using high-fidelity multi-sensor cardiac catheters did the most detailed and accurate studies of the pressure wave contour in man. The pressure waves recorded by Murgo were characterized by well-defined anacrotic notch (inflection point) in the mid-to-late part of systole, followed by a secondary wave. The contour of the pressure waves was classified according to the following criteria *(Figure 1.23):*

Type A: Peak systolic pressure (Ps) occurs in late systole after the inflection point (Pi) and (Ps-Pi) / Ps-Pd) is greater than 0.12; Pd is the minimum aortic DBP. This is invariably seen > 40 and < 65 years of age and is attributed to reflected waves from the lower body returning during early systole and extending throughout the remainder of ventricular ejection so that pressure in late systole is augmented *(Figure 1.23a).*

Type B: Peak systolic pressure also occurs in late systole following Pi, but (Ps-Pi) / Ps-Pd) is between 0 and 0.12. This is seen in subjects between the ages of 30 and 40 years as a result of intermediate pattern of wave reflection *(Figure 1.23b).*

Type C: Peak systolic pressure precedes Pi, and (Ps-Pi) / (Ps-Pd) is less than 0. (Ps-Pi) / (Ps-Pd) is termed the augmentation index (AI%) and is closely related to aortic stiffness. This pattern is seen in subjects younger than 30 years of age with a low amplitude reflected wave beginning in late systole and extending into diastole *(Figure 1.23c).*

Type D: When there is no inflection point and the AI% cannot be measured without recording blood flow and pressure simultaneously, Pi can be estimated as the point where peak flow occurs. This type of pressure wave is seen only in subjects > 65 years of age or in patients with hypertension, and was not classified by Murgo, as all his

BRACHIOCEPHALIC FLOW (MILLS et al 1970)

I   II   III

AORTIC PRESSURE (AFTER MURGO et al 1980)

A   B   C
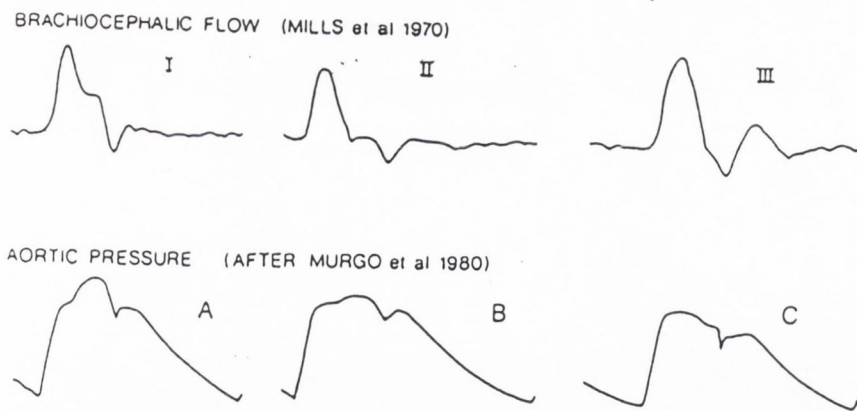
Figure 1.23. Comparison of Type A, B and C brachiocephalic flow waves with corresponding ascending aortic pressure waves recorded by Murgo et al (1980). Explanations are identical. The different flow and pressure patterns are attributable to differences in timing of wave reflection from the lower part of the body with the reflected wave returning before aortic valve closure in Type A, during valve closure in Type B and after valve closure in Type C.
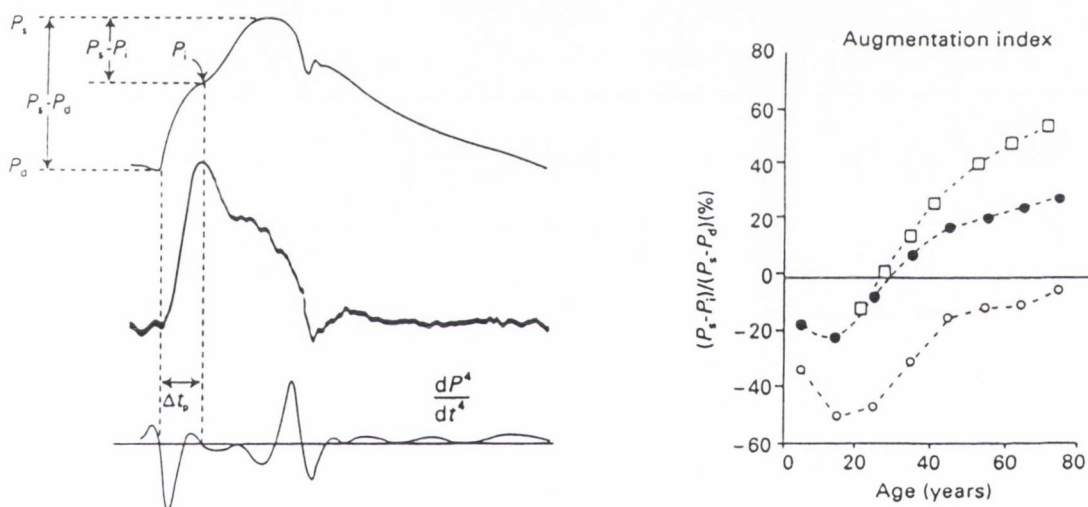
Figure 1.24. Left illustration shows the method used to calculate the augmentation index (AI = $[P_s - P_i]/P_s - P_d]$) from the pressure waveform. P_i is the inflection point (the point where the incident and reflected wave meet) which occurs at peak blood flow velocity and also at 0 crossing of the 4th derivative of pressure. Delta Tp is the travel time of the reflected pressure wave to and from the major reflecting site. Right: effects of age on augmentation index in the ascending aorta.

subjects were younger than 55 years. In this type, the reflected wave from the lower body returns shortly after aortic valve opening and blends with the upstroke of the incident wave so that no Pi occurs.

1.6.1 Quantification of Wave Reflection: Several approaches have been used to quantify wave reflection. AI%, the ratio of the augmentation of the wave to PP, is the most widely applied index of wave reflection. For its calculation, the merging point of the incident and reflected wave (Pi) needs to be identified. Kelly et al (Kelly et al. 1989b; Kelly et al. 1989a) have developed a flow-independent method by using the differential of the pressure wave to identify the Pi *(Figure 1.24).* Other approaches to quantify wave reflection include determination of the reflection coefficient (see vascular impedance) and distance to reflecting sites (O'Rourke 1982; Snellen 1980), the diastolic decay method and digital volume pulse; the latter two methods are compared to the AI% method below.

1.7. A Comparison between Systolic Pulse Wave Contour, Diastolic Pulse Wave Contour and the Digital Volume Pulse

Several methods of measuring arterial stiffness have been proposed and are either derived from the systolic part of the pressure waveform (AI% by applanation tonometry), the diastolic part of the pressure waveform (distal or small vessel compliance and proximal or large vessel compliance by windkessel method) or the digital volume pulse (DVP) percent maximal DVP amplitude (IP (DVP)) method using photoplethysmography. In this thesis, AI%, a measure of arterial stiffness derived from the systolic part of the arterial pressure waveform has been used and is described in detail in the Methods section. There have been a number of comparisons between AI% and the measures of arterial stiffness derived from the diastolic decay and the DVP method.

1.7.1 The Windkessel Arterial Model

The compliance of the entire arterial tree can also be evaluated on the basis of a single *windkessel* model, analogous to an electrical resistance capacitance system, which measures a single capacitance discharging into a single resistance (Nichols and O'Rourke 1998a). This method requires a direct recording of the arterial pulse and a measurement of CO for the calculation of the PR. Assuming that the diastolic portion of the arterial pressure waveform decays in a monoexponential fashion, the time constant of the decay (reciprocal of the pulse) is determined by the capacitance of the system and the resistance

to run off. Therefore, $C = T/R$ where C is the capacitance (representative of compliance), T is the time constant and R is the PR (Simon et al. 1979). This method is of limited use because the circulation is neither a single capacitance nor a single resistance. More importantly, the method ignores wave reflection that distorts the diastolic decay of the pressure waveform and makes it non-exponential. More complex mathematical models that have been designed for the arterial tree to account for the assumption involved in the single-chamber model with a monoexponential diastolic decay are being used (Liu et al. 1989). The Cohn Method based on this modified *windkessel* model estimates the compliance of the large proximal circulation and the small distal circulation separately (Finkelstein et al. 1988, Cohn et al, 1995).

There are problems with the *windkessel* model of the circulation and subsequently with the measurement of *arterial compliance*. The first problem arises in determining compliance at one site and the second in determining it at different sites; the major problem arises, however, in relating compliance alone to arterial function.

Firstly, the arterial wall is composed of elastin and collagen with the modulation of the arterial properties imposed by smooth muscle in the media and with the wall viscosity. The two-phase composition of the arterial wall means that the elasticity of the artery is non-linear and over the physiological pressure range, the pressure-diameter relationship is curvilinear, so the best estimate of compliance can be expressed only at a certain pressure or a given mean diameter in an elastic artery.

Secondly, in the peripheral muscular arteries, contraction or relaxation of the muscle not only changes the resting diameter for a given pressure but also alters the proportion of the stresses borne by the elastin and collegenous materials. Hence, contraction makes the artery stiffer and relaxation more compliant.

Thirdly, arterial compliance cannot be considered as 'lumped'. The values of compliance determined at one point on the arterial wall cannot be applied to the whole arterial tree. One reason is that compliance differs along the arterial system, the aorta is the most compliant, the distal arteries are less compliant and small arteries are the least compliant. But their compliance is markedly altered by changes in vasomotor tone. Also, the inertial

properties of the blood interact with the elastic properties of the wall in such a way that the pulsation generated in one artery takes a finite time to pass along the arterial wall. At any point in time, pressure is not the same at all arteries in the body. There is a delay and this can be measured from delay in the foot of the wave at two sites along the same path, as PWV (O'Rourke 1990). The whole concept of PWV makes the windkessel model inapplicable to the study of arterial compliance. PWV is not infinite, with arterial compliance lumped at one point and resistance at another, as in the windkessel model.

Finally, compliance measurements ignore wave reflection. If the wave generated by the heart passed only to the periphery, the arterial pressure and flow waves would be identical. However the two waves are quite different, with secondary fluctuations usually imposed on both. This is due to wave reflection. Some researchers have overlooked this complex phenomenon and have treated large arterial properties in terms of arterial compliance only.

Arterial compliance is an important vascular property. It interacts, however with other properties and cannot be related in any simple way to arterial function or to ventricular vascular interaction.

1.7.2 Comparison between AI% and the Diastolic Decay Method

The *windkessel* model, which defines the exchange capacities of large arteries, has been proposed to measure systemic (aortic) compliance. Validation of this model was performed in humans (Finkelstein et al, 1991). The model generates two compliances, a capacitive component (C1) and an oscillatory or reflective component (C2). The capacitive component assesses the large arterial storage capacity and the oscillatory or reflective compliance relates to the cushioning function of arteries and is a function of the arterial reflection sites that are thought to reside primarily in the small arteries and arterioles and branching points of small arteries. There is an age-dependant decline in both C1 and C2, reflecting structural or functional changes in the large conduit arteries as well as in the smaller reflecting sites (McVeigh et al, 1999). Changes have also been described in isolated systolic hypertension (ISH) (Beltran et al, 2001) and diabetes mellitus (McVeigh et al, 1993)

42

A recent study in 100 subjects (age range 19 to 77 years) with a wide range of BP (97 to 186 SBP and 52 to 104 DBP mm Hg) were studied; mean values of C1, C2 and AI% were respectively 13.8 ± 4 ml /mm Hg $\times$ 10, 5.9 ± 3.1 ml/mm Hg $\times \times$ 100 and $128.5 \pm 24.9\%$. Coefficients of variation were 32.8% for C1, 33.3% for C2 and 6.6 % for AI%. C2 was significantly and inversely correlated to AI% (r = -0.77, p<0.001)(Rietzschel et al, 2001). Avolio et al (2001) have shown that typical C-type aortic waves give the highest C2 value compared to A-type aortic waveform in the literature. They further measured aortic and radial artery pressures simultaneously in 45 humans (age range 35-84 years) at baseline and after Glyceryl trinitrate (GTN) administration to calculate AI% and C2 (measured). TF was used to calculate reconstructed aortic pressure waves and AI%. AI% (reconstructed) underestimated AI% (measured) by 0.03±0.16 but both values were correlated(r=0.64, p<0.001) and C2 and AI% were inversely correlated, (r= -0.36; p<0.01) for AI% (measured) and (r =-0.30, p<0.01) for AI% (reconstructed). Both the reconstructed and measured AI% decreased significantly after GTN but not C2. It appears that C2 is related to and reflects, at least in part, haemodynamic changes affecting central aortic BP. Given the model assumptions of windkessel and the much higher variability in C2 compared to the AI%, the latter could be a more appropriate parameter to use in the clinical setting because it is determined directly from the arterial pressure wave contour.

1.7.3 Comparison between AI% and the Digital Volume Pulse Method

Photoplethysmography: The Finapres system provides continuous non-invasive beat-to-beat recording of the arterial pulse waves from the finger artery and is widely used. The system uses a sonocontrolled method to maintain finger volume constant with the pressure recorded, as the pressure required achieving this. In this volume clamp method, the size of the finger artery as gauged with a photoplethysmograph mounted inside a cuff. It is not a recording of pressure but recording of volume change between sensors that are usually applied to the finger. The contour of the finger plethysmograph has been studied extensively. It has been shown that the finger photoplethysmograph shows systolic augmentation very similar to that which is recorded by applanation tonometry and is presumably related to aortic augmentation and possibly to ventricular and vascular hypertrophy. The finger plethysmograph shows changes with age similar to those seen in the carotid artery with applanation tonometry. It is described in terms of the ' second derivative wave' or acceleration plethysmograph' (Chowienczyk et al. 1999; Bang et al.

43

2000). MAP is slightly lower and PP higher with this method than in the brachial artery as is expected from effects of wave travel and reflection, but differences can be quite substantial with changes in vasomotor tone and HR and in arterial diseases. Though photoplethysmography has received more criticism than warranted in the past, it is being used more commonly now for the assessment of vascular stiffness.

The DVP can be recorded simply and non-invasively by photoplethysmography. The DVP exhibits a characteristic notch that can be expressed as percent maximal DVP amplitude (IP (DVP)).   A recent study (Millasseau et al 2000) explored whether a generalised TF can be used to relate the DVP to the peripheral pressure pulse and, hence, to determine whether both volume and pressure pulse waveforms are influenced by the same mechanism. The DVP was recorded by photoplethysmography in 60 subjects (10 women, aged 24 to 80 years), including 20 subjects with previously diagnosed hypertension. Simultaneous recordings of the peripheral radial pulse and digital artery pulse were obtained by applanation tonometry and a servo controlled pressure cuff (Finapres), respectively. In 20 normotensive subjects, measurements were obtained after the administration of GTN 500 microgram sublingually. TF obtained by Fourier analysis of the waveforms were similar in normotensive and hypertensive subjects. In normotensive subjects, TFs were similar before and after GTN. By use of a single generalised TF for all subjects, the radial and digital artery pressure waveforms could be predicted from the DVP with an average root mean square error of 4.4+/-2.0 and 4.3+/-1.9 mm Hg (mean+/-SD) for radial and digital artery waveforms, respectively, similar to the error between the 2 pressure waveforms (4.4+/-1.4 mm Hg). The peripheral pressure pulse is related to the DVP by a TF, which is not influenced by effects of hypertension or GTN. The IP (DVP) is influenced by pressure wave reflection (Millasseau et al, 2000). Effects of GTN on the DVP and pressure pulse may be determined by a similar mechanism. It has been proposed that response of IP (DVP) to endothelium-dependent vasodilatation can be used as a measure of endothelial function (Chowienczyk et al, 1999)

1.8 Pressure pulse in the Time and Frequency Domain

Conventionally the pulse is described in the time domain. According to this approach, the pulse is considered as a change in arterial pressure with time. Time is represented in the horizontal and pressure in the vertical axis. However, this approach has led to certain misconceptions. Clinicians have been taught to consider only systolic and diastolic BP and ignore the rest of the information contained within the arterial pressure waveform. In addition, neither the extremes of the pulse, nor its contour, are the same throughout the arterial tree. This has important implications as to left ventricular afterload and the error in its estimation when only the brachial BP is recorded. Finally, a simplistic and misleading notion has survived through generations that SBP is an index of cardiac strength and DBP a measure of arteriolar tone.

The alternative approach is purely quantitative, that is analysing the pulse in the frequency domain. The pulse is conceived as a composite wave that can be resolved into component harmonics like a musical wave *(Figure 1.25)*. In other words, the arterial pressure wave is considered as a mean value with fluctuations around the mean, rather than systolic and diastolic with fluctuations in between *(Figure 1.26)*. This approach permits separate study of steady and pulsatile phenomenon, which in turn helps understand amplification of the pulse between central and peripheral arteries, changes in pressure with age, or specific pulses in cardiovascular disease.

When a waveform is broken down into component harmonics, the lower-frequency harmonics are always of greater amplitude than the higher harmonics. Usually, 98% of the energy in the pulse is contained in the first five harmonics. The pulse can usually be characterized quite accurately from the first 5 to 10 harmonics. Valuable information is provided when harmonic analysis is used to relate frequency components of pressure and flow waves recorded at the same point *(Figure 1.27)* or corresponding frequency components of pressure waves recorded at different arterial points *(Figure 1.28)*. The latter analysis shows how individual components of compound waves are altered in travel between central and peripheral arteries.

45

1.8.1 Vascular Impedance: The term *impedance* is the measure of the opposition to flow presented by a system. Four different types of impedance are defined; however, the most frequently used in vascular mechanics are *input impedance* and *characteristic impedance*. *Input impedance* is the relationship between pulsatile pressure and pulsatile flow recorded in an artery feeding a particular vascular bed. It is described in terms of *modulus* (amplitude of pressure÷flow) and *phase* (delay between pressure and flow) plotted against frequency *(Figure 1.27)*. At zero frequency, the value of modulus is peripheral resistance and the value of phase is zero. From its value at zero frequency, modulus falls to a minimal value before rising to a low maximal value at around twice this frequency. Phase becomes negative at low frequencies (indicating that flow preceded pressure), then crosses zero at the frequency of the minimum of modulus, becomes positive and crosses zero at around the frequency of the subsequent modulus maximum. *Characteristic impedance* is the relationship between pressure and flow in artery when they are not affected by wave reflection. It is represented by the value at which modulus settles at very high frequencies. Studies of impedance provides valuable insight into several issues of vascular mechanics:

1. **Wave reflection:** Studies of impedance provide evidence that the heart sees two separate vascular beds and the whole systemic circulation can be represented by an asymmetric T system. Moreover, it allows the calculation of **A)** the reflection coefficient ($T = [Z_T - Z_C] / [ZT + Z_C]$ where Z_T is the peripheral resistance and Z_C is the characteristic impedance) thus giving information about the magnitude of wave reflection, **B)** the actual position of the resultant of all individual reflecting sites in the vascular bed (Lz, distance to the reflecting site) and **C)**, they enable unscrambling of the compound pressure and flow waves into forward-travelling and backward-travelling components *(Figure 1.18)*.
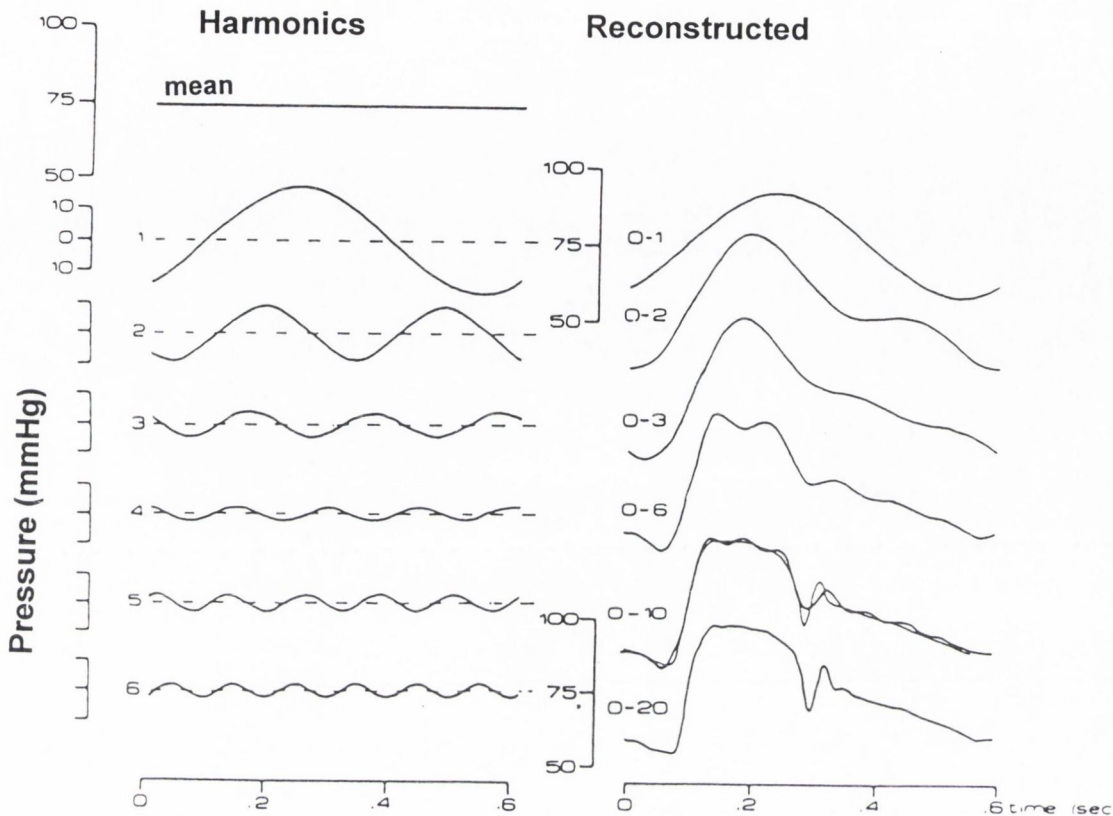
Figure 1.25. Example of a Fourier Series . Left: the mean term and first six harmonics of a pressure wave from the ascending aorta of a dog. Right: individual harmonics added to reproduce the original wave. Agreement is close with the first six harmonics added (0-6), better with the first 10 harmonics (0-10), while the recent sized wave is almost identical to the original wave (the thin line in the second bottom trace) with addition of the first 20 harmonics (0-20).
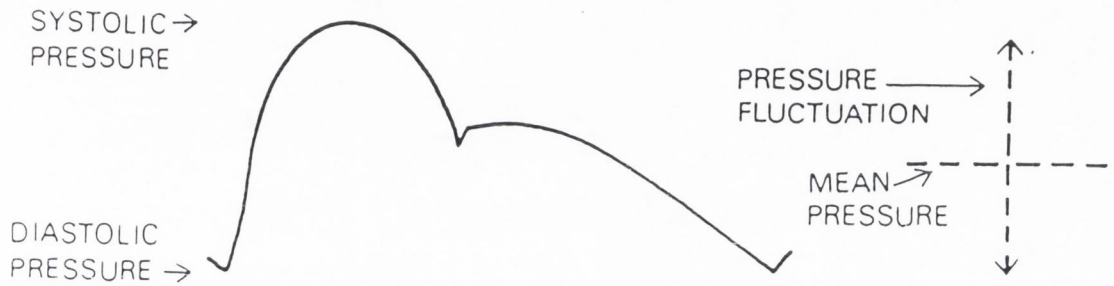
Figure 1.26. The arterial pressure pulse (centre) can be described in terms of its highest and lowest values (systolic and diastolic pressures), or in terms of its mean value (mean arterial pressure determined by peripheral vascular resistance) and the fluctuation of pressure around this mean (pulse pressure determined by ventricular ejection and arterial elastic properties).
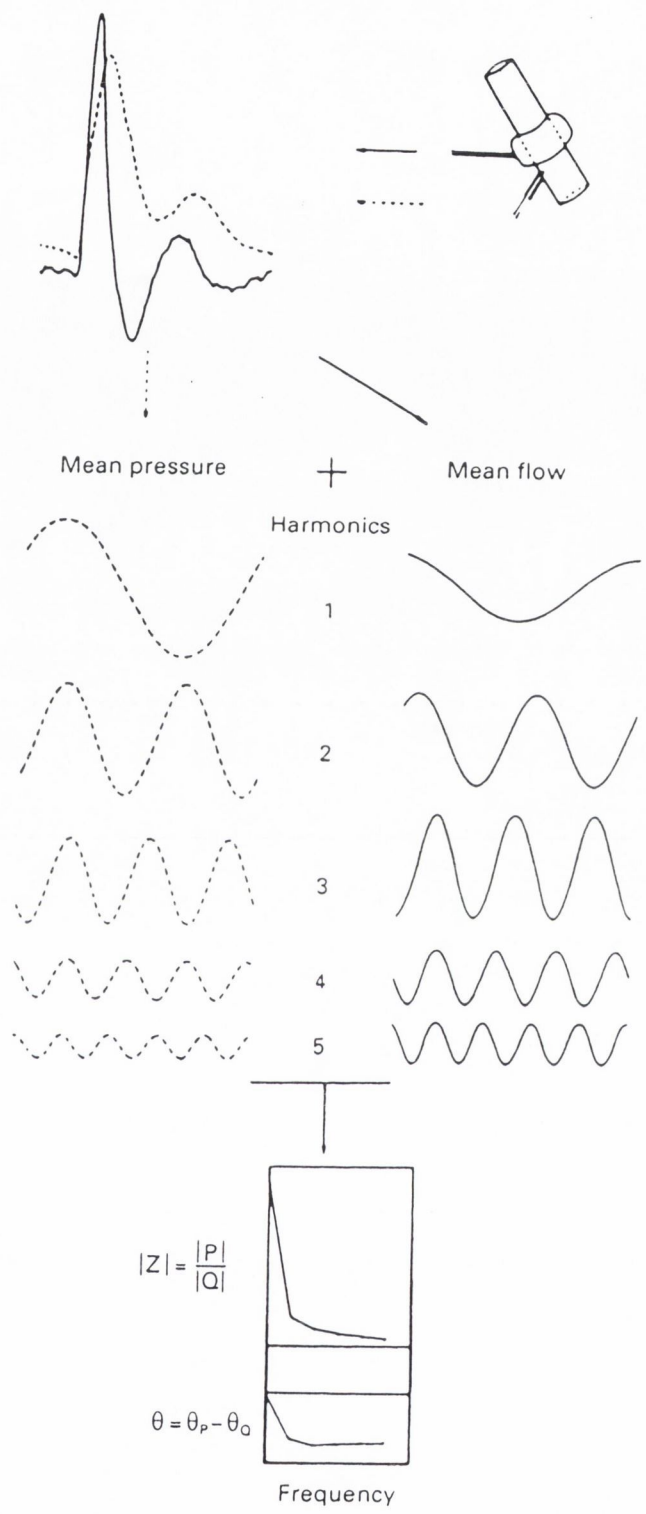
Figure 1.27. Use of Fourier Analysis to determine vascular impedance. Pressure (dotted line) and flow (solid line) measured in an artery are decomposed into mean values and a series of harmonic waves at multiples of heart rate frequency. Only the 1st to 5th are shown. Impedance modulus (top line of lower panel) is a modulus of pressure (P)/modulus of flow (Q) at the different frequencies and phase (bottom line of lower panel) is the delay between pressure and flow harmonics.

2. **Left ventricular afterload:** Vascular impedance when measured at the ascending aorta is a measure of the hydraulic load that the systemic vascular bed imposes at the ejection of the left ventricle.

3. **Different physiologic conditions:** Changes in impedance provide explanations for the mechanisms involved under different physiologic conditions such as exercise or the Valsalva manoeuvre.

4. **Effect of drugs:** Impedance provides insights into the mechanisms involved in the haemodynamic alterations observed with different drugs.

1.8.2 Pressure Transfer Function (TF):

Pressure waveforms change considerably toward the periphery. Change in amplitude of a compound wave between central and peripheral arteries is dependent on **(1)** the relative harmonic content of the central wave and **(2)** TF. TF is the relationship of pressure amplitude at two sites as a function of frequency. It is usually displayed as a graph of distal to central pressure wave amplitude expressed as a function of frequency *(Figure 1.29)*. The value of this TF or the degree of amplification depends on the difference in stiffness between central and peripheral arteries. PWV affects this TF through modification of wave reflection. An increase in PWV displaces the curve of TF toward the right as seen in the lower part of figure 1.29(TF for lower limb) where the dotted line corresponds to the older subject with increased PWV. However, TF in the upper limb in human beings changes surprisingly little with age, presumably due to the minor changes in PWV compared with that in the aorta and the lower limb (O'Rourke 1982; Snellen 1980; O'Rourke 2001; Avolio et al. 1983; Avolio et al. 1985). This finding is used to generate the ascending aortic pressure waveform from peripheral pressure wave recordings using a generalized TF.
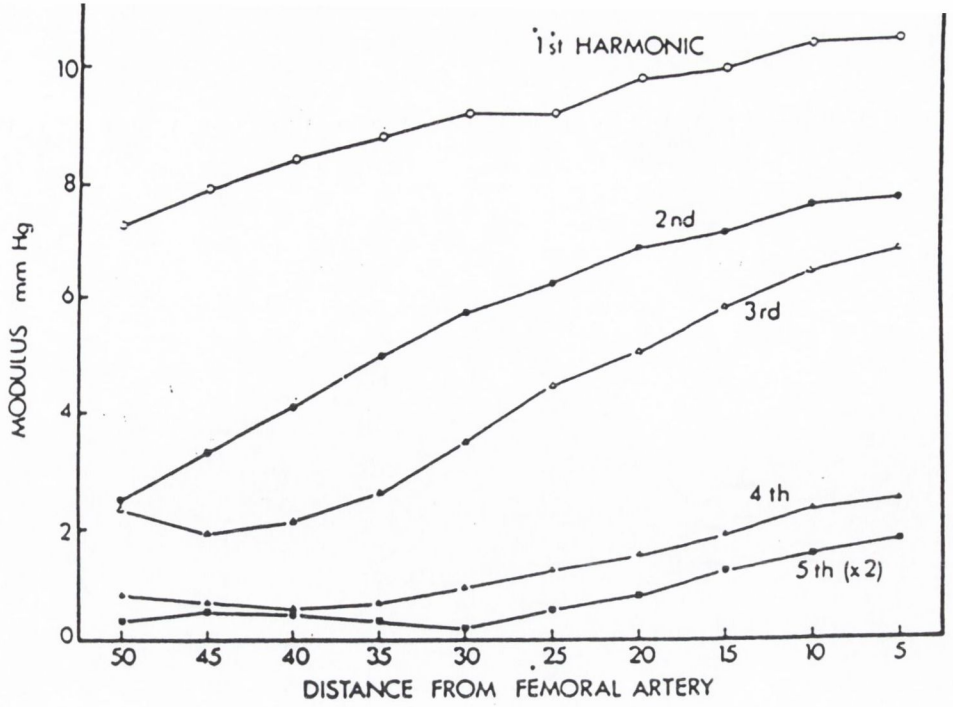
Figure 1.28. Amplitude of the first 5 harmonics of pressure waves recorded between the aortic arch and femoral artery of a wombat.
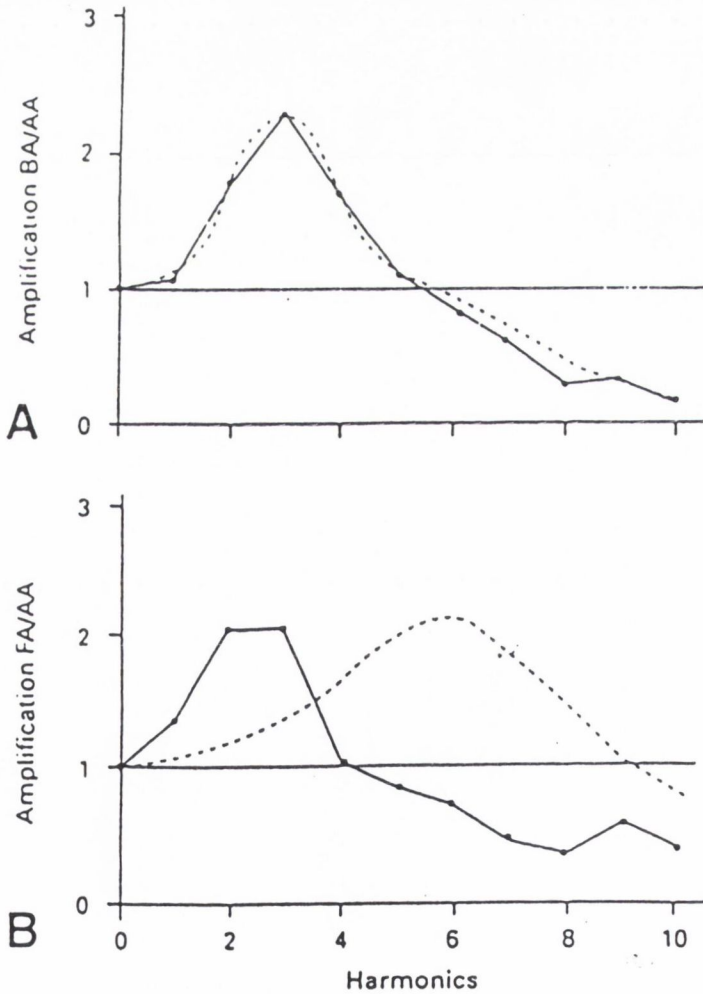
Figure 1.29. A. Amplification of the human arterial pressure wave as a function of frequency between the aortic arch and (AA) and brachial artery (BA) and **B.** between the aortic arch and femoral artery (FA). Data were obtained from Fourier analysis of pressure waves recorded simultaneously in a young male subject. The dotted lines indicate the expected changes in amplification in an older subject.

51

1.9. The Flow And Pressure Waves In Different Arteries

Ascending Aorta

Flow: The flow wave in the ascending aorta has a characteristic shape. Flow velocity rises to a peak of around 80 to 100 cm/s at some 80ms after the opening of the aortic valve, and then decreases more slowly during the latter part of systole. After a little backflow as the aortic valve shuts, there is no significant flow in the ascending aorta but only a little runoff into the coronary arteries. This type of pattern is seen consistently in man and in different animals at different HR. With age, the flow wave shows little change *(Figure 1.23)*. Even in cardiac disease, this pattern of flow is usually maintained with a few exceptions, such as aortic stenosis and hypertrophic cardiomyopathy.

Pressure: the pressure waveform shows considerable variation with age, in different disease states and in different animal species. All these variations can be explained on the basis of wave reflection. In youth the pressure wave has a rounded top and a second wave (the reflected wave) following the incisura *(Figure 1.23)*.

The Proximal Aorta And Its Major Branches

Flow: The flow wave in the descending aorta becomes more rounded than in the ascending aorta, the incisura becomes unapparent, and a wider negative wave after systole is often seen followed by a diastolic wave. In the brachiocephalic, carotid and subclavian arteries, systolic forward flow is abbreviated and is followed by flow fluctuations that are reciprocal to those in the descending aorta *(Figure 1.10)*.

Pressure: There is relatively little change in the contour of the pressure wave in the upper descending aorta and in the proximal parts of the brachiocephalic, carotid and subclavian arteries. The carotid artery pressure pulse has a configuration very similar to the ascending aortic pressure waveform. However, the augmentation features are less marked in the carotid pressure waveform *(Figure 1.10)*.

Peripheral Arteries – Lower Limb

Flow: there is a progressive fall in the amplitude of the pulsatile flow *(Figure 1.10)*.

***Pressure*:** There is a progressive increase in pulsatile pressure. MAP falls slightly, but peak SBP increases. In young subjects, the pressure waveform shows a smooth contour with a single systolic and a single diastolic wave *(Figure 1.10).*

Peripheral Arteries- Upper Limb

Flow: The flow pattern of the subclavian arteries is maintained in the upper arm *(Figure 1.10)*

Pressure: As in the lower limb, there is a progressive increase in the PP and peak SBP. The radial pressure wave contour in young subjects exhibits a prominent early systolic peak that is followed by two fluctuations: one in late systole (lower than early systolic peak) and one in diastole *(Figure 1.10).*

Effects of Ageing and Hypertension on Wave Reflection: There is a progressive increase in the PWV in humans with age irrespective of the change in BP and is attributable to the stiffening of the aortic wall, but a similar phenomenon can be brought on acutely by increasing BP; the PWV in man more than doubles between the ages of 17 and 70 years. This is a manifestation of arterial stiffening and is attributable to the fatiguing effects of cyclic stress causing fracture of load bearing elastic lamellae in the wall, and degeneration of the arterial wall *(Figure 1.30).*

These changes are not seen in the short life span of experimental animals and so far this phenomenon is unique to man. With increasing BP, the aortic wall becomes progressively stiffer as stress is transferred from the more distensible aorta to inextensible collagen; consequently the PWV increases. With increasing pressure, the reflected wave occurs earlier and moves into the systolic part of the curve. The ascending aortic pressure curve with hypertension can be readily explained on the basis of wave reflection. The first peak of the wave is transformed into a shoulder, whereas the early-reflected wave causes pressure to rise to a peak in late systole, with loss of the diastolic wave. Ascending aortic pressure waves with this abnormal pattern are regularly seen in the hypertensive individuals. However, they can also be seen in middle-aged normotensive people due to arterial degeneration and high aortic PWV. The augmentation created by wave reflection is quite substantial, averaging 21 mm Hg in a group of adult humans seen at cardiac

catheterisation and up to 50 mm Hg in hypertensive adults (Chen et al. 1996; Chen et al. 1997; Karamanoglu et al. 1993).

The features first described by Mahomed in 1874 are identical to those described in relation to the tracing of a hypertensive patient as shown in *Figure 1.4b*. *The percussion wave is usually well marked and distinctly separate from the tidal wave.... The dicrotic wave is very small and often scarcely perceptible ...the tidal wave is prolonged and too much sustained'* (Mahomed 1872). Mahomed and his successors recorded the radial pulse routinely, but they were aware that the secondary tidal wave on the radial pulse wave underestimated the amplitude of the corresponding wave in the carotid and other central arteries, which has been confirmed by Kelly et al (Kelly et al. 1989b; Kelly et al. 1989a; Kelly et al. 1989d). The technique of sphygmography is quantified now in such a way that the central aortic pressure wave contour and indices of ventricular-vascular interaction can be derived from the radial arterial pressure waveform and extends the brilliant pioneering work of Marey and Mahomed a 100 years ago.

Early Wave Reflection And Disadvantages To Left Ventricular Function:

Under normal circumstances appropriately timed wave reflection maintains aortic pressure during early diastole without boosting pressure during systole. The ill effects of early wave reflection can be readily understood by comparing pressure waves in the ascending aorta of young and old human subjects *(Figure 1.31)*. There is an increase in peak SBP, an increase in mean SBP and an increase in pressure in end-systole, all in association with a decrease in pressure throughout diastole. Elevated SBP increases myocardial oxygen demand whereas a fall in DBP tends to decrease the myocardial blood supply. All predispose to myocardial ischaemia. Furthermore, wave reflection by boosting pressure in late systole, predisposes to impaired myocardial diastolic relaxation and may further intensify ischaemia. The increase in SBP boosts left ventricular afterload, suppresses ventricular ejection and generates left ventricular hypertrophy (LVH).
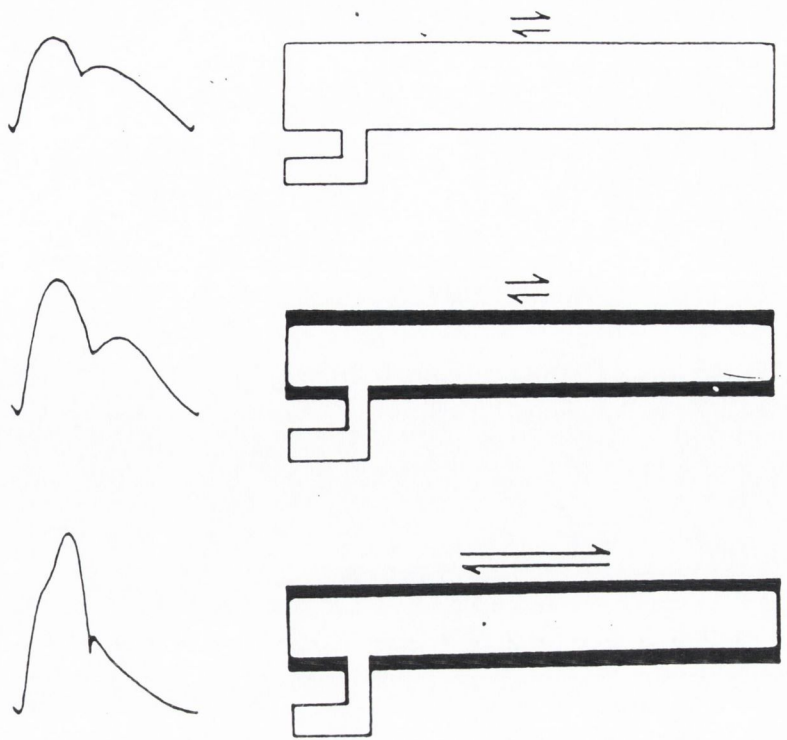
Figure 1.30. Tubular models of the arterial system with the heart at left and lumped peripheral resistance at right. Coronary arteries are represented by the 'hook'. Pressure waves in the ascending aorta are represented at left. The top model represents the normal youthful arterial tree. The central model represents the arterial system in an older person if aortic wave velocity were to remain unchanged. The bottom model represents the stiffened arterial tree of an older person with increased aortic pulse wave velocity. Reflection is represented by the diastolic wave in the first two cases and by the late systolic wave in the third.
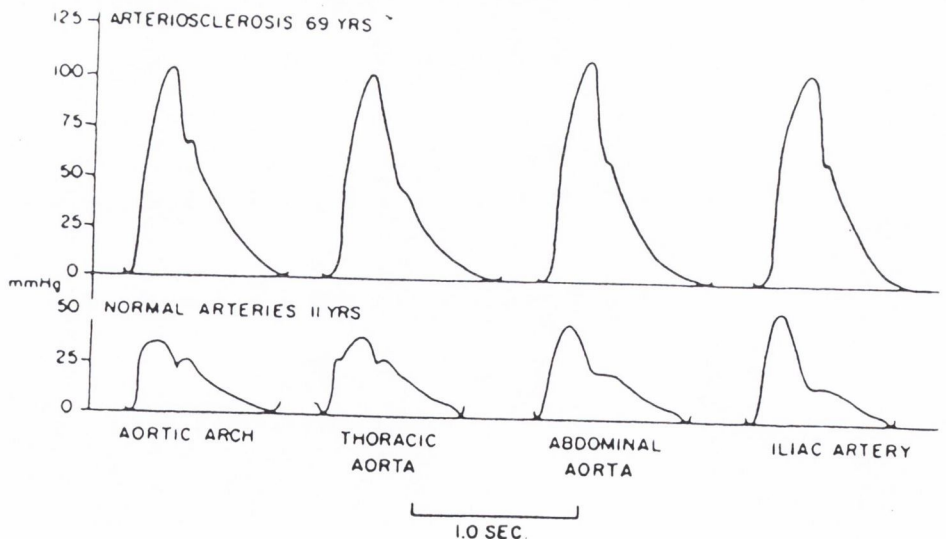
Figure 1.31. Change in shape of the arterial pressure wave between the aortic arch and iliac artery in a 69 year old arteriosclerotic man (top) and an 11 year old child.

55

1. 10. Relationship Between Arterial Stiffness, Pulse Pressure And Cardiovascular Risk

In recent years, three important haemodynamic concepts have substantially changed our conventional views on the haemodynamics of arteries: (O'Rourke and Mancia 1999; Safar et al. 2000b; Dart and Kingwell 2001)

1. The BP curve should be described as involving two different components; a steady component, MAP and a pulsatile component, PP *(Figure 1.25).*
2. The BP curve propagates at a certain velocity along the arterial tree thus causing specific changes in PP *(Figure 1.22).*
3. The influence of the above two haemodynamic mechanisms varies consistently with age *(Figure 1.22).*

PP arises as a consequence of the episodic nature of cardiac contraction and the properties of the arterial circulation. Thus while MAP is adequately described by CO and total PR, the origins of PP are more complex. PP depends upon left ventricular ejection and the properties of the arterial wall, which determine the compliance and the transmission characteristics of the arterial system. The concept that pulsatile component of BP per se has a role in cardiovascular morbidity and mortality, in addition to systolic, diastolic or mean BP, is difficult to demonstrate. Early reports from the Chicago and Framingham studies (Kannel and Stokes 1985; Franklin et al. 1997; Kannel et al. 1971) suggested that PP was not superior to SBP as a determinant of cardiovascular risk in hypertensive persons>45 years old. However, the magnitude of PP depends upon an increase in SBP, a decrease in DBP or both. The last of these is specifically observed in the presence of arterial stiffness- a condition observed mainly in elderly individuals with hypertension but also in diabetes, end-stage renal disease or both. Increased conduit vessel stiffness is an integral component of the pathophysiology of isolated systolic hypertension (ISH). Once thought to be a benign process of ageing, ISH clearly imparts an adverse prognosis and anti-hypertensive treatment effectively reduces this risk. These studies provide a link between pulse pressure, which is increased in ISH and adverse events and demonstrate that the process is amenable to treatment.

There is a rapidly growing body of evidence that confirms an association between PP and adverse clinical events. It is likely that abnormal conduit vessel stiffness mediates this adverse association. There has been considerable progress in the understanding of the nature of increased arterial stiffness and its effects on the circulation. A stiff arterial tree, given the same stroke volume and ejection rate, will produce a higher SBP, a lower DBP and a wide PP but the MAP would be unchanged (Milnor 1982; Safar et al. 1998). In the normal circulation, 85-90 % of the cardiac effort is spent in driving the blood steadily through the peripheral arterioles. The remaining 10% to 15% is 'wasted 'in making flow pulsatile. Some of the cardiac energy spent in distending the arterial tree is returned to the circulation in diastole, because of the elastic nature of the proximal aorta (*windkessel effect*) and some is dissipated as heat. Although the pulsatile losses are small they can be much higher in patients with arteriosclerosis. In experimental animals with an artificially stiffened aorta, the pulsatile energy losses can be 50% of the total (O'Rourke and Taylor 1967; Milnor 1982) and can increase myocardial oxygen consumption by 30% (Kelly 1992).

The problem of arterial stiffening is further complicated by the heterogeneity of the arterial tree. The proximal aorta is compliant and well suited to accepting the left ventricular stroke volume with a relatively low SBP. The carotid arteries are only slightly less compliant. However, as the aorta proceeds distally, it becomes stiffer with more smooth muscle in the walls resulting in the femoral, brachial and radial arteries being much more stiffer. Such an arrangement permits the proximal aorta to accept the stroke volume at a lower peak SBP (smoothing function) and returns some of the energy in diastole. The peripheral arteries increase the SBP due to reflected waves and dampen the flow pulsation in preparation for steady flow through the PR. The architecture of the arterial system is admirable; the ventricular–vascular coupling is designed to receive pulsatile flow and deliver steady flow (López et al. 2000).

1.10.1 Pulse pressure in Hypertension: Hypertension accelerates the vasculopathy of ageing and in hypertensive patients > 55 years old, the central aorta dilates and becomes less distensible. This leads to a disproportionate increase in SBP, from loss of the

cushioning function of the proximal aorta and the addition of reflected waves during systole. All this may occur without a significant increase in MAP or PR but with an associated decrease in DBP. Although the heart faces the same MAP and PR but the external work increases due to the need to raise the SBP disproportionately. It is now well established that LVH is strongly associated with an increased SBP, increased PP and PWV but is largely independent of MAP (Pannier et al. 1989; Marchais et al. 1993). Simultaneously lowering the DBP also has deleterious effects on coronary perfusion, especially in the presence of coronary stenosis (Kelly 1992). It is not surprising that over the age of 55 years, cardiovascular complications of hypertension are related better to SBP and PP than to DBP or MAP (Liu et al. 1989; Franklin et al. 1999b; Franklin et al. 1999a).

1.10.2 Increased Pulse Pressure and End-Organ Damage: A number of studies have investigated relationships between PP and surrogate end-points such as vascular structure and LVH. An important consideration in interpreting studies on PP and clinical and surrogate endpoints is the extent to which the effects of pulse pressure can be shown to be independent of the effects of reduction in MAP.

PP was the only significant predictor of wall thickness/lumen ration in subcutaneous arterioles harvested from skin buttock biopsies of normotensive and hypertensive subjects (James et al. 1995). PP remained significant in the presence of age and other clinic BP terms. In a study of hypertensive subjects, those with a PP $\geq$ 60 mm Hg had higher values for left ventricular mass than those with PP$\leq$ 60 mm Hg, despite similar MAP (Pannier et al. 1989). In a cross-sectional study of normotensive and untreated hypertensive subjects, carotid-intima media thickness was related to carotid, but not to radial PP or MAP (Boutouyrie et al. 1992). In a study of patients undergoing 24-h intra-arterial BP monitoring on the basis of elevated clinic BP, both carotid intima-media thickness and left ventricular mass were related to baseline PP after an average follow-up of more than nine years (Khattar et al. 1997). In an eight-year follow-up study of women traversing menopause, baseline SBP, but not DBP was predictive of increases in both coronary and aortic calcium scores (Kuller et al. 1999). Regression of LVH by pharmacological treatment in spontaneously hypertensive rats was dependent on changes in pulsatile load, despite similar effects on MAP (Mitchell et al. 1996).

1.10.3 Increased Pulse Pressure and Cardiovascular Risk: In the Framingham study, while SBP, DBP and PP were all positively related to outcome when entered individually, when entered in combination, the association was negative for DBP, with PP being as good a predictor as other terms for the middle-aged and elderly (Franklin et al. 1999b; Franklin et al. 1999a). The relative effects of SBP, DBP and PP were evaluated in the Hypertension detection and Follow-up Program; PP was shown to be an independent predictor of total mortality in a logistic regression model that included age, race, sex, randomised anti-hypertensive therapy, diabetes, hypertensive end-organ damage and smoking. The pulsatile component of BP, derived from PP was associated with electrocardiographic evidence of LVH in a large cross-sectional survey (Darné et al. 1989). In a perspective model from the same study, increased pulsatile component was associated with death from CHD in women over 55 years of age. In a recently published update to this study, with 19.5 years of follow-up in 19 083 men, increased PP proved to be associated with adverse cardiovascular events in men after adjusting for age, cholesterol levels and smoking. In a further analysis, higher PP predicted an increased risk both in normotensive and hypertensive men.

