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Assessment of Arterial Stiffness Using Oscillometric Arteriography

A thesis submitted to the University