The Survival and Ventricular enlargement study (SAVE) was the first to evaluate the effect of PP on outcome in post-MI patients with impaired left ventricular function (ejection fraction < 40%). A strong relationship was observed between PP and total mortality as well as recurrent MI (Mitchell et al. 1997). These observations were extended to include patients with symptomatic left ventricular dysfunction in the Studies of Left Ventricular Dysfunction (SOLVD) (Chae et al. 1999). Higher PP remained a significant, independent predictor of an adverse outcome. The SAVE and SOLVD studies confirmed that the relationship between PP and adverse events is independent of a passive increase in conduit vessel stiffness resulting from an increase in MAP alone. PP was shown to be an important predictor of the risk of congestive heart failure in the elderly with no previous history of heart failure. In a recent presentation of Framingham data, the relationship between BP and events during 20 years of follow-up was evaluated in patients over 50 years of age. After controlling for the level of SBP, DBP was shown to have an inverse relationship with adverse events at all levels of SBP. Consequently, PP was found to be

superior to either systolic or diastolic BP in predicting events in older adults (Franklin et al. 1999a; Franklin et al. 1999b; Franklin 1999). These studies raise the pertinent question of staging hypertension on the basis of either systolic or diastolic BP alone. Using current criteria, patients with a lower PP at a given SBP may be assigned a higher stage of hypertension on the basis of their DBP, despite having a lower risk of an adverse event.

Because PP is influenced by ventricular ejection and arterial stiffness, and because ventricular ejection tends to decrease with age, the question is whether PWV, a classical marker of arterial stiffness can be a marker of vascular disease and cardiovascular mortality in hypertensive patients. In a cohort of 241 patients with end-stage renal disease with an average follow-up of 11 years, only age at entry and PWV were the two dominant predictors of cardiovascular and all cause mortality (Blacher et al. 1999b). The odds ratio for a PWV grater than 12 m/sec compared with one less than 9.4 m/s were 5.4 for all cause mortality and 5.9 for cardiovascular mortality. In individuals with hypertension, the situation is far more complex as there are no longitudinal studies on arterial stiffness as a cardiovascular risk factor. However, calculation of cardiovascular risk using Framingham equations can partially resolve this problem. This was done in a study including 710 patients with hypertension, in whom the odds ratio of being in a high-risk group on the basis of presence or absence of various risk factors was assessed. The risk of any cardiovascular complication increased in parallel with the increase in PWV. Furthermore, at any given age of the individual, aortic PWV was the best theoretical predictor of cardiovascular mortality. The odds ratio of being in a group at high risk of cardiovascular mortality (5% for 10 years) for patients with PWV greater than 13.5 m/s was 7.1 (Anderson et al. 1991). These findings are in agreement with the recent longitudinal study that showed that the ratio between stroke volume and PP, an indirect marker of arterial stiffness, was an independent predictor of cardiovascular risk (De Simone et al. 1999).

In summary these findings suggest that PP and increased aortic PWV should be considered as strong predictors of cardiovascular risk regardless of whether these vascular factors are considered to have a causative role in cardiovascular disease or merely serve as markers of vascular disease already present. The former hypothesis should be tested by using specific interventional studies.

1.10.4 Interventional Studies on PulsePressure: Interventional studies have not been designed specifically to address the question of PP. However, there have been studies with ISH as the entry criterion. Since such studies have required normal DBP, they have invariably included patients with elevated PP. In the Systolic Hypertension in Europe (SYS-EUR) study with the calcium channel blocker nitrendipine, there was a significant reduction of 41% in stroke and a reduction of 26% in combined coronary events (Staessen et al. 1997). In the SHEP trial, there was a reduction of 36% in stroke and 27% in combined coronary events (SHEP Co-operative Research Group 1991). The Systolic Hypertension in China study of ISH also reported significant reductions in both stroke and combined coronary events (Staessen et al. 1997; Wang et al. 2000). A recent meta-analysis found that active treatment in patients aged ≥ 60 years with SBP≥ 160 mm Hg and DBP ≤ 95mm Hg reduced stoke by 30%, coronary events by 23% and total mortality by 18%(Staessen et al. 1997).

1.11   Epidemiology Of Arterial Stiffness

Arterial stiffness is increasingly being recognized as an important cardiovascular risk factor (Arnett et al. 1994). The aorta and the large arteries serve as major conduits and buffering organs in the arterial system. Increased aortic stiffness elevates SBP and PP with increased afterload, reduces subendocardial blood flow and augments pulsatile stress in the peripheral arteries. Arterial stiffness increases with elevated BP, however there are several other physiological as well as pathological states that can affect the stiffness of large arteries. The following is an overview of the epidemiology of arterial stiffness:

1.11.1.Physiological Factors

1. Age: Numerous population studies have shown that aortic PWV increases with age in both sexes. Relf et al. (1986) in healthy men found PWV to increase with age, independently of other cardiovascular risk factors such as high BP or increased lipid levels. Laogun and Gosling (1982) have studied the effect of age and gender on in vivo arterial compliance and found that compliance peaks around 10 years of age and thereafter declines more so in men than women until 50 years of age, thereafter the rate of decline is the same in both genders.

Avolio et al. (1985) measured aortic PWV in an urban and a rural Chinese population (age 2 months to 94 years) with a low prevalence of atherosclerosis but differing prevalence of hypertension. In both groups PWV increased significantly with age (r=0.55, p<0.05) but to a lesser extent in the population with the low prevalence of hypertension. In these studies, the aorta was affected more by age-dependent changes than the peripheral arteries.

In the Complior® study, Asmar (1999) analysed the determinants of aortic PWV in untreated hypertensives performed in about 2000 patients from 19 different countries including our centre. The results show age, followed by SBP as a major determinant of aortic PWV. Benetos et al. (1993) assessed the effects of age on local cross-sectional distensibilty coefficients at the carotid and femoral arteries. Their results showed that age was strongly correlated with arterial distensibilty at the site of the carotid but not at the femoral artery.

Frederick Akber Mahomed first pointed out the altered pressure wave contour in older individuals (Mahomed 1872). Kelly et al. (1989a) have shown that the age-dependent changes in the pressure wave contour are due to early wave reflection and increased PWV. The augmentation in late systolic pressure peak was found to be substantial and represented an increase in PP of about 25% between the ages of 30 and 60 years. Vaitkevicius et al. (1993) studied AI% as well as PWV in healthy subjects (age 21-96 years). Both measures of aortic stiffness significantly increased with age without a gender difference (AI% r =0.61 to 0.63, p<0.0001 for males; PWV r = 0.63 to 0.50 for females, p< 0.0001).

In summary, these studies have shown that arterial stiffness increase with age. The increase in arterial stiffness with age is more marked in the central elastic compared to peripheral muscular arteries. These changes have been described in different populations using different methodologies, independent of BP or any other cardiovascular risk factors. In the aorta, the greatest change occurs between 10 and 50 years, an increase of almost 60 %, but in the peripheral arteries the increase is of the order of only 20 %. Therefore, the

aorta, which is more elastic than the peripheral arteries in young age, becomes as stiff as the peripheral arteries with advancing years.

Thus the increase in aortic stiffness with age occurs gradually and continuously throughout life and is not related to increased pressure or atherosclerotic disease. The effects of repetitive cyclic stress cause fatigue fracture of the elastic load-bearing fibres of the arterial wall with dilatation of the artery. This leads to the transfer of the load to the less distensible collegenous fibres with subsequent breakdown of the elastic tissue and its replacement by collagen tissue. In addition, in advancing age, vasa vasorum flow decreases (Stefanadis et al. 1995c) which may lead to malnutrition of the outer layers of the arterial wall and induce detrimental changes (Nichols and O'Rourke 1998b).

2. Gender: Premenopausal women tend to have less stiff arteries than men until the menopause (Lehmann et al. 1992a; Rajkumar et al. 1997). Post-menopausal women have stiffer arteries than pre-menopausal women (Rajkumar et al. 1997). Elevation of aortic stiffness tends to be lower in women taking estrogens, whereas after 4 weeks of cessation of hormone replacement therapy values increase again. These effects of menopause on arterial stiffness tend to be more pronounced in hypertensive women, who already have evidence of vascular dysfunction (Karpanou et al. 1996). Dernellis et al. (1998) have suggested that estrogens may alter the elastic properties of the aorta through nitric oxide (NO) mediated endothelium dependent vasodilatation. Also, acute administration of estrogens improved arterial distensibility and reduced arterial wave reflection in post-menopausal women both with and without CHD. Longer-term effect of estrogens may involve structural changes in the arterial wall (Rajkumar et al. 1997).

Apart from the difference in hormonal status, women on average are of shorter stature than men. London et al (1995) reported a higher AI% in women compared to men as arterial wave reflection occurs earlier due to shorter effective length of the arterial system in women. In pre-menopausal women the beneficial effects of estrogens may offset these differences and furthermore, the faster HR in women tends to counteract the effects of short stature. However, not all studies have supported the gender difference in the AI% (Vaitkevicius et al. 1993).

3. Body Height: Body height has been described as an important determinant of arterial wave reflections (Smulyan et al. 1998) and thus short body height is associated with higher SBP, high PP and increased cyclic arterial stress. The generally smaller body size of woman may be responsible for increased arterial stiffness after menopause when the compensating effects of estrogens have subsided (London et al. 1995). In patients with end-stage renal disease, short body height has been found to be an important factor associated with an increase in AI% (London et al. 1992a).

4. Blood Pressure: High distending pressure expands the aorta and most of the stress is transferred from the elastin to the less distensible collegenous fibres. This per se renders the aortic wall more rigid. In vivo, in man, BP has been found to be an important, independent predictor of arterial stiffness, not only in patients with essential hypertension, but also in the normotensive population, though age-dependent changes in the arterial structure and function are difficult to separate from the pressure-dependent changes.

As far back as 1878, Moens showed that the elasticity of blood vessels as measured by PWV varied with BP. Grunmach in 1885 confirmed his observations and suggested that PWV may vary considerably with the level of BP. However, Dawson in 1917 found no consistent relationship between PWV and BP in animal studies. Bramwell et al in 1924 in a series of ingenious experiments confirmed conclusively that the velocity of the pulse increased with the BP and that the rate of propagation of the pulse wave was a mechanical phenomenon depending on the elasticity of the vessels. Sands (1924) suggested that the relationship, which occurs between DBP and PWV, may no longer be valid in patients with vascular dysfunction and described in various disease states better correlations with systolic than DBP. They suggested that factors such as 'hardening' of the vessel wall and ' muscular contraction' modify PWV in normal and abnormal subjects so that DBP is not a dominant factor. Hence in normal subjects, PWV correlates equally well with systolic or DBP but in abnormal cardiovascular states, these relationships are considerably modified. Haynes et al. (1936) analysed the relationship between PWV and BP in hypertension, arteriosclerosis, related conditions and complications. Their results showed a close relation between PP and PWV with a stronger correlation with the aortic PWV than with the arm or leg PWV.

All these studies performed in the beginning of the 20th century show that BP may influence PWV to a greater extent than structure of the vessel wall or dimensions of arteries. However, in most of these studies there are methodological pitfalls and age, as an important factor has not been taken into consideration. In fact, PWV increase with advancing age, closely following the rise in BP, which occurs as one, gets older. Eliakim et al. (1971) found higher PWV values in hypertensive subjects only after age 60. Schimmler (1975) found a definite increase in aortic PWV with MAP at all ages in a German population, though a less definite relationship was found by Avolio et al. (1983) in a Chinese community and by (Ho et al. 1983).

More recent studies performed in untreated large populations of normotensives and hypertensives with a large age range and without any cardiovascular disease found age and systolic blood pressure the most important determinants of PWV, in that order, with age being the most important determinant of aortic PWV whereas, for the peripheral PWV, the role of BP dominated (Asmar et al. 1995b). The higher correlation coefficient found between aortic PWV and SBP can be expected since the major determinant of SBP is known to be arterial stiffness of the large arteries.

5. *Hormonal State*: As considered earlier, **estrogens** may be responsible for differences in the arterial elastic properties between pre and postmenopausal women and between postmenopausal women with and without hormonal replacement therapy. Another interesting physiological state is **pregnancy** where marked hormonal changes may take place in many systems. Poppas et al. (1997) investigated the effect of pregnancy on arterial stiffness and timing of arterial wave reflection. Pulsatile arterial load was decreased beginning in the first trimester, thus helping to accommodate the increased intra vascular volume, while maintaining the efficiency of ventricular-vascular coupling. The arterial wave reflections were also significantly reduced in amplitude and were also delayed in the ascending aorta. Symptom free **growth hormone** deficient adults had significantly less distensible aortas than age and sex matched controls most likely related to increased intima media thickness (Lehmann et al. 1993). Infusion of **insulin** leads to favourable effects on the arterial pressure waveform (Westerbacka et al. 1999). Abnormalities in the

aortic pressure waveform have been reported in patients with primary **hyperparathyroidism** (Smith et al. 2000).

6. Heart Rate: Most pacing studies done in the past have shown no effect of change in HR on PWV (Eliakim et al. 1971). High HR is associated with increased aortic PWV even after adjustment for age and BP in both hypertensive and normotensive populations (Sa Cunha et al. 1997). However, the recent Complior study showed HR to be a minor determinant of PWV (1%) in untreated hypertensive patients (Asmar 1999). The AI% varies with HR due to the proportionality between ventricular ED and cardiac cycle duration (Braunwald et al. 1958; Wallace et al. 1963) the peak of the forward travelling wave would be delayed at lower heart rates and vice versa. Thus even with a fixed reflection site and same PWV, there would be an altered relationship between forward and backward travelling waves with changes in HR. AI% is thus inversely related to HR (Cameron et al. 1998; Gallagher 1994). Expressed in quantitative terms, changes in heart rate account for 4% to 6% increase or decrease in the AI% for a change of 10 beats/min in HR (Wilkinson et al. 2000a).

Moreover, increase in HR increases amplification of the pulse wave between the aorta and peripheral arteries. This is due to the greater amplification of individual harmonics at frequencies close to the maximum of 5 to 6 Hz. It should be emphasised that these changes should be taken into account in the interpretation of results after interventions that alter HR.

1.11.2 Genetic Factors: For a similar degree of risk, arterial stiffening may be more or less pronounced as a function of genetic factors. Identification of such genetic factors may be of major interest in the detection of high-risk patients. In humans, some of these factors can now be identified by studying polymorphisms of genes coding for proteins that are implicated in cardiovascular regulation (candidate genes).

1. Nitric oxide synthase gene polymorphisms: Because of the important effects of NO on BP and arterial wall tone and a possibly reduced basal release of endothelial NO in hypertension, the NO synthase gene (eNOS), which has been shown to be ploymorphic, is a putative candidate gene for hypertension and arterial stiffening.

No link has been found between hypertension and the eNOS gene (Bonnardeaux et al. 1995). Lacolley et al. (1998) evaluated the association of arterial stiffness (PWV) with two recently described polymorphisms of the eNOS gene; namely the $G^{10}T$ polymorphism and $G^{298}T$ polymorphism. They found that the distribution of $G^{10}T$ polymorphisms among hypertensives and normotensives were similar but the prevalence of $G^{298}T$ polymorphisms was higher among hypertensives than in normotensives. No association of eNOS genotypes was found with blood pressure levels or PWV.

2. *Genetic factors of the Renin-Angiotensin system:* ACE genotype may be an independent risk factor for cardiac and arterial hypertrophy and myocardial infarction (Cambien et al. 1992). Benetos et al. (1996b) found that the ACE gene insertion-deletion polymorphism is weakly associated with aortic stiffness as measured by carotid –femoral PWV in both normotensive and hypertensive subjects. In contrast, other studies have shown as association between increased intima-media thickness of the carotid artery with ACE D allele (Castellano et al. 1995). Such studies require confirmation.

1.11.3 Nutritional Factors: Peripheral PWV is increased and aortic PWV decreased after a meal in young men (Klip 1958). Also, wave reflection is reduced and the late systolic peak decreased due to dilatation of arteries in the splanchnic bed after ingestion of meals (Gallagher 1994) Chinese migrants settled in Australia for 10 years had stiffer aortas than recent migrants suggesting an unfavourable effect of the western diet on arterial stiffness (Dart and Qi 1995). PWV has been shown to be significantly lower in a population with higher fish-consumption in Japan (7.0 vs 7.7 m/sec) (Hamazaki et al. 1988) compared to low fish consumption population. Normotensive adults who had been on a low salt diet for 8 months to 5 years, showed significantly lower PWV than age and BP matched controls (Avolio et al. 1986). In genetic models of human and rat hypertension, increased sodium intake is associated with specific alterations of structure and function of conduit arteries involving extracellular matrix, but independent of BP and atherosclerosis (Safar et al. 2000a).

Chronic intake of garlic reduced aortic stiffness significantly in healthy non-smokers compared to age and BP matched controls (8.3 vs 9.8 m/sec). The difference can be

attributed to the direct NO-mediated effect of garlic on the aortic wall rather than by BP or lipids reduction (Breithaupt-Grögler et al. 1997). Moreover, studies have shown that insulin rapidly decreases wave reflection (Westerbacka et al. 1999). Oral vitamin C has been shown to significantly reduce the AI% (by $9.6\pm3\%$; $p<0.01$) compared to placebo in healthy subjects. The authors have suggested that this beneficial effect may involve protection of NO from breakdown by oxygen free radicals (Wilkinson et al. 1999). Although, elevated levels of plasma homocysteine have been related to systolic hypertension (Sutton-Tyrrell et al. 1997), there appears to b little effect on the stiffness of the carotid or femoral arteries (Smilde et al. 1998; van Guldener et al. 2000).

1.11.4 Smoking: Acutely, smoking a cigarette stiffens both central and muscular arteries in healthy adults and in men with CHD (Kool et al. 1993; Stefanadis et al. 1997b). Basal difference in terms of long-term smoking was not observed between smokers and non-smokers (Kool et al. 1993; van den Berkmortel et al. 1999). In hypertensive women, no association was found between smoking history and aortic stiffness (Taquet et al. 1993). However, the brachial artery pressure waveform of long-term smokers compared to non-smokers is significantly abnormal (McVeigh et al. 1997). Passive smoking increases aortic stiffness in middle-aged men with CHD (Stefanadis et al. 1998b). These acute effects of smoking could be due to both an increase in BP and active stiffening of the vessel due to contraction of the smooth muscle cells in the vessel wall.

1.11.5 Physical Fitness & Exercise: Aortic PWV and AI% varied inversely with maximal oxygen consumption (VO $_{2max}$) measured during treadmill exercise in male athletes (54-75 years) who had lower arterial stiffness indices (PWV –26%, AI% -36%) compared to their sedentary age-matched peers for the same level of BP (Vaitkevicius et al. 1993). Exercise training of sedentary males for 4 weeks increases their systemic arterial compliance and is independent of the fall in BP and is linearly related to overall mean maximum oxygen consumption (Cameron and Dart 1994). Moreover, muscular strength training has been found to be associated with lower systemic arterial compliance and higher PP, but unchanged carotid-femoral PWV in non-smoking male athletes training for 12 months compared to their sedentary peers (Bertovic et al. 1999). Physical fitness results in decreased augmentation of the pulse in different arteries and partially reverses

the ill effect of ageing on the arterial pulse (Vaitkevicius et al. 1993). Recent studies have shown improvement of aerobic exercise performance associated with decrease in carotid augmentation shortly after pharmacological intervention (Chen et al. 1999).

During exercise, the contour of the ascending aortic pressure waveform remains the same and aortic PP is increased proportionally to the increase in stroke volume (Kroeker and Wood 1955; Murgo et al. 1981; Rowell et al. 1968; Laskey and Kussmaul 1987). However, the most prominent change with leg exercise is the increase in PP amplification, which could be 80 mm or so greater in the radial artery than in the ascending aorta. When this amplification is not taken into account, it is most likely that misleading interpretations of phenomenon during exercise will be derived from upper limb measurements of arterial pressure (Nichols & O'Rourke 1998).

1.11.6 Disease States Influencing Arterial Stiffness

1. Hypertension: Essential hypertension combines increased PR, high PWV and early wave reflections (Ting 1993). Generally the fatiguing effects of cyclic stress seen with ageing are accelerated in hypertension. Increased smooth muscle tone, heterogeneous structural changes as well as diminished vasa vasorum flow seem to be involved in hypertension-related functional changes of arterial elastic properties as already discussed.

Aortic distensibility in the ascending, descending and abdominal aorta was significantly decreased in patients with essential hypertension compared with age-matched controls and was inversely related to SBP (Resnick et al. 1997). Borderline hypertensives have significantly higher aortic PWV than age-matched controls (7.6 m/s vs 6.8 m/s) (Girerd et al. 1989). Also, white coat hypertension is associated with increased PR and reduced arterial compliance (Soma et al. 1996). Aortic stiffness was compared in a cross-sectional approach in normotensives, untreated hypertensives and treated hypertensives and PWV was found to be lower in treated than untreated hypertensives. However, despite similar DBP, the age-dependent increase in PWV was still more prominent in well-controlled hypertensives than normotensive controls (Asmar et al. 1995a).

The aortic impedance modulus Z_i of the first harmonic of the pulse was related to the degree of augmentation and showed a close correspondence for persons both with and without hypertension (Nichols et al. 1992). However, a recent study showed that PP and PWV, but not carotid AI%, were significantly and independently associated with cardiovascular complications in essential hypertension (Asmar et al. 2001). The recent ARIC study found hypertension to be associated with carotid artery stiffness as measured with the B-mode ultrasound but the changes were BP dependent (Arnett et al. 2000).

2. Hypercholesterolaemia: In the monkey, PWV closely related to aortic stiffness and showed regression following the introduction of low cholesterol diet (Farrar et al. 1978). In man, the results are however, still controversial despite quite a number of studies. Elevated cholesterol levels, in the absence of overt atheroma, appear to be associated with reduced, rather than enhanced aortic stiffness. In a study of asymptomatic subjects with marked hypercholesterolaemia, aortic stiffness showed a less steep increase with age than for the control subjects (Dart et al. 1996). Similarly, very young subjects with familial hypercholesterolaemia showed more compliant large arteries (Lehmann et al. 1992b), whereas somewhat older adults with familial hypercholesterolaemia showed reduced large artery compliance in one study (Pitsavos et al. 1998) but not in another (Toikka et al. 1999). Asymptomatic 35-year old men showed a negative correlation between aortic stiffness and low-density lipoprotein cholesterol (Kupari et al. 1994). Chinese subjects with a low prevalence of atherosclerosis were studied and no relationship was found between cholesterol levels and PWV (Avolio et al. 1985). The authors conclude that age-dependent arterial stiffening was not due to atherosclerosis but related more to the prevalence of hypertension and salt intake.

3. Diabetes Mellitus: Diabetic patients are at high risk of developing atherosclerosis and subsequent cardiovascular events. Several trials have been performed in different diabetic sub-populations, which have been reviewed by (Lehmann et al. 1997). Glucose intolerance is associated with increased arterial stiffness in a large population of men and women and was positively associated with insulin and triglyceride levels (Salomaa et al. 1995). A positive family history of non-insulin dependent diabetes mellitus (NIDDM) is associated with decreased arterial distensibility in young normoglycaemics (Hopkins et al. 1996). Furthermore, in the ARIC study, carotid artery stiffness was positively correlated with

70

plasma glucose concentration (Arnett et al. 2000). Abnormalities of the brachial artery pressure waveform, consistent with increased arterial stiffness, can be detected early in the course of NIDDM before complications ensue (McVeigh et al. 1994). It is unlikely that atherosclerosis per se accounts for the stiffer aortas in NIDDM patients as arterial stiffness can be detected before the onset of frank diabetes (Salomaa et al. 1995). However, it has been argued that increased PWV in diabetic subjects is a marker of early atherosclerosis (Woolam et al. 1962). Young insulin dependent diabetics (IDDM) have elastic aortas compared to older NIDDM patients though the latter have increased aortic stiffness compared to age-matched controls (Lehmann et al. 1997). No reduction in distensibilty of the elastic and muscular arteries was found in patients with uncomplicated Type I diabetes (Kool et al. 1995). Diabetes appears to blunt the protective effects of female sex hormones. In female IDDM and NIDDM patients, but not male, aortic stiffness was higher compared to age and gender-matched controls (Lehmann et al. 1997). Patients with type I diabetes have increased augmentation in the central pressure waveform and unfavourable subendocardial ratio (Brooks et al. 1999; Wilkinson et al. 2000b).

Whether the changes in arterial stiffness in diabetes are simply a marker of occult atheroma or are causally involved in parthenogenesis, is still unclear. Moreover the validity of measuring arterial stiffness in the diabetic patients in clinical practice remains to be established.

Coronary Artery Disease: Aortic distensibilty in normotensive patients with CHD and angiographically normal aortas was found to be significantly lower as compared to age-matched controls with chest pain but with normal left ventricular function (Stefanadis et al. 1990). Moreover patients with two and three-vessel disease have stiffer aortas than patients with one-vessel disease (Triposkiadis et al. 1993). Non-invasive assessment of cardiovascular risk can give more information, it is however not a definitive diagnostic test for CHD (Cameron et al. 1996).

4. Cerebrovascular Disease: PWV measured in stroke patients showed stiffer aortas compared to age and sex-matched controls but BP was higher in the former. Calculation of a BP independent index of aortic compliance did verify the observation the stroke patients have stiffer aortas (Lehmann et al. 1995).

5. *Renal Failure And Dialysis*: Aortic PWV was significantly increased in patients on haemodialysis compared to age and BP matched controls (11.13m/s vs 9.65 cm/sec) (London et al. 1992a). Blacher et al. (1999b) calculated mortality rates in end-stage renal disease patients (n=200, mean age 51.7 years, follow-up for 56 months) and found aortic stiffness to be a strong and independent predictor for all-cause and cardiovascular mortality. Patients with end-stage renal disease also have increased wave reflection as compared to age and BP matched controls (London et al. 1994).

6. *Atherosclerosis*: Considering the complex relationship between atherosclerosis, age, BP and the other cardiovascular risk factors, which influence arterial stiffness, it is difficult to analyse the complex relationship between arterial stiffness and atherosclerosis *per se*. It is recognized that coronary atheroma is often accompanied, even at an early age by disease in the large blood vessels, particularly the thoracic and abdominal aorta (Vihert 1976; McGill et al. 1997). Indeed one explanation for the observed relationship between elevated inflammatory risk markers such as C-reactive protein and future coronary events (Kuller et al. 1996) can be such that this elevation is marker of widespread atheromatous process. The aorta and major arteries are known to stiffen with age but several studies have found that the presence of widespread atheroma amplifies this event. Thus measurement of stiffness in the proximal ascending aorta, the aortic arch and the abdominal aorta has been shown to be elevated in CHD (Hirai et al. 1989; Stefanadis et al. 1990; Dart et al. 1996; Gatzka et al. 1998). Similarly arterial compliance, largely contained within the aorta, can be shown to be significantly lower in such patients (Cameron et al. 1996). A recent study has shown that elevation in PP in patients with CHD was closely associated with an increase in the electrocardiographically measured arterial stiffness (Gatzka et al. 1998). PWV was related to atherosclerotic risk factors (Asmar et al. 1995b) and more recently, the Rotterdam study looked at the association between indicators of atherosclerosis and PWV in an elderly population and found a close relationship between intima-media thickness of the carotid artery and PWV (Van Popele et al. 1998).

The demonstration of an association between arterial stiffness and atherosclerotic processes including CHD may suggest the possibility that a rise in PP due to degenerative changes in the arterial wall could in turn cause amplify the subsequent development of

atherosclerosis (Avolio et al. 1998) and then initiate a vicious cycle inducing further repetitive arterial wall damage. However, despite a plausible association between PP and atherosclerosis, whether high PP could actually cause atherosclerosis is not established. Changes in arterial stiffness may begin early in the development of experimental atherosclerosis (Hiromoto et al. 1996) and can be exacerbated by mechanical damage (Hayashi and Matsumoto 1994).

1.11.7 Effect of Antihypertensive Agents on Arterial Stiffness: A number of studies have been carried out to compare the effects of different agents and classes on arterial stiffness, but some additional factors need to be considered. Thus, the non-linearity of arterial elasticity means that a reduction in distending pressure will, *parri passu*, leads to a reduction of arterial stiffness. There may also be heart rate effects on arterial properties (Mangoni et al. 1996; Lang et al. 1994; Wilkinson et al. 2000a). An additional consideration in many studies is the location and class of artery under investigation. Thus many studies have just examined the muscular or peripheral arteries, such as the brachial artery, which may not be representative of the proximal vessels. The pathophysiological consequences of changes in arterial stiffness largely relate to a change in central, rather than peripheral pressures.

1. Angiotensin –converting enzyme inhibitors: In a placebo-controlled crossover study of the acute effects of the angiotensin-converting enzyme (ACE) inhibitor, quinapril, in patients with essential hypertension, there were increases in carotid artery distensibilty and aortic compliance (measured by PWV) and a reduction in arterial wave reflection. The carotid artery effects were all attributable to the BP reduction, but the changes in PWV and arterial wave reflection were in part independent of blood pressure changes (Topouchian et al. 1998). Pulse pressure was reduced by a similar amount at both carotid and brachial sites. In an invasive study with intra-venous captopril, large artery compliance was lower in hypertensive compared to normotensive subjects and was increased by captopril but not to the values found in normotensive subjects (Ting 1993). In another acute study, neither dihydralazine nor perindoprilat significantly altered carotid-femoral PWV in patients with mild to moderate hypertension (Benetos et al. 1990). However, while there was a strong correlation between change in blood pressure and PWV

in the dihydralazine group, no such correlation was found with perindoprilat, which was interpreted as implying a direct arterial effect of the ACE inhibitor. Ramipril reduced aortic PWV in hypertensive subjects treated for 42 days. Although BP was lowered, there was no relationship between the change in PWV and change in mean, systolic or diastolic BP, suggesting a BP independent effect (Benetos et al. 1991). Three months treatment with perindopril increased brachial artery compliance in comparison with placebo in a single-blind study in hypertensive subjects without tangential tension, again suggesting a direct effect on arterial wall properties (Asmar et al. 1988). In a single-blind cross-over comparison of eight weeks treatment with the ACE inhibitor lisinopril or the calcium channel blocker, nifedipine in elderly hypertensive patients, aortic PWV was reduced significantly more by lisinopril, despite an equal reduction in MAP by both drugs, suggesting an arterial wall effect of ACE inhibitors (Shimamoto and Shimamoto 1995).

2.   *Calcium Channel Blockers:* In a double-blind, cross-over comparison of 12 weeks treatment with the calcium channel blocker felodipine or the diuretic hydrochlorthiazide in subjects with mild to moderate hypertension there was more pronounced falls in carotid-femoral as well as peripheral PWV with felodipine. However, felodipine was also associated with greater BP reduction (Asmar et al. 1993). The finding of brachial artery dilatation with felodipine, but not with hydrochlorthiazide, however, indicated direct arterial wall effects of the calcium channel blocker. Brachial-radial PWV was improved by another dihydropyridine, nicardipine, but not by the beta-aderenoceptor antagonist atenolol in hypertensive patients treated for eight months (De Cesaris et al. 1992). In a parallel group comparison of hydrochlorthiazide with nitrendipine in subjects with mild to moderate hypertension treated for two months, there was no significant difference in effects on muscular radial artery compliance (Khder et al. 1998). Effects of nitrendipine (as well as perindopril) in hypertensive subjects appeared to be determined by angiotensin II receptor I genotype. Thus, whereas PWV change with perindopril was more marked in those with a C allele, only those with the AA allele showed a PWV effect with nitrendipine (Benetos et al. 1996a).

3. *Alpha- and beta-adrenoceptor antagonists:* In an acute invasive study in which aortic PWV was inferred from the first minimum of the impedance modulus (Zi) PWV was unaffected by a beta-adrenoceptor antagonist but was reduced by combined alpha- and beta-blockade (Ting et al. 1991). As discussed, in a comparative study with nicardipine, atenolol did not affect peripheral PWV in long-term treatment of hypertensive patients (De Cesaris et al. 1992). However, in another study, atenolol reduced aortic PWV as well as BP (Kelly et al. 1989c). In other studies of beta-adrenoceptor antagonists, whereas six-weeks treatment with metoprolol had no significant effect on brachial-radial or carotid-femoral PWV in hypertensive subjects (Trimarco et al. 1987), four weeks treatment with bisoprolol reduced both peripheral and central PWV (Asmar et al. 1991). And in an eight-week study of hypertensive subjects, comparing atenolol with fosinopril, arterial wave reflection in the ascending aorta was reduced significantly more by fosinopril than by the beta-adrenoceptor antagonist (Ting et al. 1995). However, no adjustment was made for lower HR in the atenolol-treated subjects and as discussed above, AI% increases at lower heart rates.

4. *Diuretics And Nitrates:* Acute administration of isosorbide dinitrate to untreated hypertensive subjects increased compliance at carotid, brachial and femoral sites when measured with an echocardiography-tracking device (Laurent et al. 1992). Nitrate therapy also produced a fall in BP and in wave reflection as assessed by central pressure augmentation (Laurent et al. 1992). A single oral dose of nicornadil reduced BP and peripheral PWV in hypertensive patients (Levenson et al. 1986).

In a comparison of a potassium-losing and potassium-sparing diuretic in hypertensive subjects treated for six weeks, both lowered BP from baseline but did not significantly affect PWV (Laurent et al. 1990).

Despite a considerable body of work, there is still uncertainty over the direct effects of a number of anti-hypertensive agents on large artery properties. However, a number of studies have suggested that ACE inhibitors and to a lesser degree, calcium channel blockers do exert direct arterial wall effects and induce changes not solely due to the passive effect of blood pressure lowering. The newer technique of tonometry and wall tracking do allow examination of arterial wall properties at different pressures but the

studies are limited to accessible peripheral arteries whereas the major contributor to systemic arterial compliance is the aorta, which cannot be studied directly by these techniques. In this respect assessment of central pressure changes by using generalized TF to estimate central arterial pressure waveform and pressures from peripheral recording sites is very promising.

1.12 Aims

Arterial stiffness is increasingly being recognized as an independent cardiovascular risk factor. Arterial pulse wave analysis is a recent development and well established in the assessment of stiffness. My primary objective was to establish the technique of pulse wave analysis in an Irish setting and explore both a healthy and hypertension population for the major determinants of the arterial pulse waveform and possible differences between healthy and diseased population. In the course of this work, epidemiological information was published which established the prognostic value of the measurement of pulse wave velocity and this was then incorporated into the later studies. Specific questions that I wished to address included:

a.   What are the possible determinants of arterial stiffness assessed by AI%, PWV and pulse pressure amplification in healthy and hypertensive populations?

b.   How do common dietary factors such as caffeine and alcohol affect arterial stiffness?

c.   What are the acute effects of smoking on arterial stiffness in a healthy population? Does passive smoking have a similar effect?

d.   GTN decreases arterial wave reflection by the endothelium-independent mediated vasodilator by donating NO in muscular arteries. Does sildenafil, which also modulates this system, have a similar effect?

e.   Do the most recent group of antihypertensive agents, the ATII receptor antagonists affect arterial stiffness and if so, is the effect primarily due to their hypotensive action or possible a direct vascular effect? Furthermore, does the combination of an ACE inhibitor and an angiotensin II receptor antagonist confer any benefit over either drug used singly?

CHAPTER TWO
METHODOLOGICAL CONSIDERATIONS FOR MEASURING ARTERIAL STIFFNESS

2.1. Introduction

PP arises as a consequence of episodic cardiac contraction and the elastic properties of the arterial tree, in contrast to MAP, which is determined by PR and CO. The elastic properties of the arterial tree, or arterial stiffness, can be considered either in terms of a windkessel model giving the measurements of compliance and distensibilty or as a propagative model of the arterial tree, allowing to study the properties of the pressure waves in terms of contour, amplitude, changes between central and peripheral arteries and the speed of travel of the wave between two sites.

2.2. Distensibilty and Compliance

Compliance of a localized arterial segment is simply the increase in volume (V), diameter (D) or cross-sectional area (A), assuming no change in the length of the arterial segment for a given increase in pressure (ΔP) that is

$$C = \Delta V / \Delta P \text{ (cm3. mmHg}^{-1})$$

where ΔV = change in volume and ΔP = change in pressure

for one unit of length, volume = cross-sectional area, depending on diameter, thus

$$C = \Delta D / \Delta P \text{ (cm3. mmHg}^{-1})$$

Aortic as well as brachial pressure, where ΔD = change in diameter and ΔP = change in pressure.

Compliance does not take into account for the resting or diastolic dimensions before distension begins; By comparison, distensibilty includes the value for the resting diameter or cross-sectional area and thus represents change in these variables as a percentage. Thus

$$\text{Distensibility (D)} = \Delta V / \Delta P . V \text{ (mm Hg}^{-1})$$

where ΔV = *change in volume*, ΔP = *change in pressure*, V = *baseline volume*

for one unit of length, volume = cross-sectional area, depending on diameter, thus:

$$D = \Delta D / \Delta P . D \text{ (mm Hg}^{-1})$$

Investigators have used several different methods to derive the distensibility of the arterial wall (Table 2.1).

Table 2.1. Methods for calculating distensibilty of arteries using different non-invasive techniques.

Moens-Korteweg	$PWV = \sqrt{E.h / 2\rho r} = \sqrt{1/\rho DC}$ (m/s)
Bramwell-Hill	$PWV = 0.357. \sqrt{V/(\Delta V/\Delta P}$ (m/s)
Transit time technique	$PWV = L/T$ (m/s)
Elastic (Peterson) modulus	$E_\rho = (D. \Delta P/\Delta D) = 1/(2.DC)$ (pa)
Young's modulus	$E = \rho (PWV)^2 /Dh = (D/h) DC$ (Pa)
Distensibility coefficient	$DC = (\Delta A/A)/\Delta P \approx 2. (\Delta D/D)/\Delta P$ (Pa^{-1})
Compliance coefficient	$CC = (\Delta V/L)/\Delta P = \Delta A/\Delta P \approx \pi. D. \Delta/2\Delta P (mm^2/kPa)$

A more compliant wall would have a larger increment in diameter or cross-sectional area for a given increase in pressure than a less compliant one. By comparison, a more distensible artery would so the same but from a certain baseline diameter, it would undergo a greater percent change in diameter or cross-sectional area for a given PP. These measurements have been made accurately in the peripheral arteries, such as brachial, carotid and radial with the use of high frequency ultrasound. Several methods have been described in literature (Table 2.2).

Table 2.2.   Non-invasive methods for the assessment of arterial compliance and distensibilty used by different investigators.

√Windkessel model	√Continuous wave Doppler (Olsen et al, 1979)
√A-Mode echo (Arndt et al, 1968) √Pulsed wave Doppler (Safar ME, 1981)	
√B-mode echo (Barth et al, 1988) √Multi-gate pulsed Doppler (Reneman et al, 1986)	
√M-mode echo (Roman MJ et al, 1992) √ Doppler-tracking (Hoeks et al, 1990)	

Special ultrasound techniques are also available for measuring wall thickness (Girerd et al. 1994; Roman et al. 1992) that allow for calculation of wall stress and characterization of the arterial wall as a material as well as its behaviour as a visco-elastic tube (Laurent 1995). Similar studies have been performed in the thoracic aorta but are more difficult as it is relatively inaccessible (Isnard et al. 1989). Recently, dimensional changes of the thoracic aorta have been measured from images obtained by trans-oesophageal ultrasound

probe (Girerd et al. 1994) but direct measurements have also been made (Stefanadis et al. 1995b) .

In this thesis, the propagative model of the arterial tree is used. The main estimate of arterial stiffness was the measurement of the amplitude and timing of wave reflection, the *AI%*, using *pulse wave analysis*. The other estimate of arterial stiffness that I used was *carotid-femoral PWV* measurement. I had the privilege to be invited to spend one week in training on the technique and application of PWV with Prof. Ronald Asmar and his team at Institute Cardiovasculairé and Prof. Safar in Hospital Broussais in Paris. PWV measurement was not available in my initial work so it has not been incorporated in my earlier work. Both these methods have been described below.

2.3   Arterial Pulse Wave Analysis

'Pulse wave analysis' has been the used in this thesis to study the dynamic interaction between the left ventricle and the arterial system by analysis of the pressure waveform. The radial artery pressure waveform has been utilized to derive the aortic pressure waveform by the technique of applanation tonometry.

1. Applanation Tonometry: Applanation tonometry is based upon the same principle as used to record intra-ocular pressures, i.e., when two curved surfaces are flattened, circumferential pressures are equalized. In practice a probe with a Millar micromanometer at its tip is used to flatten the artery. This works best for superficial arteries overlying bone, such as the radial or carotid, when the waveform can be recorded with a high degree of accuracy *(Figure 2.1).* Under optimal conditions for applanation the pressure wave measured non-invasively is virtually identical to that recorded from intra-arterial recordings (Kelly et al. 1989b; Chen et al. 1996). In practice, the pressure required to applanate the radial artery ' the hold-down pressure' does vary, so the systolic and diastolic BP cannot be measured reliably. However, in most cases, the contour and amplitude of the pressure waveform (pulse pressure) can be determined reliably.
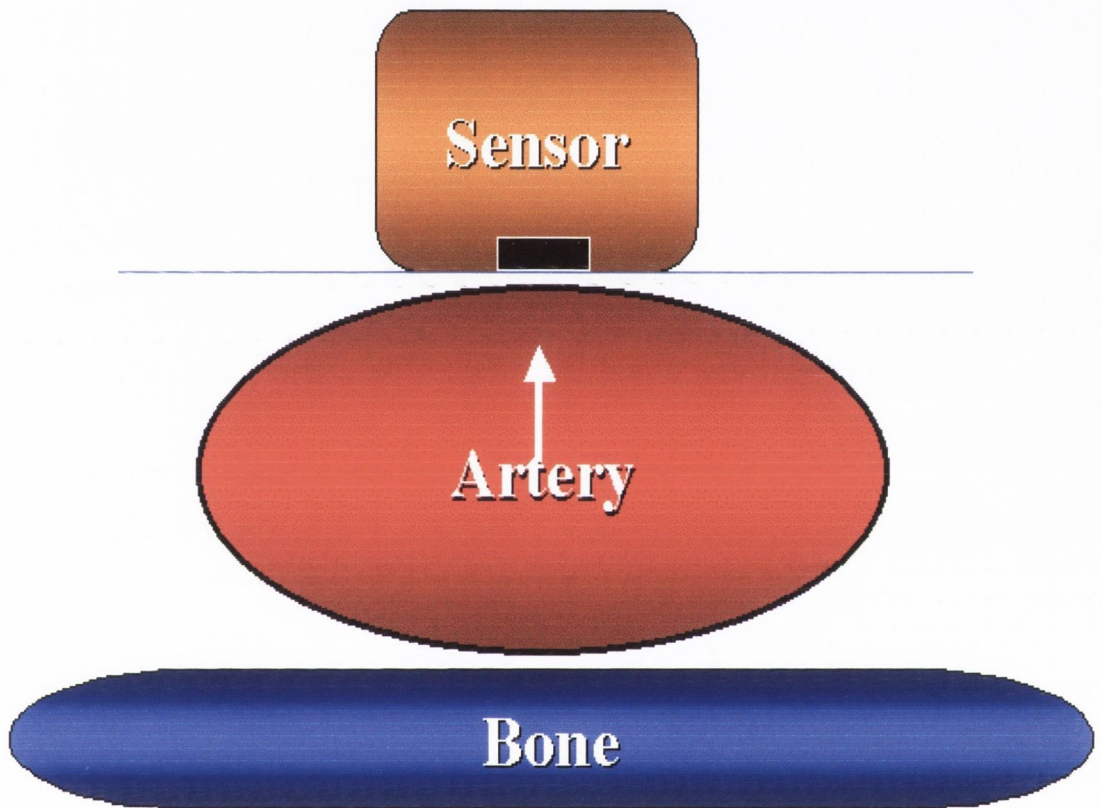
Figure 2.1. The Technique of applanation tonometry. Flattening of the
curved surface of a pressure-containing structure causes a balancing of
the inherent circumferential forces so that the force registered is the
radially directed intra-arterial pressure.

2. Calibration of Radial Artery Pressure Waveform: The values of SBP and DBP are set in the system, assuming that MAP is identical throughout the arterial tree (Kelly et al. 1989b). We have thus, for radial tonometry, set SBP as well as DBP in the radial artery trace to the values determined by values taken from brachial sphygmomanometry.

3. Derivation of the AorticPressure Waveform: A micromanometer-tipped probe (Sphygmocor™, PWV Medical, Australia) was applied to the skin overlying the radial artery and the peripheral radial pulse continuously recorded. For accurate recordings the micromanometer must be applied with light pressure to the vessel wall so that the transmural forces within the vessel are perpendicular to the arterial surface. The arterial pressure waveform is modified during its transit from the ascending aorta to the peripheral vessels, thus the central aortic pressure waveform blood pressure indices (systolic and diastolic BP in mm Hg) are derived from radial tonometry and the peripheral brachial BP assuming that the MAP is identical throughout the arterial tree using a previously validated mathematical transfer function within the software package (Sphygmocor™) The logic behind this is that a recorded peripheral waveform can be broken down into its harmonic components, and from there, the harmonic components of the aortic waveform can be produced and ultimately the aortic waveform can be synthesized. It has been discussed previously how the harmonic components of peripheral and central waveforms are related to each other in amplitude and phase through a relationship termed transfer factor (see pressure pulse in time and frequency domains). This is affected by many factors and differs between individuals and different conditions. However, for the TF of the upper arm in adults, these variations are relatively small and a generalized TF (applicable to all adults under all conditions) can generate the ascending aortic pressure waveform. Different investigators have determined this generalized TF with remarkably similar results despite differences in age, sex, size and clinical status of subjects (Chen et al. 1997; Karamanoglu et al. 1993; O'Rourke 1970; Nichols and O'Rourke 1998b). Using the generalized TF, the corresponding series of aortic pressures waves are derived and displayed below the radial waves in the report. Each wave series is then ensemble-averaged to give a single radial and corresponding aortic pressure waveform. Inflection

points on the waves (wave foot, incisura, first and second peak) are determined from differentials of the pressure pulse. Time to the first (incident wave) and second systolic peak (reflected wave) from the wave foot are given, together with AI% which is calculated as height of the systolic peak divided by PP *(Figure 1.24)*. The AI% is positive if the second peak is greater than the first peak and negative when the second peak is smaller than the first peak. It is also possible to estimate the different periods of the heart (LVED, diastolic time) and the subendocardial viability ratio (SVR), which gives an estimate of subendocardial perfusion. It is calculated as tension time index/diastolic duration X 100(Buckberg et al. 1972). The time it takes for the reflected wave to arrive back at the heart is given as ΔTr *(Figure 2.2)*. The validity of these derived measurements have been confirmed by simultaneously performed direct intra-arterial measurements (Kelly et al. 1989a; Wilkinson et al. 1998a; Chen et al. 1997). This technique has been found to be reproducible in both healthy and diseased populations (Liang et al. 1998; Siebenhofer et al. 1999; Wilkinson et al. 1998b).

Pulse wave analysis using applanation tonometry is a simple non-invasive technique that provides both an accurate recording of the peripheral pressure waveform and a reliable determination of the aortic pressure waveform, thus enabling valuable insights into arterial and ventricular function.
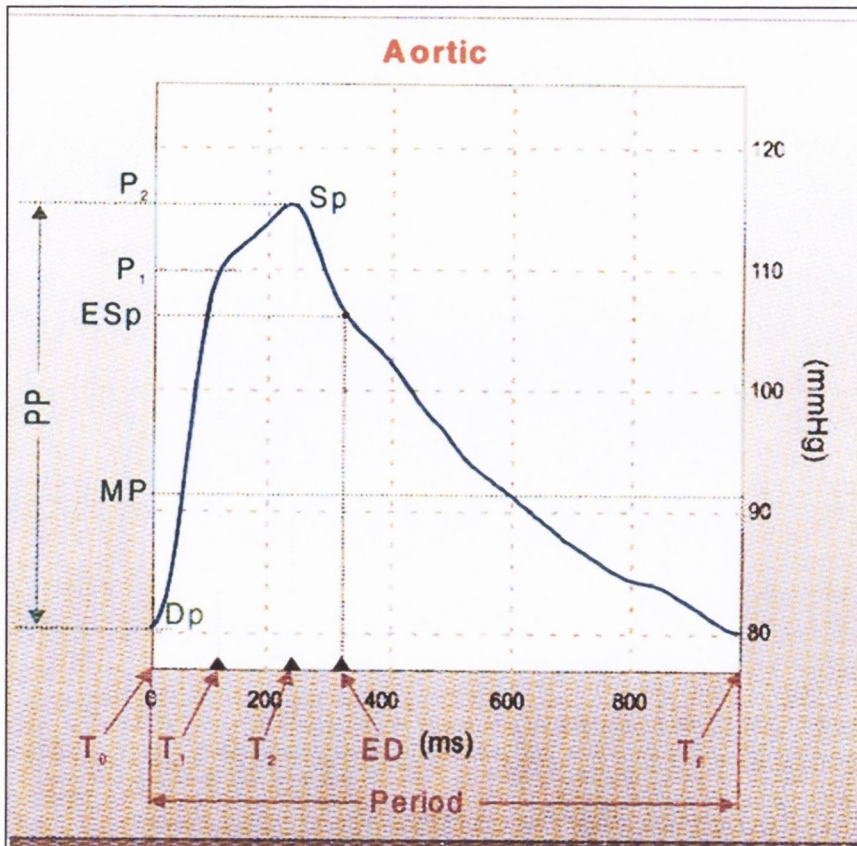
Figure 2.2. Features of the arterial pulse.

Table 2.3. Description of parameters measured in the aortic pressure waveform.

Calculated parameters	Abbreviation	Units
Period	Tf	msec
Ejection duration	ED	msec
Systolic Pressure-	Sp	mm Hg
Diastolic Pressure	Dp	mm Hg
Pulse pressure	PP	mm Hg
Time to 1st Peak-Aortic	T_1	msec
Time to 2nd Peak-Aortic	T_2	msec
Pressure at T_1	P_1	mm Hg
Pressure at T_2	P_2	mm Hg
End-systolic pressure	ESP	mm Hg
Mean Pressure	Mp	mm Hg

2.4   Pulse Wave Velocity Measurement

PWV in this thesis was measured using the Complior® system (Colson®, France), which relies upon automated calculation of PWV based on computerized algorithms.

1. Principle: The pressure wave generated by ventricular ejection is propagated throughout the arterial tree and the speed determined by the elastic and geometric properties of the arterial wall and the blood density. Since, fluid is contained in a series of elastic conduits, energy propagation occurs through the wall and not through the incompressible blood (Avolio et al. 1998). The material properties of the arterial wall, its thickness and the lumen diameter thus become the major determinants of PWV.

This concept has been formalized in a mathematical model in which PWV is given by **Moens-Korteweg equation:**

$$PWV = \sqrt{E.h / 2\rho R}$$

or by the **Bramwell-Hill equation**

$$PWV = \sqrt{\Delta P.V/(\Delta V/ P} \ (m/s)$$

where E=elastic modulus, R= radius of the artery, h= wall thickness, ρ = blood density and ΔV and ΔP the changes in volume and pressure respectively.

2. Calculation: PWV is calculated from measurement of pulse transit time and the distance travelled by the pulse between two recording sites:

$$PWV= Distance/Time \ (meters/seconds)$$

This system uses the pressure signal recorded by a pressure-sensitive external transducer (TY-306 pressure transducer (Fakuda Co); this transducer has a large frequency bandwidth form less than 0.1 Hz to 100 Hz which covers the principal frequency harmonics of the pressure wave at different HR and thus allows the application of PWV measurement *(Figure 2.3).*

3. Technique: With the Complior® system, pressure waveforms are digitalized at different rates according to the distance between the recording sites; the sampling acquisition frequency for carotid-femoral PWV is 500 Hz. The two pressure waveforms are stored in a recirculating memory buffer, half of which is displayed at any time.
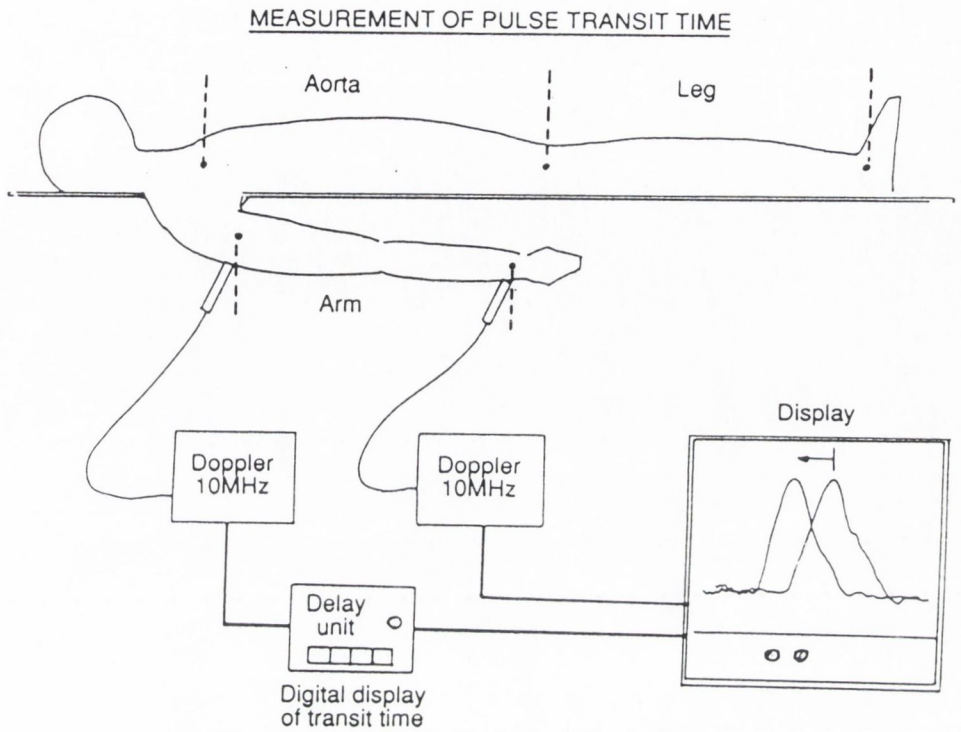
Figure 2.3. Method of measuring pulse wave velocity. Pressure waves are recorded simultaneously along the path of wave travel from the heart. The delay from the foot of the proximally-recorded to the distally-recorded waves is measured, and wave velocity is calculated as the distance between sites divided by the delay. Wave velocity is expressed in centimeters per second. It is always considerably higher than peak flow velocity in the aorta or other arteries.

Processing analysis automatically adjusts the gain of each waveform for an equality of the two signals. A maximum of 588 data points per waveform are displayed at any time covering a capture time of 0.735-1.47 seconds.

When the operator observes a pulse waveform of good quality on the computer screen, digitalisation is suspended and calculation of the time delay between the two pressure upstrokes is initiated. Two vertical lines are drawn on the computer display to indicate the position of the maximal rate of change of the pressure waveforms. The delay between the two pulse waves is determined by performing a calculation between the data of the two waveforms. The correlation is performed on the initial rise of the pulse until after the true pulse peak. The correlation algorithm is the executed. The correlated waveform is then displayed and the calculated mean PWV is printed out. The Complior® has been validated (Asmar et al. 1995b) and used in many large clinical trials and epidemiological studies (Asmar et al. 1999). A detailed review of the methodology and clinical applications of PWV has been extensively dealt by (Asmar 1999).

2.5 Reproducibility of Methods

The literature suggests that pulse wave analysis; measurement of AI% and PWV measurement have a low variability with high inter-observer agreement. The co-efficient of variation with the technique repeated in the same individual on one occasion was less than 4% and when repeated over time less than 5% for both these techniques.

2.6 Statistical Methods

Given the nature of the data, comprising of a large database, with a series of descriptive studies, comparative crossover studies, treatment and time interactions, a variety of statistical methods were employed. Details are given for each particular study.

2.7 Ethical approval

Ethical approval was granted by the institutional ethics committee for all the studies in this thesis.

CHAPTER THREE

DETERMINANTS OF ARTERIAL STIFFNESS

THE DETERMINANTS OF ARTERIAL STIFFNESS

3.1. Introduction

The mechanical characteristics of the systemic vasculature are a major influence on cardiac afterload and coronary perfusion. Indices of arterial compliance and distensibility have therefore been proposed as surrogate markers of cardiovascular risk and also as a potential therapeutic target (Nichols and O'Rourke 1998b; Safar et al. 2000b; Dart and Kingwell 2001). AI% in the ascending aorta is a means of quantifying the distensibilty of the aorta through the effect of mechanical properties on the timing of the reflected wave and hence the magnitude of augmentation of aortic pressure (Murgo et al. 1980b; Chen et al. 1997; Kelly et al. 1989a; Kelly et al. 1989b). It has been used to define age-related vascular changes (Avolio et al. 1985; Kelly et al. 1989d) and the effect of different cardiovascular risk factors on the arterial pulse wave (Asmar et al. 2001; Roman et al. 1992), monitor acute changes in arterial haemodynamics with exercise (Vaitkevicius et al. 1993), HR changes (Wilkinson et al. 2000a) and different therapeutic modalities (Asmar et al. 1991; Kelly et al. 1989c; London et al. 1996)

In view of its potential for epidemiological studies and clinical use, a number of groups have used arterial pulse wave analysis to obtain pressure waveforms and AI% to assess aortic properties as determined by the AI%. Initially, carotid artery applanation was used showing good approximation of results from non-invasively determined carotid pressure waveforms with invasively determined central aortic waveforms (Chen et al. 1996). Use of radial applanation tonometry with derivation of a surrogate central aortic pressure waveform by using a generalized TF has been described (Karamanoglu et al. 1993). Recently a good association between this technique and results based on directly recorded central pressure waveforms were noted (Chen et al. 1997).

The use of radial rather than carotid applanation tonometry to obtain a pressure waveform has since been proposed as a preferable method on the basis that it is an easier technique and more amenable to adequate applanation. The drawback of this approach has been the established difference between temporal pressure wave shapes between the two sites. However, the aortic BP was linearly related to brachial BP in a group of treated hypertensive patients (Cameron et al. 1998). Despite these earlier reports, still there is

paucity of information about the determinants of AI% in both an untreated hypertensive as well young normotensive population using this technique. In addition PP amplification (AMP), which may also be an important determinant of arterial elastic properties, hitherto been ignored, is considered here. In this section, I report the major determinants of AI% in a normotensive and untreated hypertensive population. Later in the course of the studies, the measurement of PWV also became available and in a subgroup of hypertensive and normotensive populations, it has also been analysed for its determinants and its relationship with AI% and AMP. I have also looked at the relationship between PWV and transit time of the reflected wave (ΔTr), calculated from the derived aortic pressure waveform (see Methods).

3.2. Materials & Methods

1. Subjects: The study was carried out in 416 subjects, including 188 patients with essential hypertension (aged 50±14 years) and 228 healthy subjects (aged 25.8±10, mean±SD). The hypertensive population consisted of never-treated patients referred to the Hypertension Clinic at St. James's Hospital, Dublin. The diagnosis of essential hypertension was established by detecting a sustained high blood pressure (>140 mmHg SBP or >90 mm Hg DBP, or both on the basis of repeated sphygmomanometric measurements and confirmed by sustained high levels of ambulatory BP (>135 mm Hg systolic or >85 mm Hg diastolic or both on 24 hour BP monitoring (Space Labs®) in the absence of clinical or laboratory evidence suggesting that a patient had secondary forms of hypertension. I also studied 228 healthy young normotensive subjects. This group comprised largely of healthy medical students and a small proportion of older normotensive subjects drawn from hospital/university personnel and patients suspected of hypertension or white-coat hypertension. The patients were examined in the context of the assessment protocol of the Hypertension Clinic. All the subjects gave informed consent and the study had institutional ethics committee permission.

2. Research Plan: Due to the heterogeneity of the study populations and the different level of information available for both groups (for hypertensive patients, I had to rely on information recorded in the patient charts for some of the data), I carried out two different

analyses. In the first study, I looked at the normotensive population to assess the determinants of AI% in a healthy population. In the second study, I studied the determinants of AI% in the hypertensive subjects and analysed any possible relationships between the cardiovascular risk factors and AI%, as the determinants of AI% may be different in a healthy vascular tree from one that operates under high distending pressure.

The subjects were classified as smokers if they smoked regularly which in most cases were more than 15 cigarettes per day for last 15 years. Alcohol excess was defined for males as greater than 21 units per week and for females greater than 14 units per week. The studies were carried out in a quiet room at 23°C, with the subject in the supine position. The patients were allowed to relax for at least 15 minutes before the haemodynamic measurements were made.

3. Derivation of the aortic pressure waveform: Augmentation in the ascending aorta was originally defined as the ratio of the difference between the pressure at the first systolic shoulder and DBP to that between pressure at the second inflection point and DBP, P_2 / P_1 (Murgo et al. 1980b). If the ratio is greater than 100, the waveform is classified as type A and if less than 100, as type C (See Introduction). A quantitative augmentation pressure **AP**, defined as the difference between the late and early systolic peaks, is also provided by automatic analysis. In this study I have used the ratio of AP and PP expressed as a percentage, the **AI%** (Kelly et al. 1989a; Kelly et al. 1989b). This gives both negative and positive values. Although numerically different values are obtained with the two methods (as there are no negative values in the Murgo method) a one-to-one mapping occurs between the two definitions and both provide a quantitative expression of pressure wave contour. (Sphygmocor®, PWV Medical™, Australia).

Immediately after recording BP with a validated automated oscillometric BP monitor (Omron® HEM 705-CP), the same arm was used for applanation tonometry. A high-fidelity micromanometer (BPAS-1, PWV Medical ®, Sydney, Australia) was used to flatten the radial artery and the radial pulse continuously recorded. The central aortic waveform was derived from radial tonometry by using a previously validated TF relating peripheral to central waveform within the software package (Sphygmocor®, PWV

Medical™, Australia) as previously described in Methods.   The validity of the derived AI% has been confirmed by simultaneous direct central aortic measurements (Chen et al. 1996; Kelly et al. 1989b) and is highly reproducible in both healthy and diseased populations (Liang et al. 1998; Siebenhofer et al. 1999; Wilkinson et al. 1998b). AMP was calculated as the difference between the measured brachial SBP and the derived aortic SBP.

4. *Pulse wave Velocity Measurements:* Carotid-femoral PWV was determined according to the foot-to-foot method using the Complior ® device. The simultaneous recording by two pressure sensitive transducers of the carotid and femoral waveform and measurement of the time delay of successive records from the foot of each wave divided by the distance between the transducers allows calculation of PWV (metres/sec). The distance travelled by the pulse wave was measured over the body surface with a tape measure as the distance between the recording sites and fed into the software and 10 consecutive waves were sampled.   The validity of the Complior ® has been established (Asmar et al. 1995b).

5. *Statistical Analysis:* Associations between AI% and categorical variables including sex, smoking status, alcohol excess are examined using analysis of variance (ANOVA). Associations between AI% and continuous variables including age, BP, body height and weight, body mass index (BMI) and waist-hip ratio are examined using correlation. Age is also categorised and analysed as three age bands: <30 years, 30-50 years and >50 years. Multiple regression, using a forward stepwise approach, is used to examine the significance ($p<0.05$) of any variables found to be significant in the univariate analysis. To allow for the effect on AI% on the known pressure dependence of vascular compliance, only DBP was used in multiple regression analysis. The reason for this is that minimum distending pressure is more appropriate index of pressure dependence than either SBP or MAP, which are themselves contributed to by systolic pressure augmentation and hence are not independent predictors. Results from the multiple regression are presented as partial regression coefficients and their 95% confidence intervals. The percentage of variance explained is presented for each model. *Significance* for univariate and multiple regression analysis is taken as <0.05. Statistical analyses were performed using JMP version 3.0 (SAS Institute Inc), and plots were performed using PRISM.

3.3. Results

3.3.1. Relationship between Different Measures of Wave Reflection and Blood Pressures

There was the expected close association (all subjects n= 416) between AI% and AP(r = 0.92, p<0.0001) and AI% and P_2/P_1(r = 0.98, p<0.0001) (***Figure 3.1***).

3.3.2. Comparison of Measured Brachial and Derived Aortic Pressures

Figure 3.2 shows the relationship between brachial BP measured oscillometrically and the assigned (post-transformation) values representing aortic BP. The slope of the association between measured brachial and derived SBP and DBP did not differ significantly from unity; however, the derived DBP was consistently greater than that measured (mean difference 1.3 mm Hg±0.06).

3.3.3. The Distribution Of Augmentation Index%, Pulse Pressure Amplification and Pulse Wave Velocity According to Age Groups

The AI%, AMP and PWV were also studied according to age groups. Results are shown in ***Figure 3.3***. As expected, the AI% and PWV were significantly greater in the older age groups and the AMP significantly lower. As the age bands 2 and 3 comprised of older subjects most of who were hypertensive, we analysed the data adjusting for BP and the above relationships were still valid.

AP vs AI
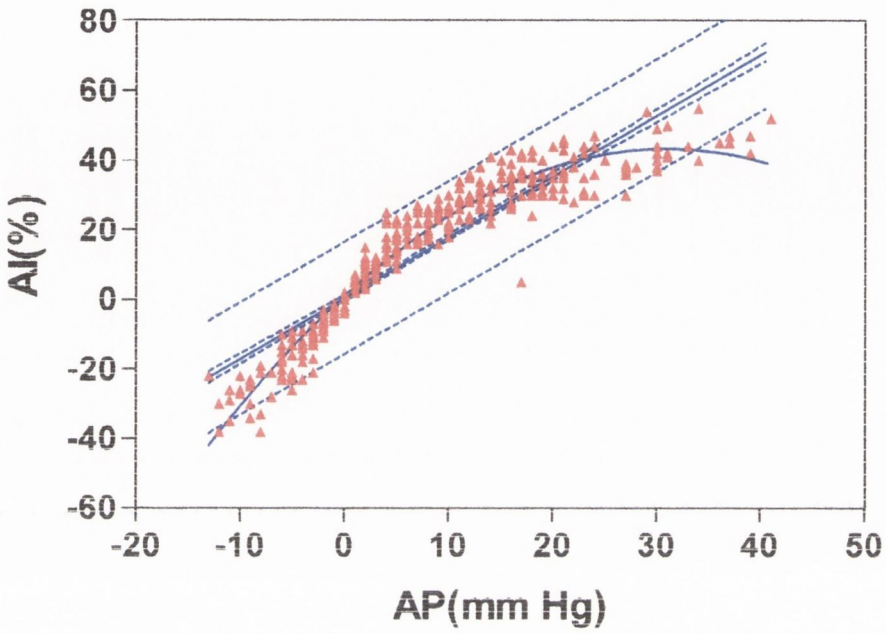
AI% vs P2/P1
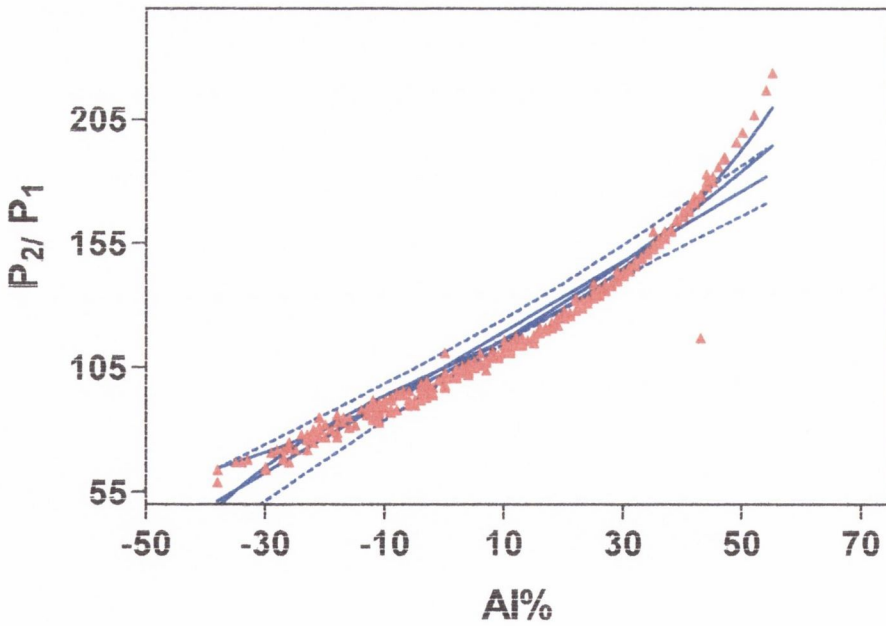
Figure 3.1. Univariate association (95% confidence intervals of the line and 95% prediction interval of AI% with AP and P_2/P_1 in the total group of normotensive and hypertensive subjects(n=416).

PSBP vs CSBP
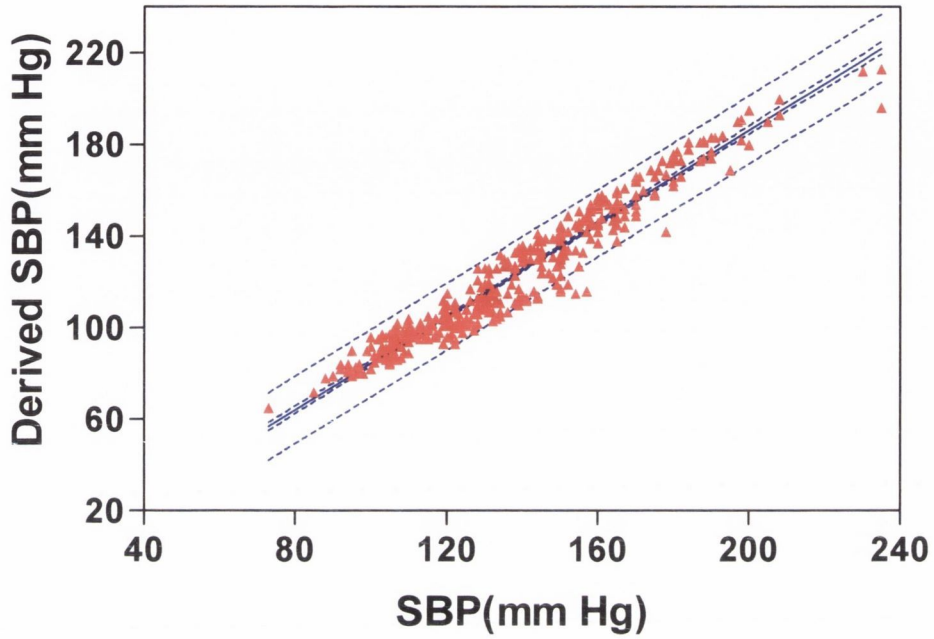
PDBP vs CDBP
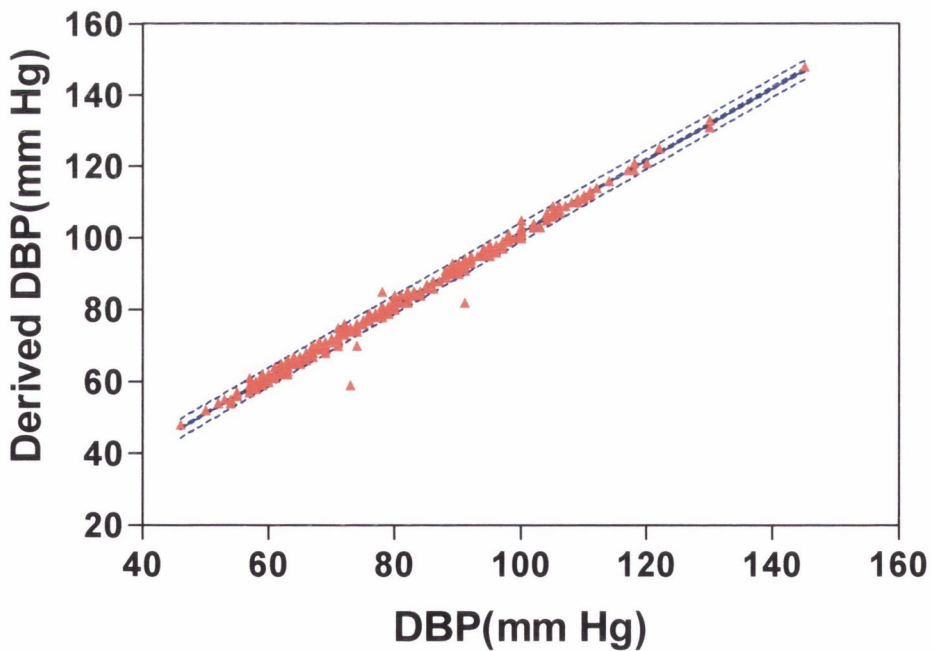
Figure 3.2. The comparison of derived aortic SBP and measured brachial SBP in the total group of normotensive and hypertensive subjects(n=416).
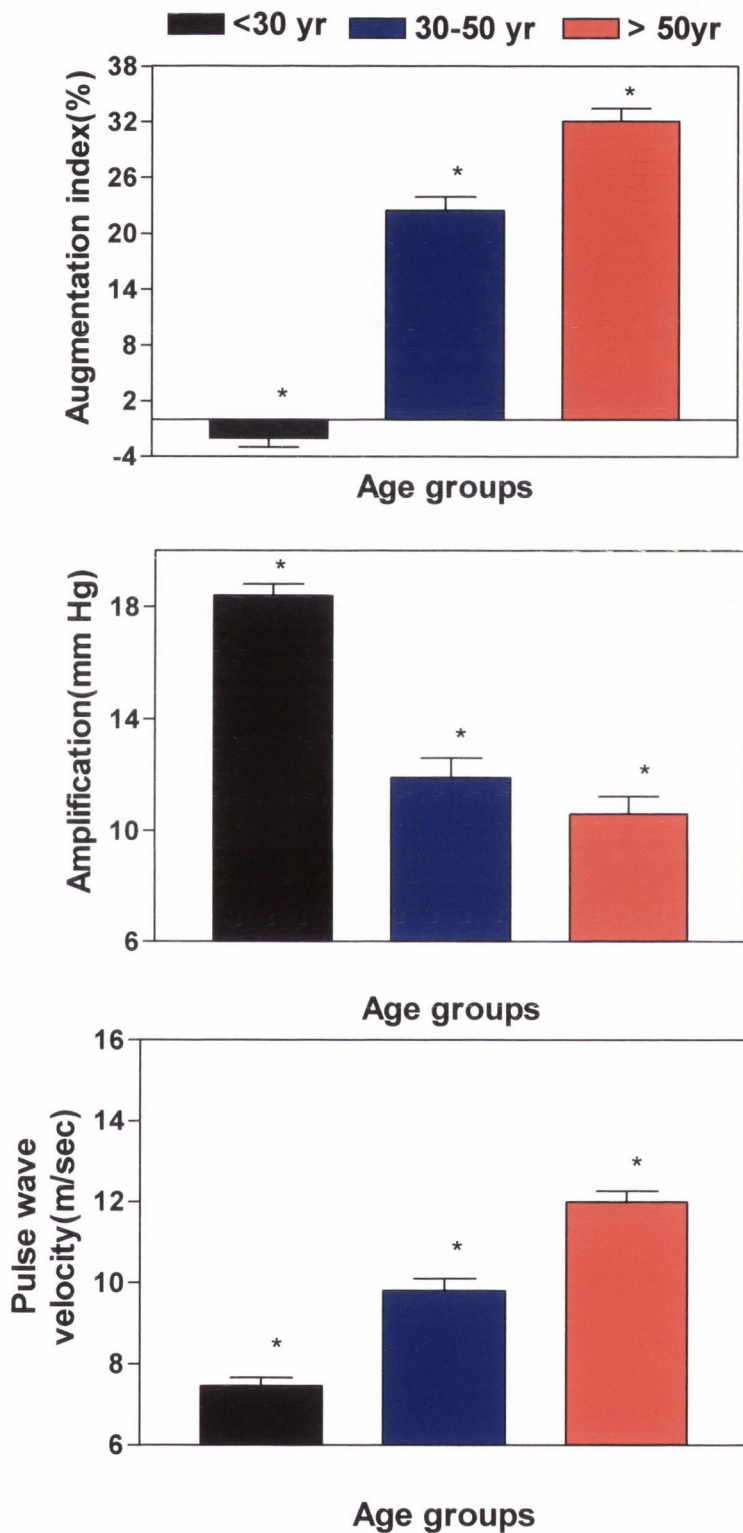
Figure 3.3. The distribution of AI%, AMP and PWV according to the three different age groups in the combined normotensive and hypertensive populations(mean±sem, *p<0.05)

3.3.4. Determinants of Augmentation Index in the Normotensive Population

The demographic data for the normotensive population is given in **Table 3.1**. For the normotensive group, the age was similar across the two genders. The males had significantly higher weight, height and waist-hip ratio as compared to females. The SBP was significantly higher in males (124 ± 1.2 vs 111 ± 1.2, p<0.001) but there was no difference in HR or DBP between the two genders. The females had a significantly higher AI% than males (2.2 ± 1 vs -4.3 ± 1, p<0.001) but the PWV was higher in males (8.1 ± 0.3 vs 7.36 ± 0.3, p<0.03) compared to females *(Figure 3.4)*.

Table 3.1. Demographic and haemodynamic characteristics of the normotensive population (228)

	Males (n=118)	Females (n=110)	Significance
Age (years)	26.3±0.9	25±0.9	NS
Body weight (kg)	7.4±1.2	63±1.3	P<0.001
Body height (cm)	175±1.3	165±1.3	P<0.001
BMI (kg/m^2)	25.3±0.2	25±0.2	NS
Waist/Hip ratio	0.86±0.01	0.78±0.01	P<0.001
Brachial SBP (mm Hg)	124±1.2	111±1.2	P<0.001
Brachial DBP (mm Hg)	70±0.8	69±0.9	NS
Aortic SBP (mm Hg)	106±1	96.4±1	P<0.001
Aortic DBP (mm Hg)	70.2±0.9	72.6±0.9	NS
HR (min^{-1})	68±1	69±1	NS
AI (%)	-4.3±1	2.2±1	P<0.001
PWV (m/sec)	8.1±0.3	7.36±0.3	P<0.05
AMP (mm Hg)	20±0.5	14.7±0.3	P<0.0001

Values are presented as mean±SEM, NS > p 0.05.
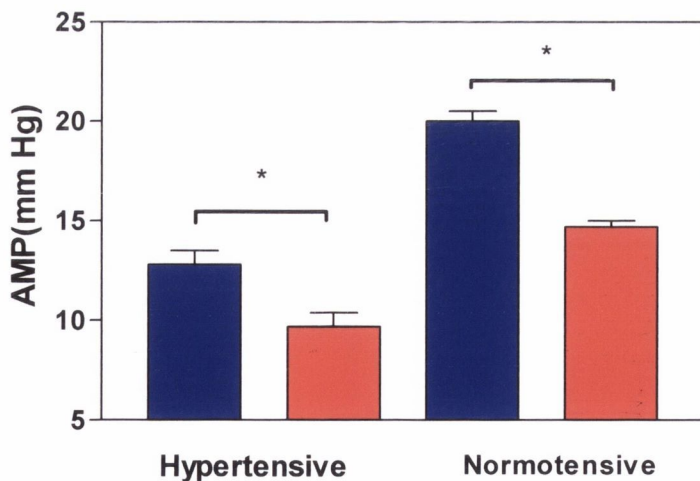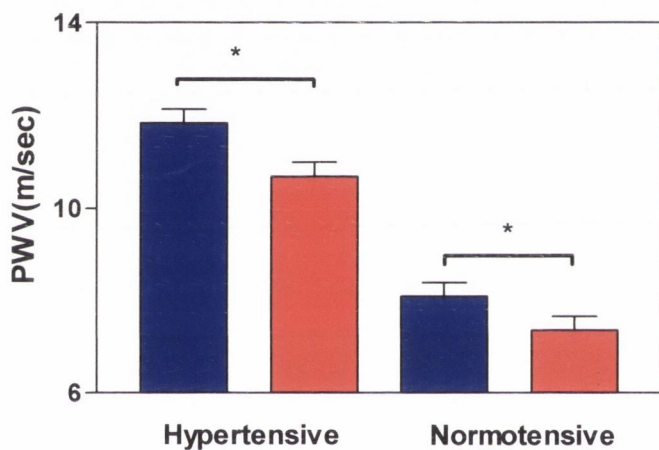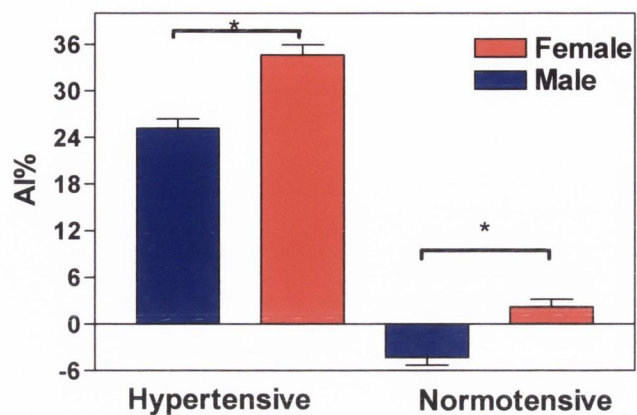
Figure 3.4. The AI%, PWV and AMP in males versus females in the
hypertensiove group(n=188) and in the normotensive group(n=228).
Data presented as Mean±SEM,*p<0.05.

Table 3.2. Life-Style Factors in the normotensive population (where data incomplete, number surveyed in parenthesis).

	Total (228)	*Males (118)*	*Females (110)*
Smoking	49(193)	22(99)	27(94)
Fm History of hypertension	41(137)	19(72)	22(65)
Alcohol excess	61(183)	32(99)	29(84)
Fitness Level			
Active Sports	54	36	18
Occasional exercise	85	38	47
Couch potatoes	44	23	21

Table 3.3. Univariate associations of quantitative variables with AI% in the normotensive subjects (n=228)

Variable	Number	Correlation	P-value
Age (yr)	228	0.52	<0.0001
Body height (cm)	199	-0.18	<0.01
Body weight (kg)	200	-0.02	NS
BMI (kg/m^2)	199	0.07	NS
Waist-Hip ratio	147	0.13	NS
Brachial SBP (mm Hg)	228	0.03	NS
Brachial DBP (mm Hg)	228	0.24	<0.0005
Aortic SBP (mm Hg)	228	0.236	<0.0005
Aortic DBP (mm Hg)	228	0.236	<0.0005
HR (minute^{-1})	228	-0.07	NS
AMP (mm Hg)	228	-0.55	<0.001
PWV (m/sec)	82	0.44	<0.0001
ΔTr (msec)	174	-0.1	<0.05

Table 3.2 shows the distribution of categorical variables in the healthy normotensive population sub grouped by gender. *Table 3.3* shows the univariate associations between AI% and the quantitative variables in this particular population. The AI% was significantly higher in females compared to males *(Figure 3.4)*. There was a significant positive correlation of AI% with age, brachial DBP, aortic systolic and diastolic BP and PWV and an inverse association with height, AMP and ΔTr *(Figure 3.5 & 3.6)*. There was no correlation of AI% with heart rate *(Figure 3.6)*.

Table 3.4. Univariate association of categorical variables with AI% in the normotensive subjects.

Variable	Number	Mean AI%	95%CI	P value
Sex				
Male	119	-3.1±1.4	-5.06-(-0.33)	P<0.0001
Female	110	2.6±1.4	0.1-5.3	
Smoking				
Smokers	48	0.66±2	3.26-4.58	P<0.01
Non-smokers	128	-5.3± 1.2	-9.22-(-1.4)	
Alcohol excess				
Yes	61	-2±1.9	-3.72-(-1.72)	NS
No	117	-3±1.3	-5.5-(-0.4)	
Fitness level				
0	42	5.8±2	1.88-9.72	
1	82	-0.4±1.4	-2.34-3.1	P<0.0001
2	54	-14±1.7	-17-(-10.7)	

Values are presented as mean±SEM and 95% CI, NS > p 0.05.

Smokers had a higher AI% compared to non-smokers in the normotensive group *(Figure 3.8)* while there was no effect of alcohol excess. The level of physical fitness had a significant effect on AI% in both genders; a graded effect was seen with the lowest AI% in the active sportspersons and the highest in the sedentary individuals *(Figure 3.9)*.

As gender effect on AI% was significant, I analysed the univariate relationships of AI% with both categorical and quantitative variables separately. The univariate relationships between the AI% and the quantitative relationships for both genders are given in **Table 3.5.** Males had a higher correlation of AI% with age, but not with height compared to females. The females showed a significant correlation of AI% with HR while the male subjects did not. Both genders did not show any relationship of AI% with brachial SBP. The relationship of AI% with the categorical variables is given in **Table 3.6.** The male smokers had a significantly higher AI% as compared to male non-smokers, but no such effect was observed in the females **(Figure 3.7).** Both males and females had a significantly lower AI% if they were physically active **(Figure 3.8).**

Table 3.5. Univariate associations of quantitative variables, with AI% in the male and female normotensive subjects separately.

Variable	*Male*				*Female*	
	N	R	P	N	R	P
Age (yr)	119	0.60	<0.0001	110	0.49	<0.0001
Body height (cm)	109	-0.06	NS	95	-0.21	<0.05
Body weight (kg)	106	0.09	NS	94	0.009	NS
Brachial SBP (mmHg)	119	0.03	NS	110	0.07	NS
Brachial DBP (mmHg)	119	0.22	<0.01	110	0.31	<0.0007
Aortic SBP (mmHg)	119	0.29	<0.0001	110	0.38	<0.0001
Aortic DBP (mmHg)	119	0.21	<0.05	110	0.31	<0.001
Heart Rate (min^{-1})	119	-0.19	<0.05	110	-0.04	NS
AMP (mm Hg)	119	-0.48	<0.0001	110	-0.61	<0.0001
PWV (m/sec)	48	0.48	<0.0005	34	0.51	<0.001

Values are presented as mean±SEM, NS > p 0.05.

A forward stepwise regression analysis was performed in the young healthy subjects. Age was the most important predictor of AI% in the healthy normotensives followed by sex and brachial diastolic BP **(Table 3.7).**
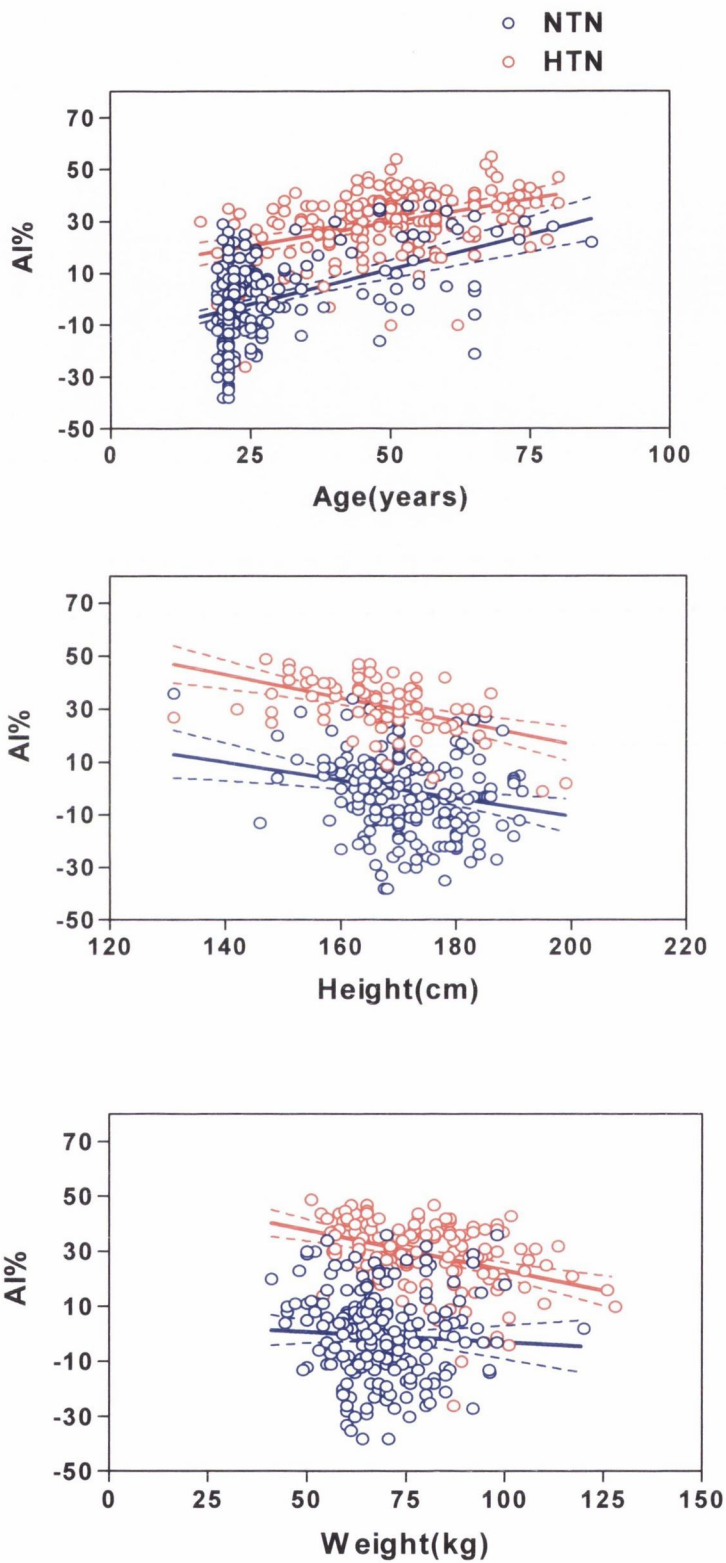
Figure 3.5. Univariate association (95% confidence intervals of the line) of AI% with age, height and weight in the normotensive (n=228) and hypertensive subjects (n=188).
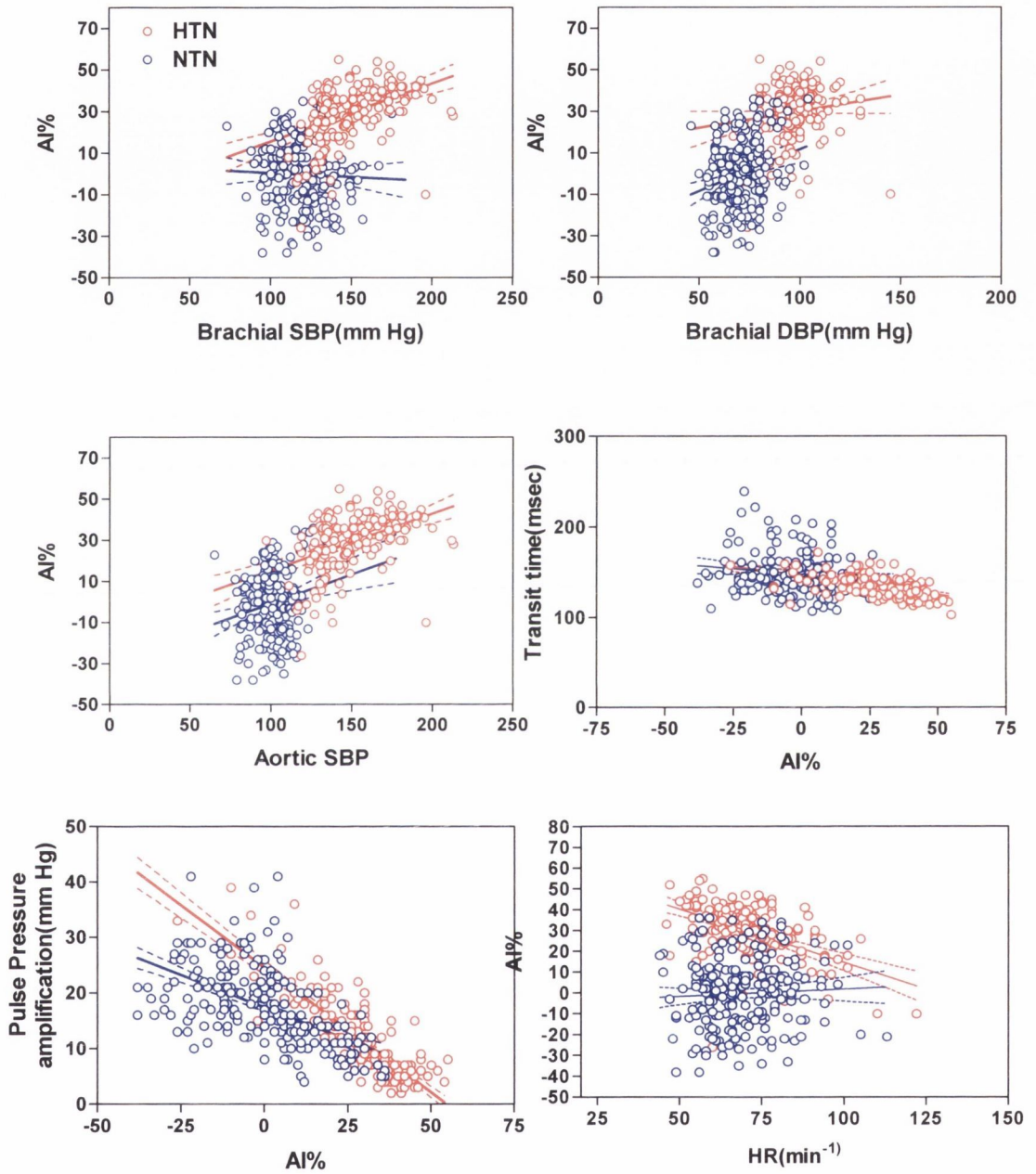
Figure 3.6. Univariate association (95% confidence intervals of the line) of AI% with brachial and aortic SBP, brachial DBP, AMP, HR and ΔTr in the normotensive (n=228) and hypertensive (n=188) subjects.
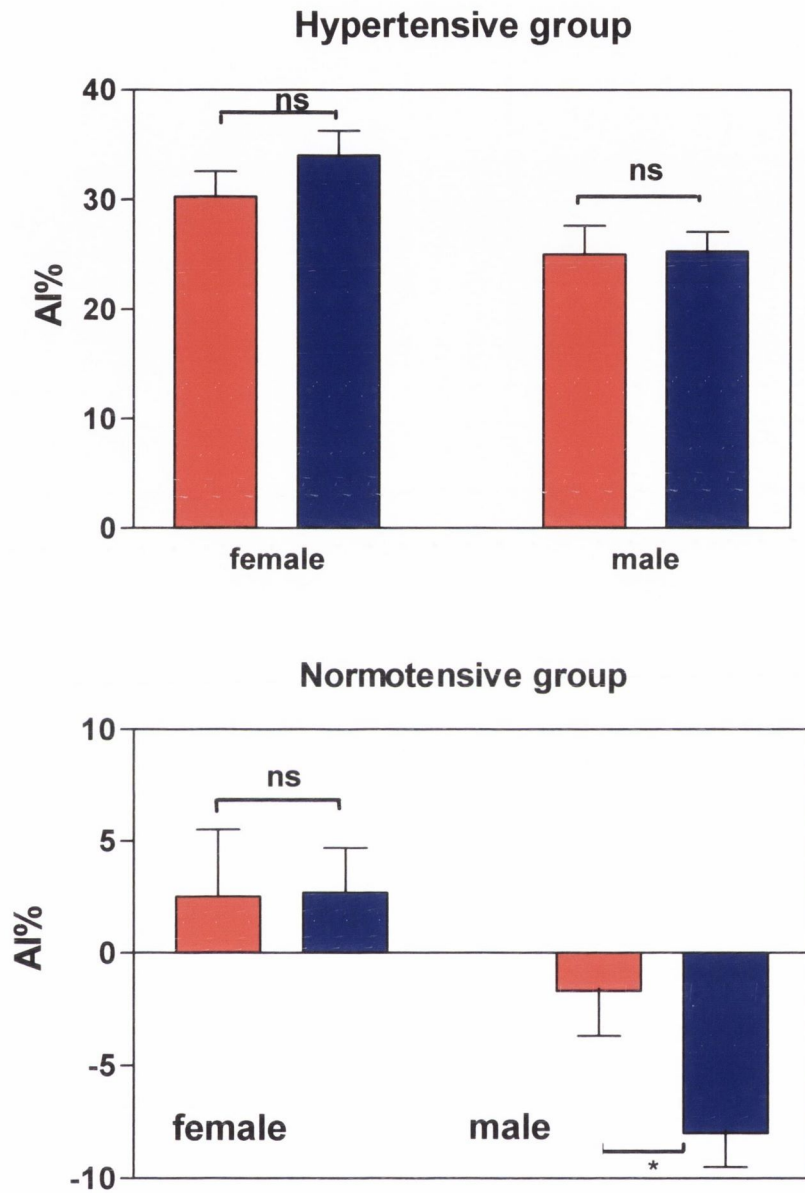
Figure 3.7. The impact of smoking on the AI% in both genders in the hypertensive (n=134) and normotensive(n=176) subjects. Data presented as mean±SEM,*p<0.05,ns=>0.05.
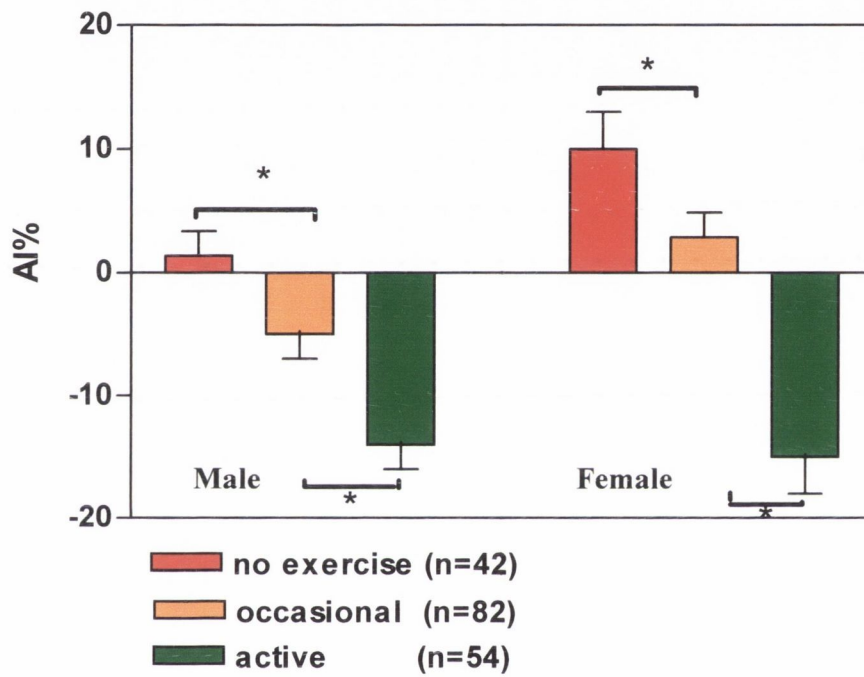
Figure 3.8. The effect of physical fitness on AI% in both male and females normotensive subjects (n=178). Data presented as mean±SEM,*p<0.05.

Table 3.6. Univariate associations of AI% to categorical variables in the male and female normotensive subjects separately.

		Male				Female		
Smoking	N	AI%	CI%	P	N	AI%	CI%	P
Yes	21	-1.7±2	-2.2-5.62		27	2.5±3	6.8-18.8	
No	75	-8±1.5	-11-(-5)	<0.05	66	2.7±2	11.1-18.5	NS
Alcohol excess								
Yes	32	-6.8±2	-10-(-2.9)		29	3±3	5.9-17.7	
No	64	-6.7±1	(-8)-(-4.7)	NS	53	1.2±2	12.3-20	NS
Fitness level								
0	22	1.4±2	2.5-5.3	P<	20	10±3	4.2-15.8	P<
1	35	-5±2	-9-(-1)	0.001	47	2.9±2	1-6.8	0.001
2	36	-14±2	-18-(-10)		18	-15±3	-21-(-9)	

Values are presented as mean±SEM, NS > p 0.05.

Table 3.7. Partial regression coefficients for forward stepwise linear regression for AI% in the normotensive subjects.

Predictor Variable	*Partial regression coefficients*	*RSquare*	*P value*
Constant	39		
Age (yr)	0.68	0.20	<0.0001
Sex (m-f)	-4.0	0.26	<0.0001
Brachial DBP (mm Hg)	0.29	0.28	<0.01

3.3.5. Determinants of Augmentation Index in the Hypertensive Population

Demographic and haemodynamic characteristics for the hypertensive patients are summarized in *Table 3.8* with the groups divided by gender. There was no difference between the males and females in terms of age, BP and HR. The males were heavier and taller and had a higher waist-to-hip ratio than females. The AI% was significantly higher in females (34.5 ±1 vs 25.2±1, p<0.001) compared to males *(Figure 3.4)*. Also, the

PWV was significantly higher in males (11.84±0.3 vs 10.7±0.3, p<0.03) and AMP (12.8± 0.7 vs 9.7±0.7, p<0.5) compared to females *(Figure 3.4)*.

I looked at the AI% in relation to the cardiovascular risk factors (*Table 3.9*) and found no association between AI% and smoking status, alcohol excess, and family history of hypertension, CHD, LVH, diabetes, hyperlipidaemia and cerebrovascular accident. The relationship was also analysed between the levels of total cholesterol, low-density lipoprotein concentration, total triglycerides, high-density lipoprotein (HDL) and serum glucose in 90 cases. The only significant correlation was observed between HDL and AI%(r = 0.27, p<0.005).

Table 3.8. Demographic and haemodynamic characteristics of hypertensive patients

	Men (n=107)	Women (n=81)	Significance
Age (years)	50±1.5	49.8±1.6	NS
Body weight (kg)	88±1.6	70.5±1.6	P<0.001
Body height (cm)	172.7±1.5	159.8±1.5	P<0.001
BMI (kg/m^2)	27.9±0.6	26.9±0.6	NS
Waist/Hip ratio	0.91±0.01	0.83±0.01	P<0.005
Brachial SBP (mm Hg)	160.8±2	162±2	NS
Brachial DBP (mm Hg)	98±1.2	96±1	NS
Aortic SBP (mm Hg)	148±2	152.6±2	NS
Aortic DBP (mm Hg)	99.6±1	97.4±1.3	NS
HR (minute^{-1})	71.5±1	71±1	NS
Augmentation index (%)	25.2±1	34.5±1	P<0.001
Pulse wave velocity (m/sec)	11.84±0.3	10.7±0.3	P<0.04
AMP (mm Hg)	12.8±0.7	9.7±0.7	P<0.003

Values are presented as mean±SEM, NS > p 0.05.

A univariate analysis was performed between the quantitative variables and the AI% in the total hypertensive population *(Table 3.10)* and with both genders separately *(Table 3.11)*. There was no correlation between the brachial systolic and diastolic BP, aortic diastolic BP and AI% in the hypertensive subjects. In contrast to the normotensive group, the

106

hypertensive subjects analysed separately showed a very significant correlation between HR and AI%(r = -0.46, p<0.0001) *(Figure 3.6)* and there was no difference between the aortic and brachial SBP between the two genders. There was no significant difference in the AI% either between smokers and non-smokers *(Figure 3.6)* or between individuals who took alcohol excessively or not.

I also analysed the determinants of AI% in the male and female hypertensive subjects separately. Age had a stronger correlation with AI% in the male group while height was quite significantly related to AI% in the females only. There was no correlation between the brachial SBP and AI% in both genders. However, the aortic SBP significantly correlated with the AI% in both genders. The DBP was significantly correlated to AI% in the female hypertensives only. The HR inversely correlated to the AI% in both genders but the relationship was stronger in females.

Table 3.9. Cardiovascular risk profile in hypertensive population as recorded from patient charts (where data incomplete, number surveyed in parenthesis).

	Total (188)	*Men (107)*	*Women (81)*
Smoking	42(134)	26(77)	16(57)
Hyperlipidaemia	40(116)	24(63)	16(53)
Diabetes	5(121)	3(67)	2(54)
Obesity	48(122)	31(67)	17(55)
Alcohol excess	45(128)	36(78)	11(57)
Fm History HTN	62(123)	28(68)	34(55)
Fm History CHD	44(120)	23(67)	21(53)
Gout	9(130)	9(74)	0(56)
Renal Calculi	6(130)	5(74)	1(56)
CHD	6(126)	3(72)	3(54)
CVA	4(124)	3(71)	1(53)
PVD	1(126)	0(72)	1(54)
LVH	28(105)	18(60)	10(45)
Renal Failure	5(126)	4(72)	1(54)

Again, there was no relationship between AI% and smoking and alcohol status in the two genders. However, the correlation between HDL and AI% was observed for females only.

Table 3.10. Univariate associations of quantitative variables with AI% in the hypertensive subjects (n=188)

Variable	Number	Correlation	P-value
Age (yr)	188	0.40	<0.0001
Height (cm)	39	-0.30	<0.05
Weight (kg)	123	0.40	<0.0001
BMI (kg/m^2)	39	0.06	NS
Waist-Hip ratio	25	-0.23	NS
PSBP (mm Hg)	188	0.14	NS
PDBP (mm Hg)	188	0.14	NS
CSBP (mm Hg)	188	0.43	<0.0001
CDBP (mm Hg)	188	0.17	NS
HR(minute^{-1})	188	-0.49	<0.0001
AMP	188	-0.84	<0.0001
PWV (m/sec)	42	0.08	NS

Table 3.11. Univariate associations of quantitative variables, with AI% in the male and female hypertensives separately.

Variable	Male			Female		
	N	R	P	N	R	P
Age (yr)	97	-0.47	<0.0001	77	-0.36	<0.001
Body Height (cm)	17	-0.30	NS	22	-0.41	<0.005
Body Weight (kg)	66	0.15	NS	57	0.44	<0.0001
Brachial SBP (mmHg)	97	0.16	NS	77	0.09	NS
Brachial DBP (mm Hg)	97	0.15	NS	77	0.27	<0.01
Aortic SBP (mm Hg)	97	0.46	<0.0001	77	0.36	<0.001
Aortic DBP (mm Hg)	97	0.14	NS	77	0.23	<0.05
HR (minute^{-1})	97	-0.47	<0.0001	77	-0.61	<0.0001
AMP (mm Hg)	97	0.86	<0.0001	77	0.80	<0.0001

I carried out a stepwise regression analysis for the hypertensive subjects separately. HR, height and age were the most important predictors of AI% in the hypertensive population in that order (*Table 3.12*)

Table 3.12. Partial regression coefficients for forward stepwise linear regression for AI% in the hypertensive subjects (188).

Predictor Variable	Partial regression coefficients	R Square	P value
Constant	67		
Heart Rate (min^{-1})	-0.49	0.41	<0.0001
Height (cm)	-0.05	0.52	<0.0001
Age (yr)	0.18	0.63	<0.005

3.3.6. Determinants of Pulse Pressure Amplification in the Normotensive Population

The normotensive population was analysed separately for the determinants of AMP. The univariate relationships of AMP with quantitative variables are given in *Table 3.13* showing no relationship with HR and much strong correlations with BP. The associations of categorical variables with AMP were also studied *(Table 3.14)* showing a significant difference in the AMP between the two genders *(Figure 3.4)* but there was no effect of smoking or alcohol excess on AMP in the healthy subjects.

I also performed a forward stepwise regression analysis for AMP in this healthy group and SBP, DBP followed by age were the most important predictors *(Table 3.15)*.

Table 3.13 Univariate associations of quantitative variables with AMP in the normotensive group.

Variable	Number	Correlation	P-value
Age (yr)	228	-0.34	<0.0001
Body Height (cm)	199	0.23	<0.001
Body Weight (kg)	200	0.18	P<0.01
Brachial SBP (mm Hg)	229	0.57	<0.0001
Brachial DBP (mm Hg)	228	-0.25	<0.0001
Aortic SBP (mm Hg)	228	0.18	<0.005
Aortic DBP (mm Hg)	228	-0.25	<0.001
HR (minute^{-1})	228	-0.006	NS
AI%	228	-0.55	P<0.0001
PWV (m/sec)	82	-0.03	=0.72

Table 3.14. Univariate associations of categorical variables with AMP in normotensive subjects.

Variable	Number	Mean AMP	95%CI	P value
Sex				
Male	119	20±1.5	17-22.9	P<0.0001
Female	110	14.7±0.6	13.5-15.8	
Smoking				
Smokers	48	16.5± 0.9	14.7-18.2	NS
Non-smokers	141	18.5± 0.5	17.5-19.4	
Alcohol excess				
Yes	61	18±0.6	16.8-19	NS
No	117	18.5±0.6	17.3-19.6	
Fitness level				
Couch Potatoes	42	16.4±0.9	14.6-18.1	<0.0001
Occasional sports	83	16.5±0.7	15.1-17.8	
Active sports	54	22.6±0.8	21-24.1	
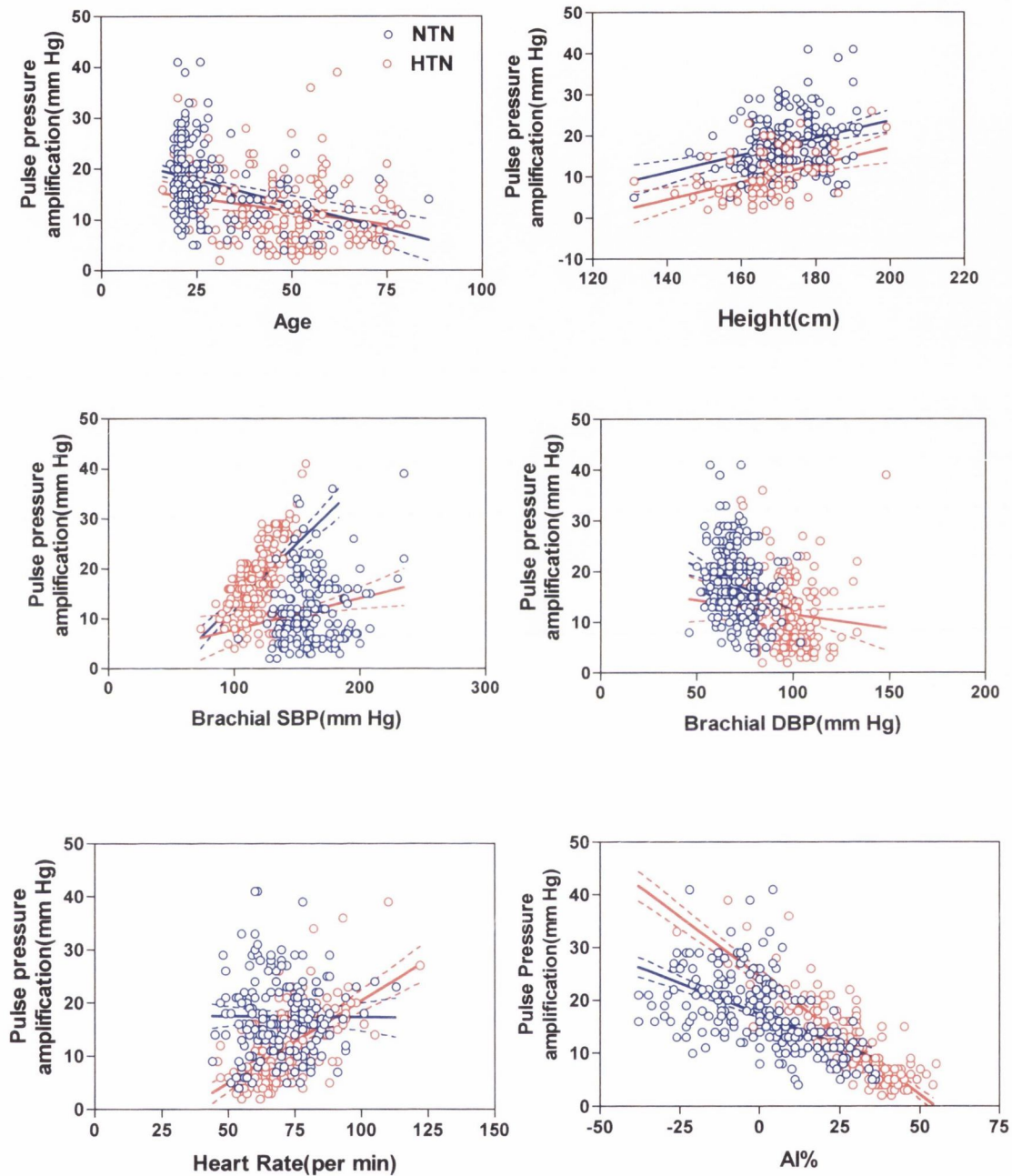
Figure 3.9. Univariate associations (95% intervals of the line) of AMP with age, height, brachial SBP and DBP, heart rate and AI% in normotensive (228) and hypertensive subjects (188).

Table 3.15. Partial regression coefficients for forward stepwise linear regression for AMP in the normotensive group.

Predictor Variable	Partial regression coefficients	R Square	P value
Constant	0.43		
PSBP (mm Hg)	0.42	0.49	<0.0001
PSBP (mm Hg)	-0.39	0.78	<0.0001
Age (yr)	-0.18	0.85	<0.0001

3.3 .7. Determinants of Pulse Pressure Amplification in the Hypertensive Population

AMP was also analysed in the hypertensive group separately. *Table 3.16* shows the univariate associations between the quantitative variables and AI% in the hypertensive group.

Table 3.16. Univariate associations of quantitative variables with pulse pressure amplification (AMP, mm Hg) in the hypertensive group.

Variable	Number	Correlation	P-value
Age (yr)	188	-0.20	<0.01
Body height (cm)	39	0.42	<0.01
Body weight (kg)	123	0.28	P<0.01
Brachial SBP (mm Hg)	188	0.19	<0.01
Brachial DBP (mm Hg)	188	-0.13	NS
Aortic SBP (mm Hg)	188	-0.14	<0.05
Aortic DBP (mm Hg)	188	-0.10	NS
Heart Rate (minute^{-1})	188	0.55	<0.0001
AI%	188	-0.84	P<0.0001
PWV (m/sec)	42	-0.06	NS

Table 3.17. Univariate associations of categorical variables with AMP in male and female subgroups of both hypertensive subjects.

	Male				Female			
	N	AMP	CI%	P	N	AMP	CI%	P
Smokers	24	12.6±1	10.6-14.5		16	13.3±1.4	10.5-16	
Non-smokers	50	13±1	11-15	NS	40	8.9±0.9	7.1-10.3	<0.01
			Alcohol excess					
Yes	34	11±1	9-12.9		11	12±1.5	9-14.9	
No	39	15±0.9	13.2-16.7	<0.05	45	9.24±0.7	7.8-10.6	NS

Values are presented as mean±SEM and 95% CI, NS > p 0.05.

Table 3.18. Partial regression coefficients for forward stepwise linear regression for AMP in the hypertensive group.

Predictor Variable	*Partial regression coefficients*	*R Square*	*P value*
Constant	12.6		
Heart Rate (min^{-1})	0.27	0.30	<0.0001
Sex (f-m)	-0.57	0.36	0.05
PDBP (mm Hg)	-0.22	0.43	<0.0001
PSBP (mm Hg)	0.16	0.53	<0.001
Age (yr)	-0.12	0.61	<0.01

Table 3.17 shows the relationship of categorical variables with AMP in the hypertensive population. The males with history of alcohol excess had significantly lower AMP than males without such history. The female smokers had a significantly higher AMP than female non-smokers in this group. The partial regression coefficients for the hypertensive population for AMP are given in *Table 3.18* showing HR, sex and DBP to be the most important predictors for AMP in a hypertensive group followed by SBP and age.

3.3. 8. Determinants of PWV in the Total Group of Hypertensive and Normotensive Subjects

PWV was measured in only 130 out of the 416 subjects studied. However, I looked at associations with both the quantitative and categorical variables with PWV *(Table 3.19 & 3.20)*. PWV is most strongly correlated to age followed by SBP *(Figure 3.10)* and the somewhat stronger relationship with aortic as opposed to brachial SBP. It is also positively related to AI% *(Figure 3.10)* and DBP, followed by waist-hip ratio, body weight, and HR in that order. It was inversely related to AMP and ΔTr *(Figure 3.9)* and did not correlate to body height. Among the categorical variables, gender was important, as males have significantly higher PWV than females *(Figure 3.4)*. However, smoking status and alcohol intake did not have any impact on PWV *(Table 3.20)*.

We performed a stepwise regression model for PWV with either systolic or diastolic BP or both in the equation and the results are as shown in *(Table 3.21)*. Thus age and SBP explain almost 50% of the variance in PWV followed by gender.

Table 3.19. Univariate associations of quantitative variables with PWV in the combined group of both normotensive and hypertensive subjects (n=130)

Variable	Number	Correlation	P-value
Age (yr)	130	0.77	<0.0001
Body Height (cm)	130	0.03	NS
Body Weight (kg)	130	0.39	<0.0001
BMI (kg/m^2)	130	0.39	<0.0001
Waist-Hip Ratio	130	0.38	<0.0001
Brachial SBP (mm Hg)	130	0.75	<0.0001
Brachial DBP (mm Hg)	130	0.64	<0.0001
Aortic SBP (mm Hg)	130	0.77	<0.0001
Aortic DBP (mm Hg)	130	0.65	<0.0001
Heart Rate (minute^{-1})	130	0.26	<0.01
AI%	130	0.62	<0.0001
AMP (mm Hg)	130	-0.32	<0.001
ΔTr (msec)	100	-0.40	<0.0001

Table 3.20. Univariate associations of categorical variables with PWV in the combined group of both normotensive and hypertensive subjects.

Variable	Number	Mean AI%	95%CI	P value
Sex				
Male	72	9.68±0.3	9.1-10.2	P<0.05
Female	58	8.66±0.3	8-9.2	
Smoking				
Smokers	27	8.92±0.46	8.02-9.82	NS
Non-smokers	69	8.9± 0.28	8.36-9.44	
Alcohol excess				
Yes	26	9.26±0.47	8.34-10.18	NS
No	64	8.74±0.3	8.16-9.32	

Table 3.21. Partial regression coefficients for forward stepwise linear regression for dependent variable, PWV in the combined group of normotensive and hypertensive subjects.

Predictor Variable	*Partial regression coefficients*	*R Square*	*P value*
Constant	1.01		
Age (yr)	0.07	0.6	<0.0001
SBP (mm Hg)	0.04	0.74	<0.0001
Sex (f-m)	-0.3	0.75	<0.01

The results of our combined analysis showed considerable difference in the PWV between the two genders and I decided to look at the two groups separately.

We also analysed the relationship of smoking status and alcohol excess on PWV in males and females separately. PWV was higher in male smokers compared to non-smokers (9.66 ± 0.6 vs 8.9±0.4) but the difference was not statistically significant. In males with excessive alcohol intake, PWV was 9.72±0.58 vs 8.8±0.42 in those with social intake of alcohol. In the female group, smokers had a lower PWV (8.1±0.66 vs 8.89±0.42) than

non-smokers, though not significantly so. In females with high alcohol intake, PWV was 8.3±0.8 vs 8.64±0.4 in those with a low intake of alcohol.

Table 3.22. Univariate associations of quantitative variables with PWV in the male and female subgroups of both normotensive and hypertensive subjects.

Variable	Male			Female		
	N	R	P	N	R	P
Age (yr)	70	0.77	<0.0001	55	0.81	<0.0001
Body Height (cm)	70	0.02	NS	55	-0.27	<0.05
Body Weight (kg)	70	0.38	<0.0008	55	0.32	<0.01
BMI (kg/m^2)	70	0.42	<0.001	55	0.42	<0.001
Waist-Hip Ratio	70	0.37	<0.001	55	0.31	<0.05
Brachial SBP (mm Hg)	70	0.72	<0.0001	55	0.79	<0.0001
Brachial DBP (mm Hg)	70	0.60	<0.0001	55	0.75	<0.0001
Aortic SBP (mm Hg)	70	0.74	<0.0001	55	0.82	<0.0001
Brachial DBP (mm Hg)	70	0.60	<0.0001	55	0.76	<0.0001
Heart Rate (min^{-1})	70	0.27	<0.01	55	0.14	NS
AI%	70	0.64	<0.0001	55	0.70	<0.0001
AMP (mm Hg)	70	-0.39	<0.0001	55	-0.46	<0.0005
ΔTr (msec)	61	-0.40	<0.001	39	-0.51	<0.001

There is a higher correlation of PWV with age in both genders separately and the correlation with AI% in higher when the two genders are analysed separately *(Table 3.22)*.

3.3. 9. Determinants of Pulse Wave Velocity in the Hypertensive Population

In the hypertensive subjects, 44 PWV measurements were available so it was not possible to look at all the relationships. PWV significantly correlated with age(r= 0.49, p<0.01), weight (r = 0.36, p<0.05), brachial SBP(r = 0.47, p<0.001), aortic SBP(r = 0.46, p< 0.001) but there was no correlation with HR or DBP. In this small sample, I did not observe any relationship between PWV and AI% or AMP. The PWV was significantly higher in the male subjects compared to females (11.8±0.3 vs 10.8± 0.3, p<0.04) but there was no difference in the PWV according to smoking status or alcohol intake. Though the number

116

of observations was small, I did perform a forward stepwise regression on PWV in the hypertensive group and found age followed by SBP as the significant predictors of PWV.

3.3.10 Determinants of Pulse Wave Velocity in the Normotensive Population

In the normotensive population, 82 subjects had their PWV measured and I performed a univariate analysis with both quantitative and categorical variables to se if there was any difference in the relationships compared to the hypertensive population. PWV was positively related to age($r=0.71$, $P<0.0001$), body weight($r =0.29$, $p<0.001$), brachial SBP ($r= 0.63$, $p<0.0001$) and DBP($r=0.36$, $p<0.001$). The correlation with aortic SBP was much stronger than brachial SBP($r=0.75$, $p<0.0001$). PWV was also positively correlated to AI ($r= 0.44$, $p<0.0001$) and HR ($r=0.44$, $p<0.001$). The PWV was significantly higher in males compared to females (8.66 ± 0.3 vs 7.5 ± 0.3, $p<0.01$) *(Figure 3.1)*. There was no relationship between PWV and either smoking status or alcohol intake. In a forward stepwise regression model, age followed by SBP predicted the PWV in the normotensive subjects.
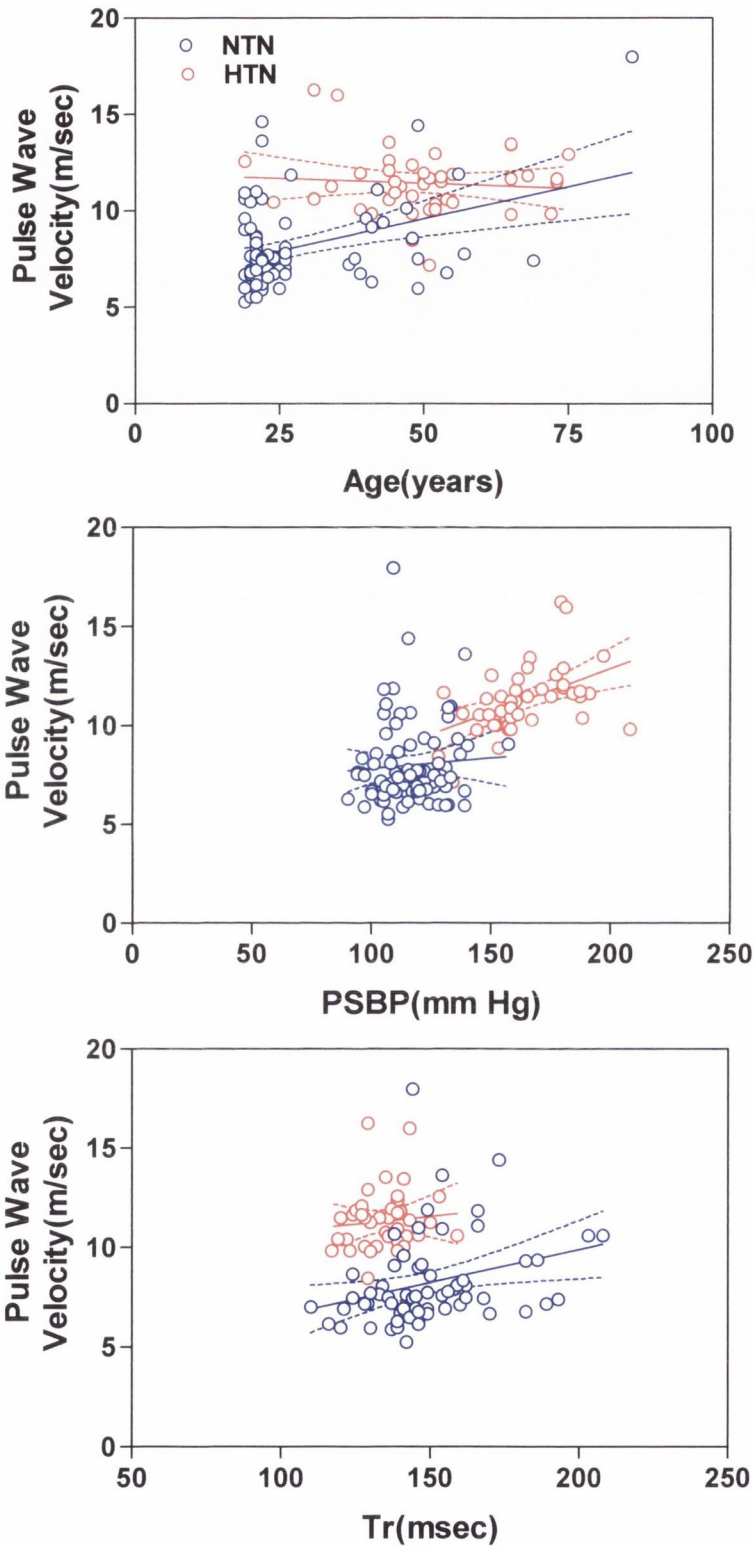
Figure 3.10. Univariate association (95% confidence interval of the line) of PWV with age, brachial systolic blood pressure and transit time of the reflected wave in the normotensive(n=82) and hypertensive(n=48) subjects.

118
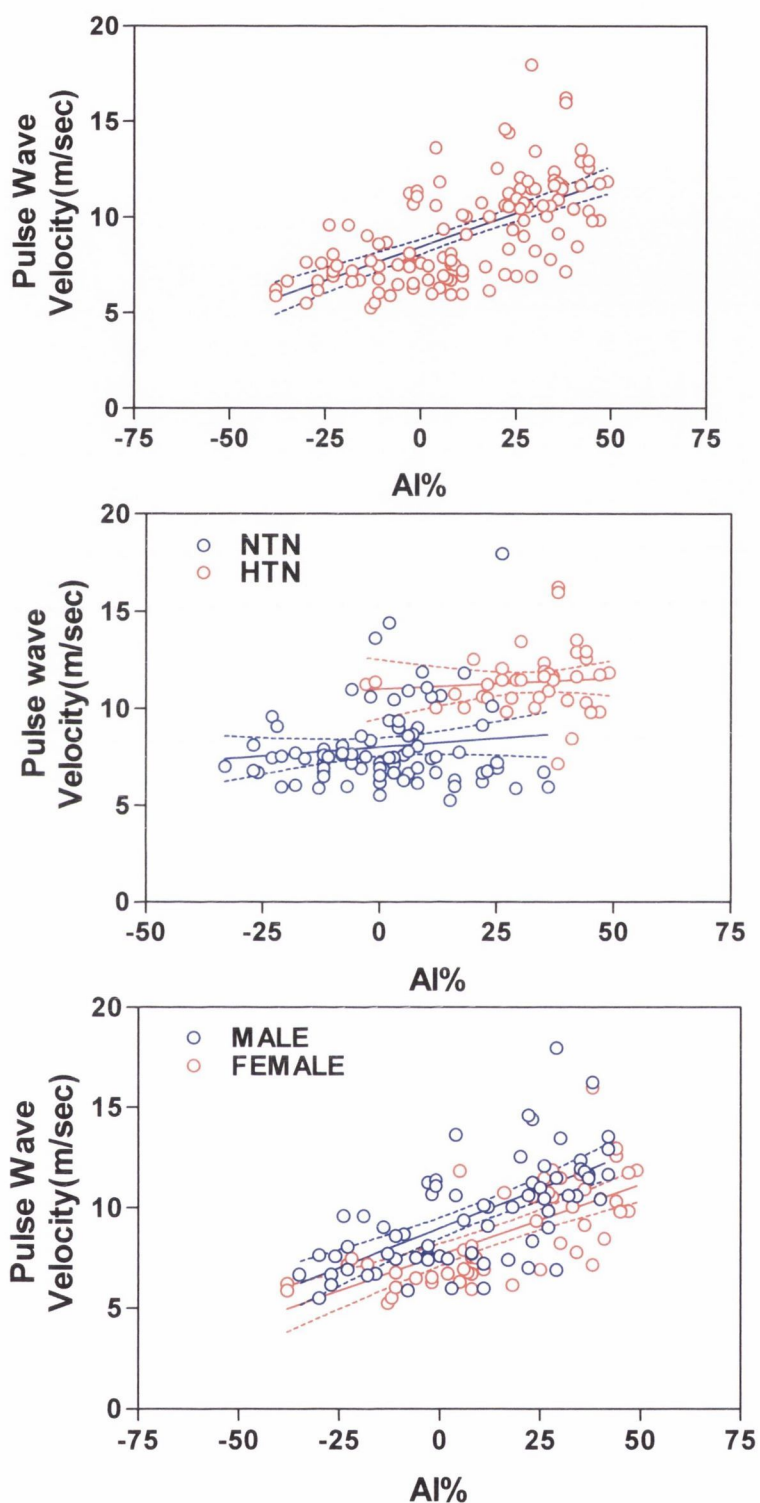
Figure 3.11. Univariate association(95% confidence interval of the line) of AI%
and PWV in the combined group of normotensive and hypertensive subjects
(n=130) (top panel), in the hypertensive(n=48) and normotensive(n=82) subjects
(middle panel) and in male(n=72) and female(n=58) subjects of the total population
(bottom panel).

3.4. Discussion

The major findings in this study are:

- There is a high degree of correlation between AI% and the other calculated indices of arterial wave reflection i.e. AP and P_2/P_1 *(Figure 3.1)*.
- There is linear relationship between the measured brachial BP and the derived aortic BP values *(Figure 3.2)*.
- There is a significant effect of age, independent of BP on AI%, PWV and AMP in both genders *(Figure 3.3)*.
- There is a significant difference in the AI%, PWV and AMP between the two genders *(Figures 3.4)*.
- Resting HR is an important determinant of the AI% in the hypertensive subjects but not in the young normotensive subjects *(Figure 3.6)*.
- Males show an effect of smoking or alcohol excess on AI% but not females (*Figures 3.7*).
- The level of physical fitness in the healthy population significantly affects AI% in both males and females *(Figure 3.8)*.
- The two measures of arterial stiffness i.e., AI% and PWV correlated significantly with each other and the correlations are higher with both genders studied separately *(Figures 3.10)*.
- This is the first study describing the determinants of AMP in a large population, its relationship with other stiffness indices and the effect of gender, smoking status and alcohol excess on this variable *(Figure 3.4 & 3.9)*.
- The transit time (ΔTr) was significantly and inversely related to PWV r =-40 *(Figure 3.10)* explaining 16% of the variability in PWV.

3.4.1 Relationship between Measured and Derived Blood Pressures

There is a clear association between AP and the dimensionless AI%, therefore the use of AI% *(Figure 3.1)* may be preferable as numerical scaling is not required (Kelly et al. 1989a; Vaitkevicius et al. 1993; Cameron et al. 1998).

In the current study, the difference in diastolic BP fall was within the range of measurement resolution and is inconsequential. SBP is amplified with peripheral progression. The strong relationship between measured brachial and derived aortic SBP *(Figure 3.2)* implies that in the practical case, equivalent results may be obtained by assessment of the pressure waveform from the carotid or subclavian artery with the use of directly measured brachial pressures adjusted, if necessary using the linear regression equation obtained.

3.4.2 The Effect of Ageing on Augmentation Index, Pulse Wave Velocity and Pulse Pressure Amplification

In the total group of healthy and hypertensive subjects, three age bands were created, Less than 30, between 30 and 50 and greater than 50 years of age. There is a marked difference in the AI%, PWV and AMP between the three groups *(Figure 3.8)*. The differences persisted even when the data was normalized for BP. These results are consistent with published work showing the effect of age on AI% (Kelly et al. 1989a), PWV (Asmar 1999) and AMP (Nichols and O'Rourke 1998b).

3.4.3 Resting Heart Rate And Stiffness Indices

In the present study, I observed different relationships between resting HR and the measures of stiffness i.e., AI%, PWV and AMP depending on the study population. HR correlated significantly and inversely to AI% in the hypertensive group but not in the young normotensive population *(Figure 3.5)*. For PWV, there was significant positive correlation with HR in the total study group but not in the hypertensive and normotensive groups separately probably because of small numbers. AMP was inversely related to HR in the total study group and the hypertensive subjects, but not in the normotensive population *(Figure 3.7)*.

There are considerable changes in the contour of the pulse with changes in HR because of the change in duration of ventricular ejection that affects the timing of the merging of the incident with the reflected wave. There is not much information on the relationship between resting HR and AI%, however dynamic changes in HR have shown that for every 10 beats increment, the AI% changes by 4-6%(Wilkinson et al. 2000a). Therefore

interventions that result in HR changes should take this factor into account e.g., beta-blockade, exercise.

The effect of heart rate on PWV has been a subject of much debate. A recent study has shown that high HR was significantly associated with increased PWV, even after adjusting for BP and age (Sa Cunha et al. 1997; Morcet et al. 1999) in both normotensive and hypertensive populations.

I found the resting HR to be inversely related to AMP, which can be explained by harmonic analysis; increase in HR increases AMP of the pulse wave between the aorta and the peripheral arteries. This is due to greater amplification of individual harmonics at frequencies close to the maximum of 5 to 6 Hz.

3.4.4 Effect of Smoking and Alcohol Excess on Augmentation Index

The effect of smoking and alcohol excess on AI% was observed to be gender dependent, with females showing no effect. In the total study group, males who smoked and had a history of alcohol excess had a significantly higher AI% as compared to males with a healthier life-style *(Figure 3.6)* These effects were not seen in the hypertensive group though the healthy subjects analysed separately showed a significant difference of smoking but not of alcohol excess on the AI%*(Figure 3.6).* The impact and possible mechanisms of the effects of smoking and alcohol on AI% are discussed at length in chapters 4 & 5.

3.4.5 The Effect of Fitness Level on Augmentation Index

The young normotensives were graded into three groups depending on self-reported levels of fitness. The group 0 were '*couch potatoes*', group 1 exercised occasionally and group 2 were active sportspersons. In both males and females, there was a graded effect of exercise on AI%, the highest AI% seen in Group 0 and the lowest in group 2, which clearly shows the favourable effect of regular exercise in both males and females *(Figure 3.7).* Physical fitness results in decreased augmentation of the pulse in different arteries and partially reverses the ill effect of ageing on the arterial pulse (Vaitkevicius et al. 1993). The mechanisms involved are not clear, but a plausible explanation could be a generalized

endothelium-mediated dilatation of muscular conduit arteries that results in decrease of the intensity and in changes in timing (through decrease of PWV) of wave reflection. On the other hand, the possibility that these changes are due to a causal relationship and not due to an adaptive response cannot be excluded; subjects with arterial systems less affected by ageing process may have a better performance and are likelier to be involved in regular exercise. Recent studies have shown an improvement of aerobic exercise performance associated with decrease in carotid augmentation shortly after pharmacological intervention (Chen et al. 1999). Regular aerobic exercise has great potential then of being used as a non-pharmacological intervention in subjects with stiff arteries. This issue has recently been addressed and it was noted that regular aerobic exercise attenuates age-related reductions in central arterial compliance and restores levels in previously sedentary healthy middle-aged and older men (Tanaka et al. 2000). This may be one mechanism by which habitual exercise may lower the risk of cardiovascular disease in e.g., hypertensive population.

3.4.6 Gender Difference in Stiffness Indices

The finding of higher AI% in women is consistent with published literature (Hayward and Kelly 1997; London et al. 1992b; London et al. 1995) *(Figure 3.8).* The multiple regression analysis showed that the difference in AI% between the two genders could be partly explained by body height (Smulyan et al. 1998). Contrasting with the brachial SBP, the aortic SBP was not different in men and women, a finding in accord with previous studies (London et al. 1995). Body height is an important factor that determines the augmentation of arterial pressure wave on account of timing of wave reflection. Shorter persons have increased augmentation and vice versa (London et al. 1992b; London et al. 1995). In contrast to earlier studies, which have failed to show a significant correlation between height and AI% separately in males and females (Table 3.5) (Hayward and Kelly 1997), I found AI% to be significantly associated with height in both genders. Increased systolic pressure augmentation and resultant increase in left ventricular afterload and development of LVH may explain why short stature has been reported in some studies to be a risk factor for cardiovascular disease (Herbert et al. 1993).

3.4.7 Correlation of Augmentation Index with Pulse Wave Velocity

I was able to show in this study that AI% and PWV are significantly correlated in both hypertensive or normotensive subjects *(Figure 3.11)*. This is in agreement with earlier work (Marchais et al. 1993; Brown 1999). However, this study differs from the above in that I observed this relationship in older hypertensive and young normotensive, in contrast to the previous studies, which examined patients with end-stage renal disease and healthy normotensive subjects respectively. This information is very useful as it provides support for the use of AI% to assess arterial stiffness, as it is the easiest and quickest method of measuring arterial stiffness currently available. However, the much higher correlation of the AI% and PWV within the genders *(Table 3.19 & 3.23) (Figure 3.11)* than overall suggests that sex is a major confounding variable and different normal ranges should be calculated for men and women in future studies.

The PWV was significantly higher in males compared to females in all groups *(Figure 3.4)*. This is in agreement an earlier study (Asmar et al. 1999), which showed higher PWV for age in men than in women. It has been suggested that the PWV is higher in males compared to females in adult life but similar in children and the elderly (Asmar 1999).

The AMP was significantly higher in males compared to females in all groups *(Figure 3.8)*. The significant association of AMP with height and HR makes it difficult to separate the gender effect on AMP from the height and HR interaction.

3.4.8 Pulse Pressure Amplification

This is the first study describing AMP in a large population. We found AMP to be age-dependent, decreasing with age *(Figure 3.3)*. It was also significantly higher in males compared to females in both populations *(Figure 3.4)*. It positively correlated with HR, body height, and waist-hip ratio and negatively with AI%, PWV and DBP, which are new findings from this study *(Figure 3.9)*. Smoking and alcohol excess also decreased AMP, but in males only. The hypertensives had a significantly lower AMP than the normotensive subjects.

Transmission of arterial pressure to the periphery is accompanied by AMP. Mechanisms proposed to account for this include reflection from distal sites Murgo et al. (1980b) as well as nonlinearity in the forward travelling wave from the heart (Jones et al. 1992). This leads to aortic-brachial differences that diminish with age due to the effect of arterial stiffening in producing aortic systolic pressure augmentation (O'Rourke et al. 1992b). As brachial PP is inversely related to body height, the suggestion is that this phenomenon is also affected by transmission length (Asmar et al. 1997).

The phenomenon of AMP may have important consequences when considering clinical end-point studies, which have mostly relied on brachial artery measurements, whereas important pathophysiological consequences of a change in PP, such as LVH and coronary perfusion are related to aortic pressure. The AMP is especially of importance when considering young individuals, as the disparity between aortic and peripheral BP may be as much as 20 mm Hg (Panca et al. 1992) AMP has been looked at in smaller studies only observing the effects of anti-hypertensive drugs on arterial stiffness (Topouchian et al. 1998) but to date no work has been carried out in large numbers as in the present study.

3.4.9 Relationship of Pulse Wave Velocity to Transit Time of Reflected Wave

The number of subjects who had both their PWV and transit time measured was relatively small, only 100 which included both hypertensive and normotensive subjects. The correlation is significant, r=-0.40 which thus explains about 16% of the variance in PWV. Keeping in view the smaller number of subjects studied, it may be acceptable to use it in situations when PWV is not available, though with caution as such a weak correlation suggests that there may be different factors determining PWV and transit time of the reflected wave.

3.4.10 The Determinants of Arterial Stiffness Indices

The determinants of AI% were different in the hypertensive subjects with heart rate and height being the most important followed by age. In the normotensive subjects, age was the most important followed by diastolic BP and sex. This highlights that depending on the distending pressure; AI% could be determined by totally different factors in a normotensive and hypertensive population. In contrast, PWV is predicted almost 50% by

age followed by systolic BP regardless of which group is studied. Because of the large differences in AI% between men and women, the analyses was also performed on the two genders separately. There was a significant difference in the AI%, PWV and AMP between males and females *(Figure 3.3).*

Databases such as this provide a springboard on which to explore the determinants of arterial stiffness. Controlled as well as observational studies are needed to further explore these relationships. As an example of such a use, I have explored the issue of spurious systolic hypertension of youth.

Pseudo Systolic Hypertension in Young Men – Elastic Not Stiff Arteries

3.5   Introduction

During medical student practicals we noted a number of individuals with high systolic but normal DBP by current WHO definitions (Guidelines Sub-Committee and World Health Organisation - International Society of Hypertension. 1999) who have ISH (BP > 140/<90mm) and could be disadvantaged in relation to life assurance or fitness to work. The concept of pseudo hypertension, described by Osler, where cuff pressure exceeds simultaneously measured intra-arterial pressure is almost exclusively considered in relation to stiff arteries in the elderly (Zweifler and Shahab 1993).   However there is epidemiological data showing a the rise in SBP in people in their twenties which has been attributed by O'Rourke (Nichols and O'Rourke 1998b) to extreme amplification of pressure wave from the aorta to the brachial artery owing to highly elastic arteries.

3.6   Materials and Methods

We therefore compared brachial, measured by an automated oscillometric method (Omron 705 CP) to aortic BP in our 174 medical students.   Following 15 min supine rest, aortic blood pressure and arterial wave reflection were recorded from radial applanation tonometry using the generalised TF (Karamanoglu et al. 1993) with a commercially available software system (Sphygmocor PWV) to generate the aortic pressure waveform. Systolic amplification was calculated by subtracting the aortic from the brachial pressure. Subjects also independently completed a questionnaire with regard to smoking habits, alcohol intake and participation in physical sports.   Weight and height were recorded.

3.7   Results

Eleven individuals (including 4 studied on 3 occasions) with brachial SBP ≥140 mm but normal brachial diastolic and normal aortic pressures were compared with the other 163 subjects and, because they were exclusively male also with the normotensive male sub-group (Table 3.23).   Their aortic SBP although well within the normal range, was higher than that of the normotensives including the male sub-group, as was their systolic pressure amplification (*Figure 3.12*).   **Figure 3.13** shows waveform analysis in a pseudosystolic

hypertensive male showing high brachial artery SBP and normal aortic SBP with an aortic pressure waveform showing normal wave reflection in diastole.

Table 3.23 Comparison of subjects (mean±SEM), demographic data and haemodynamic measurements (mmHg), with pseudo-systolic hypertension to all normotensive subjects (*p<0.01) and male normotensives, (^f p<0.01)

	Pseudo systolic Hypertension (Males)	Normotensives		
		All	Male	Female
Number	11	163	76	87
Age (years)	23.5±0.92	22±0.2	22±0.48	22±0.27
Height (cm)	179±1.85	171.4±0.7	176.7±0.8	166.56±0.93
Weight (kg)	78.9±1.79	67.5±1.1	74.8±1.7	61±0.9
Smokers	0*(0%)	41(26%)	19(15%)^f	22(18%)
Active Sports	11*(100%)	40 (25%)	33(41%)^f	17(22%)
Brachial systolic BP	147.3±2*	114.8±0.96	120.5±1.3^f	109.8±1.2
Brachial diastolic BP	70±2.2	68.4±0.71	69.9±1	67±0.98
Aortic systolic BP	115.9±1*	96.8±0.9	100±1.5^f	94±0.9
Aortic diastolic BP	70±2.5	69.6±0.7	71±1	68.3±0.9
Heart rate/min	61.1±1.9	69.5±1.2	68.3±1.65	70.7±1.7
Amplification	31.4±1.5*	17.9±0.7	20.5±1.36^f	15.7±0.55

The characteristic finding in our observational study was all individuals with pseudo systolic hypertension were male, non-smokers, involved in active sports with high arterial pulse pressure amplification in comparison with the whole group and with the other males.
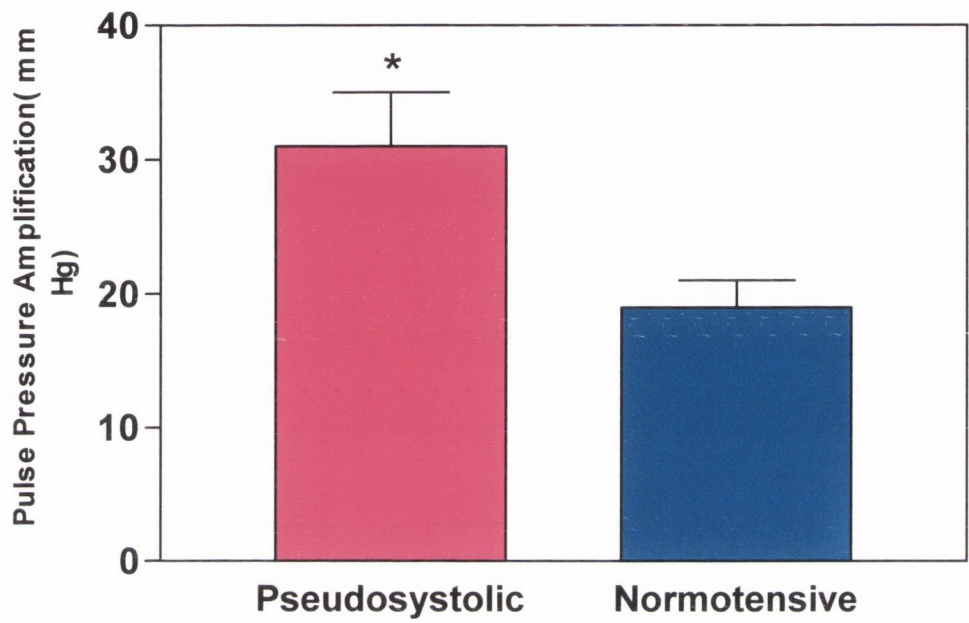
Figure 3.12. The pulse pressure amplification in normotensive (n= 76) versus pseudo-systolic hypertensive (n=11) male subjects(Total n=163, mean±SEM, *p<0.05)
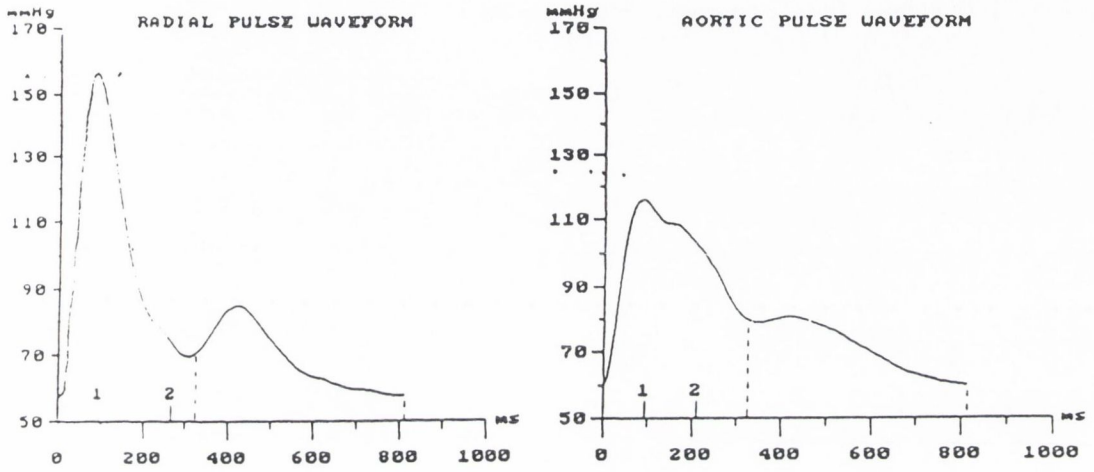
Figure 3.13. Radial and aortic pressure waveforms in a healthy 22 year old male with blood pressure 155/71 mm Hg recorded in the brachial artery (left) and 115/71 mm Hg in the aorta (right) indicative of pseudo-systolic hypertension of youth.

3.8 Discussion

Pseudo-hypertension is attributed to non-compressibility of the brachial artery and is largely seen in elderly subjects with arterial stiffness and primarily affects DBP. Systolic pressure amplification is an important index of the elasticity of arteries and is reduced with ageing (Karamanoglu et al. 1993). A finding of a high degree of amplification suggests highly elastic arteries. It is interesting that all these subjects were non-smokers and involved in active sports as smoking decreases arterial elasticity and exercise may enhance it (Nichols and O'Rourke 1998b). It is likely therefore that the increased amplification is responsible for the SBP being elevated in the peripheral arteries. Although we do not have long term follow up data, we expect that individuals with such pseudo systolic hypertension may not be at increased risk. However, there is indeed need for caution in interpreting these results, as the derived aortic SBP was still higher in the young men with pseudo-systolic hypertension compared to young individuals with normal brachial SBP. Further studies with 24 hour ambulatory blood pressure monitoring, some measure of CO and long-term follow-up is needed to confirm a positive prognosis of these individuals. The gold standard would be of course to perform aortic catheterisation in these subjects to confirm our results but that is not possible in clinical practice. We still would recommend that otherwise healthy young men with ISH should have their aortic SBP measured to exclude such pseudo systolic hypertension.

There is considerable epidemiological data and public interest on the effect of dietary factors such as salt, caffeine and alcohol on BP in different populations and races throughout the world. Salt intake, additionally has been shown to increase arterial stiffness independent of BP. Like salt, caffeine and alcohol are consumed worldwide but are not generally perceived as being pharmacologically active. Caffeine is the most widely consumed beverage in the world in the form of coffee, tea and various soft drinks however coffee perhaps is the biggest source of caffeine in our diet. The epidemiological data on the long-term effects of caffeine are controversial despite showing acute pressor effects in various studies with substantial increases in PR and serum catecholamines. However, a recent suggestion is that caffeine might increase systolic blood pressure in the long-term without effecting DBP, suggesting a role for large arterial stiffness rather than increased peripheral resistance. However, there is no information on the effects of caffeine on arterial stiffness.

In the past two decades, a series of studies have suggested that moderate consumption of alcohol, particularly wine appreciably reduce the risk of heart disease. The French paradox, which suggests the high intake of red wine may be the underlying factor in the low incidence of heart disease in the French people despite having a high cardiovascular risk profile has led to heightened interest in the possible beneficial effects of red wine on the cardiovascular system. Recent studies have looked at endothelial dysfunction and red wine, both with and without alcohol. While recent cross-sectional analysis suggests that alcohol intake may lead to increased arterial stiffness, no study has looked at the acute effects of alcohol and red wine on arterial stiffness.

The purpose of this chapter was to explore these issues in the context of arterial stiffness. Therefore I performed two separate double-blind randomised crossover studies:

1. The acute effects of caffeinated and decaffeinated coffee on arterial stiffness and the aortic pressure waveform in healthy subjects

2. The acute effects of red wine, with and without alcohol on the aortic pressure waveform and arterial stiffness in healthy subjects.

CHAPTER FOUR

EFFECT OF CAFFEINE AND ALCOHOL ON ARTERIAL STIFFNESS

Acute Effect Of Caffeine On Arterial Stiffness And Aortic Pressure Waveform

4.1   Introduction

There is conflicting evidence on the effect of caffeine on systemic haemodynamics(Robertson et al. 1984; Gorbbee et al. 1990; Jee et al. 1999). In acute studies it has been shown to increase blood pressure and peripheral vascular resistance but tolerance rapidly develops (Robertson et al. 1981). The pressor effect of caffeine is predominantly due to its action on the resistance vessels rather than an increase in cardiac output (Pincomb et al. 1985). While the increase in sympathetic nervous system activity, serum adrenaline and renin have been causally linked; the acute pressor effect is also seen in adrenalectomised patients (Smits et al. 1986). Vasoactive hormones such as aldosterone vasopressin and arterial natriuretic peptide however are not increased by caffeine (O'Rourke and Frohlich 1999; Nussberger et al. 1990). Caffeine is a non-selective antagonist of adenosine at both A_1 and A_2 receptors. Adenosine A_1 receptor stimulation inhibits the release of norepinephrine at the sympathetic smooth muscle junction while A_2 receptors have a direct vasodilatory effect (Ohnishi et al. 1988; Smits et al. 1987).

Caffeine is one of the most widely consumed vasoactive substances with 80% of adults in the United States having a daily intake of 200-300 mg (Gilbert 1984). A recent meta-analysis suggests that chronic coffee consumption may increase SBP (Jee et al. 1999). The acute effects of caffeine on large artery properties however are not described. As early wave reflection and PWV, measures of arterial stiffness, are associated with an increase in SBP, we examined the effect of caffeine on these properties of large arteries.

4.2   Materials and Methods

1. Subjects: Seven healthy volunteers (4 female) aged 26±6 years (mean, ±SD) participated in this study that had Institution Ethics Committee Approval.

The subjects were given 250 ml of either freshly brewed caffeinated or decaffeinated coffee in a randomised double blind crossover protocol on two separate days at least one week apart. The caffeine content of the 250 ml of coffee determined by high-pressure

liquid chromatography (Kreiser and Martin 1980) was 150±5 mg and under 2 mg for caffeinated and decaffeinated coffee respectively. All subjects had abstained from smoking, alcohol or caffeine containing beverages in the 12 hours prior to the study. The haemodynamic measurements were performed in a quiet room at 20-23°C. The subjects were recumbent throughout the study. After a stable baseline, haemodynamic measurements were made in the supine position at baseline, and 30, 60 and 90 minutes following ingestion of coffee.

2. Blood Pressure Measurements: Brachial BP and HR were measured in the left arm with an automated digital oscillometric BP monitor (Omron® Model 705-CP). A mean of three readings was taken at each measurement time.

3. Derivation of the aortic pressure waveform: Immediately after recording BP, the same arm was used for applanation tonometry. A high-fidelity micromanometer (BPAS-1, PWV Medical ®, Sydney, Australia) was used to flatten the radial artery and the radial pulse continuously recorded. The central aortic waveform was derived from radial tonometry by using a previously validated TF relating peripheral to central waveform within the software package (Sphygmocor®) as previously described (Mahmud and Feely 2001b). The validity of the derived AI% has been confirmed by simultaneous direct central aortic measurements and is highly reproducible in both healthy and diseased populations (Wilkinson et al. 1998b; Siebenhofer et al. 1999).

4. Pulse wave Velocity Measurements: Carotid-femoral PWV was determined according to the foot-to-foot method using the Complior ® device. The simultaneous recording by two pressure sensitive transducers of the carotid and femoral waveform and measurement of the time delay of successive records from the foot of each wave divided by the distance between the transducers allows calculation of PWV (metres/sec). The distance travelled by the pulse wave was measured over the body surface with a tape measure as the distance between the recording sites and fed into the software and 10 consecutive waves were sampled. This method of evaluating the covered distance by superficial measurement may introduce a margin of error since the direction of blood flow in the carotid artery is opposite to that in the abdominal aorta. However, PWV is measured separately in the

same subjects; such error therefore does not affect the results. The validity of the Complior ® has been established (Asmar 1999).

6. Statistical Analysis: The effect of coffee on time-dependent patterns of evolution of blood pressure, PWV and AI% was tested by repeated measures two-way analysis of variance ANOVA.

In addition, since BP influences arterial haemodynamic parameters, we carried out a complimentary analysis on these variables from baseline over time, adjusted for BP at the time they were measured. Comparison of the area under the BP time curve, corrected to baseline reading, by the trapezoidal rule was made by Wilcoxon Rank Sum test. Correlations were examined by least square regression analysis. The results are presented Mean±SEM *$p < 0.05$.

4.3   Results

There was no significant difference in the baseline haemodynamic values for both days.

1. Acute Effects of Caffeine on Blood Pressure and Heart Rate: The changes in brachial systolic and diastolic BP over the 90 minutes following both caffeinated and decaffeinated coffee are shown in *Figure 4.1.* There was an increase in brachial diastolic blood pressure over 90 min but not in brachial SBP. However the integrated change in BP, represented by the area under the systolic and diastolic BP time curve, was greater following caffeinated than decaffeinated coffee ($p < 0.05$). There was no significant change in HR following either caffeinated or decaffeinated coffee *(Figure 4.1).*

Aortic systolic and diastolic BP increased with time following caffeinated but not decaffeinated coffee ($p < 0.05$). Moreover, the effect of caffeine was more pronounced on aortic than brachial SBP ($p < 0.05$) as shown by a larger increase in the area under the BP time curve for aortic than brachial BP *(Figure 4.2).*

2. Acute effects of Caffeine on Pulse wave velocity: Following caffeinated coffee PWV increased progressively from 7.2±0.4 to 8.0±0.6 at the end of 90 minutes (p<0.05). No change was seen with decaffeinated coffee *(Figure 4.3)*. No correlation was found between change in PWV and increase in BP and changes in aortic PWV were restricted after adjustment for SBP changes in the peripheral brachial artery and in the aorta. The increase in PWV remained significant after adjustment for changes in BP at 30 minutes (p=0.02 for systolic, p=0.06 for diastolic) and 60 minutes (p<0.05 for both) following baseline but not at 90 minutes.

3. Acute effects of Caffeine on Arterial Wave Reflection: The AI% increased from −5.1%±7.6 to +5.28%±5.6 following caffeine over 90 minutes (p<0.01). There was no significant effect of decaffeinated coffee on the AI%*(Figure 4.3)*. The increase in the index showed a trend to significance after adjustment for BP at 60 min (p = 0.056 for systolic, p = 0.07 for diastolic).
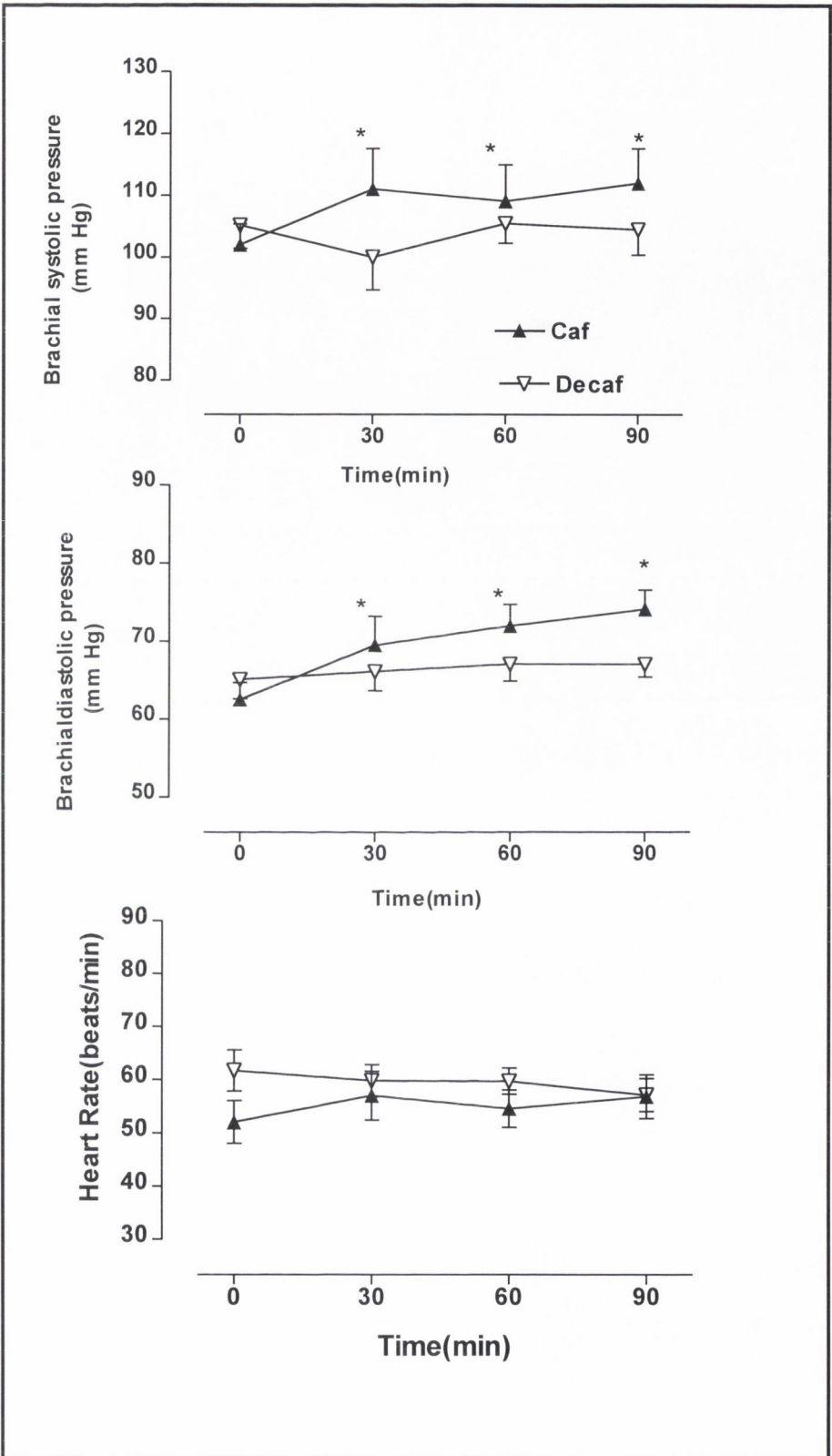
Figure 4.1. Acute changes in brachial blood pressures and heart rate following the ingestion of caffeinated and decaffeinated coffeee in 7 healthy subjects (mean±SEM, *p<0.05 from baseline)
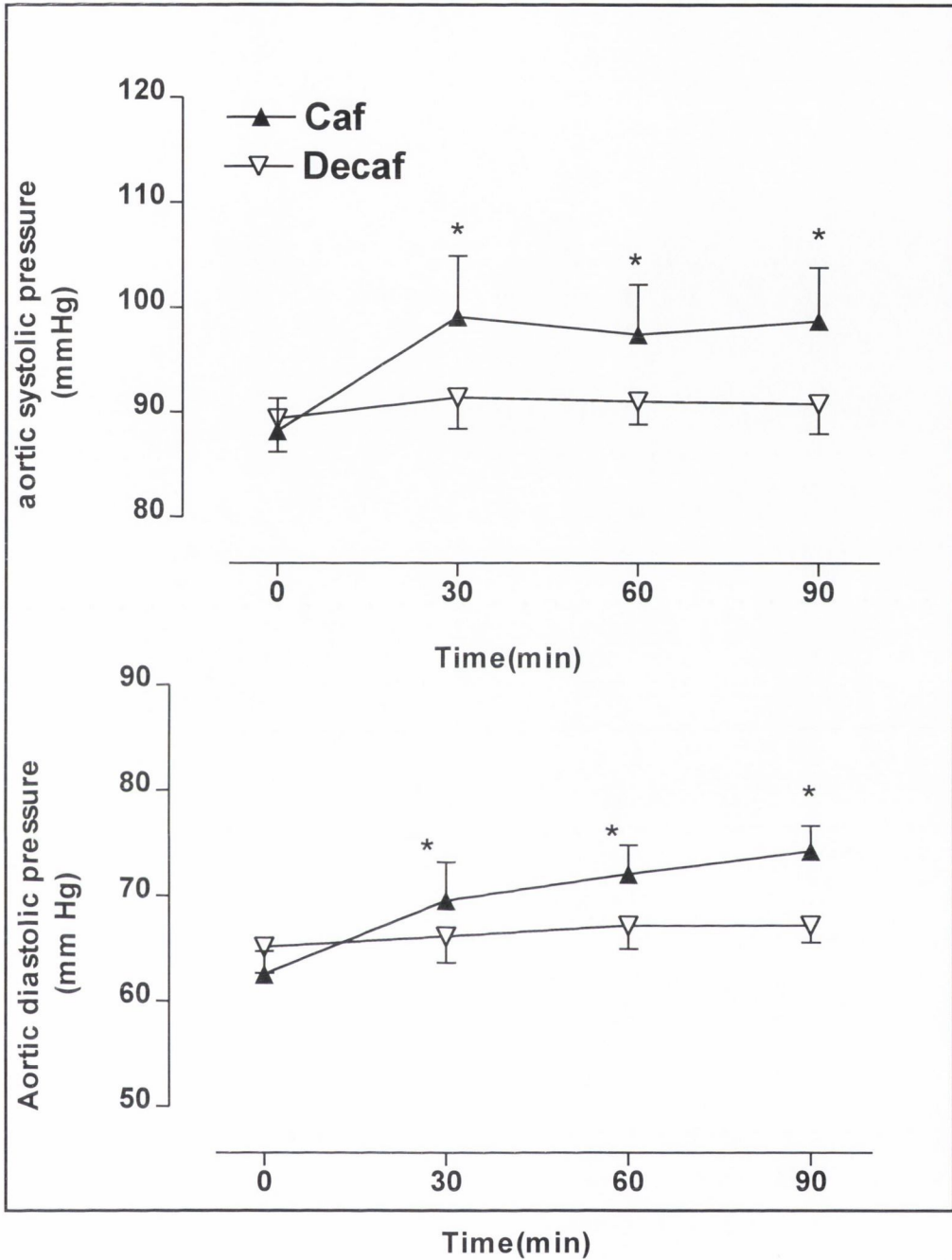
Figure 4.2. Acute changes in aortic blood pressures following the ingestion of caffeinated and decaffeinated coffeee in 7 healthy subjects (mean±SEM, *p<0.05 from baseline)

138
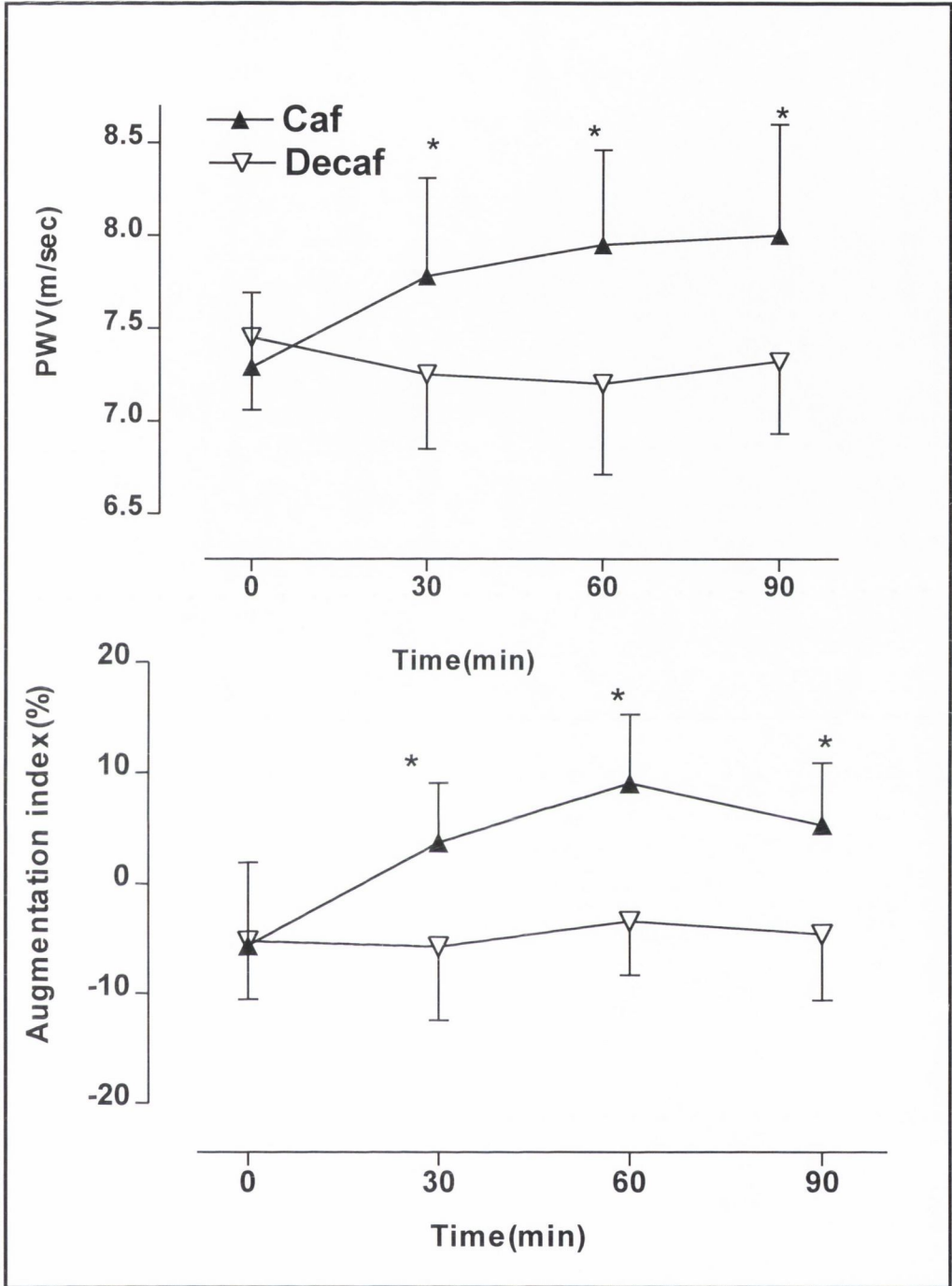
Figure 4.3. Acute changes in pulse wave velocity and augmentation index following the ingestion of caffeinated and decaffeinated coffeee in 7 healthy subjects (mean±SEM, *p<0.05 from baseline)

4.4 Discussion

This study demonstrates an acute effect of coffee on aortic waveform, BP and vascular stiffness. We believe this can be attributed to caffeine, as the effect was not seen with decaffeinated coffee. These results not only suggest the possibility that this action of coffee may contribute to its 'hypertensive effect' but also emphasize the importance of controlling caffeine intake in studies on vascular stiffness.

While we have shown an acute effect on arterial stiffness it is interesting to note that a recent meta-analysis (Jee et al. 1999) shows that tolerance to coffee is incomplete. During chronic ingestion of coffee, systolic but not diastolic BP is elevated. Chronic arterial stiffness is associated particularly with increased SBP rather than diastolic (O'Rourke and Kelly 1993; Breithaupt-Grogler and Belz 1999; McVeigh et al. 1997). The deterioration of the elastic properties of the aorta in this study as shown by increased PWV and arterial wave reflection may be one of the mechanisms underlying the pressor effects of caffeine hitherto overlooked. While it is not possible to definitely conclude in part due to the small number of subjects that this effect is independent of the effect of caffeine on BP, we did not find any correlation between the increase in variables and when adjusted for changes in BP, the effect of caffeine on PWV, but not arterial wave reflection, remained significant. Furthermore, the greater increase in aortic SBP than in the brachial SBP, as reflected by a comparison of the area under time pressure curves suggest an effect additional to sympathetic peripheral vasoconstriction. Increased PWV and arterial wave reflection are the probable explanation for this finding. Also these results may suggest an underestimation of the pressor effects of caffeine, when the effects on aortic pressure are not specifically measured. The increased PWV is considered to reflect increase in aortic stiffness while the effect on AI% or arterial wave reflection, may in part be due to either increased PWV or an increase in PR with vasoconstriction of the peripheral muscular arteries or both.

We also noted a pressor effect on peripheral BP - the magnitude of which is smaller than that seen in some other studies. This can be largely attributed to the dose (150 mg) administered. We chose this dose as it better represents the more usual social intake of

caffeine rather than a 250mg dose used in other studies, which is more equivalent to the total daily dose (Kreiser and Martin 1980). We did not measure the levels of caffeine, which may through an examination of concentration-effect relationship have strengthened our conclusion that it is the caffeine in the coffee that is responsible for increasing BP and arterial stiffness in this study. While the number of subjects is relatively small, the study was randomised and double blind. Furthermore the results are internally consistent as the surrogate markers of vascular stiffness were measured simultaneously by two independent methods – applanation tonometry and PWV.

The published data (Nurminenen et al 1999) relating to intake of coffee and caffeine on blood pressure in man was recently reviewed from MEDLINE and Current Contents databases searched from 1966 to April 1999. Acute intake of coffee and caffeine increases blood pressure and caffeine is regarded as the main active component. The pressor response is strongest in hypertensive subjects. Repeated administration of caffeine showed a persistent pressor effect in some studies, whereas in others chronic caffeine ingestion did not increase blood pressure. Epidemiological studies (Asmar et al. 1995b) have also produced contradictory findings regarding the association between blood pressure and coffee consumption. However during regular use tolerance to the cardiovascular responses develops in some people, and therefore no systematic elevation of BP in long-term and in population studies can be shown. Overall they concluded (Asmar et al. 1995b) that regular coffee may be harmful to some hypertension-prone subjects and a more recent meta-analysis (Jee et al. 1999)shows a significant chronic effect on SBP.

This issue of different sensitivity is of particular importance has recently been specifically addressed (Nurimen et al. 1999). The acute effects of caffeine on arterial blood pressure were compared in 5 male hypertension risk groups, optimal BP, high-normal BP, stage 1 hypertension and treated hypertensives (Nurimen et al. 1999). Caffeine, approximately 250 mg raised both systolic and diastolic BP, but the strongest response to caffeine was observed among diagnosed men, followed by the stage 1 and high-normal groups and then by the normal and optimal groups. Diagnosed hypertensive men had a pre-to-post drug change in BP that was 150% greater than in the optimal group. The acute BP elevation with caffeine is also enhanced in borderline hypertension (Hartley et al. 2000). Also

caffeine seems to have an additive pressor effect with stress in male medical students with a family history of hypertension and high normal blood pressure(Pincomb et al. 1996). Both systolic and diastolic BP was affected. In contrast, in the chronic studies, the effect of caffeine seems to be predominantly systolic (Jee et al. 1999). Also in normotensive middle-aged male habitual caffeinated coffee drinkers switching to decaffeinated coffee led to a significant reduction in systolic but not diastolic mean ambulatory BP (Shephard et al. 2000). In this context it should be noted that the effects of chronic vascular stiffness manifest predominantly as changes in SBP (Superko et al. 1994).

The cardiovascular effects of caffeine are largely mediated through blockade of both A_1 and A_2 adenosine receptors (Smits et al. 1987; Nichols and O'Rourke 1998a; Daniels et al. 1998). Inhibition of phosphodiesterases is also seen but only in pharmacological doses. That caffeine may have vascular effects is also suggested in a recent study where it attenuated the increase in forearm blood flow following exercise (Daniels et al. 1998). In that study caffeine also induced an increase in angiotensin II. The main novel finding in our study is that caffeine acutely stiffens the aorta and impedes the function of the peripheral muscular arteries also, which may be an additional vascular mechanism for the hypertensive effect of caffeine. On the other hand there may be some tolerance also to this effect of caffeine, an issue that requires longitudinal study.

There is now epidemiological data to show that PWV is an independent risk factor for total and cardiovascular mortality (Blacher et al. 1999b; Kreiser and Martin 1980). Recently measurement of PWV in hypertension and patients at cardiovascular risk has been recommended (Daniels et al. 1998). In addition arterial wave reflection is commonly assessed in vascular studies. Therefore our findings are also important because caffeine intake is ubiquitous and studies conducted on vascular stiffness should control for its intake.

The Acute Effect of Red Wine with Alcohol and without Alcohol on Arterial Stiffness

4.5   *Introduction*

The medicinal benefits of alcohol, particularly wine, have been suggested for millennia. As recently as the 1830's William Stokes studied the effect of wine on the pulse in typhus fever (Stokes 1839).   Over the last two decades, a series of studies from around the world have demonstrated that moderate alcohol and wine consumption appreciably reduce the risk of cardiovascular disease (Friedman and Kimball 1986; Fuchs et al. 1995; Gaziano et al. 1993) and decrease overall mortality rates (Thun et al. 1997).   Alcohol may also have other positive effects for certain diseases and conditions reducing the risk of diabetes (Perry 1995), dementia (Orgogozo et al. 1997) and renal stones (Curham et al. 1996). Since ancient times the regular consumption of wine has been closely associated with diet, particularly in Mediterranean countries (Willet 1994).   However, it was the epidemiological studies looking into factors linking diet to CHD that threw up the so-called French paradox (Renaud and de Lorgeril 1992).   Despite a high intake of saturated animal fat in France epidemiological studies suggest that consumption of alcohol in the region of 20-30 g (2-3 units) per day, can reduce the risk of CHD by at least 40%.   The paradox is not that the mortality rate from CHD is low in France, but that it is low despite the high level of risk factors (serum cholesterol, SBP and consumption of dairy products) and the relatively low consumption of fruit and vegetables by the French people. Furthermore using surrogate indices of vascular diseases such as the ankle brachial index as a measure of impaired lower extremity circulation, it was noted that those with the higher alcohol intake had less peripheral vascular disease (Jepson et al. 1995).   Also in a study of 22,000 male physicians, alcohol of all types was associated with a lower risk of peripheral vascular disease (de Lorimier 2000).

Numerous mechanisms have been advances to explain the cardio-protective effect of wine and include an effect on platelet aggregation, increases in HDL cholesterol and an antioxidant effect particularly of flavonoids which are found in significant amounts in grape products (de Lorimier 2000).   Recently it has been shown that red wine may improve endothelial dysfunction (Agewell et al. 2000).   On the other hand increasing

consumption of alcohol, particularly above 4 drinks per day, is associated with increased BP (de Lorimier 2000; Beilin et al. 1999). Curiously, however, BP may decrease acutely following alcohol (Kojima et al. 1993). This and the common experience of flushing with alcohol suggest it may have a direct vascular effect but the effect may vary throughout the vasculature. Therefore to further explore the vascular effects of alcohol, we compared the effect of red wine and de-alcoholised red wine on large artery properties, particularly arterial stiffness in healthy volunteers.

4.6   Materials and Methods

1. Study Population
We studied eight (5 female) healthy non-smokers, age range 21-40 years (weight 70±3.9 kg, height 1.7±0.2 metres, mean±SEM). Subjects had no major risk factor for CHD and were not taking any prescription drugs, over the counter medications or vitamin supplements. The average alcohol intake in the subjects was 10 ±3.8 units/week. The protocol was approved by the Institutional Ethics Committee and informed consent was obtained from all subjects.

2. Study Protocol:
The study consisted of two visits, at least one week apart. Subjects were asked to abstain from alcohol in the 24 hours prior to each study visit. Each subject was studied fasting having abstained from all caffeine containing beverages in the 12 hours prior to the study and was given 500 ml of red wine (0.8g/kg ethanol) or 500 ml of red wine without alcohol in a double-blind randomised cross-over fashion. Each drink was to be consumed within 10minutes. The studies were carried out in a quiet room at 20± 1^{0}C with subjects in the supine position. After a supine rest of 15 minutes, baseline haemodynamic measurements were made. Subsequent readings were taken 30, 60 and 90 minutes following ingestion of either drink.

3. Blood Pressure Measurements:
Brachial BP and HR were measured in the left arm with an automated digital oscillometric BP monitor (Omron® Model 705-CP, Japan). A mean of three readings was taken at each measurement time.

4. *Derivation of the aortic pressure waveform*: Immediately after recording BP, the same arm was used for applanation tonometry. A high-fidelity micromanometer (BPAS-1, PWV Medical ®, Sydney, Australia) was used to flatten the radial artery and the peripheral radial pulse continuously recorded. The central aortic waveform was derived with radial tonometry by using a previously validated TF relating peripheral to central waveform within the software package (Sphygmocor®, PWV, Sydney) as previously described (Mahmud and Feely 2001b). The validity of the derived AI%, a measure of arterial wave reflection, has been confirmed by simultaneous direct central aortic measurements (Kelly et al. 1989b; Chen et al. 1996) and is highly reproducible in both healthy and diseased populations (Wilkinson et al. 2000a; Siebenhofer et al. 1999).

5. *Pulse wave Velocity Measurements*: Carotid-femoral PWV was determined according to the foot-to-foot method using the Complior ® device (Colson/Dupont Medical, Pantin, France). The simultaneous recording by two pressure sensitive transducers of the carotid and femoral waveform and measurement of the time delay of successive records from the foot of each wave divided by the distance between the transducers allows calculation of PWV (metres/sec). The distance travelled by the pulse wave was measured over the body surface with a tape measure as the distance between the recording sites and fed into the software and 10 consecutive waves were sampled. This method of evaluating the covered distance by superficial measurement may introduce a margin of error since the direction of blood flow in the carotid artery is opposite to that in the abdominal aorta. However, PWV is measured separately in the same subjects; such error therefore does not affect the results. The validity of the Complior ® has been established (Asmar et al. 1995b).

6. *Statistical Analysis*: The data was analysed with JMP version 3(version for Windows, SAS Institute, Cary, NC). The difference between the baseline haemodynamic values was analysed with one-way analysis of variance. Haemodynamic changes were studied by two-way analysis of variance for repeated measures applied to a crossover design and testing treatment and period effect. As there was no significant difference between the two groups, it was assumed that there was no carry over effects and further data analysis was based on ignoring the order in which subjects received their treatment.

145

In addition, since BP may influence arterial haemodynamic parameters, we carried out a complementary analysis on PWV and AI% adjusted for the changes in BP at the time they were measured. Values are expressed as mean±SEM and p<0.05 considered significant.

4.7   Results

There was no significant difference in the baseline haemodynamic parameters on the two study days *(Table 4.1)*.

Table 4.1:   Baseline haemodynamic measurements on each study day (BP – blood pressure mmHg, PWV and AI %; mean±SEM

	Red wine with Alcohol	*Red Wine without alcohol*
Brachial SBP	109.6±3.2	108.8±4.4
Brachial DBP	67.7±1.9	65.7±2.7
Aortic SBP	93.5±3.7	92.5±3.7
Aortic DBP	67.2±2	66.6±2
Heart Rate (beats/min)	58.6±2.8	59.3±2.9
PWV (m/sec)	7.6±0.2	7.5±0.2
AI (%)	5.1±2.6	4.5±1.8

1. Changes in Blood Pressure and Heart Rate

There was a significant decrease in brachial systolic and diastolic BP following ingestion of red wine with alcohol only *(Figure 4.4)*.   The decrease in BP occurred at 30 minutes following baseline and was more marked by 90 minutes compared to de-alcoholised wine (p<0.01).   SBP decreased from 109.6±3.2 mmHg to 104±4 mm Hg at 90 minutes following red wine with alcohol.   The DBP decreased from 67.7±1.9 mmHg to 63±1.6 mmHg at 90 minutes following red wine with alcohol.   HR increased significantly after red wine with alcohol but not with de-alcoholised wine (p<0.02) from 58±2 beats/min at baseline to 62.3±3 at 90 minutes *(Figure 4.4)*.
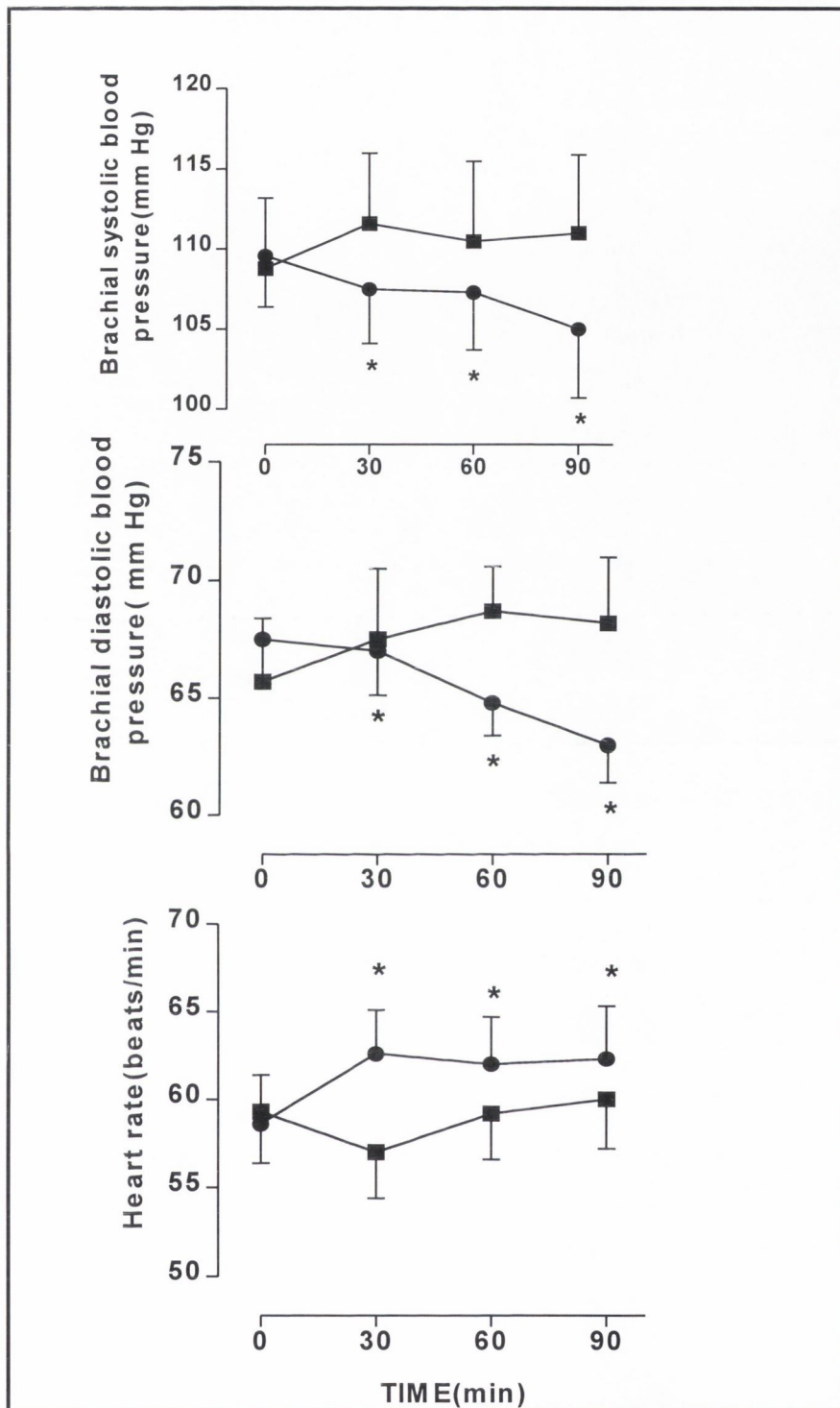
Figure 4.4. Brachial systolic and diastolic blood pressure and heart rate after ingestion of red wine with alcohol (0.8g/kg) compared to red wine without alcohol in 8 healthy young subjects (mean±sem, *p<0.01)
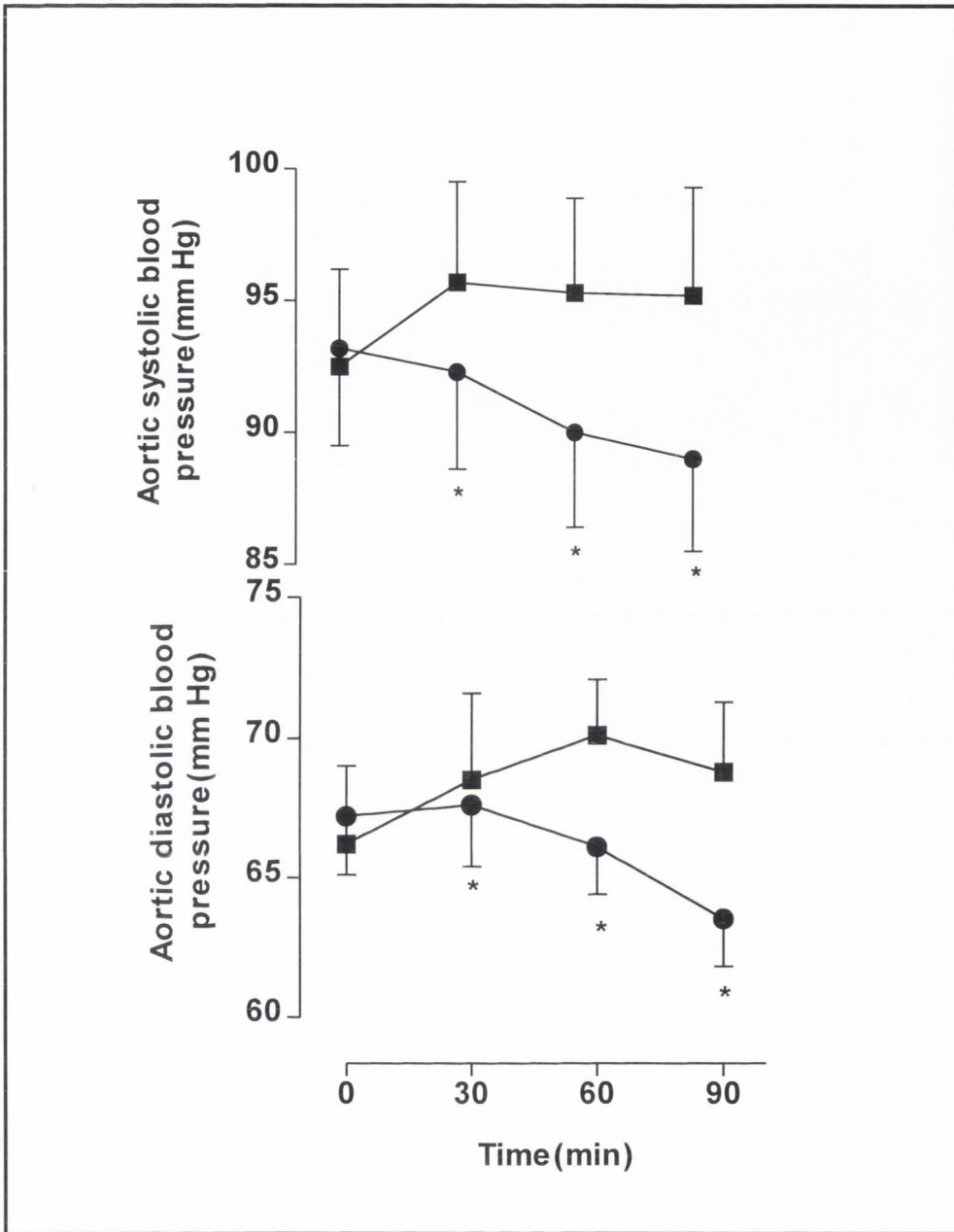
Figure 4.5. Aortic systolic and diastolic blood pressure after ingestion of red wine with alcohol (0.8g/kg) compared to red wine without alcohol in 8 healthy subjects (mean±sem,*p<0.01)
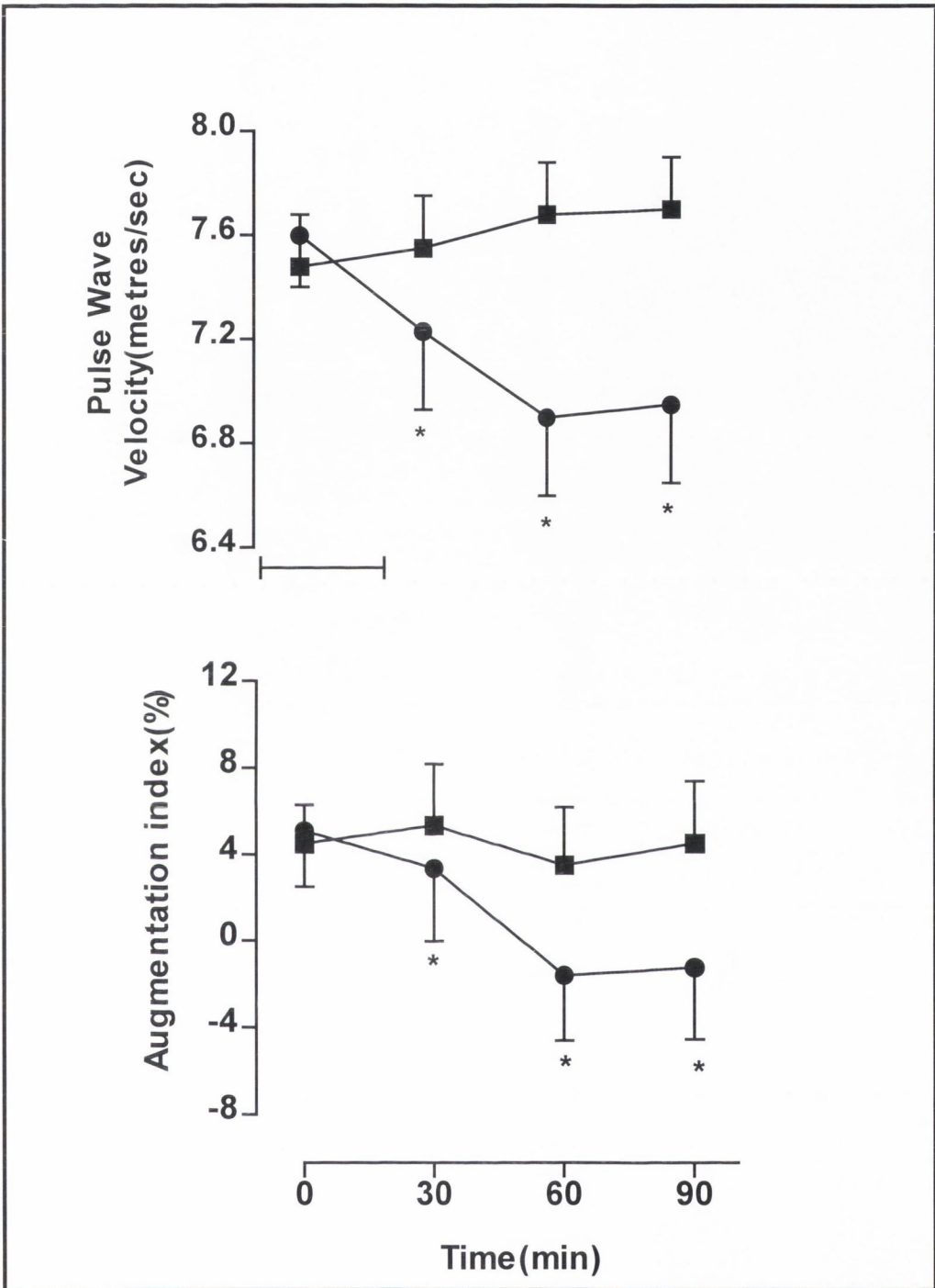
Figure 4.6. Pulse wave velocity and augmentation index following red wine with alcohol (0.8 g/kg) compared to red wine without alcohol in 8 healthy subjects(mean±sem,*p<0.01)

2. Changes in Aortic Pressures and Arterial Wave Reflection

Both aortic systolic and diastolic BP decreased significantly following red wine with alcohol, aortic SBP decreased from 93.2±3.7 at baseline to 89±3 at 90 minutes (p <0.05) and aortic diastolic pressure from 66±1.7 to 62±2 mmHg (p<0.05) at 90 minutes. No such changes were seen with the red wine without alcohol *(Figure 4.5)*.

AI% decreased significantly from 5.1±2% at baseline to −1.25±3 (p<0.05) at 90 minutes following red wine with alcohol only *(Figure 4.6)*. The decrease in AI% was still significant (p<0.05) when corrected for the fall in BP.

3. Changes in Pulse Wave Velocity

At baseline, PWV was significantly (p<0.05) correlated to brachial SBP (r = 0.59) and DBP (r=0.46). The PWV decreased significantly following ingestion of red wine with alcohol from 7.6±0.2 m/sec at baseline to 6.9±3 m/sec at 90 minutes (p<0.001) but no change in PWV was seen with de-alcoholised wine *(Figure 4.6)*. The change in PWV was still significant even when adjusted for blood pressure changes.

4.8   Discussion

In this study, acute ingestion of red wine with alcohol improved PWV and decreased arterial wave reflection in the ascending aorta, along with decreased aortic and brachial BP. The changes in arterial stiffness were still significant when corrected for the fall in BP.

The effect of alcohol on BP has been recognized for some time and chronic alcohol consumption is a major risk factor for hypertension. While the effect is seen in both genders it is more marked in women and increases with age and is additive to that of obesity (Beilin et al. 1999). Heavy drinking patterns are an important factor. There is linearity for the effect of alcohol on BP over a wide range of daily alcohol intake. Studies using ambulatory BP monitoring confirm a pressor effect when 4-6 drinks are taken throughout the 24 hours but the effect is usually greater on the day following alcohol withdrawal (Beilin et al. 1999). In addition, the association between alcohol consumption and elevated BP is contributed to by a significant white coat effect (Ryan and Howes 2000). Of interest, in inbred strains of mice, heritability accounts for some 60% of the

pressor response to acute alcohol exposure (Hatton et al. 2000). In this context there is evidence in man that apolipoprotein E phenotype determines the effect of alcohol (> 24 g/week) intake, particularly if > 115g/week, on BP(Kauma et al. 1998). On the other hand, abstinence in daily alcohol drinking is effective in lowering BP and BP variability in non-treated hypertensive patients who consume more than 80 g per day (López et al. 2000). A recent 12-year follow up study of Japanese men showed odds ratio for the development of hypertension of 2.39 where the alcohol intake was greater than 46 g/day having made adjustment for age, BMI and other confounding factors (Tsuruta et al. 2000). However, in a follow up of some 17,000 subjects (NHANES III), SBP was positively and DBP negatively associated with alcohol intake (Hajjar et al. 2001).

In our study we observed that acutely, only red wine with alcohol produced a reduction in BP. This has been seen in previous studies (Kojima et al. 1993). On the other hand some studies have shown no change in BP following alcohol and these may in part be attributed to the amounts of alcohol, population under study and the duration of observation (O'Callaghan et al. 1995). In hypertensives, acute alcohol intake initially lowered BP through systemic vasodilatation (Kawano et al. 1992). In normal men given 15g, 30g and 60g of alcohol, for the higher dose mean BP, using 24 hour ambulatory BP monitoring, was 4/2 mmHg lower in the period immediately following alcohol but 7/4 mmHg higher at night (Rosito et al. 1999). In a recent study where healthy subjects were given 1g per kg body weight of alcohol, it had no effect on BP. However when the subjects were exposed to stepwise increases in lower body negative pressure, sufficient to produce hypotension, alcohol potentiated the orthostatic effects (Narkiewicz et al. 2000). We excluded caffeine from our study as it has been shown that caffeine may itself have a pressor effect and itself acutely increases arterial stiffness (Mahmud and Feely 2001a). Of interest combining alcohol and caffeine generally offset the pressor effects observed with either of these substances administered alone (Rush et al. 1989).

There may also be a variation in protective effect of alcohol on the vasculature. While alcohol intake is associated with a reduced incidence of peripheral vascular disease its effect on carotid atherosclerosis is less clear (Kieckl et al. 1998). Kieckl found a J shaped relationship in the progress of atherosclerosis using carotid ultrasound (Kieckl et al. 1998) and in the ARIC study of 45-64 year old men and women after adjustment for age, body

mass index, smoking, cholesterol and diabetes, there was no relationship between current alcohol intake and either carotid artery wall thickness or distensibility (Demirovic et al. 1993). On the other hand several epidemiological studies have suggested that CHD mortality can be reduced by moderate consumption of alcohol, particularly red wine (Jackson et al. 1991). Nonetheless, it is unclear how the moderate consumption of red wine provides the protective effect against CHD. Although the cardio-protective effects of most alcoholic beverages are attributed to ethanol-induced elevation of HDL (Gaziano et al. 1993) and decreased platelet aggregation (Renaud and de Lorgeril 1992; de Lorimier 2000), factors other than ethanol in wine may be responsible for some of its cardio-protective effects. There are certain antioxidant compounds that are only found in red wine and not in spirits, such as phenols including flavinoids (anthocyanins, quercetin) and non-flavinoids like resveratol (de Lorimier 2000). There is also epidemiological evidence (Hirvonen et al. 2001) from the ATBCCP. Study of 25,372 male smokers that those in the highest quantile of flavinol and flavone intake had significantly less non-fatal myocardial infarction than those in the lowest quantile. The antioxidant properties of these phenols in red wine may counter the pro-oxidant activity of alcohol, decrease platelet aggregation and intracellular adhesion molecules, increase HDL cholesterol and inhibit the oxidation of LDL cholesterol (de Lorimier 2000).

Recently attention is focused on the effect of constituents of wine on endothelial dysfunction, an important prognosticator of cardiovascular events (Suwaidi et al. 2000). In healthy volunteers a high fat diet induced endothelial dysfunction as shown by reduced dilatation of the brachial artery. Loss of endothelial function was not seen when this diet was supplemented with wine for 30 days (Wuevas et al. 2000). In a separate study 12 healthy subjects drank 250 ml of red wine with or without alcohol and brachial artery dilatation was measured. After red wine the resting brachial artery diameter, resting blood flow and heart rate increased significantly but not after de-alcoholised wine (Agewell et al. 2000). Brachial dilatation was significantly greater after de-alcoholised red wine than drinking red wine with alcohol. It was suggested that the increased brachial diameter and flow were attributed to alcohol. These haemodynamic changes may have concealed an effect on flow-mediated dilatation, which increased significantly only after de-alcoholised red wine (Agewell et al. 2000). Acutely alcohol increases the blood flow velocity in the optic nerve head in part due to the production of acetaldehyde a metabolite

152

of ethanol (Kojima et al. 2000). That the non-alcohol constituents of red wine produce an effect is further supported by a study on coronary flow velocity reserve (Kauma et al. 1998) before and after vodka, white wine and red wine. Only red wine increased the coronary flow vascular reserve (Lendingham and Laverty 1996) suggesting that polyphenols found in red wine may have vasodilating effects on the coronary micro-vessels. In patients with CHD, purple grape juice improved flow mediated vasodilatation using high resolution brachial artery ultrasonography, and also prevented LDL oxidation, which led the authors to suggest that flavonoids in the purple grape products may prevent cardiovascular events independent of alcohol content (Stein et al. 1999).

In stroke prone spontaneously hypertensive rats, extracts of wine phenolics improve aortic biomechanical properties (Mizutani et al. 1999). In addition they attenuated the expected elevation of BP in these animals possibly by increasing vaso-relaxation activity and improved aortic elasticity. Our results showing reduced large artery stiffness with red wine may in part be attributed to such an effect but on the other hand the effect was not seen with de-alcoholised wine. However as the study was designed to look at the vascular effects of alcohol rather than of red wine and it is difficult in the absence of a placebo to exclude an effect of the red wine on arterial stiffness. Nonetheless, an almost identical baseline ob both study days and absence of an effect of de-alcoholised wine compared to baseline argues against such an effect. I did not measure the levels of alcohol in this study, which would have given additional information about concentration-effect relationships. While the hypotensive effect of alcohol is a plausible explanation for reduced PWV and wave reflection the effect was seen when controlled for changes in BP, suggesting a direct effect of alcohol on large vessels. It is also possible that such action may in part explain the clinical observation in patients with hypertrophic obstructive cardiomyopathy that acutely alcohol appears to reduce left ventricular outflow tract obstruction (Flores-Ramirez et al. 2001).

It is clear therefore that depending on the vascular territory, the amount of alcohol and its vehicles, wine, beer, etc. may have disparate and opposite effects. Epidemiologically, there is evidence in a 9 year longitudinal study of middle aged normotensive Japanese men, that chronic intake of large amounts of alcohol is associated with increased PWV – arterial stiffness (Nakanishi et al. 2001) This is in contrast to this study showing decreased

PWV following acute alcohol intake . It is possible that the effects of alcohol on PWV are somewhat akin to its haemodynamic effects on BP acutely reducing pressure but in the long term, BP may increase. Acutely alcohol may reduce arterial stiffness but subsequently vessels may stiffen with continued alcohol intake particularly more than 4 units/day (Nakanishi et al. 2001). The effect of alcohol on arterial stiffness needs also to be examined some hours to 24 hours following ingestion of alcohol to exclude a late 'pressor' effect. Such information is also required if one is to exclude the influence of dietary factors in the study of arterial stiffness.

CHAPTER FIVE
THE EFFECT OF ACTIVE AND PASSIVE SMOKING ON ARTERIAL STIFFNESS

Both active and passive smoking are major cardiovascular risk factors. The patho-physiological mechanisms underlying the damage induced by smoking include endothelial dysfunction, sympathetic activation and lipid peroxidation but there is paucity of data on the effect of active and passive smoking on arterial wave reflection in the ascending aorta, a surrogate marker of arterial stiffness. In this chapter I describe the acute effects of smoking one cigarette in both smokers and non-smokers and effects of passive smoking in healthy non-smokers on arterial stiffness.

The Acute Effect Of Active Smoking On Arterial Stiffness In Smokers And Non-Smokers

5.1. Introduction

Smoking is a major risk factor in the development and progression of cardiovascular disease (Jacobs et al. 1999). Despite extensive research, the patho-physiological mechanisms that are responsible for smoking-related vascular damage have not been elucidated. In addition to alterations in haemostatic factors, endothelial function and blood lipids (van den Berkmortel et al. 1999) and changes in arterial elastic properties may play an important role, as some investigators have demonstrated reduced compliance of both large and medium-sized arteries immediately after smoking one cigarette (Kool et al. 1993; Giannattasio et al. 1994; Stefanadis et al. 1998b).

(McVeigh et al. 1997) demonstrated abnormalities in the brachial artery pressure waveforms of chronic smokers compared to non-smokers using invasive methods. However, the acute effects of cigarette smoking on arterial wave reflection in the ascending aorta in either chronic or naïve smoker are not known. Therefore, we studied the acute effects of smoking one cigarette on arterial wave reflection and PWV in healthy smokers and non-smokers by arterial pulse wave analysis.

5.2. Materials and Methods

1.Subjects

The study group comprised of 28 healthy volunteers, 11 of whom were smokers (Table 5.1). None of the participants had systemic hypertension, diabetes mellitus, and hypercholesteremia or CHD and were not taking any medications. The smokers smoked

15 cigarettes / day for 6-10 years. The subjects gave informed consent and the study had institutional ethics committee permission.

2. Study Design

The subjects were studied in the fasting state, having abstained from caffeine-containing beverages, alcohol and smoking in the 12 hours prior to the study. The studies were carried out in a quiet room at 22°C. Subjects rested in a supine position for 15 minutes before the baseline haemodynamic measurements were obtained. Subsequently, each subject smoked a cigarette (nicotine content 1.2 mg) within 5 minutes using a previously described standardised protocol (Kool et al. 1993). Subsequent haemodynamic measurements were made at 5, 10 and 15 minutes following baseline.

3. Blood Pressure Measurements:

Brachial BP and HR were measured in the left arm with an automated digital oscillometric BP monitor (Omron® Model 705-CP). A mean of three readings was taken at each measurement time.

4. Derivation of the aortic pressure waveform:

Immediately after recording BP, the same arm was used for applanation tonometry. A high-fidelity micromanometer (BPAS-1, PWV Medical™, Sydney, Australia) was used to flatten the radial artery and the peripheral radial pulse continuously recorded. The central aortic waveform was derived from radial tonometry by using a previously validated transfer factor relating peripheral to central waveform within the software package (Sphygmocor®). AI% was calculated from the aortic pressure waveform as the height of the late systolic peak divided by PP and is a measure of wave reflection in the ascending aorta. The validity of the derived AI% has been confirmed by simultaneous direct central aortic measurements (Kelly et al. 1989d) (Chen et al. 1996) and is highly reproducible in both healthy and diseased populations (Liang et al. 1998; Siebenhofer et al. 1999; Wilkinson et al. 1998b).

5. PWV Measurements:

Carotid-femoral PWV was determined according to the foot-to-foot method using the Complior ® device. The simultaneous recording by two pressure sensitive transducers of the carotid and femoral waveform and measurement of the time delay of successive records from the foot of each wave divided by the distance between the transducers allows calculation of PWV (metres/sec). The distance travelled by the

156

pulse wave was measured over the body surface with a tape measure as the distance between the recording sites and fed into the software and 10 consecutive waves were sampled. This method of evaluating the covered distance by superficial measurement may introduce a margin of error since the direction of blood flow in the carotid artery is opposite to that in the abdominal aorta. However, PWV is measured separately in the same subjects; such error therefore does not affect the results. The validity of the Complior ® has been established (Asmar et al. 1995b).

6.Statistical Analysis:

Results were analysed with JMP Version 3.1 (Statistical Analysis for Windows Version). The differences between the two groups were analysed by one-way analysis of variance. The changes in the haemodynamic parameters over time were analysed by analysis of variance of repeated measures, testing for the effect of time and interaction between time and treatment. For AI%, which is strongly influenced by HR changes, we analysed the trend over time for the raw AI% values and also the AI% corrected for HR changes (Wilkinson et al. 2000a). All results are expressed as Mean±SEM, $p<0.05$ considered significant.

5.3. Results

The demographic data for the smokers and non-smokers is given in Table 5.1.

Table 5.1. Demographic data for smokers and non-smokers (mean±SEM).

	Smokers	*Non-smokers*
Number (Female)	11(5)	17(9)
Age (years)	22±1	22±1
Weight (Kg)	66±3	67±3
Height (cm)	174±4	170±2.3

There was no difference in the aortic and brachial BP and HR between the two groups. The PWV was higher in the smokers but the difference was not statistically significant. The AI% however, was significantly higher in the smokers at baseline compared to non-smokers **(Table 5.2).**

Table 5.2. Baseline haemodynamic parameters in the smokers and non-smokers. Mean ± SEM, *p<0.05.

	Smokers	*Non-smokers*
Brachial SBP (mm Hg)	114±2	114±3
Brachial DBP (mm Hg)	67±1.5	66±1.6
Aortic SBP (mm Hg)	98.3±1.5	95±2.3
Aortic DBP (mm Hg)	67.5±1	66±2
Heart Rate (beats/min)	57±2	62±2
PWV (meters/sec)	7.28±0.2	7.1±0.2
AI (%)	6.45±5*	-11±3.7

1. Changes in brachial blood pressure and heart rate

Short-term smoking caused a significant increase in brachial SBP (p<0.01) DBP (p<0.01) and HR (p<0.001) thathad come back to baseline 15 minutes after smoking in both smokers and non-smokers. However, as seen in *Figure 5.1*, diastolic blood pressure (ans systolic for smokers only) was still significabtly higher compared to baseline in both smokers and non-smokers at 15 minutes.

2. Changes In Aortic Blood Pressures And Wave Reflection

The aortic systolic and diastolic BP increased significantly following smoking one cigarette in both smokers and non-smokers (p<0.01). The greatest changes were seen in the first five minutes after smoking *(Figure 5.2)*. AI%, which is HR dependent, decreased significantly in both smokers and non-smokers following smoking due to marked HR increments. Applying a correction factor (Wilkinson et al. 1999) for changes in HR, the AI% actually increased significantly following smoking in both smokers and non-smokers *(Figure 5.3)*.
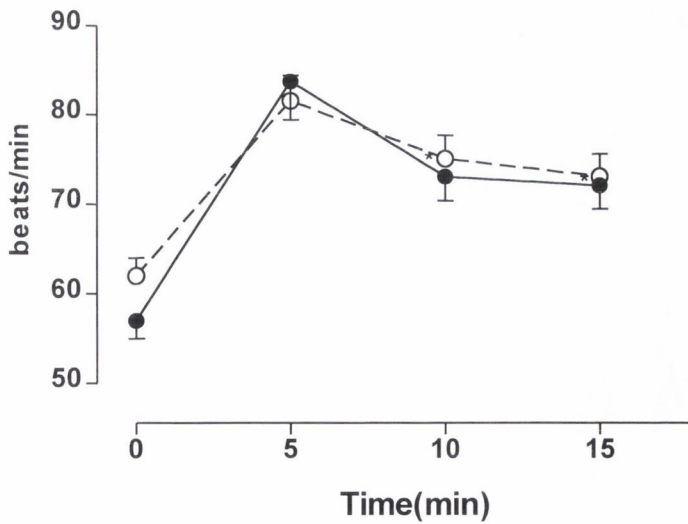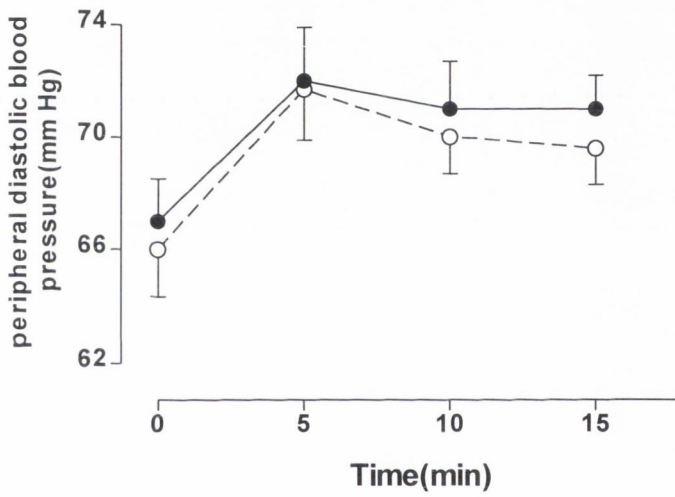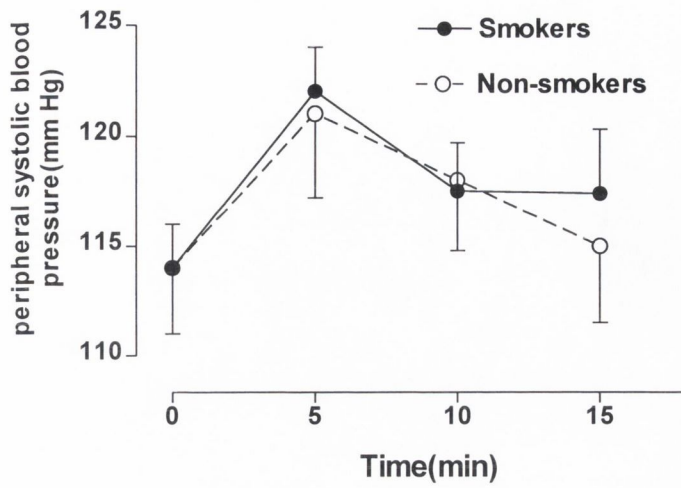
Figure 5.1. Changes in brachial systolic and diastolic blood pressure and heart rate at 5, 10 and 15 minutes after smoking one cigarette in healthy smokers and non-smokers(n= 28, mean±sem)
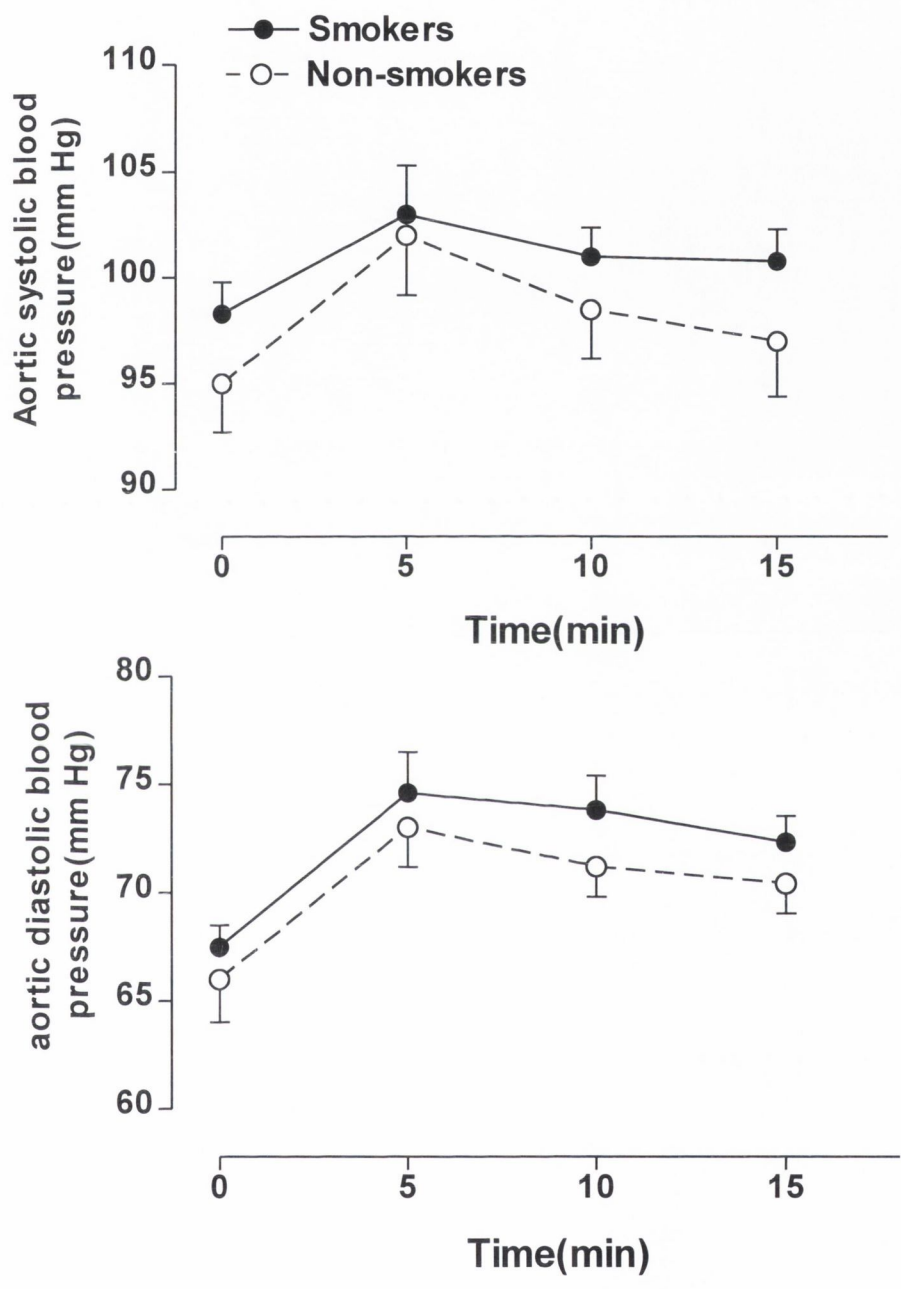
Figure 5.2. Changes in aortic systolic and diastolic blood pressures at 5, 10 and 15 minutes after smoking one cigarette in healthy smokers and non-smokers(n=28, mean±sem)
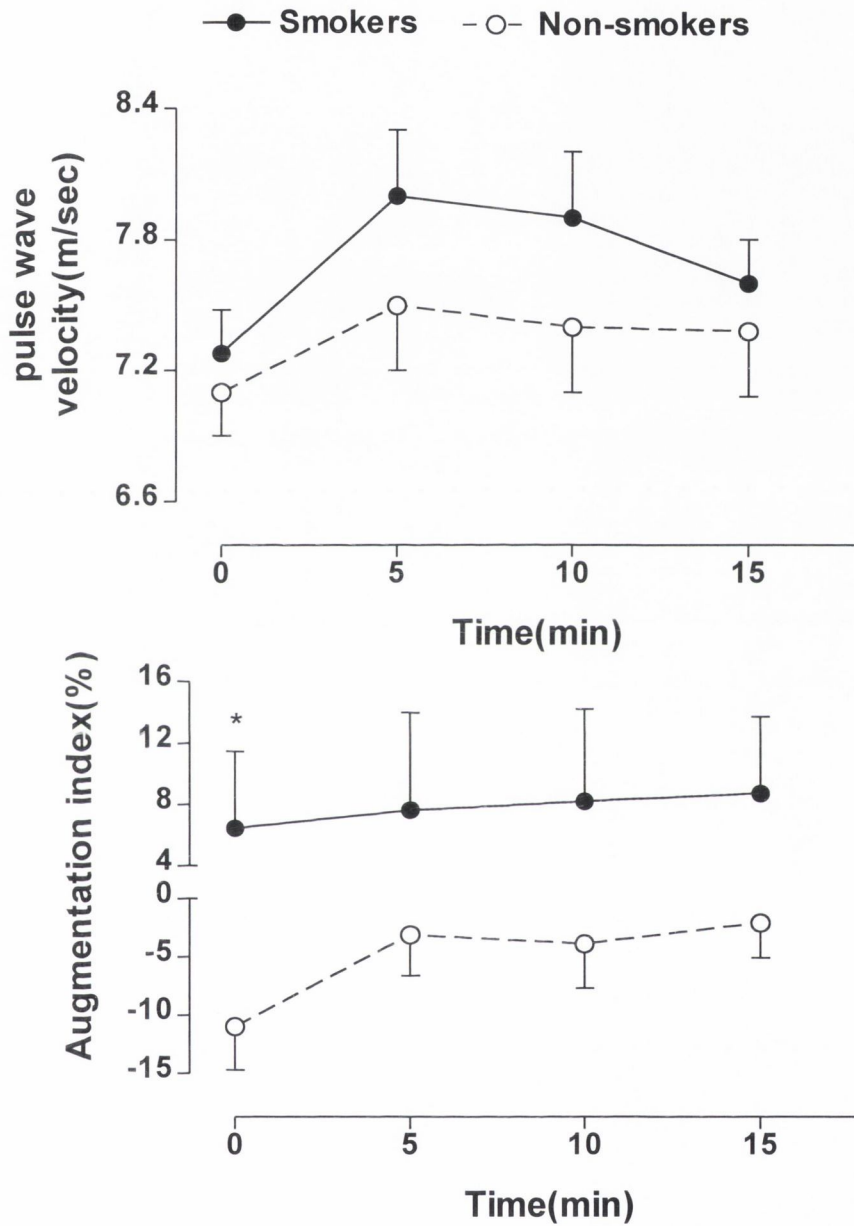
Figure 5.3. Changes in pulse wave velocity and augmentation index at 5, 10 and 15 minutes after smoking one cigarette in healthy smokers and non-smokers (n=28, mean±sem, *p<0.05smokers vs non-smokers)

3. Changes in PWV

PWV increased significantly in both smokers and non-smokers from baseline (p<0.01). The greatest response to smoking was observed in the first five minutes following smoking *(Figure 5.3).* The changes persisted for the entire 15 minutes of the study.

5.4. Discussion

We have shown in this study a significant effect of smoking on large artery properties

1) Smokers have a higher AI% at baseline, compared to non-smokers, suggesting increased arterial wave reflection in their aorta.

2) Acute cigarette smoking increases stiffness of the aorta as shown by increased PWV and also increased amplitude of wave reflection in the ascending aorta.

3) There was no difference between smokers and non-smokers in responsiveness to smoking. Both in response to smoking, showed increase in AI% when corrected for HR changes and PWV compared to non-smokers.   Whether such an effect is due to down regulation of nicotine receptors, impaired endothelial function, or other mechanisms, remains to be elucidated.   Of note chronic smoking is associated with lower BP rather than hypertension.

Epidemiological studies have provided clear evidence to associate smoking with almost all forms of arterial disease. The earliest epidemiological studies to demonstrate the association between smoking and cardiovascular disease include Framingham and Sir Richard Doll's classic study of British doctors (Doll and Peto 1976). The patho-physiology of smoking-induced cardiovascular diseases is not fully elucidated but a number of mechanisms have been known suggested such as changes in haemostatic factors (Levenson et al. 1987; Haire et al. 1989), changes in blood lipids (Morrow et al. 1995; Freeman and Packard 1995), changes in endothelial factors (Celermajer et al. 1993), changes in arterial vessel wall function (Kool et al. 1993; Wollersheim et al. 1993; Stefanadis et al. 1997b) and arterial wall structure (Howard et al. 1994; Liang et al. 2001).

162

changes in arterial vessel wall function (Kool et al. 1993; Wollersheim et al. 1993; Stefanadis et al. 1997b) and arterial wall structure (Howard et al. 1994; Liang et al. 2001).

The effect of smoking on arterial haemodynamics has been evaluated in numerous studies including healthy non-smokers, healthy smokers and in smokers and non-smokers with other cardiovascular risk factors. (Wollersheim et al. 1993) described increased arterial stiffness of the popliteal artery and a tendency towards stiffening of common femoral and carotid arteries in 13 habitual smokers by measuring the pressure-strain elastic modulus ultrasonographically. Also, an invasive study showed abnormalities of the brachial artery pressure waveform in smokers (McVeigh et al. 1997). Yet (Kool et al. 1993) and (van den Berkmortel et al. 1999) found no difference in arterial compliance between smokers and non-smokers. In contrast, in the present study, we have observed a much higher AI% in smokers compared to non-smokers who have been matched for age, weight, height and haemodynamic status. This suggests that chronic smokers as young as 22 years old have evidence of increased wave reflection in the ascending aorta which is well-recognized as a global estimate of arterial stiffness. This study confirms the earlier observation by (McVeigh et al. 1997) that smokers have abnormal brachial artery waveforms compared to non-smokers and a recent study showing increased stiffness index in smokers compared to non-smokers (Liang et al. 2001). Some studies have shown no difference between smokers and non-smokers in terms of arterial mechanical properties (Kool et al. 1993) and (van den Berkmortel et al. 1999) which measured regional compliance in both central and peripheral arteries in smokers and non-smokers. However, both these studies relied on brachial blood pressure measurement, assuming that pressure is identical in all arteries which is not the case, thereby causing a deviation from the real compliance and distensibilty coefficient. Also, regional compliance does not give any estimate of the totality of the effect of smoking on all the arterial territories, which is much more reliably obtained by measuring arterial wave reflection in the ascending aorta.

Studies on the acute effects of smoking have been much more in concordance. T The effect of smoking on forearm haemodynamics in healthy smokers (10-15 cigarettes/ day / 10 years) showed a significant increase in BP, HR and PWV, a decrease in forearm blood flow and unchanged PR (Brunel et al. 1992). This increase in PWV was transient and peaked at 15 minutes after smoking (Belin et al. 1990; Failla et al. 1993; Kool et al. 1993)

163

showed that acute cigarette smoking decreases arterial compliance in both large elastic and medium-sized muscular arteries. More, recently, (Stefanadis et al. 1997b) using invasive methods, showed decreased aortic compliance acutely after smoking one cigarette in men with CHD.

In the present study, AI%, an index of arterial wave reflection in the ascending aorta, decreased following smoking in contrast to PWV which increased immediately after smoking. Arterial wave reflection depends on the timing of ventricular ejection. Due to the proportionality between ejection time and cardiac cycle duration (Braunwald et al. 1958; Wallace et al. 1963), the peak of the forward travelling wave occurs earlier in the cardiac cycle at faster HR. Thus even with a fixed reflection site and PWV, there would be an altered relationship between forward and backward waves. AI% is therefore lower at higher HR and vice versa. (Wilkinson et al. 2000a) recently demonstrated a linear relationship between AI% and HR in a pacing study in which in HR increased in increments from 60 to 110 beats/min. The AI% decreased by 4 percent for every 10 beats / minute increment in HR. Applying this correction factor, we were able to show in the present study, that the AI% increased in parallel with the PWV. However, the correction has to be interpreted with caution. We have seen previously (unpublished observations) that even with HR increments of 60 beats/min during sustained isometric exercise, the AI% still increased significantly. There may be different mechanisms whereby HR changes alter AI% and these need to be further explored.

There could be several mechanisms underlying the increase in arterial stiffness. In affirmation of previous studies, we have shown increments in BP, HR and arterial stiffness parameters in the first five minutes after smoking. These acute changes have been attributed to an increase in circulating and local catecholamines; plasma catecholamines start to rise at 5 minutes, are maximal at the end of a 10-minute smoking period and return to baseline levels 30 minutes after the start of smoking (Cryer et al. 1976). That may explain the still higher BP and HR at the end of the 15 minutes study period.

The concomitant increase in plasma catecholamines with haemodynamic changes is compatible with the contention that haemodynamic changes are due to symapatho-neuronal stimulation during smoking. Nicotine stimulates sympathetic ganglia, resulting in

the release of norepinephrine from post-ganglionic nerve terminals. In addition, nicotine also increases the central nervous system sympathetic neural discharge (Winniford 1990). Vasopressin is also released in response to smoking and may contribute to vascular effects of smoking, since a vasopressin antagonist can blunt the nicotine-induced vasoconstriction in skin blood vessels (Winniford 1990). It has been shown that sympathetic activation decreases arterial compliance in medium-sized muscular arteries (Boutouyrie et al. 1994). Sympathetic activation can lead to increased smooth muscle tone leading to alteration in the load borne by elastic vis a vis non-elastic tissues(Dobrin and Rovick 1969)and changes in the vasa vasorum which have been shown to affect large artery properties(Stefanadis et al. 1993; Stefanadis et al. 1995c). Smoking may impair arterial elastic properties by impairing endothelial function. NO plays a vital role in the regulation of basal vascular tone and BP. Organic nitrates such as GTN are metabolised by the vasculature to NO and so act as an external source of NO and in humans, GTN profoundly altars the pulse pressure waveform and decreases the AI%(Yaginuma et al. 1986). Thus arterial stiffness and wave reflection may be under the same regulatory control of endogenous mediators such as NO. It has been shown in animal experiments that inhibition of NO synthase in the rabbit is associated with augmented wave reflection (Matz et al. 1994). In diabetes, a condition associated with endothelial dysfunction, abnormalities of the pulse wave consistent with increased vascular stiffness are detectable early in patients with no clinical evidence of cardiovascular disease (Cockcroft et al. 1997). Smokers have impaired endothelial function (Celermajer et al. 1993) and acutely, cigarette smoking leads to impaired endothelium-mediated vasodilatation (Lekakis et al. 1997). Similar changes have been seen acutely following nicotine chewing gum (Sarabi and Lind 2000). Acute cigarette smoking also increased endothelial cell counts in venous blood immediately after smoking tobacco and the response was greater than with non-tobacco cigarettes (Davis et al. 1985). Smoking impairs the endothelial NO production from saphenous vein rings and from platelets (Powell 1998) and smoking may lead to reduced tetrahydrobiopterin concentrations. When tetrahydrobiopterin concentrations are low, induction of NO synthase by smoking generates hydrogen peroxide instead of NO, leading to oxidative vascular damage. In keeping with this observation, high concentrations of the anti-oxidant vitamin C improve the endothelial dysfunction observed in brachial artery and forearm vessels of smokers (Powell 1998). Acute vitamin C administration also decreases arterial wave reflection in the ascending aorta (Wilkinson et al. 1999).

165

It has been proposed that smoking predisposes to atherosclerosis through a sum of its acute effect (Klein 1984). The observation that smoking causes an increase in arterial stiffness that is maintained for at least 15 minutes leads to the conclusion that habitual smoking could be associated with increased stiffening of the arteries for long periods of the day, even without permanent arterial damage; it is likely that after multiple acute exposures, some lasting damage on arterial elastic tissues may accrue. This persisting deleterious effect on the arterial mechanical properties could contribute to acute cardiovascular events and to accelerated atherogenesis.

Effect Of Passive Smoking On The Aortic Pressure Waveform In Healthy Subjects

5.5 Introduction

Passive smoking is associated with the development of atherosclerosis, coronary artery disease and stroke (Jiang et al. 1999; Bonita et al. 1999). However, the mechanisms by which passive smoking may be related to increased cardiovascular risk are unclear.

Among possible mechanisms, the effect of passive smoking on the mechanical properties of the arterial wall may play an important role in generating cardiovascular dysfunction. Active smoking alters endothelial function (Celermajer et al. 1993), induces coronary vasoconstriction (Moliterno et al. 1994) and increases the stiffness of both muscular and elastic arteries (Kool et al. 1993; Giannattasio et al. 1994; Stefanadis et al. 1997b) . More recently, long-term passive smoking has been shown to impair endothelium-dependent vasodilatation in healthy adults (Celermajer et al. 1996) which is partially reversible (Raitakari et al. 1999) . Also, a recent invasive study in men with CHD has shown that acute exposure to passive smoking impairs elastic properties of the aorta (Stefanadis et al. 1998b).

As early wave reflection is detrimental to optimal ventricular-vascular function and associated with different cardiovascular risk factors, we hypothesized that acute exposure to passive smoking may also lead to detrimental effects on the aortic pressure waveform in healthy adults.

5.6 Materials and Methods

1. Subjects

We studied 21 healthy subjects (10 males, 11 females) aged 26±5 years with normal BP (BP< 140/90 mmHg). Six of these subjects also acted as controls (4 male, 2 female) aged 28±6 years. All the participants were non-smokers who were exposed to environmental tobacco smoke only very occasionally in their daily lives. None of the subjects were taking any medications. The study had institutional ethics committee approval.

2. Study Design

The subjects were studied fasting, having abstained from alcohol or caffeine-containing beverages in the 12 hours prior to the study. All haemodynamic measurements were made in the supine position with a 15-minute rest before baseline. A mean of three readings was taken. After stable baseline measurements, a series of 15 cigarettes (nicotine content: each 1.2 mg) were lit at a sufficient rate to maintain carbon monoxide level between 25-30 ppm (confirmed by Multiwarn II, Draeger Instruments, UK) in an unventilated room over one hour. Controls were studied in an identical manner breathing room air.

3. Blood Pressure Measurements

The haemodynamic measurements were performed in a quiet room at 20-23°C and after resting for 15 minutes. Brachial BP and HR were measured in the left arm with an automated digital oscillometric BP monitor (Omron® Model HEM 705-CP). A mean of three readings was taken.

4. Derivation of the aortic pressure waveform

Immediately after taking the final BP reading, the same arm was used for applanation tonometry. A high-fidelity micromanometer (BPAS-1, PWV Medical®, Sydney, Australia) was used to flatten the radial artery and the peripheral radial pulse continuously recorded. The central aortic waveform is derived from radial applanation tonometry and the brachial BP, assuming that MAP is identical at both sites by using a previously validated mathematical transformation, the TF, within the software package (Sphygmocor®, PWV Medical™, Australia). The aortic waveform was further analysed to yield central systolic and diastolic BP and calculate the AI%, which reflects the degree of wave reflection in the ascending aorta. The derived aortic waveform reflects the sum of both incident and reflected waves. The early systolic peak (P_1) is the primary wave and corresponds to the timing of peak flow and the late systolic peak (P_2) is the result of reflected waves returning from the peripheral site and causing an increase in amplitude of aortic SBP and PP. AI% is the difference between the two peaks expressed as a percentage of PP. The validity of these derived values has been confirmed by

simultaneous direct central aortic measurements and is highly reproducible in both healthy and diseased populations (Wilkinson et al. 1998b; Fuchs et al. 1995).

Statistical Analysis

The statistical analysis was performed with SAS (Statistical Analysis System) software version for Windows. Data is expressed as mean±SEM. The time dependent patterns of haemodynamic changes were studied by analysis of variance of repeated measures, testing for gender and period effect. $P<0.05$ was considered significant.

5.7   Results

There was no change seen in BP, HR or AI% in the control group.

1. *Effects Of Passive Smoke Exposure On Blood Pressure And Heart Rate*

There was a clear difference in the haemodynamic response of male and female subjects following passive smoking *(Table 5.3)*. The brachial SBP increased significantly over time in the male subjects only, from 124.4±3 mmHg at baseline to 136.9±3.4 mmHg at the end of 60 minutes (p=0.001) but not in the females (p>0.2). The aortic SBP also increased over time in the male subjects, 110.2±3.4 mmHg at baseline to 123.1±4.3 mmHg at the end of the 60 minutes following exposure to passive smoking (p=0.001) but no change was seen in the female subjects (p>0.2). There was no significant change in the brachial or aortic diastolic BP or HR over time in both males and females.

2. *Changes In The Aortic Pressure Waveform*

A pattern of characteristic abnormalities was observed in the radial and aortic pressure waveforms following passive smoking in males. As expected, at baseline the reflected waves occurred during diastole but gradually moved to the systolic portion of the BP curve and increased in amplitude as exposure to passive smoking continued *(Figure 5.4)*. This movement of the reflected wave from diastole to systole was associated with a significant rise in the AI%, from −1.7±5.2 at baseline to 14±4.8% at the end of 60 minutes (p<0.001) in males but not in the female subjects though a transient fall was

Table 5.3: Brachial, aortic blood pressure and heart rate at baseline and 15, 30 and 60 minutes after exposure to passive smoking in male and female subjects. Mean±SEM, (*$p<0.05$)

Time	Brachial BP (mm Hg)				Aortic Blood Pressure (mm Hg)				Heart Rate(per min)	
Min	Systolic		Diastolic		Systolic		Diastolic			
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0	124.4±3	114±3.7	79.2±2.6	77.6±3.2	110.2±3.4	100.4±3.2	80.2±2.7	78.6±3.1	68±4.6	73±3.5
15	125±2.8	115.6±5.2	79.1±3.2	76.2±2.9	109.2±2.8	98.9±4	79.9±3.2	75.5±3.7	69.2±4.7	75±1.8
30	133.9±3.4*	114.2±4.2	82.7±4	72.7±3.3	117.6±3.8*	96.1±3.6	83.7±4	76±3.7	66.4±3.9	76.3±2.3
60	136.9±3.4*	113.7±4.1	87.9±5.3	73.6±2.2	123.1±4.3*	98.5±3	89.1±5.2	74.5±2.3	67.3±4.2	71.9±1.9
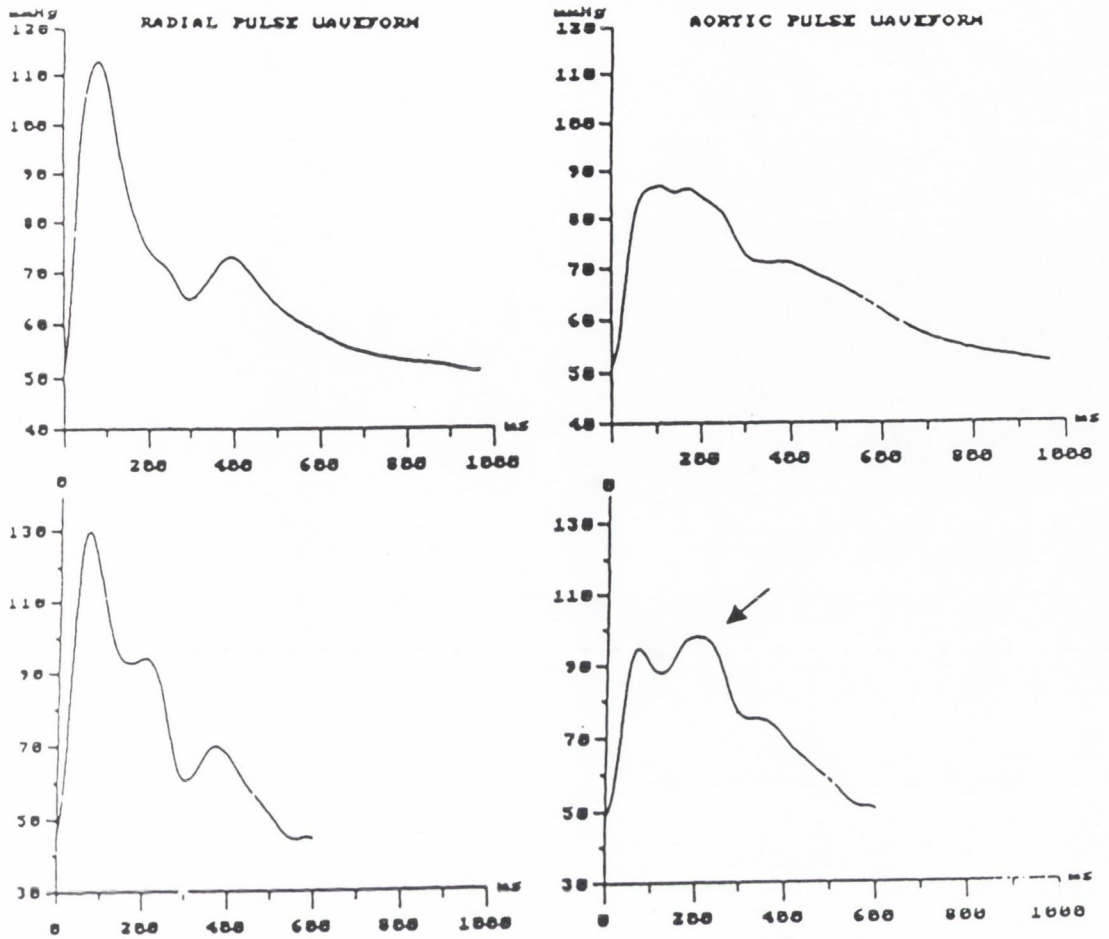
Figure 5.4. Radial and aortic pressure waveforms as controls (top panel) and after 60 minutes of exposure to passive smoking (bottom panel) with increase in amplitude of the 'reflected wave' (↓) in the aortic pressure wave during systole.

seen at 15 minutes in females (p=0.02) *(Figure 5.5).* The changes in the AI% in males appeared at 15 minutes, preceding the rise in both brachial and aortic SBP, which occurred from 30 minutes onwards.

Figure 5.5. Arterial wave reflection, measured as augmentation index increased significantly (*p<0.001) in male subjects only during exposure to passive smoking over 60 minutes.
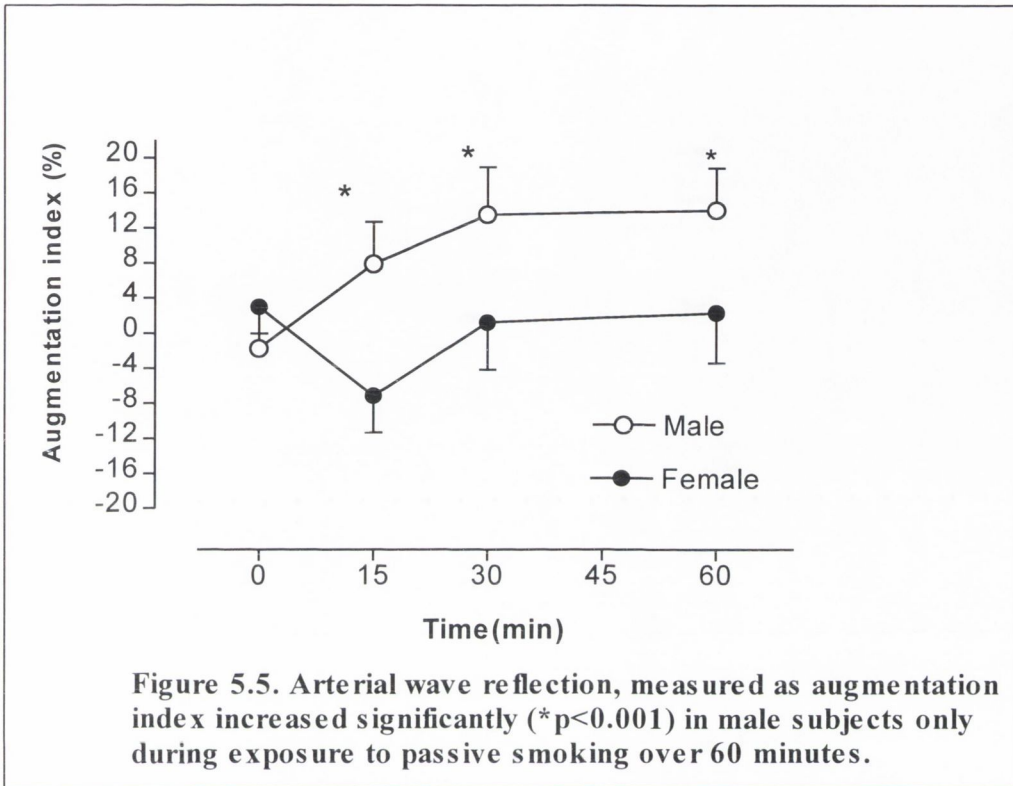
5.8   *Discussion*

Active cigarette smoking is accepted as a cause of CHD.   Recently exposure to passive smoking has also been identified as a major cardiovascular hazard, including stroke (Jiang et al. 1999; Bonita et al. 1999), with major public health implications (Wells 1988).   There is a compelling association between passive smoking and cardiovascular disease but the mechanisms underlying are unclear.

The response of BP and HR to acute environmental tobacco smoke exposure has been controversial; Some studies have shown an increase in BP and HR following passive smoking(Raitakari et al. 1999; Wilbert 1978) whereas others have observed no such effect (Kato et al. 1999; Hausberg et al. 1997).   The SBP increased significantly in the present study only in the male subjects after 30 minutes of exposure to passive smoking. The DBP showed a trend towards an increase but there was no increment in HR.

172

Investigators have shown an acute increase in arterial stiffness in both elastic and muscular arteries after smoking one cigarette (Calermajer et al. 1993; Moliterno et al. 1994; Kool et al. 1993)and abnormalities of the brachial artery pressure waveform is seen in chronic active smokers(McVeigh et al. 1997). Recently, Stefanadis et al have shown using invasive methods, that acute exposure to passive smoking impairs elastic properties of the aorta in middle-aged and older males with coronary artery disease (Raitakari et al. 1999). The important finding in our study is that the deleterious vascular effect of passive smoking on the aortic pressure waveform is also seen in healthy young subjects within 15 minutes of exposure.

The AI%, a manifestation of early wave reflection, increased at 15 minutes and was still elevated at 60 minutes following exposure to environmental tobacco smoke in males. The arterial pressure waveform in both the radial artery and the aorta showed abnormalities in the male subjects *(Figure 5.4)*. This phenomenon is relevant to left ventricular performance; early return of reflected waves and a late systolic peak in the central pressure waveform is associated with higher vascular impedance, an index of left ventricular afterload (Murgo et al. 1980a).

It was not possible to determine the exact mechanism whereby passive smoking produced this effect and to what extent it is related to smoke-induced changes in BP. The elevated vascular tone could be due to sympathetic nervous system activation and passive smoking increases sympathetic drive (Hausberg et al. 1997). Animal and human evidence suggest that sympathetic activation stiffens both elastic and muscular arteries (Boutouyrie et al. 1994). Among the structural constituents of the arterial wall, smooth muscle cells are the components subject to both acute and active changes. The underlying process is likely to be smooth muscle contraction because contracted muscle is less distensible than relaxed muscle. The increased wave reflection in our study could also be due to endothelial dysfunction as it has been shown that passive smoking is associated with a dose-related impairment of endothelium-dependent dilatation in healthy subjects (Celermajer et al. 1996)and endothelial dysfunction may be related to arterial stiffness (Cockcroft et al. 1997). However, short-term exposure to passive smoking has not been shown to affect either endothelium-dependent or independent vasodilatation in healthy adults (Kato et al.

1999). Other possible mechanisms for the acute detrimental effects on the vascular wall following exposure to tobacco smoke include platelet activation (Jiang et al. 1999; Davis et al. 1985), impaired endothelial release of prostacyclin (Nadler et al. 1983)and increased vasopressin release (Benowitz 1988). The observation that changes in wave reflection were seen at 15 minutes prior to the increase in blood pressure suggests that more than one mechanism may be involved.

The female subjects in the study did not show any harmful effects on the arterial waveform when exposed to tobacco smoke. We noted an acute fall in the AI% in female subjects at 15 minutes, probably reflecting a compensatory increase in distensibility at the level of small arteries. This was followed by the AI% returning to the baseline in females for the remainder of the study period while continuing to rise steadily throughout in the male subjects. All the female subjects in this study were young and pre-menopausal women. These results support the possibility that the sensitivity of the female arterial wall to smoking is attenuated at least in the younger age group. Brachial artery flow-mediated dilatation was not decreased in female current compared with former smokers whereas it was decreased in male current smokers compared to former smokers, suggesting a gender difference in arterial function induced by smoking (Celermajer et al. 1993). Furthermore, marked differences have been observed in the influence of smoking on intima-media thickness between healthy men and women; smoking was associated with increased intima-media thickness in male subjects only (Gariepy et al. 2000). The mechanism of this relative arterial protection in women from the adverse effects of smoking is unknown but circulating estrogens may have a role. It is well known that pre-menopausal females are protected from CHD(Lerner and Kennel 1986). There is also a gender difference in the shape of the arterial pressure waveform, the AI% being higher in females than males (Hayward and Kelly 1997). Furthermore, hormone-replacement therapy has a favourable effect on aortic pressure waveform in post-menopausal women (McGrath et al. 1998; Stefanadis et al. 1999). The adverse effects of smoking on endothelium, arterial distensibility, plasma fibrinogen and oxidative stress may be blunted endogenous oestrogens in pre-menopausal women.

While there is evidence (Jarvis et al. 2000)that the exposure of children to passive smoking is decreasing in England there is nonetheless increasing concern about the

deleterious effects of passive smoking to which the majority of the population are exposed. The levels used in our study mimic the intensity of acute exposure in real life situations (Scherer 1990; Scherer 1989)such as a smoky bar and are within the guidelines for maximum allowable exposure to passive smoking (Office of Air Quality. and United States Environmental Protection Agency (EPA). 1990). The effect of this level of exposure on the vasculature is considerable, producing abnormalities of aortic pressure waveform that we would commonly see in mild hypertension (Mahmud and Feely 2001b). We know that such a level of exposure also impairs the elastic properties of the aorta in patients with CHD (Raitakari et al. 1999). While burning cigarettes as used in our study produced side-steam smoke, as opposed to exhaled smoke (main-stream), it has also been shown that actively smoking one filtered cigarette also causes an acute deterioration of aortic elastic properties (Stefanadis et al. 1997b). We now need further information on the effects of lesser degrees of exposure to passive smoking, especially in the high-risk populations such as patients with hypertension, diabetes and CHD, including post-menopausal women.

CHAPTER SIX

MODULATION OF NITRIC OXIDE AND ARTERIAL STIFFNESS-EFFECTS OF GLYCERYL TRINITRATE AND SILDENAFIL CITRATE

NO is an important regulator of vascular tone and has been implicated in the pathogenesis of hypertension and endothelial dysfunction. Nitrates were the first pharmacological group used in the late 19th century to study drug effects on the pulse wave and are still used to document endothelial dysfunction. Increasingly drugs are being developed to modulate the effects of nitric oxide, either directly or indirectly, in the case of sildenafil. I therefore explored the effect of GTN and sildenafil on the arterial pressure waveform.

The Effects Of Nitroglycerin On The Aortic Pressure Waveform - Underestimation Of Vasodilator Effects By Brachial Pressure Measurements

6.1 Introduction

Nitroglycerin, an effective therapy for angina pectoris, is also used in acute left ventricular failure and hypertensive emergencies. At normal therapeutic doses, it has little or no effect on arteriolar tone and peripheral resistance, but quite marked effects on venous tone. In higher doses, nitroglycerin dilates both the large arteries and arterioles and consequently decreases PR and arterial stiffness (Kelly et al. 1990). However, there is substantial evidence that nitroglycerin even at lower doses, may decrease aortic SBP despite having much lesser effect on brachial SBP (Kelly et al. 1990; Yaginuma et al. 1986; Murgo et al. 1980b).

In adult man, early wave reflection from peripheral sites augments SBP in the aorta and hence increases left ventricular afterload (Murgo et al. 1980b; O'Rourke et al. 1992b; Nichols and O'Rourke 1998a); such early wave reflection is due to arterial stiffening (Avolio et al. 1983; Nichols and O'Rourke 1998a). Nitroglycerin dilates peripheral arteries in the same way as it dilates coronary arteries (Kelly et al. 1990). Arterial dilatation causes a decrease in wave reflection and in ascending aortic systolic pressure (Yaginuma et al. 1986; Takazawa et al. 1995).

Invasive studies with nitroglycerin have consistently shown a substantial reduction in peak SBP in the ascending aorta and left ventricle of adult man (Yaginuma et al. 1986;

Takazawa et al. 1995). However, under clinical conditions, a fall in SBP measured in the brachial artery is not always observed with the conventional doses of nitroglycerin, even though a favourable effect is seen in the relief of angina and left ventricular afterload.

The purpose of this study was to explore the effects of nitroglycerin on the ascending aortic pressure waveform in healthy subjects.

6.2   Materials and Methods

We studied 8 healthy subjects (22.5±2 years, mean±SEM), 3 females and 5 males. None of the subjects were taking any medications and all were non-smokers. The subjects were studied in the fasting state, having abstained from caffeine containing beverages and alcohol in the 12 hours prior to the study.

The studies were carried out in a quiet room and the subjects allowed relaxing for 15 minutes before taking the baseline haemodynamic measurements. The brachial BP was measured in the left arm with a digital oscillometric BP monitor (Omron® Model HEM 705-CP) and a mean of three readings was taken.

Derivation of the aortic pressure waveform: Immediately after recording BP, the same arm was used for applanation tonometry.   A high-fidelity micromanometer (BPAS-1, PWV Medical ®, Sydney, Australia) was used to flatten the radial artery and the peripheral radial pulse continuously recorded.   The central aortic waveform was derived from radial tonometry by using a previously validated TF (Chen et al. 1997; Kelly et al. 1989b) relating peripheral to central waveform within the software package (Sphygmocor®).   The validity of the AI%, which is a measure of wave reflection in the ascending aorta, has been confirmed by simultaneous direct central aortic measurements (Kelly et al. 1990; Karamanoglu et al. 1993) and is highly reproducible in both healthy and diseased populations (Liang et al. 1998; Siebenhofer et al. 1999; Wilkinson et al. 1998b).

Each subject was given 400 μg of sublingual nitroglycerin (nitrolingual®) and haemodynamic measurements were made at baseline and at 5-8 minutes following nitroglycerin, as this has been shown to correspond to the greatest change from a type A to

177

a type C waveform (Murgo et al. 1980b). Results were analysed with the Student t-test for paired samples and expressed as mean±SEM, p<0.05 was considered significant. The subjects gave informed consent and the study had institutional ethics committee permission.

6.3   Results

Data from all the subjects is summarized in Table 6.1. There was little change in DBP in either the aorta or the brachial artery following nitroglycerin administration. The average decrease in DBP at the two sites was not significant (-2.7 mm Hg aortic p=0.3; -1.3 brachial artery, p=0.5).

In contrast to DBP, aortic SBP decreased significantly in all subjects following nitroglycerin. The fall was considerable, ranging from 8.1 to 16.3 mm Hg, averaging 12.2±2 mm Hg. However the decrease in brachial was significantly less than in the aortic SBP, ranging from +0.7 to −12 mm Hg, averaging 6±2 mm Hg. In one of the eight subjects, a decrease of 8 mm Hg in aortic SBP was observed without any reduction in brachial SBP.

Table 6.1. Haemodynamic changes following 400µg nitroglycerin in healthy subjects (n=8, mean±SEM, *p<0.05 compared to control). BP = blood pressure, mm Hg unless stated otherwise.

	Control	*Post-Nitroglycerin*
Brachial systolic BP	*114.7±5*	*108±5**
Brachial diastolic BP	*62.5±2*	*61.2±3*
Aortic systolic BP	*98±5*	*86±4**
Aortic diastolic BP	*62.7±2*	*60.7±3*
Augmentation index (%)	*3±2*	*-14±2**
Heart rate (bpm)	*72.6±6*	*78±6.5**

The fall in aortic SBP was accompanied by a substantial and significant fall in the AI% from 3±2% to -14±2% following nitroglycerin. In subjects who had waveforms characterized by a late systolic peak separated from the first peak by a 'shoulder' on the

upstroke of the wave, nitroglycerin reduced or eliminated the late systolic peak *(Figure 6.1)*. Changes in contour of the aortic pressure waveform are subtler than the marked fall in the AI%. The study subjects were all young individuals, with the initial systolic pressure peak in the aortic pressure waveform being dominant and with a secondary wave on the diastolic down slope. This appeared to be reduced by nitroglycerin so that the whole pressure waveform assumed a more tented or triangular appearance during systole *(Figure 6.1)*.

6.4   *Discussion*

Nitroglycerin is usually classified as a venodilator since in the more usual "lower" dosage it causes little or no change in PR. The haemodynamic changes observed in this study however cannot be entirely explained on the basis of venodilatation with a decrease in venous return and stroke volume. Venodilatation might cause a small decrease in MAP. However, it cannot account for the substantial and preferential fall in aortic SBP compared to brachial SBP. It cannot also explain the marked decrease in the AI% following nitroglycerin with changes in contour of the aortic pressure wave.

The magnitude of aortic SBP fall observed in this study is less than reported previously (Murgo et al. 1980b; Yaginuma et al. 1986; Takazawa 1987; Kelly et al. 1990). Murgo et al. (1980), Yaginuma et al. (1986) and Takazawa (1987) (Yaginuma et al. 1986; Takazawa 1987) did not measure brachial BP at the same time as they measured the ascending aortic pressures and assumed that the BP fall they observed in the ascending aorta was accompanied by a similar degree of SBP reduction in the brachial artery. However, (Kerry et al. 1990) also measured the brachial SBP at the same time as they measured the ascending aortic SBP, and found significantly greater reduction in the latter. More recently, (Takazawa et al. 1995) have also shown by using the fourth derivative wave that brachial BP reduction underestimates the vasodilator effects of nitroglycerin. In contrast to previous studies (Murgo et al. 1980b; Yaginuma et al. 1986; Takazawa 1987; Kelly et al. 1990), which have shown modest albeit significant reductions in the systolic pressure augmentation in the aortic pressure waveform, the present study demonstrated substantial reduction in the AI%. Our study population comprised of young healthy subjects in contrast to older subjects with vascular dysfunction studied in earlier work.
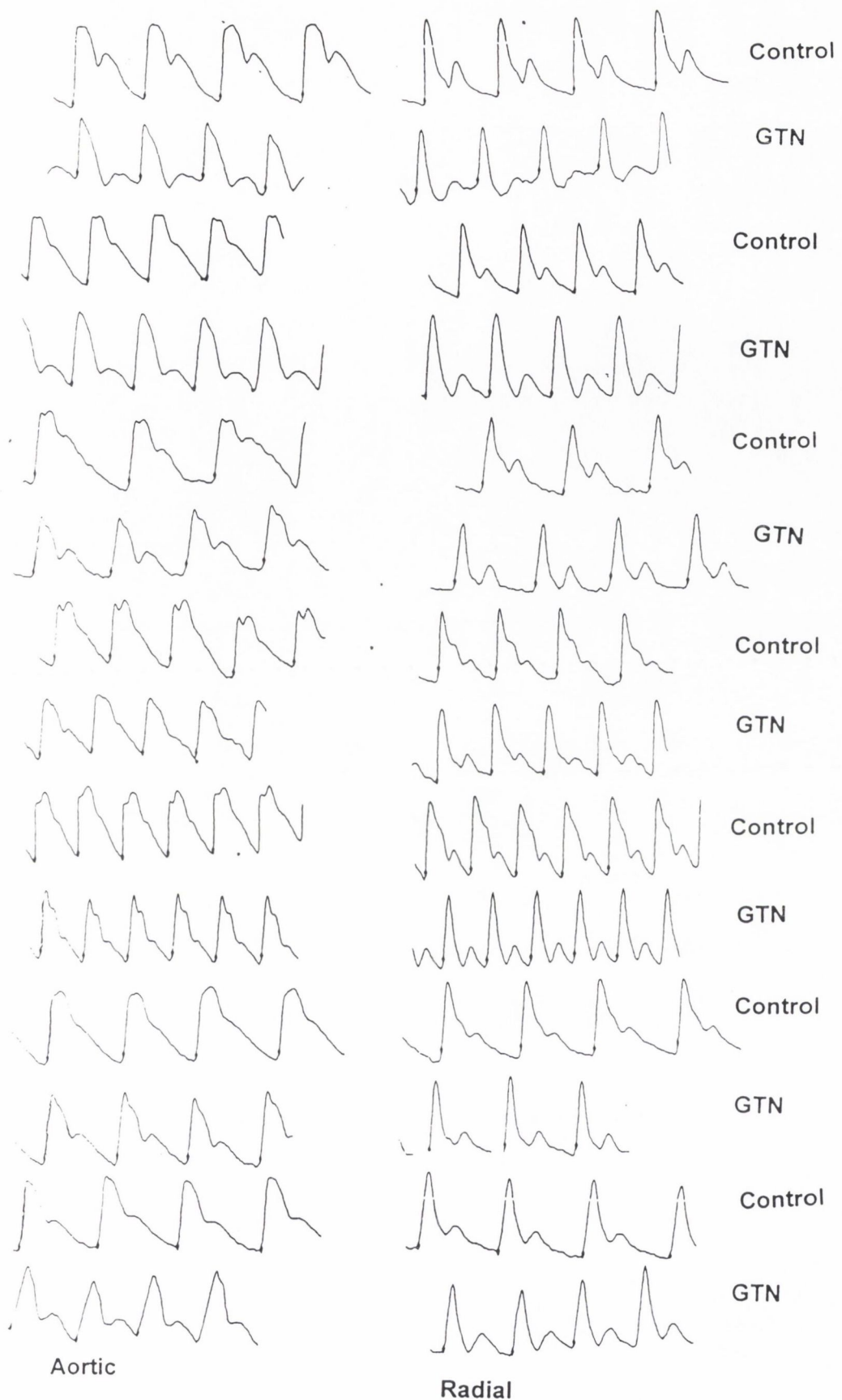
Figure 6.1. (Left panel) pressure waves in the ascending aorta of 7 subjects before (control) and after (GTN) sublingual nitroglycerin. (Right panel) pressure waves directly recorded in the radial artery of the same 7 subjects before (control) and after (GTN) sublingual nitroglycerin

Since the reduction in aortic SBP is not always apparent in the peripheral pressure waveform, estimates of pressure using sphygmomanometry may underestimate the beneficial effects of a drug on left ventricular afterload. Wave reflection is responsible not only for the augmented pressure peak in the ascending aorta of adult man (Yaginuma et al. 1986; Murgo et al. 1980b; O'Rourke 1982; Nichols and O'Rourke 1998b) but also for the secondary wavelet on the descending limb of the peripheral arterial pressure wave such as brachial or radial (Murgo et al. 1980b; Nichols and O'Rourke 1998b). The reflected wave returning predominantly from the lower body (Murgo et al. 1980b; O'Rourke 1982) corresponds in timing to the late systolic peak of the aortic pressure wave and actually generates the peak SBP. However, in a peripheral artery of the upper limb — further from the reflecting sites in the lower body — it occurs relatively later, after SBP has peaked in the aorta. Reduction in amplitude of wave reflection therefore would be expected to reduce the peak of SBP in the aorta without having much effect on the pressure peak in the brachial or radial artery (Kelly et al. 1990).

The haemodynamic effects of nitroglycerin are dependent on the vascular territory. There is little effect on the largest, the aorta (Feldman et al. 1981; Nichols and O'Rourke 1998b) and in the smallest vessels, the arterioles (Kelly et al. 1990). The effects are probably graded, as with the coronary circulation (Nichols and O'Rourke 1998b) with greatest effects seen in the medium-sized conduit arteries (Simon et al. 1982). Such variable action of nitroglycerin on vessels of different calibre is essential to explain its effects on wave reflection (Yaginuma et al. 1986; Fitchett et al. 1988; Takazawa 1987). The mechanism suggested for the preferential effect of nitroglycerin on medium-sized muscular arteries may be to do with the greater availability of sulfhydryl donors in the smaller arteries and thus greater activation of guanylate cyclase by nitroglycerin leading to greater vasodilatation.

In conclusion, this study conforms the earlier findings of an effect of nitroglycerin on wave reflection which may be responsible for the reduction in left ventricular afterload, as well as increasing myocardial blood supply (Nichols and O'Rourke 1998b). Furthermore, it highlights both the limitations of brachial artery pressure readings a guide to effects of vasodilator agents such as nitroglycerin and the benefits of complementing

sphygmomanometry with arterial pulse wave analysis. Furthermore, this area warrants closer study to provide new and different drugs designed to reduce wave reflection when the aorta is stiffened but preserving perfusion pressure and arteriolar tone, thus avoiding 'steal'.

Effect Of Sildenafil On Blood Pressure And Arterial Wave Reflection In Treated Hypertensive Men

6.5   Introduction

Sildenafil, a selective inhibitor of phosphodiesterase type 5(PDE5) is now widely used in the treatment of erectile dysfunction.   Post marketing surveillance initially revealed a number of serious cardiovascular events, including 69 deaths reported to the Food and Drug Administration (FDA) temporally associated with the use of sildenafil (Cheitlin et al. 1999) which raised questions about possible adverse cardiovascular effects.   A recent commentary in the Journal has drawn attention to these and other answered questions (Tomlinson 1999).   Since then a further analysis of spontaneous reports to the FDA up to February 1999 shows 401 deaths (219 cardiac, 140 sudden) over 10-11 months in 4-5 million men or 8.5-deaths/million men/month, which is well within the expected rate for such patients (Kloner 2000).   Data from a preliminary review of 5,000 users in England yields a standardised mortality ratio from CHD at 30% lower than expected (Shakir et al. 2001).

Marked hypotension has been noted when sildenafil was used concomitantly with nitrates. In addition sildenafil may itself have a hypotensive effect.   In healthy subjects, sildenafil 50 mg decreased BP (mmHg) by a mean of 7.7 systolic (SBP), 4.5 diastolic (DBP) and in addition decreased forearm vascular resistance (European Agency for Evaluation of Medicinal Products and Committee for Proprietary Medicinal Products 1998). This suggests that the vasodilatory effects of sildenafil are not confined to penile vasculature. While sildenafil has been given to hypertensive patients without adverse consequence, those were largely on monotherapy and the recent American College of Cardiology/American Heart Association consensus statement (Cheitlin et al. 1999) draws attention to potential hazards in patients on multi-drug antihypertensive therapy and advises "until adequate studies are done in these subgroups sildenafil should be prescribed with caution".   The actions of sildenafil are in part mediated through enhancement of the effects of NO (Jackson et al. 1999; Morales et al. 1998) . Nitrates given acutely markedly alter the aortic pressure waveform reducing aortic BP, delaying wave reflection in the aorta and reducing arterial stiffness(Westling et al. 1984, (Kelly et al. 1990).)   We hypothesised that sildenafil which effected a fundamental regulator of vascular tone, i.e.,

be expected to have systemic effects on arterial function. We therefore studied the effects of sildenafil on BP and early wave reflection, in treated hypertensive men, including those on multi-drug regimens, who requested sildenafil.

6.6   *Materials And Methods*

1. Patients

Eight men aged 57-65 years, with erectile dysfunction for 1-6 years and hypertension (initial diagnosis > 140/90 mmHg and ambulatory 24 hour > 130/85 mmHg) for more than 1 year on chronic antihypertensive therapy, 4 monotherapy, 3 on dual therapy, 1 on 5 agents (amlodipine 5, diuretic 4, ACE inhibitor 3, angiotensin receptor antagonist 2, diltiazem 1) participated in this study.   A secondary cause was not discovered in any of the patients.   None had overt CHD or were receiving nitrates. Three patients had previous cerebrovascular events, one patient had atrial fibrillation and another was a non-insulin dependent diabetic managed on diet alone.   Concomitant prescriptions included aspirin (3), Warfarin (2) and Pravastatin (2).   Erectile dysfunction was attributed to the underlying vascular events, diabetes and psychological causes.   The withdrawing of diuretics had not been effective. The patients gave informed consent and the institutional ethics committee approved the study.

2. Study Design

While maintaining their anti-hypertensive medications, patients were given orally either sildenafil 50 mg or placebo in a randomised single-blind cross-over fashion on two separate occasions, at least fifteen days apart. Doses of all medication was unchanged and given at the same time some 4 hours before sildenafil/placebo on both study days. The haemodynamic measurements were carried out in the supine position in a quiet room at room temperature of $20°\pm0.5°C$. BP, HR and AI% were measured at baseline and at fifteen-minute intervals for two hours following sildenafil or placebo. Adverse events were recorded at both study visits. The primary haemodynamic measurements were made at 60-90 minutes following placebo/sildenafil when peak drug levels are achieved (European Agency for Evaluation of Medicinal Products and Committee for Proprietary Medicinal Products 1998; Viagra (sildenafil citrate) package insert 1998).

3. Blood pressure and Heart Rate Measurements

BP was measured with an automated digital oscillometric device (Omron ®, Model HEM-705CP) to avoid observer bias. Each reading was taken immediately before pulse wave analysis. The HR was recorded at baseline and every thirty minutes for two hours following sildenafil/placebo by a 12-lead electrocardiogram.

Derivation Of The Arterial Pressure Waveform

The aortic BP and the AI% in the aorta were measured by pulse wave analysis (PWA) using Sphygmocor® (BPAS-1, PWV Medical ™, Sydney, Australia). A high fidelity micromanometer (SPC-301: Miller Instruments, Texas, USA) was used to flatten the radial artery. When the surfaces are flattened, circumferential pressures are equalised and an accurate pressure waveform can be recorded; this technique is termed applanation tonometry. The software allowed on-line recording of the peripheral waveform. After 20 sequential waveforms had been acquired, the integral software was used to generate an average peripheral and corresponding aortic waveform. The aortic waveform was then analysed further to determine the AI% which is defined as the difference between the first (P_1) and second peaks (P_2) of the aortic pressure waveform, expressed as a percentage of PP, and the LVED was measured from the foot of the pressure wave to the diastolic incisura. The method has been validated and found highly reproducible (Wilkinson et al. 1998b; Siebenhofer et al. 1999).

Statistical Analysis

Brachial blood pressures, aortic BP, AI% and HR were analysed using two approaches:

1) Deriving the area under the effect curve following sildenafil or placebo, which was calculated using the trapezoidal rule from the baseline readings on the individual study days.

2) Analysing individual data following 75 minutes, which has been shown to correlate with the time of peak blood levels of sildenafil, and also the extent of maximal reduction in BP again corrected to the individual baseline values on each study day.

These data were analysed by ANOVA for repeated measures and Wilcoxon rank Sum Test. Correlations were examined by linear regression analysis. All results are expressed as mean±SEM and $p < 0.05$ considered significant.

6.7 Results

Although BP varied throughout on both study days, the lowest readings were seen with sildenafil. There were significant reductions in systolic and diastolic BP as recorded in the brachial artery and aorta following sildenafil *(Figure 6.2 & 6.3)*. The extent of individual maximum reductions from baseline in brachial SBP (24±10 vs 6±8, $p < 0.05$) and DBP (8±5 vs 3±2, $p < 0.05$) occurred on the sildenafil study day. Following sildenafil both brachial BP (systolic 127.7±6 vs 144.8±5, $p < 0.05$; diastolic 76.6±2 vs 86±2, $p < 0.01$) and aortic BP (systolic 116±5 vs 133.5±5 and diastolic 78±2 vs 87.5±2) were lower at ($p < 0.01$) 75 minutes. On average brachial BP was some 17/11 less 75 minutes following sildenafil than placebo.

The AI%, a measure of wave reflection was lower *(Figure 6.3)* at 90 minutes ($p < 0.05$). For the entire 120 minutes the area under the BP, both brachial and aortic, and AI% time curve (by the trapezoidal rule corrected for baseline reading) was significantly lower ($p < 0.05$) with sildenafil. There was no correlation between the extent of fall in BP and the extent of fall in AI%. The shape of the radial and aortic pressure waveform was changed *(Figure 6.4)*, height of the late systolic peak reduced by sildenafil.

Despite a pressure of 90/66 mmHg on treatment with sildenafil and another patient exhibiting a 48/23 fall, none complained of hypotensive symptoms although 2 had flushing and nasal congestion. No significant change in LVED or HR was noted.
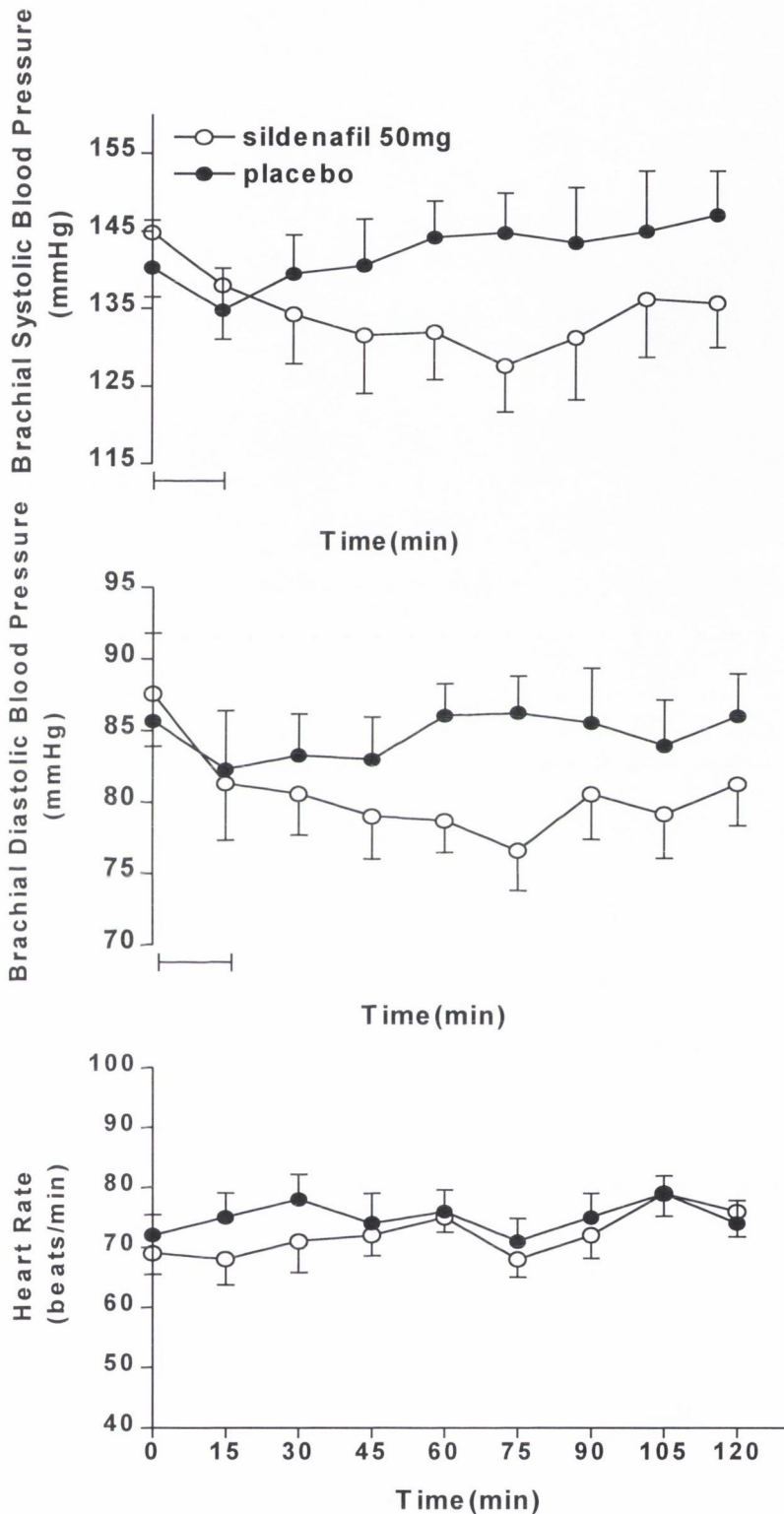
Figure 6.2. Changes in brachial blood pressures and heart rate
from baseline and every 15 minutes for 2 hours following oral
sildenafil 50 mg or placebo in treated hypertensive men. Data
presented as mean±SEM, n=8)
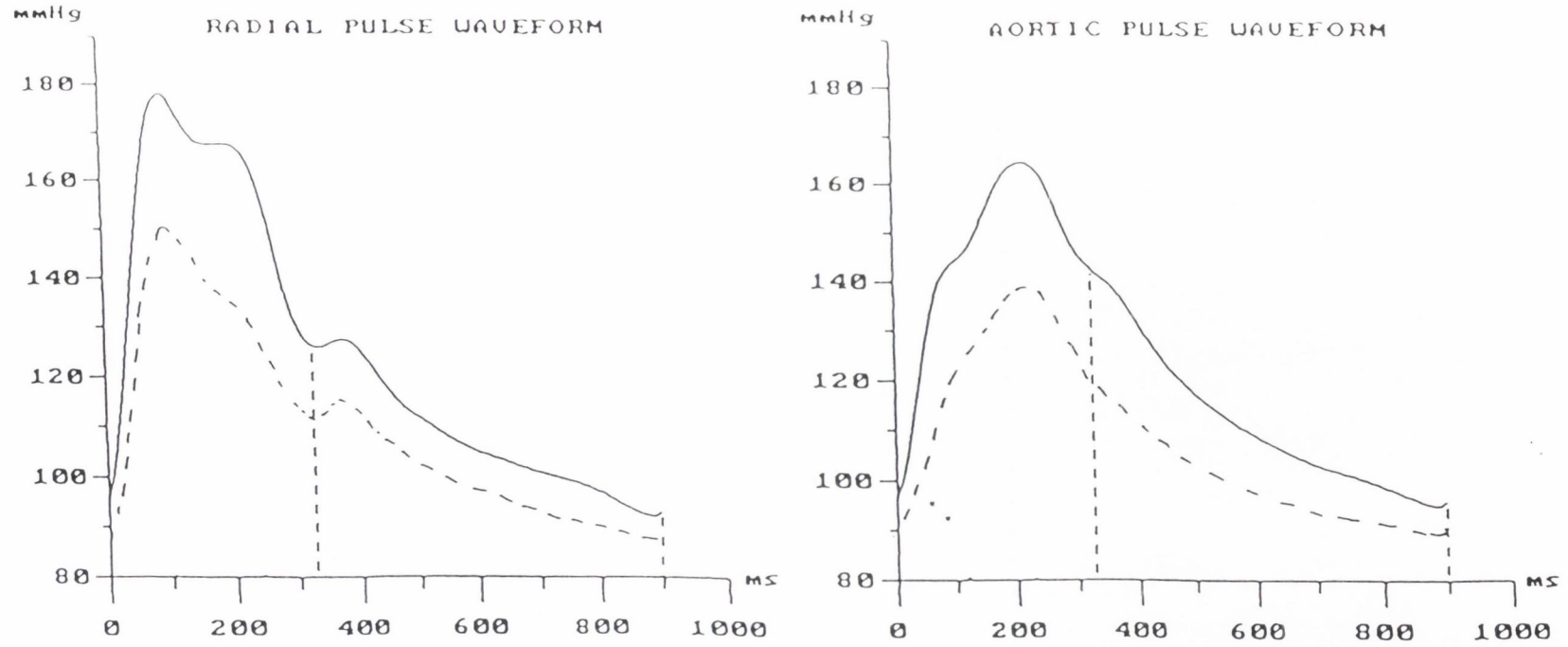
Figure 6.4. The radial and aortic pressure waveforms before (solid line) and at 90 minutes (broken line) after sildenafil 50 mg in a hypertensive subject.

6.8   *Discussion*

Sildenafil in now frequently prescribed for men with erectile dysfunction irrespective of its aetiology, which is often difficult to diagnose with certainty (Tomlinson 1999). The relaxation of vascular smooth muscle in the corpus cavernosum that is essential for penile erection is mediated by NO, which activates guanylate cyclase to produce cGMP, decreasing intracellular calcium. Sildenafil, a highly selective inhibitor of PDE5, is responsible for the degradation of cGMP, thus enhances the effects of NO, endogenous or exogenous. The actions of nitroglycerin and other NO donors are mediated via the NO-cGMP pathway, after their conversion or release of NO. NO decreases PR and BP in healthy humans. Exogenously acting nitrates, acting through NO-cGMP pathway can also cause modest reduction in systemic BP. Nitrates in addition have shown to improve arterial compliance and also have shown a marked reduction in the intensity of early wave reflection in the aorta despite no change in systemic BP (Kelly et al. 1990).

Our results show that sildenafil 50 mg acutely lowers BP, both brachial and aortic, without a compensatory rise in HR.   The hypotensive effects could be direct or secondary to a drug interaction.   Also we found a beneficial effect of sildenafil on arterial wave reflection.

1. *Effect Of Sildenafil On Brachial Blood Pressure*

BP fluctuated on both study days and there was a marked variation in the time and extent of maximal fall in BP with sildenafil.   In one individual there was a reduction of 48/23 mmHg but in this and other studies (Boolell et al. 1996; Herrman et al. 2000) hypotensive symptoms were not seen.   We used a validated oscillometric device to record BP.   While a recent study (Van Popele et al. 2000) suggested that another type of oscillometric monitor may give higher readings than a random zero sphygmomanometer, the latter is not without its own drawbacks and as the same device was used throughout our study we believe the findings are valid.

This study shows that in comparison to placebo, sildenafil has a significant effect on BP in treated hypertensive men *(Figure 6.2 & 6.3).* In healthy male volunteers administration of a single dose of up to 200 mgs did not produce "clinically" significant effects on resting

190

HR or BP (Cheitlin et al. 1999; Viagra (sildenafil citrate) package insert 1998) nonetheless in other volunteer studies sildenafil produced a transient modest reduction in systolic (8-10 mmHg) and diastolic (5-6 mmHg) BP. The peak effect occurred at one hour after dose, coincident with peak plasma concentrations, and BP returned to baseline about 4 hours after dose (Cheitlin et al. 1999). The hypotensive effect appears to be neither age dependent nor dose related. In normal volunteers intravenous administration of 20-80 mgs produced a significant decrease in PR and systemic vascular resistance index. Sildenafil has been noted to have both arteriodilator and venodilator effects on the peripheral vasculature. In 8 patients with stable angina sildenafil reduced systemic and pulmonary artery pressures and CO consistent with mixed arterial and venous vasodilatory effects. More recently in 14 men with severe CHD a significant reduction in systemic arterial and pulmonary arterial pressure was seen without any effect on pulmonary wedge pressure, CO or coronary blood flow (Herrman et al. 2000).

2. Effect Of Sildenafil On Aortic Blood Pressure

Sildenafil significantly decreased both systolic and diastolic BP in the aorta as compared to placebo. The peak effect occurred at 75 minutes after the administration of sildenafil. Though the number of subjects is small, there is a suggestion that the fall in the aortic SBP may be more than that in the brachial artery *(Figure 6.3)*, which however, did not reach statistical significance. This preferential effect on the aortic SBP compared to the brachial SBP is well known for nitrates (Kelly et al. 1990).

3. Effect Of Sildenafil On Heart Rate

Despite an overall reduction in BP and marked falls of BP in individual subjects, HR was unchanged. In clinical practice dizziness (2%) and postural hypotension at less than 2% have been reported rarely and at a similar rate to placebo treated patients (Cheitlin et al. 1999; Viagra (sildenafil citrate) package insert 1998). While flushing is commonly reported with sildenafil (ten times more frequently than with placebo) tachycardia or palpitations are not more frequent (Cheitlin et al. 1999; Viagra (sildenafil citrate) package insert 1998; Boolell et al. 1996). In an interaction study with GTN, for a fall in SBP of greater than 25 mmHg the change in HR was negligible (Webb et al. 1999). Of interest, a recent study has shown that sildenafil 100 mg in normotensive subjects causes a marked

increase in sympathetic activity, both at rest and during stressful stimuli (Philips et al. 2000).

4. Drug Interactions With Sildenafil

Interactions between drugs are either kinetic or dynamic in origin. While sildenafil is metabolised mainly by cytochrome P450 and particularly CYP3A4, drugs that inhibit this enzyme including cimetidine and erythromycin may increase its level in blood. A recent pharmacoepidemiological study has shown that the prescribers are careful in avoiding co-prescribing with interacting drugs particularly nitrates(Williams and Feely 2001). We are unaware that any of the antihypertensive drugs in our study, calcium channel blockers, diuretics, ACE inhibitors or concomitant therapy have such an effect. In an interaction study with the calcium antagonist, amlodipine (Webb et al. 1999) in hypertensive men, the mean maximal reduction in BP with sildenafil 100 mgs was some 8/7 mmHg greater in comparison with placebo. A similar pharmacodynamic interaction has been seen with another calcium antagonist lercanidipine (Hedner et al. 2000). The pharmacokinetics of neither amlodipine nor lercandipine was altered in these studies. We have now shown a fall in BP of somewhat greater magnitude in patients on a variety of antihypertensives, which is in keeping with a direct hypotensive effect of sildenafil.

The cardiovascular response to sexual activity has recently been reviewed (Stein 2000). Initial studies by Masters and Johnson with young subjects in laboratory conditions, reported heart rate and blood pressure responses at near maximum exercise levels that do not reflect the less strenuous (moderate exercise) response seen in more recent studies using ambulatory recordings in middle aged men. A modest BP lowering effect of sildenafil during sexual intercourse, which usually produces SBP in the region of 150-180 mmHg (El Sakka Al 1996), may explain why hypotensive symptoms have not been commonly reported. On the other hand in untreated hypertensives (155/87 mmHg) during coitus the HR reached 124/min and SBP increased by 50%. Overall the risk of myocardial infarction after sexual activity is very low from 0.01% over a year for low risk individuals engaging in weekly sexual activity to 0.1% for high-risk individuals (Kimmel 2000).

5. Effect Of Sildenafil On Arterial Wave Reflection

As arteries stiffen, especially in hypertension, PWV increases and the localisation of the sites of wave reflection may move proximally causing the reflected wave to increase in amplitude and return to the ascending aorta earlier, during systole (O'Rourke and Kelly 1993). The AI%, a measure of early wave reflection was significantly elevated in our subjects which is compatible with the degree of arterial stiffness seen in hypertension (O'Rourke and Gallagher 1996; Mahmud and Feely 2001b).

We believe our data suggests a direct effect of sildenafil on large artery function. We noticed a change in the arterial pressure waveform *(Figure 6.4)* and a significant fall in the AI% *(Figure 6.3)*. We did not see any relationship between the extent of fall in BP and AI% although the study was not sufficiently powered to examine BP independent effects. Also not all antihypertensive drugs have a beneficial effect on arterial stiffness in reducing arterial wave reflection despite the same level of BP reduction (Chen et al. 1995; Ting et al. 1991). ACE inhibitors, but not alpha or conventional beta-blockers have been shown to reduce arterial wave reflection. Sildenafil significantly reduced early wave reflection and by inference arterial stiffness. Furthermore nitrates markedly reduce early wave reflection and showed a preferential effect on aortic than brachial BP (Westling et al. 1984; Kelly et al. 1990). One caveat in the present study is studying the effects of sildenafil at the time of peak effect of the other anti-hypertensive drugs. It would be of interest to measure the activity of sildenafil at the trough effects of the anti-hypertensive drugs which may help separate the BP lowering effects of sildenafil from its effects on arterial stiffness. Not withstanding, this was a placebo-controlled study and I did not observe any change in arterial stiffness following placebo.

The fall in AI% seen with sildenafil could be due to a number of mechanisms e.g. delaying wave reflection by decreasing PWV, shorter LVED or by reducing the intensity of wave reflections. The reduction in AI% could be due to decrease in PWV, which was not measured in the present study. However in vitro studies have shown little vasodilatory effect of sildenafil on the aorta unless combined with nitrates (Wallis et al. 1999). The LVED did not show any significant change. A decreased in AI% could also be due to reduction in the intensity of wave reflection. Sildenafil increases cGMP levels in muscular arteries in vitro and decreases forearm vascular resistance in healthy humans (European

Agency for Evaluation of Medicinal Products and Committee for Proprietary Medicinal Products 1998). The principal sites of wave reflection are where small muscular arteries terminate in high resistance arterioles. Clinical studies have shown that wave reflection intensity, can be reduced by drugs that dilate small peripheral conduit arteries (Yaginuma et al. 1986). A direct effect of sildenafil on the vascular smooth muscle in the small muscular arteries could lead to vasodilatation and decrease in the amplitude of wave reflection. The extent of the effect of sildenafil on arterial wave reflection was in part independent of alterations in BP. The most likely mechanism of sildenafil is a direct effect on vascular smooth muscle cells in the small muscular arteries, increasing nitric oxide in the vessel wall and causing active vasodilatation, leading to a reduction in amplitude of wave reflection in the aorta.

NO decreases PR and BP in man (Webb et al. 2000). While sildenafil preferentially inhibits PDE5, which is concentrated in the genitalia, it is also found throughout the systemic vasculature (Wallis et al. 1999). Also in vivo sildenafil has been shown in cardiac tissue to increase cyclic adenosine monophosphate (cAMP), which has vasodilatory effects, and there is evidence of "cross talk" between cGMP and cAMP-dependent signal transduction pathway (Stief et al. 2000). The effect of sildenafil on arterial function seen in this study is not, therefore unexpected. It does however point to additional therapeutic targets for inhibition of PDE5.

CHAPTER SEVEN

THE EFFECTS OF DRUGS ON ARTERIAL STIFFNESS THAT MODULATE THE RENIN-ANGIOTENSIN SYSTEM

Conventionally, the main focus of antihypertensive therapy has been simply a reduction in BP. In recent years, three important haemodynamic considerations have substantially changed our conventional views on the treatment of hypertension (Safar et al. 1994; Nichols and O'Rourke 1998b). Firstly, the BP curve should be described as involving two different components: a steady component, the MAP, and a pulsatile component, the PP. Secondly, the BP curve propagates at a certain velocity along the arterial tree, thus causing specific changes in PP. Thirdly, the influence of these two haemodynamic mechanisms varies consistently with age. Epidemiologically, it is now appreciated that the greatest burden from hypertension is in the older population, particularly from SBP. Indeed in patients over the age of 60 years in the Framingham study SBP is the stronger determinant of cardiovascular risk than either MAP or DBP (Franklin et al. 1999b). The demonstration that measures of arterial stiffness, particularly PWV, may be an important prognosticator not only of cardiovascular events but total mortality in the hypertensive (Laurent et al. 2000) and end-stage renal disease population (Blacher et al. 1999a) has provided a new therapeutic target for antihypertensive agents i.e. arterial stiffness.

The purpose of these studies was to explore the potential role of angiotensin II receptor antagonists in reducing arterial stiffness. As mentioned before, there are difficulties in separating the BP lowering effects from the possible direct effects of antihypertensive drugs on large artery function. In exploring the role of ATII receptor antagonists on arterial stiffness, I examined the issue from a number of different perspectives:

1. Comparison with an antihypertensive drug known not to affect arterial stiffness (i.e. diuretic)

2. Comparison with an antihypertensive agent known to reduce arterial stiffness by a similar mechanism i.e. ACE inhibitor

3. The possible mechanisms involved were further explored by examining the combination of an ATII receptor antagonist and an ACE inhibitor. All the above were conducted in untreated hypertensive patients.

4. Finally I examined their potential role in reducing arterial stiffness in the more usual "add-on" situation by examining the effect of an ATII antagonist in poorly controlled long standing hypertensives, receiving at least three drugs (including an ACE inhibitor), who showed evidence of increased arterial stiffness. To demonstrate that the action on arterial stiffness was a class effect,

studies were conducted with two different ATII receptor antagonists, losartan and valsartan.

Comparison Of The Angiotensin II Receptor Antagonist Losartan With Hydrochlorthiazide On Arterial Stiffness

7.1 Introduction

For the same BP reduction, antihypertensive drugs do not cause the same arterial changes in hypertensive patients (Safar et al. 1990).   Also, there may be a spectrum of activity on different components of the vasculature e.g. elastic aorta or more muscular arteries.   ACE inhibitors, calcium channel blockers, alpha (some beta) blocking agents decrease arterial stiffness, however, no significant change is observed following conventional beta-blocking agents such as propranolol, the vasodilatory drug, dihydralazine and the diuretic compound, hydrochlorthiazide (Dart and Kingwell 2001).   ATII receptor antagonists are being increasingly used in the hypertensive population.   While their efficacy in reducing BP is no greater than standard antihypertensives, a greater degree of patient acceptability is likely to result in increasing use.   It is unlikely for ethical reasons that they will ever be compared as monotherapy against placebo to establish their effect on morbidity and mortality in the hypertensive population.   Increasingly surrogate markers will be used in the assessment of new antihypertensive agents.   To date the effect of ATII receptor antagonists on arterial stiffness has not been explored.   In this study, we hypothesized that if an ATII receptor antagonist reduced arterial stiffness, in addition to lowering blood pressure of magnitude similar to seen with a diuretic e.g. hydrochlorthiazide which is known not to affect arterial stiffness, a direct arterial effect of the ATII receptor antagonist may be implied.

7.2 Materials and Methods

1. Subjects

Eleven patients (5 men and 6 women) with essential hypertension completed the study. All subjects, age (57 ± 3, mean$\pm$SEM) range 47-69 years and mild to moderate hypertension > 140/90 mmHg, confirmed by ambulatory BP monitoring > 130/85 mmHg,

and were not on any antihypertensive treatment.   None of the patients had any other cardiovascular conditions, diabetes or renal disease and were not taking any medications. Informed consent was obtained from all subjects and the study had institutional ethics committee permission.

2.Study Design

Patients participated in a single-blind randomised crossover study.   The patients were initially randomised into two groups: that in-group A received an ATII receptor antagonist, losartan 50 mgs for four weeks.   Patients in Group B were given the diuretic, hydrochlorthiazide 12.5 mgs for four weeks.   At the end of 4 weeks of treatment the patients had a four-week washout period with patients in Group A switching to the diuretic, hydrochlorthiazide and those in Group B to the ATII receptor antagonist, losartan.

3.Study Protocol

The patients were studied in the morning in the fasting state apart from medication which was taken at a standardized time 2 to 4 h earlier, and having abstained from caffeine containing beverages, smoking and alcohol in the 12 hours prior to the study.   Studies were carried out in a quiet room at 23°C and all haemodynamic measurements were made after a supine rest of 15 minutes.   BP and HR were measured in the left arm with an automated digital oscillometric BP monitor (Omron® HEM Model 705-CP).   A mean of three readings was taken.

4.Derivation of arterial pressure waveform

Immediately after recording BP, the same arm was used for applanation tonometry.   A high-fidelity micromanometer (BPAS-1, PWV Medical®, Sydney, Australia) was used to flatten the radial artery and the peripheral radial pulse continuously recorded (Kelly et al. 1989b).   The central aortic waveform was derived from radial tonometry by using a previously validated TF relating peripheral to central waveform within the software package (Sphygmocor® PWV Medical ™, Australia) as described earlier.   The validity of the derived AI% has been confirmed by simultaneous direct central aortic measurements

and is highly reproducible in both healthy and diseased populations (Wilkinson et al. 1998b; Siebenhofer et al. 1999).

5.Measurement Of Pulse Wave Velocity

Carotid-femoral PWV, a classic index of arterial stiffness, was determined according to the foot-to-foot method using the Complior® device.   The simultaneous recording by two pressure sensitive transducers of the carotid and femoral waveform allows measurement of the time delay of successive records from the foot of each wave divided by the distance between the transducers and calculation of PWV (metres/sec).   The distance travelled by the pulse wave was measured over the body surface with a tape measure as the distance between the recording sites and fed into the software and 10 consecutive waves were sampled.   This method of evaluating the covered distance by superficial measurement may introduce a margin of error since the direction of blood flow in the carotid artery is opposite to that in the abdominal aorta but this is not considered significant (Asmar 1999). Moreover, PWV was measured repeatedly in the same subjects; and any minor error therefore does not affect the results.   The validity of the Complior® has been established (Asmar et al. 1995b).   The observer was blind to the form of therapy.

Statistical Evaluation

The statistical analysis was performed using JMP, Version 3 (Statistical Analysis System for Windows).   Baseline values of groups A and B were analysed with one-way analysis of variance.   Haemodynamic changes were studied by two-way analysis of variance for repeated measures applied to a crossover design and testing treatment and period effect. As there was not significant difference between the two groups, it was assumed that there was no carry over effects and further data analysis ignored the order in which subjects received their treatment.   Values are expressed as mean±SEM, $p < 0.05$ was considered significant.

7.3 Results

There was no difference in baseline values for the two groups (Table 7.1). Following drug treatment, brachial BP (systolic and diastolic) decreased in both groups ($p<0.001$) and there was no different in BP fall between the two groups. The AI% decreased significantly following losartan only ($p<0.001$) but not after hydrochlorthiazide. Similarly, PWV decreased significantly with losartan ($p<0.001$) but not following hydrochlorthiazide.

Table 7.1: **Blood pressure, both brachial and aortic SBP and DBP (mmHg) PWV and AI% in 11 hypertensive patients before and following losartan 50 mg or hydrochlorthiazide 12.5 mg in a single-blind randomised cross-over study (mean±SEM, *p<0.05 from baseline).**

	Baseline	*Losartan*	*Baseline*	*Hydrochlorthiazide*
Brachial SBP (mmHg)	164±6.6	147±6*	167±4.2	152±5*
Brachial DBP (mmHg)	93.6±2.3	86.7±3.2*	93.5±2.9	89.8±3.2*
Aortic SBP (mmHg)	156.6±6.8	137±5.9*	158.8±54	144.7±4.4*
Aortic DBP (mmHg)	94.6±2.1	87.7±2.9*	94±2.7	91±3*
PWV (m/sec)	11.75±0.6	10.3±0.5*	11.53±0.6	11.77±0.6*
AI (%)	38±1.5	31.6±1.9*	34±2	34±1*

7.4 Discussion

In this study, hydrochlorthiazide and losartan caused a similar reduction in BP, thus it was possible to compare the drug induced arterial changes for the same BP changes at brachial and aortic sites. The treatment with losartan, in addition, produced a significant reduction in arterial wave reflection and PWV, demonstrating a decrease in arterial stiffness. As is well established (Kelly et al. 1989b; London et al. 1992c; Nichols and O'Rourke 1998b) the modification of PP between central and peripheral arteries depends principally on non-uniform elasticity of arteries and peripheral pulse wave reflection (Avolio et al 1983). Several reports have previously shown that the AI% is increased in hypertensive subjects and reflects substantial changes in the amplitude and timing of wave reflection. Due to increased stiffening of arteries, PWV increases and the reflected wave returns earlier from

peripheral reflecting sites to the heart in systole instead of diastole with increased amplitude, resulting in increased left ventricular afterload and compromised coronary perfusion pressure (Nichols & O'Rourke 1998). One of the most important findings of this study was that losartan significantly reduced wave reflection in the aorta compared to hydrochlorthiazide. Significant modification in wave reflection has been documented following ACE inhibitors (Topouchian et al. 1998; Ting 1993; Benetos et al. 1991; Dart and Kingwell 2001). All of these studies clearly indicate that captopril and related compounds alter substantially the timing of wave reflection and PWV along the arterial wall, causing the reflected wave to move from the systolic part of the BP curve to diastolic part. In this study since these changes occurred with losartan only, the weight of the evidence suggests that this particular arterial change was also related to the renin-angiotensin system in this case, by directly blocking the angiotensin II type 1 receptor. As mentioned in Chapter 1 and demonstrated in Chapter 3 there is a strong relationship between PWV and SBP, however the present study demonstrates that reduction in BP with hydrochlorthiazide is not associated with reduced PWV suggesting that some of the effects of antihypertensive drugs may be a direct action on large artery function. The finding that losartan reduced PWV and AI%, provides evidence for such an effect for ATII receptor antagonists. We measured the haemodynamic response to losartan and hydrochlorthiazide at the time of peak effect of the drugs to show the maximum effect on BP and arterial stiffness. However, it can be argued that haemodynamic measurements at trough levels may have helped to separate blood pressure lowering effects from the effects on arterial stiffness.

Longitudinal studies have indicated that in hypertensive subjects, low sodium intake is associated with a larger brachial artery diameter and in the elderly hypertensives, sodium overload causes a reduction in arterial compliance and distensibility unrelated to BP changes (Safar et al. 1994). In contrast, most of the studies in man have shown diuretics to have no effect on the stiffness of arteries (Kool et al. 1993; Khder et al. 1998). The contribution of counter-regulatory mechanisms, possibly related to the activation of the renin-angiotensin and sympathetic nervous system by diuretics might explain the differences between the clinical and experimental changes observed with diuretic compounds. A note of caution should be added because the short duration and small dose of diuretic treatment there may be an underestimation of the effect of hydrochlorthiazide.

Nonetheless it led to a significant reduction in BP. In this study, the possible interaction between diuretics and the renin-angiotensin system following drug treatment was simply deduced from the pharmacological intervention and did not involve other information on the status of the renin-angiotensin and sympathetic nervous system or on the degree of salt and water depletion. However, whether such information is more useful than a clear-cut pharmacological study remains the subject of debate and requires to be more extensively documented (Safar and Levy 1993). The possible mechanisms whereby the ATII receptor antagonist may reduce arterial stiffness are discussed in greater detail in the next section where the effect of ATII receptor antagonism is compared to that of ACE inhibition.

In conclusion, the results of this study show that a similar BP reduction, the ATII receptor antagonist caused significant arterial changes resulting in reduced arterial wave reflection and PWV along the aortic wall. It may be suspected that the modest arterial changes which are observed following diuretic treatment along might be due to the concomitant stimulation of the renin-angiotensin and sympathetic nervous system induced by salt and water retention.

Reduction in Arterial Stiffness with angiotensin II receptor antagonists – comparative to ACE inhibition and may be additive

7.5 Introduction

Hypertensive cardiovascular complications often result from alteration in medium sized arteries such as the coronary arteries and the intra and extra cranial cerebral arteries. Antihypertensive drug therapy prevents about 40% of strokes in treated individuals, whereas the prevention of ischaemic heart disease is much less effective (Collins et al. 1990) Thus optimisation of drug treatment is a major goal in the clinical management of hypertension(Safar and London 1998). Arterial stiffness is emerging as a therapeutic target in its own right. An improvement in arterial stiffness may result from an effect on the arterial wall for example through actions on smooth muscle cell tone, or on the wall structure. However, because the relationship between pressure and volume is curvilinear due to the visco-elastic nature of arteries, BP reduction itself could account for the improved arterial stiffness. Therefore, it is important to determine whether the improvement in arterial stiffness is the "passive" result of BP reduction or partly independent of changes in BP. Since delaying arterial wave reflection and decreasing arterial stiffness is now a logical strategy in the management of hypertension, the effects of antihypertensive drugs on arterial haemodynamics is of considerable interest.

Two major classes of antihypertensive drugs, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists, reduce BP by inhibiting the action of angiotensin II. The ACE inhibitors suppress the conversion of angiotensin I to angiotensin II, whereas the ATII receptor antagonists inhibit the binding of angiotensin II with the AT_1 receptor site. There are, however, differences in their profile of activity. ACE inhibitors also block the breakdown of vasoactive bradykinin but may not block the local production of angiotensin II in the vessel wall by non-ACE pathways (e.g. chymase, urease). ACE inhibitors have been repeatedly shown to lower BP and to selectively decrease arterial stiffness both in humans and animal experiments, beyond that expected from the decrease in BP alone (Levy et al. 1988; Topouchian et al. 1998). We would show

that an ATII receptor antagonist when added to a regimen of more than three antihypertensives, including an ACE inhibitor, in poorly controlled hypertension, has a beneficial effect on arterial wave reflection and PP amplification (Mahmud and Feely 2001b). The effect of monotherapy with an ATII receptor antagonist on large arterial function however has not been described. Furthermore, while both ATII receptor antagonists and ACE inhibitors target the same pressor system, due to their different sites of action they may have additive or synergistic effects. The purpose of the study was to explore the effects of an ATII receptor antagonist on large artery properties particularly arterial stiffness, in comparison and combination with an ACE inhibitor.

7.6   Materials and Methods

1.Subjects

Twelve patients (8 men and 4 women with essential hypertension completed the study. All subjects, age range 27-72 years (49 $\pm$3, mean$\pm$SEM) had mild to moderate hypertension > 140/90 mmHg, confirmed by ambulatory BP monitoring >130/85 mmHg, and were not on any antihypertensive treatment. None of the patients had any other cardiovascular conditions, diabetes or renal disease and were not taking any medications. Informed consent was obtained from all subjects and the study had institutional ethics committee permission.

Patients participated in a group single blind randomised crossover study. The patients were initially randomised into two groups; those in group A received an ATII receptor antagonist valsartan, initially 80 mg for two weeks and increasing to 160 mg for the subsequent two weeks. Patients in Group B were given an ACE inhibitor, captopril 50 mg initially for two weeks and increased to 100 mg for the subsequent two weeks. At the end of 4 weeks of treatment the patients had a two-week wash-out period with patients in Group A switching to the ACE inhibitor, captopril and those in Group B to valsartan and the treatment was continued for another 4 weeks and at the end of this period, captopril 100 mg and valsartan 160 mg were combined for a further 2 weeks in all the patients.

2.Study Protocol

The patients were studied in the morning in the fasting state apart from medication which was taken at a standardized time 2 to 4 h earlier, and having abstained from caffeine containing beverages, smoking and alcohol in the 12 hours prior to the study. The patients were studied in a quiet room at 23°C and all haemodynamic measurements were made after a supine rest of 15 minutes. BP and HR were measured in the left arm with an automated digital oscillometric BP monitor (Omron® HEM 705-CP). A mean of three readings was taken.

3.Derivation Of Arterial Pressure Waveform

Immediately after recording BP, the same arm was used for applanation tonometry. A high-fidelity micromanometer (BPAS-1, PWV Medical®, Sydney, Australia) was used to flatten the radial artery and the radial pulse continuously recorded (Kelly et al. 1989b). The central aortic waveform was derived from radial tonometry by using a previously validated TF relating peripheral to central waveform within the software package (Sphygmocor® PWV Medical ™, Australia) as previously described (Mahmud and Feely 2001b). The validity of the derived AI% has been confirmed by simultaneous direct central aortic measurements and is highly reproducible in both healthy and diseased populations (Wilkinson et al. 1998b; Siebenhofer et al. 1999).

4.Measurement Of Pulse Wave Velocity

Carotid-femoral PWV, a classic index of arterial stiffness, was determined according to the foot-to-foot method using the Complior® device. The simultaneous recording by two pressure sensitive transducers of the carotid and femoral waveform allows measurement of the time delay of successive records from the foot of each wave divided by the distance between the transducers and calculation of PWV (metres/sec). The distances travelled by the pulse wave was measured over the body surface with a tape measure as the distance between the recording sites and fed into the software and 10 consecutive waves were sampled. This method of evaluating the covered distance by superficial measurement may introduce a margin of error since the direction of blood flow in the carotid artery is opposite to that in the abdominal aorta but this is not considered significant (Asmar et al. 1999). Moreover, PWV was measured repeatedly in the same subjects; and any minor

error therefore does not affect the results. The validity of the Complior ® has been established (Asmar et al. 1995b). The observer was blind to the form of therapy.

5.Statistical Evaluation

The statistical analysis was performed using JMP, Version 3 (Statistical Analysis System for Windows). Baseline values of Groups A and B were analysed with one-way analysis of variance and did not show any statistical difference. Haemodynamic changes were studied by two-way analysis of variance for repeated measures applied to a crossover design and testing treatment and period effect. As there was no significant difference between the two groups and it was assumed that there was no carry over effects, further data analysis was based on ignoring the order in which subjects received their treatment. In addition, since BP may influence arterial haemodynamic parameters, we carried out a complementary analysis on PWV and AI% adjusted for the changes in BP at the time they were measured. In order to test for possible differences between aortic and brachial BP measurements, a three-way analysis of variance (i.e. subject, treatment, site) was carried out. Values are expressed as mean±SEM, $p<0.05$ considered significant.

7.7 Results

There was no difference in baseline values for the two groups. Both drugs produced a significant reduction in both brachial and aortic BP ($p<0.01$), PWV ($p<0.001$) and AI% ($p<0.001$) (Table 7.2). PP, both aortic and brachial BP decreased with both therapies ($p<0.01$). There was no relationship between the reduction in BP and PWV or AI% in individual patients.

While the reduction in BP and AI% with combined therapy was greater than with individual therapy, only the effect on PWV of combined therapy was statistically greater ($p<0.05$) than the effect of monotherapy. In comparing individual therapy this is largely attributable to the addition of captopril ($p = 0.09$) than valsartan ($p=0.57$).

Table 7.2. Mean (±SEM) BP and arterial stiffness (PWV, AI%) following ATII receptor antagonist valsartan 160 mg for four weeks, an ACE inhibitor captopril 100 mg for four weeks and their combination for two weeks in a randomised crossover study in never-treated hypertensive patients (*p<0.01 from baseline and monotherapy versus combination ^{f}p<0.05)

	Valsartan 160 mg		Captopril 100 mg		Combined 160mg/ 100mg
	Before	*After*	*Before*	*After*	
Brachial SBP	157±3.8	133.6±4.3*	157±3.4	138±4.4*	129±3.4
Brachial DBP	95.7±2.6	82.3±2.3*	97.3±2.7	84.7±2.2*	80±1.7^f
Brachial PP	59.8±2.4	50.8±2.7	60±3	53.7±2.6	48.7±2.6
HR/min	75.3±3.5	74.5±3.9	75±3.8	74.5±4	74±4
PWV m/sec	11.4±0.48	9.9±0.25*	11.1±0.2	10.3±0.35*	9.3±0.26^f
AI x %	24.1±4.2	18.6±5*	27±4.4	17.3±4.6*	13.6±4.1
Aortic SBP	147.1±4.2	122.5±4.7	148±4	124±4.2	117.8±3.4
Aortic DBP	96.7±2.6	83±2.3*	99.3±2.7	85.7±2.2*	81±1.7^f
Aortic PP	46.2±2.8	36.9±2.8	59±2.7	36.6±2.5	34.7±2.2

mmHg unless stated otherwise

1. Blood Pressure and Pulse Wave Velocity

Brachial systolic and diastolic BP decreased significantly with both valsartan and captopril (p<0.001) from baseline. *Figure 7.1* shows the mean fall in brachial systolic and diastolic BP following valsartan, captopril and their combination from baseline. Carotid-femoral PWV also decreased significantly following both drugs (p<0.001). At baseline PWV was significantly correlated to peripheral SBP (r=0.52, p<0.0001). There was a significant decrease in brachial systolic and diastolic BP with the combination therapy versus monotherapy (p<0.001). The decrease in PWV was almost significant comparing combination therapy to monotherapy (p<0.059). The changes in PWV were independent of BP (p> 0.5). *Figure 7.2* shows the change in PWV after valsartan, captopril and a combination from baseline.

2. Pulse Pressure and Augmentation Index

Brachial and aortic PP decreased significantly with both drugs (p<0.001). At baseline the aortic PP was significantly lower (p<0.001) compared to brachial PP. Following treatment, the decrease occurred to the same extent in the brachial and aortic PP, with no interaction between treatment and site of measurement. AI% decreased following both

captopril (p<0.001) and valsartan (p<0.001) from baseline but there was no difference between the two treatments.   The decrease in AI% following combination therapy was
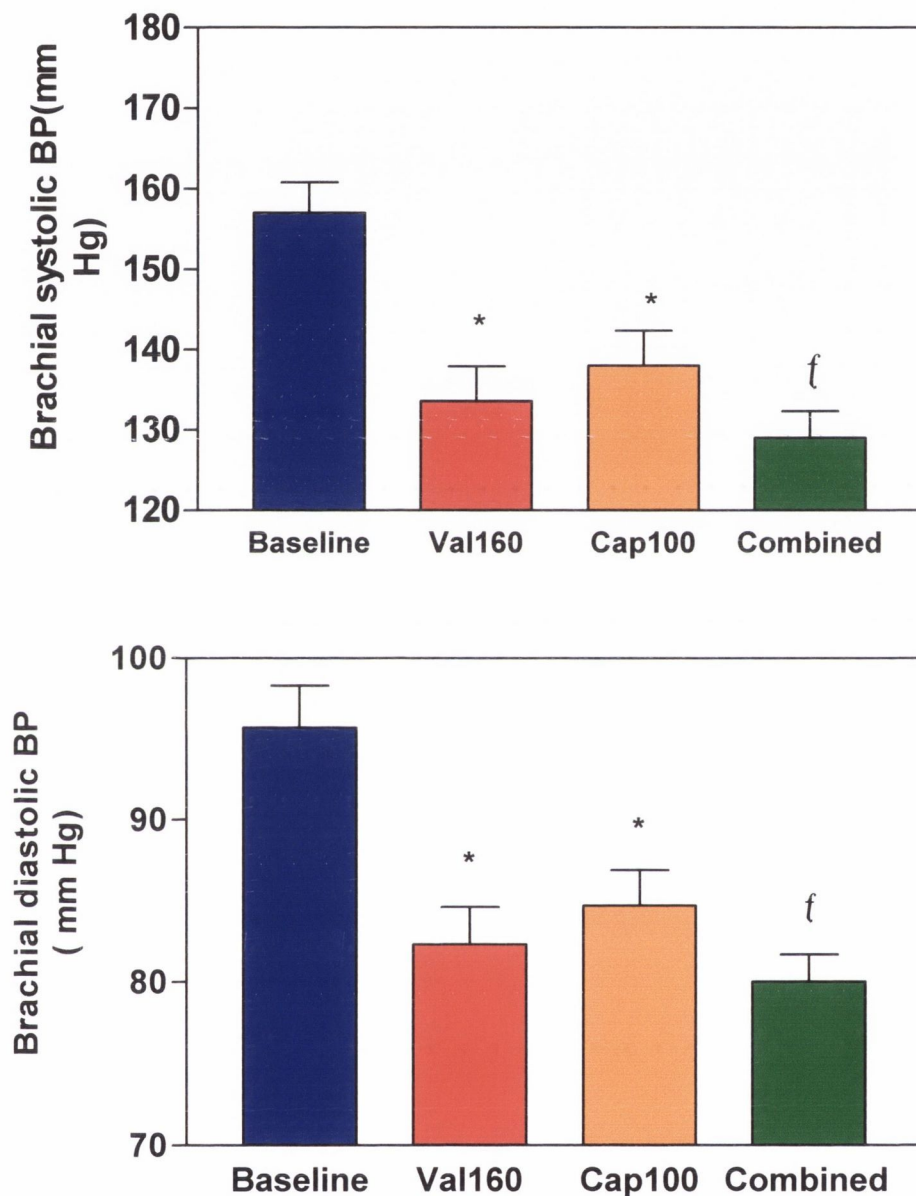
Figure 7.1. Brachial blood pressure after an ATII receptor antagonist valsartan 160 mg for four weeks, an ACE inhibitor captopril 100 mg for four weeks and their combination for two weeks in a randomised crossover study in never-treated hypertensive patients (*p<0.05 from baseline and p∫ monotherapy versus combined treatment.
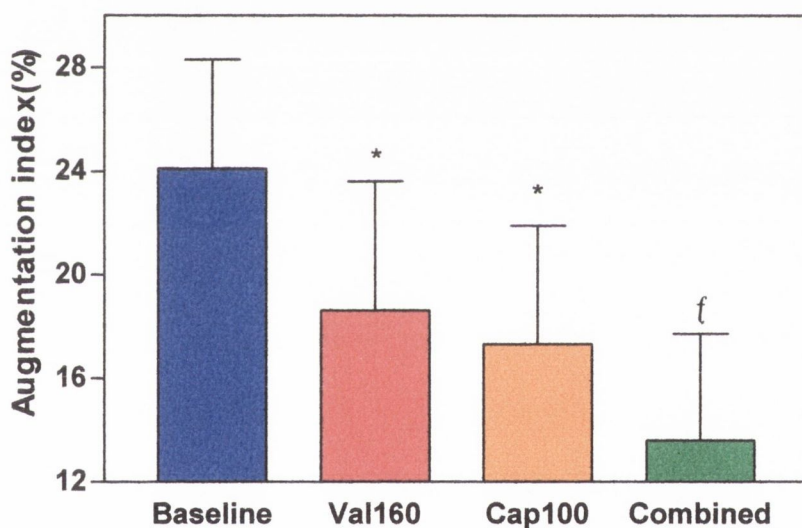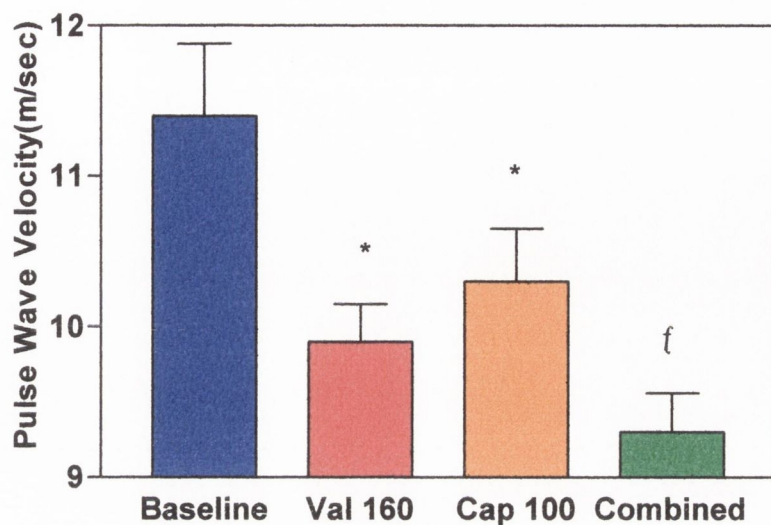
Figure 7.2. Brachial blood pressure after an ATII receptor antagonist valsartan 160 mg for four weeks, an ACE inhibitor captopril 100 mg for four weeks and their combination for two weeks in a randomised crossover study in never -treated hypertensive patients (*p<0.05 from baseline and p ʄ monotherapy versus combined treatment.

not significant compared to monotherapy (p>.05). The changes in AI% were independent of BP reduction. There was no significant change in the ΔTr or LVED with any treatment from baseline. **Figure 7.2** shows the changes in AI% following treatment with valsartan, captopril and their combination from baseline.

7.8   *Discussion*

The novel findings in our study were that following an angiotensin II receptor antagonist.

1. The reduction in AI% and PWV was independent of BP, suggesting that the decrease in arterial stiffness is mediated both by the drug effect as well as the BP reduction.

2. The PP amplification was maintained but reverted towards lower BP values.

3. ATII receptor blockade is as effective as an ACE inhibitor in reducing arterial stiffness and

4. There may be an additive effect of the ATII receptor blocker and ACE inhibitor when used in combination shown by a tendency towards lower PWV values seen with combination therapy versus monotherapy.

The main novel finding in this study is an effect of ATII receptor antagonism on large artery function – arterial wave reflection and PWV – reducing arterial stiffness. Furthermore the effect was not related to the extent of fall in BP and remained significant for changes in BP. Of interest in a recent study on the survival of patients with end-stage renal failure (Guerin et al. 2001) insensitivity of PWV to a decrease in BP was an independent predictor of mortality.   ACE inhibitors, which reduced PWV, had a favourable effect on survival, independent of BP changes.

The important effect of ATII receptor blocker was an alteration of the effect of wave reflection. Such a reduction can theoretically be achieved by three mechanisms: delaying wave reflection (increased ΔTr, due either to decreased PWV or reflection at more distal sites), structuring the LVED and reducing the intensity of wave reflection (decreased reflection co-efficient (Murgo et al. 1980b). Valsartan did not change the LVED, and the improvement in timing was related to the longer ΔTr in association with decreased PWV. Nevertheless, the reduction of AI% was mainly independent of changes in aortic PWV and ΔTr suggesting that the intensity of wave reflection was the principal mechanism.   The principal sites where normally wave reflection occurs are arterial terminations where muscular conduit arteries terminate in high resistance arterioles and MAP drops as a result of vasomotor time and resistance (Nichols and O'Rourke 1998b).   The effect of valsartan on arterial wave reflection was in part independent of alterations in BP and changes in

aortic PWV suggesting the reduction in the intensity of arterial wave reflection occurs along the arterial tree in the peripheral arterioles. There is evidence that wave reflection intensity can be reduced by drugs that dilate small peripheral arteries in contrast to arteriolar dilating drugs (Yaginuma et al. 1986; Simkus and Fitchett 1990).

Hypertension induces structural changes in arteries particularly destruction of elastin, increase in collagen and vascular smooth muscle cell hypertrophy (Stanley et al. 2000). Decreased arterial compliance in hypertension is largely due to increased deposition of extracellular matrix protein particularly collagen within the wall. Both mechanical strain and ATII have been shown to increase matrix protein expression and the effect of strain is attenuated by ATII receptor antagonist (Stanley et al. 2000). Angiotensin II through AT_1 receptor may induce abnormal structure of the arterial wall in part through its effects on extra-vascular matrix protein and collagen synthesis (Ford et al. 1999). Increased collagen content has been found in small resistance arteries of hypertensive patients and collagen is some 400-1000 times stiffer than elastin (Stanley et al. 2000; Sharif et al. 1998). In 17 hypertensive patients randomised to either atenolol or losartan for one year the media/lumen ratio of small arteries and the slope of the elastic modules versus stress were reduced by losartan only. As the degree of antihypertensive effect was similar it was concluded that ATII receptor blockade might reduce stiffness and produce structural changes in subcutaneous resistance arteries (Stanley et al. 2000).

The second important effect of valsartan was a significant decrease in aortic PWV. The decrease in PWV was in parallel to the decrease in arterial wave reflection and pressure in the aorta. These changes in PWV were independent of BP reduction and were still observed after adjustment for baseline values of PWV and BP alteration. The arterial pressure – volume relationship is curvilinear, arteries being stiffer at high pressure and arterial stiffness decreases with BP reduction. Therefore it is difficult to ascertain whether the improvement in PWV with antihypertensive therapy is the 'passive' result of BP reduction or is a consequence of pressure independent alterations of the arterial wall. Both animal and human studies have shown that both acute and chronic administration of ACE inhibition improves large artery compliance independently of BP changes (Topouchian et al. 1998; London et al. 1996; Levy et al. 1988). This improvement was related to vascular smooth muscle relaxation and decreased arterial wall thickness, with

reversion of smooth muscle cell hypertrophy and decreased collagen content (Levy et al. 1988). The mechanisms responsible for alterations in arterial stiffness in humans are difficult to analyse.

There is evidence that ATII blockade may reduce vascular stiffness by both structural and functional changes. There is experimental evidence in genetically hypertensive rats that valsartan produces reversal of cardiac and vascular hypertrophy even when BP is not reduced to normotensive levels (Lendingham and Laverty 1996). In hypertensive patients with LVH, valsartan was as effective as enalapril in causing regression (Nalbantgil et al. 2000). In normotensive volunteers, angiotensin II infusion increases the AI% and aortic PP but not brachial PP (Wilkinson et al. 2001). The ATII receptor blocker candesartan improved tonic NO release and reduced vasoconstriction to endogenous endothelin I in the forearm of hypertensive patients after 12 months therapy (Stanley et al. 2000). Of interest 4 weeks treatment with the ATII receptor antagonist losartan improved endothelial dysfunction in patients with CHD by increasing the bio-availability of NO (Hornig et al. 2001). The Val-Heft study adds support to such strategy.

Whereas MAP is stable along the arterial tree, PP increases markedly from central to peripheral arteries. This PP gradient is observed in both normotensive and hypertensive populations. Because PP amplification is the consequence of progressive decrease in cross-sectional area and the increase in stiffness of muscular arteries and as it is largely influenced by the timing of arterial wave reflection, it is logical to expect that vasodilatation may modify PP amplification. We would show that an ATII receptor antagonist when added to antihypertensive drug regimen of at least three drugs, including an ACE inhibitor, led to improvement in PP amplification.

The rationale for adding ATII receptor blockers to ACE inhibition is the different range of activities and observations that the recommended doses of ACE inhibitors provide only partial inhibition of ACE. The pressor response to angiotensin I in patients with heart failure was blunted by valsartan but increased linearly with ascending doses despite treatment of ACE inhibitors at maximum doses. Greater ACE inhibition was seen when captopril was increased to 300 mg/day (Jorde et al. 2000). More recently it has been shown in resistance arteries from patients with CHD that the inhibition of either ACE or

211

chymase pathways alone has no effect on ATII generation and both pathways must be blocked before the vasoconstrictor action of angiotensin I is inhibited (Petrie et al. 2001).

As the number of subjects studied was relatively small and there was no washout period prior to studying the treatments, the additive effects must be interpreted with caution. There are theoretical reasons for supporting such a possibility as there is now considerable evidence for non-ACE dependent angiotensin II production both by local tissue bound ACE and non-ACE enzyme conversion. It has been estimated that 30-40% of angiotensin II generation in the kidney is non-ACE dependent when the renin system is stimulated by a low salt diet (Hollenberg and Sever 2000). We measured the haemodynamic response to valsartan and captopril at the time of peak effect of the drugs to show the maximum effect on BP and arterial stiffness. However, it can be argued that haemodynamic measurements at trough levels of the drugs may have shown helped to separate BP lowering effects from effects on arterial stiffness.

There is preliminary data also to suggest a greater degree of LVH after 8 weeks in patients who received losartan in addition to enalapril 40-80 mg day titrated to achieve a target BP (Nesukay et al. 2000).

Favourable Effects on Arterial Wave Reflection and Pulse Pressure Amplification of adding Angiotensin II Receptor Blockade in Resistant Hypertension

7.9 Introduction

It is common to see patients who have poorly controlled hypertension despite taking multiple anti-hypertensives. We have previously shown that monotherapy with ACE inhibitors and ATII receptor antagonists reduce arterial stiffness in never treated-hypertensive patients. Moreover, there is a suggestion that the two when combined, may confer an additional additive effect on BP and arterial stiffness. However, ACE inhibitors and ATII receptors are more commonly used as add on therapies in patients who are not well controlled on existing anti-hypertensive medication. We therefore studied the effect of adding valsartan (Holwerda et al. 1996), an ATII receptor antagonist on AP and PP amplification in poorly controlled hypertensive patients, already on ACE inhibitors as done in clinical practice.

7.10 Materials and Methods

1.Patients:

Eighteen patients (6 men and twelve women) participated in the study. The mean age (Mean $\pm$ SD) was 56 ± 9 years, (range 41 to 69). All the patients had inadequately controlled essential hypertension (>160 /95 mm Hg) for the last 6 months and were on at-least three anti-hypertensives always including an ACE inhibitor, diuretic (n=16), calcium antagonists (n= 12), beta-blocker (n= 6) and alpha-blocker (n=4). The average dosages of ACE inhibitors being used were captopril 100mg and enalapril 20mg. The mean AP in the patients was 21 ± 8mm Hg. An age–matched group of 6 hypertensives with an AP of 10 ± 5mm Hg was also studied concurrently over a two-week period to show that the AP did not change over time. Informed consent was obtained from all the patients and the study had institutional ethics committee permission.

213

2.Study Design:

The patients were studied in the fasting state and abstained from caffeine containing beverages and smoking in the 12 hours prior to the study. The patients were studied at baseline and then at 2 hours and 2 weeks following the oral administration of 80mg of valsartan, an angiotensin II, and ATII receptor antagonist.

3.Blood Pressure Measurements:

The haemodynamic measurements were made in a quiet room at 20-23°C and after a rest of 15 minutes in the recumbent position. Brachial BP and HR were measured in the left arm with an automated digital oscillometric BP monitor (Omron®, Model 705-CP). A mean of three readings was taken.

4.Derivation of Arterial Wave Reflection

Immediately after taking the final BP reading, the same arm was used for applanation tonometry. A micromanometer-tipped probe (Sphygmocor™ ,BPAS-1;PWV Medical, Sydney , Australia) was applied to the surface of the skin overlying the radial artery and the peripheral radial pulse continuously recorded (Kelly et al. 1989b). The central aortic waveform is derived from radial tonometry and the brachial BP, assuming that MAP is equal at both sites (Nichols and O'Rourke 1998b) by using a previously validated mathematical transformation within the software package (Sphygmocor™)(Panca et al. 1992; Karamanoglu et al. 1993; O'Rourke et al. 1992a). The aortic waveform was further analysed to yield the central systolic and diastolic BP and calculate the AP and AI%, both reflecting the degree of wave reflection and arterial stiffness *(Figure 2.2, Chapter 2).* The derived aortic waveform represents the sum of both incident and reflected waves. The early systolic peak (P_1) is the primary wave and corresponds with the timing of peak flow and the late systolic peak (P_2) is the result of the reflected wave returning from the peripheral site and causing an increase in amplitude of the pulse and aortic SBP. AP is the difference between the early and late systolic peak when expressed in absolute terms in mm Hg while AI% is the difference between the two peaks expressed as percentage of total pulse height. The validity of these derived values has been confirmed by simultaneous direct central aortic measurements (Karamanoglu et al. 1993; Chen et al. 1997) and is highly reproducible in both healthy and diseased populations. The within-

214

observer difference AI% is 0.49±5.37% and the between-observer difference is 0.23±3.80% for the AI% (Wilkinson et al. 1998b) and the between-observer difference for the Aug is 0.1±2.1 (Siebenhofer et al. 1999). The pulse height variability (% of mean pulse height) was always less than 5% in our study.

5.Statistical Analysis

The statistical analysis was performed through SAS (Statistical Analysis system for Windows) software. Time-dependent patterns of BP changes, arterial wave reflection and PP amplification were tested by analysis of variance (ANOVA) of repeated measures. To adjust for the effect of calcium-channel blockers, the haemodynamic changes were analysed by two-way ANOVA of repeated measures, testing time and treatment interaction. We also correlated the effect of BP change to the fall in AP at each time point by ANOVA. Data is expressed as Mean±SD and $p<0.05$ considered significant.

7.11   Results

The addition of valsartan was well tolerated. One patient complained of dizziness and no patient withdrew from treatment.

1.Blood Pressure and Heart Rate

Both brachial SBP and DBP decreased significantly with valsartan ($p < 0.001$) at 2 hours and 2 weeks after starting therapy (Table 1.). There was no change in HR. There was no change in BP, PP amplification or AP in the control group of hypertensives.

2. Pulse.Pressure and Early Wave Reflection

At baseline, aortic PP was significantly lower than brachial PP ($p<0.01$). Both brachial ($p< 0.05$) and aortic PP ($p<0.01$) decreased significantly following valsartan (Table 7.4). This decrease *(Figure 7.3)* occurred to a greater extent in the aortic than in the brachial PP ($p < 0.05$). There was a significant decrease in the amplitude of wave reflection, the AP decreased from a baseline value of 21±8 versus 11±7 at two hours to10±5 at the end of two weeks ($p<0.01$). The AI% decreased from a baseline value of 34.8±6 versus 22.1±7 at two hours to 21.9±8 at the end of two weeks ($p<0.001$).

Table 7.3. BP and HR at baseline, at two hours and 2 weeks following the addition of ATII receptor blocker valsartan to a drug regimen of more than three anti-hypertensive drugs including an ACE inhibitor in patients with poorly controlled hypertension (n=18, mean ± SD, *p<0.01 compared with baseline).

	Brachial		Aortic		Heart Rate
	Systolic	Diastolic	Systolic	Diastolic	Per Min
Baseline	174±10	101±13	165±12	102±12	63±8
2 hours	156±16*	93±16*	145±12*	95±14*	65±11
2 Weeks	149±18*	87±16*	135±16*	89±14*	66±9

When the results were analysed separately to adjust for the group on calcium channel blockers, the AP decreased from a baseline value of 18.1±4.7 versus 10.1±3.9 at 2 hours to 11.2±6.3 at the end of 2 weeks (n=12, p<0.01) in the group taking calcium channel blockers. In the group not on calcium channel blockers, the AP was 23.75±11 at baseline and decreased to 13±10 at two hours and 8.5±3.1 at two weeks (n=6, p<0.001). There was a significant positive correlation in individual subjects between the fall in BP and the fall in AP (r=0.59,p<0.001) when compared between baseline and two weeks.

Table 7.4: Brachial and aortic PP amplification, AP (mm Hg) and AI% at baseline, 2 hours and 2 weeks following the addition of valsartan, an AII receptor antagonist to a drug regimen of more than three drugs including an ACE inhibitor in patients with poorly controlled hypertension (n=18, mean ±SD, *p < 0.01 compared with baseline).

	Brachial PP (mmHg)	Aortic PP (mmHg)	Amplification (mmHg_	AP (mmHg)	AI%
Baseline	72±14	66±15	8±3	21±8	34.8±8
2 hours	64±18*	50±13*	12±7*	11±7*	22.1±7*
2 weeks	61±9*	46±12*	14±5*	10±5*	21.9±8*

3.Pulse Pressure Amplification

Valsartan induced a greater fall in aortic SBP than in brachial PP (p<0.05) with PP amplification increasing to normal values (p < 0.001) *(Figure 7.4).*
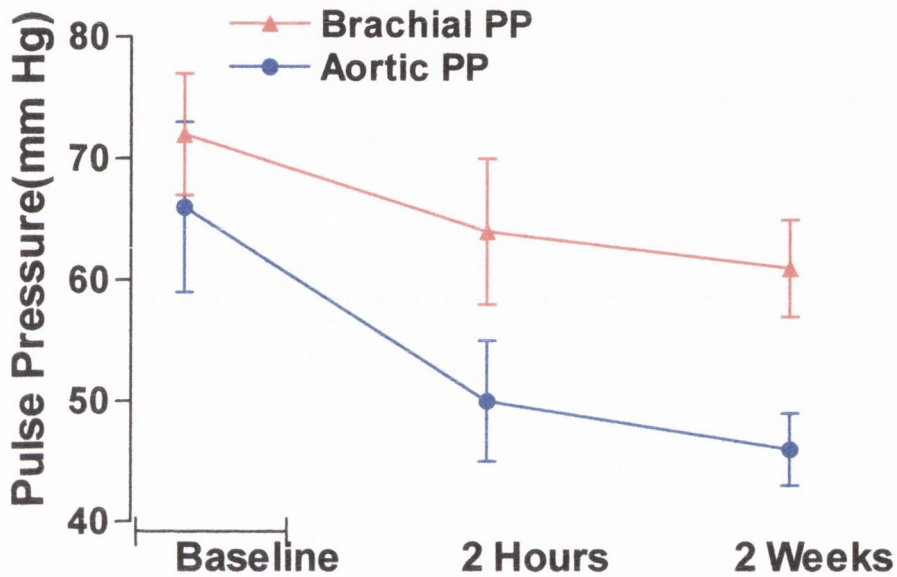
Figure 7.3. Brachial and aortic PP at baseline, 2 hours and two weeks following the addition of an ATII receptor antagonist , valsartan 80 mg in a group of hypertensive pateints already on more than three anti-hypertensive including an ACE inhibitor(n=18, Mean ± SD, *p<0.01).
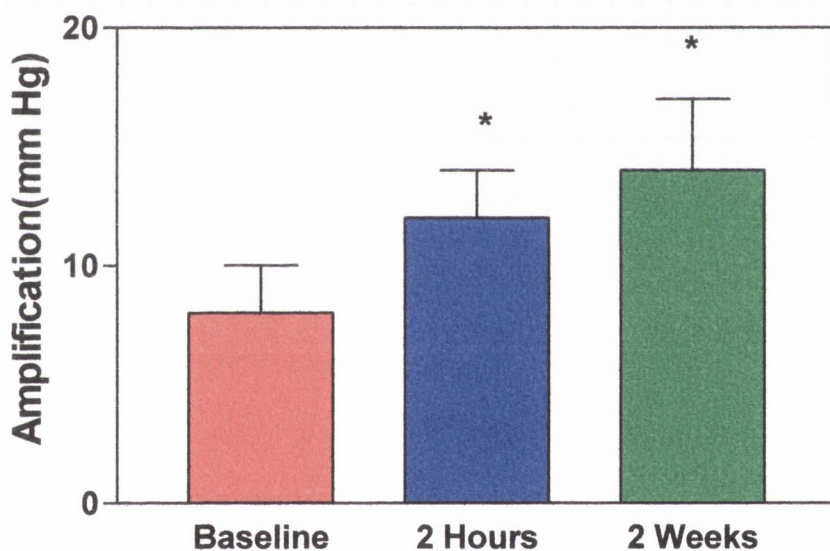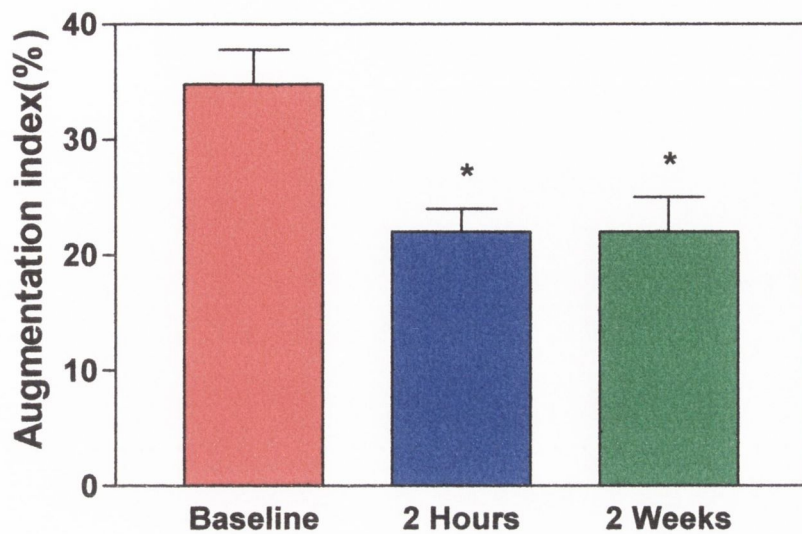
Figure 7.4. AI% and PP amplification (mm Hg) at baseline, 2 hours and 2 weeks following the addition of valsartan, an ATII receptor antagonist to a drug regimen of more than three anti-hypertensive drugs including an ACE inhibitor in patients with poorly controlled hypertension (n=18, mean±SEM, *p<0.05 from baseline)

7.12 Discussion

As large artery stiffness increases in older subjects, SBP rises and DBP falls, with a resulting increase in PP. The normal greater amplification of PP between central and peripheral arteries gradually also decreases as a result of the augmentation of the central PP by early wave reflection. There is now increasing evidence that PP is an indicator of large-artery stiffness and an independent predictor of the risk of CHD, a relationship that is even stronger than with MAP in middle-aged and older subjects (Franklin et al. 1999a).

Our results show the effects of adding an ATII receptor antagonist to an ACE inhibitor on arterial wave reflections, PP and PP amplification in poorly controlled hypertensives:

i)   Both SBP and DBP decreased significantly with no major adverse effects.

ii)   There was a significant decrease in AI% in the ascending aorta.

iii)   The aortic PP decreased significantly more than brachial PP with a significant increase in PP amplification.

1. Valsartan-induced effects on arterial wave reflection

The present study showed that in patients with poorly controlled hypertension, an ATII AT_1 receptor antagonist valsartan, when added to patients already on an ACE inhibitor, induced a marked reduction of arterial wave reflection, producing a fall in late systolic peak in the aorta, associated with a reduction in both the AP and AI%, which is largely due to fall in BP but may partly be due to the effects of the drug on the arterial wall.

There has been a recent surge in interest in drugs that not only reduce blood pressure but also modify the blood pressure curve. In this setting, nitrates(Kerry et al. 1990), calcium-channel blockers (Pannier et al. 1994), vasodilating beta-blockers (Kelly et al. 1989c) and ACE inhibitors (Ting 1993; London et al. 1996; Topouchian et al. 1998) have all been shown to decrease arterial wave reflection in the ascending aorta. This is in contrast to drugs that have no effect on arterial wave reflection and arterial stiffness despite the same extent of BP reduction. For instance, neither alpha-blockers (Ting et al. 1991) nor conventional beta-blockers (Chen et al. 1995) improve arterial wave reflection. Some beta-blockers might actually increase the amplitude of reflected waves acutely (O'Rourke

and Kelly 1993). Arteriolar dilators have also shown only a modest effect on wave reflection (O'Rourke and Kelly 1993).

The suggestion from our results is that ATII receptor antagonists may also have a similar effect as ACE inhibitors and may provide additional benefit on arterial stiffness and arterial wave reflection when used in combination with an ACE inhibitor. However, the AP and AI% depend on three factors: wave reflection, PWV and sites of wave reflection. As PWV has not been measured in this study, we cannot attribute the effects of ATII receptor antagonist wholly to decrease in wave reflection. Comparative studies incorporating both PWV and arterial wave reflection measurements are needed to specifically answer this question.

2.Pulse Pressure Amplification

Haemodynamic studies have shown that whereas MAP is relatively stable along the arterial tree, PP increases markedly from central to peripheral arteries. It has been found using invasive haemodynamic methods, that the radial aortic pressure difference in normotensive subjects aged 48-77 years approximates +12 to20 mm for SBP and −1 mm for DBP (Panca et al. 1992; O'Rourke and Kelly 1993). In our study population of hypertensive patients the mean amplification for SBP was 8 (± 3mm, SD) mm Hg.

As PP amplification is due to the progressive decrease in cross sectional area and the increase in early wave reflection on going from large to small arteries, it is to be expected that drugs with vasodilator properties would modify PP amplification. Nitrates modify PP amplification favourably due to their preferential effect on aortic more than brachial pressure (Kerry et al. 1990). In genetically hypertensive rats that have equal central and peripheral pressures, giving ACE inhibitors or calcium-channel blockers restores the pulse pressure gradient but hydralazine does not (Tsoucaris et al. 1995). However, there is evidence that ACE inhibitors, by causing an equal decrease in brachial and aortic PP may not actually increase PP amplification, despite reducing wave reflection in patients with essential hypertension (Topouchian et al. 1998) although some studies have shown a restoration of pulse pressure gradient in patients with end-stage renal disease (London et al. 1996). In the present study, we have observed that valsartan when added to an ACE

inhibitor, preferentially decreased aortic PP, favourably modifying PP amplification, restoring it to normal values.

3. Additive Effects of ACE Inhibition and Angiotensin II Receptor Blockade

The important finding in our study is that angiotensin II receptor blockade when added to ACE inhibitor, has beneficial haemodynamic effects not only on BP (Table1.) but also on the important components of the BP curve, namely, arterial wave reflection and PP amplification (Figure 3). Both ATII receptor antagonists and ACE inhibitors affect the same pressor system, but because acting at different sites, they might have additive or synergistic effects. There are a number of theoretical reasons to support this view; Firstly, ACE inhibitors may not be able to maintain a continuous decrease in plasma ATII levels, which is due to i) reactive rise in plasma renin and ATI secondary to the interruption of ATII feedback on renin release and ii) the presence in the heart and blood vessel walls of other ATII forming pathways not responsive to ACE inhibitors. This phenomenon can be neutralised by ATII blockade. Secondly, the reactive rise in plasma ATII induced by ATII receptor blockade can be strongly inhibited by ACE inhibition. Therefore, addition of an ATII receptor antagonist to an ACE inhibitor might help to achieve a more powerful block of the renin-angiotensin system. Finally, the ancillary properties of both ACE inhibitors, e.g., interruption of kinin metabolism and interaction with NO, and of ATII receptor antagonists, e.g., interaction with NO and prostaglandins may be active when the two agents are used singly, but may be synergistic when the groups are combined (Richer et al. 1998) . Additive effects on BP and renin release in sodium-depleted normotensive adults (Azizi et al. 1995) and on exercise capacity in heart failure patients (Hamroff et al. 1999) has been described recently when an ACE inhibitor is combined with an ATII receptor antagonist. It may be argued that 12 of the patients were already on calcium channel blockers, which also have favourable effects on arterial wave reflection. However, we have shown that the 6 patients not taking calcium channel blockers also had a significant decrease in AP.

The favourable effect on arterial wave reflections and PP amplification could represent either a structural change in the vessel walls involving reduction of growth of vascular smooth muscle cells in the vessel walls, as ATII promotes vascular hypertrophy independent of any BP changes (Su et al. 1998). Moreover, ATII receptor antagonists have

221

been shown to cause a BP independent regression of LVH in hypertensive patients (Thürmann et al. 1998). However, the duration of our study is too short to ascribe the beneficial effects to actual structural changes in the vessel wall and the fall in AP was in part attributable to the decrease in BP. Furthermore, there was no difference in the haemodynamic effects of valsartan between two hours and two weeks suggesting that a functional vasodilatation could be the main mechanism for the reduction in arterial stiffness and not an actual structural change. Also, it suggests that the haemodynamic effects of ATII receptor antagonists recorded acutely after taking the drug reflects the long time effects of the drug.

There may be a functional change in the vessel wall, especially in the endothelium. The endothelium is known to release vasoactive substances like NO and endothelin-1 (ET-1). Changes in vascular tone can alter arterial stiffness (Cockcroft et al. 1997). GTN, a source of NO, has been shown to cause changes in the arterial waveform indicative of reduced arterial stiffness (Wilkinson et al. 1998b). NO modulates the generation of ATII and also acts as a functional antagonist at vascular smooth muscle cells (Blantz et al. 1998). Thus blocking the effects of ATII on vascular smooth muscle cells may lead to improved endothelial function. This antagonism between NO and the ATII system may explain the beneficial haemodynamic effects observed with the addition of ATII blockade to ACE inhibition leading to an improvement in endothelial dysfunction.

The small number of patients in our study as well as the fact that they were a select group of resistant hypertensives already on three drugs means that these results may not apply to all grades of hypertension. We need larger long-term comparative studies with agents producing similar hypotensive effect to extend such observations to hypertension in general. It may be argued that not all patients were on the maximum doses of ACE inhibitors, diuretics or calcium channel blockers, however the purpose of the study was look at the effect of adding an ATII antagonist to patients already on therapy including an ACE inhibitor with resistant hypertension as this reflects more what is generally recommended (Ramsay 1999) and is happening in clinical practice. We measured the haemodynamic response to valsartan at the time of peak effect to show the maximum effect on BP and arterial stiffness. However, it can be argued that haemodynamic

222

measurements at trough levels may have helped to separate the BP lowering effects of valsartan form its effects on arterial stiffness.

In conclusion, the addition of angiotensin II blockade to an ACE inhibitor in the setting of patients with poorly controlled hypertension significantly lowers BP without any major adverse effects. Perhaps of greater interest are the favourable vascular effects reducing arterial wave reflection and increasing PP amplification. However, the data presented here is not definitive and we need larger, randomised clinical trials to answer this question.

CHAPTER EIGHT

DISCUSSION

General Discussion

Arterial pulse wave analysis using applanation tonometry is a major advance over the mechanical sphygmograph used by Marey, Mahomed, McKenzie and others over 100 years ago. Their descriptions in the 19th century were accurate but lacked quantification. This led to the disappearance of the sphygmograph from clinical practice as stated by Kelven *"....when you can measure what you are speaking about and express it in numbers you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind"*. It is that quantification to the measurement of PWV and AI% that allows us to confirm the hypothesis of our pioneers and explore fully the use of this barometer of arterial function. However our era is characterized by the unexpected combination of 'high tech' recording and 'low-tech' interpretation. Therefore at the outset of these studies on the arterial pulse in Ireland, the concept appeared "quaint" to many of my colleagues, and it was a challenge to develop 'high tech' interpretation with a 'low tech' technique.

Looking forward, the increasing epidemiological data is providing a sound bases for the measurement of PWV (Laurent et al. 2000; Blacher et al. 1999b; Blacher et al. 1999c; Blacher et al. 1999a). It is hoped in time such information will be available for AI% and already its measurement has been incorporated into a number of longitudinal and interventional studies. It is clear that PWV and AI% measure different components of the arterial tree. I found PWV and AI% to be significantly correlated (r=0.62, n=130). The fact that this is not unity, as they assess different segments of the arterial tree, should not be viewed as a disadvantage but an opportunity for greater insight into the function and health of the elastic aorta and the muscular conduit arteries separately. Both these arterial segments not only differ in their pressure load but also in the relative amount of elastic, collagen and smooth muscle in their walls, suggesting that their response to pathological conditions and therapy may be quantitatively different. The high correlation of these two indices supports to the use of radial applanation tonometry, which is at present the easiest and quickest of the available methods for the assessment of arterial stiffness. This should encourage the evaluation of arterial stiffness in prediction of vascular complications by inclusion of AI% measurements in day-to-day clinical practice and large outcome trials.

The correlation of PWV and AI% increases when the two genders are analysed separately (male =0.64; female =0.70) suggesting that different ranges may need to be derived for males and females. Gender effects are quite apparent in stiffness indices. PWV was significantly higher in males in all groups than females whereas AI% was significantly higher in females. Thus not only do males and females differ in the elastic properties of the arteries depending upon which arterial segment elastic or muscular is studied, but also that disease processes may result in changes in the arterial properties differently in both genders. Support for this view is found in my studies on smoking, both active and passive. Here in healthy young subjects an effect on AI% was seen in males only. Although there were no HR differences between genders, AI% significantly correlated to HR in the hypertensive and not in normotensive population who show a stronger association with BP. Changes in HR are responsible for producing considerable alterations in the contour of the pressure pulse due to changes in LVED which effect wave timing and merging of the incident and reflected wave. When quantified, changes in HR account for 4% to 6% in aortic augmentation (Wilkinson et al. 1998b). I observed in studies with active smoking that though the PWV increased following smoking one cigarette, AI% decreased significantly due to marked tachycardia. These HR changes should therefore be taken into account in the interpretation of results after interventions that alter HR, that need to extend beyond pacing e.g. sympathetic and parasympathetic blockade.

One of the more notable features in my study of healthy young subjects was the finding of a group of subjects with spurious or pseudo systolic hypertension. These individuals have very elastic arteries as shown by very low values of AI% and a high degree of PP amplification giving rise to the falsely high values of brachial SBP. All these subjects were males, non-smokers and active sportsmen. I observed the beneficial effect of physical fitness in young normotensive subjects that showed a significantly low AI% with regular exercise compared to their sedentary peers and this was true for both genders. In this context a recent study (Tanaka et al. 2000), which reports that aerobic exercise can blunt an age-related stiffening of large arteries is pertinent. Thus middle-aged and older sedentary subjects after 3 months walking briskly or jogging for 40-45 minutes per day, 4-6 days per week can restore their arterial compliance. The potential use of such "therapy" in individuals who are intolerant or who do not wish to take pharmacological therapy for hypertension or are "addicted" to nicotine may perhaps provide an alternative and

attractive form of therapy without adverse effects or high acquisition costs. Programmed exercise may remarkably change our approach to the prevention and treatment of vascular disease associated with arterial stiffness.

Perhaps the most worrying aspect of these studies has been the finding of arterial stiffness in otherwise healthy young smokers, all males. The fact, that they are medical students who are aware of the detrimental effects of cigarettes and should have a more sensible and positive approach to health promotion is even more worrying. While the addiction to nicotine is strong and I have shown that active cigarette smoking increases arterial stiffness, it is important to ascertain whether nicotine replacement therapy also increases arterial stiffness. This would also help us understand the role of nicotine versus carbon monoxide in causing stiffening of the arteries in smokers. The finding that passive smoking was associated with increased arterial stiffness is perhaps not surprising but that it can be shown non-invasively increases its relevance. The fact that females did not show any response to passive smoking again highlights the lack of any effect of long-term smoking on arterial stiffness in healthy young females compared to females in the normotensive population. My study confirms a recent study, which also showed a gender difference in the response to long-term smoking, females escaping the detrimental effects of smoking in the long-term (Gariepy et al. 2000). The fact that females appear relatively protected is of particular interest in that not only does it provide evidence for a direct hormonal influence on the vascular wall but suggests the possibility of using oestrogens as protection against arterial damage due to smoking.

While it was hoped that arterial stiffness might be a therapeutic target, until recently there was little scientific evidence to justify a study in this field. With the report (Blacher et al. 1999b) that PWV is the single most important prognosticator, of greater importance than any single measure of BP alone, for long term all cause and cardiovascular mortality in patients with chronic renal failure, highlights the observation that lowering BP in such patients is of importance only when PWV was reduced as well (Guerin et al. 2001). This will provide confidence for those who wish to undertake such trials. However, pharmacological modulation may not always involve drug therapy but should also include dietary or possibly lifestyle changes. In this context my studies with alcohol have raised the dichotomous nature of this approach as acutely I observed a decrease in arterial

226

stiffness but in the population database of healthy normotensive subjects, alcohol excess was associated with stiff arteries in males. In this regard I showed that caffeine, the most widely consumed beverage in the world, acutely stiffens arteries but studies need to be undertaken to ascertain its long term effects on arterial stiffness.

Our traditional view of hypertension being primarily diastolic and the focus of clinical trials mean we ignore the bulk of the hypertensive population. For the majority, hypertension is primarily systolic with a wide PP and stiff arteries. Therapy aimed at reducing DBP alone has resulted in a large population with a wide PP and poorly controlled SBP. Thus non-invasive arterial pulse wave analysis can be seen as an important diagnostic tool determining the risk status of the patient but also making predictions with regard to the choice of therapy. Thus in patients with ISH or disproportionate increase in SBP, drugs could be developed that could target several other mechanisms such as reducing left ventricular ejection rate, decreasing arterial stiffness or delaying the timing of wave reflection. An active decrease in arterial stiffness may be obtained independently of a decrease in MAP with drugs specifically relaxing vascular smooth muscle in the walls of the large arteries. Another possible mechanism is the slowing and reduction of arterial wave reflection through vasodilatation and reduced PWV. The latter alteration reduces SBP more in the aorta than in the peripheral artery thus restoring the normal relationship of wave reflection to ventricular ejection as I have shown in studies on GTN, sildenafil and also the preferential decrease in aortic SBP seen where valsartan, an ATII receptor blockade is added to an ACE inhibitor in resistant hypertension. The work on sildenafil raises the possibility that drugs not primarily designed as antihypertensive agents may be re-evaluated for their BP lowering effects and furthermore implies widening our horizons for the development of new antihypertensive agents that preferentially decrease systolic rather than diastolic BP.

Another goal of drug treatment of hypertension should be to modify arterial stiffness independently of BP through a change in vasomotor tone, a change in arterial structure or a combination of both. The data in regard to the ATII receptor antagonists is of interest in this regard showing that some of its effects are independent of BP. Because aortic collagen accumulation in hypertension is reduced by blockade of the AT_1 receptor, inhibition of the renin-angiotensin system by ACE inhibition either alone or together

appears to be a favourable combination. The vasopeptidase inhibitor Omipatrilat, which has a preferential effect on SBP, may be another choice. Increasingly the majority of patients require two or more antihypertensive drugs; we now need to look at additive and possibly synergistic effects of antihypertensive agents on arterial stiffness.

Finally it is clear from my epidemiological database and the literature that we cannot explain all the variables known to influence arterial stiffness. At best we can account for the major determinants – age, BP, height, gender etc. that would explain up to 50% of the variability. As arterial stiffness is a therapeutic end-point in its own right, we must now combine our functional studies with a structural extrapolation of the determinants of arterial stiffness. Endothelin and angiotensin have significant mitogenic effects favouring vascular hypertrophy and stiffness (Powell 1998). Collagen degradation by matrix metalloproteinases (MMPs) is increasingly being implicated in conditions with altered elasticity including aortic aneurysm (Iishi et al 2000) and such changes may be the basis of increased arterial stiffness. Therefore we need to explore elastic and collagen sub-types and the genes that regulate vascular tone such as angiotensin, NO, endothelin etc. together with studies on the collagen matrix particularly MMPs.

The next wave of exploration of arterial stiffness is likely to come from an integrated, functional and molecular approach. Proceeding with one, while neglecting the other is so reminiscent of the consequences of the development of the sphygmomanometer with the demise of arterial pulse wave analysis. In future, with complimentary studies, it may unravel the mystery behind *"by his pulse you will know him"*.

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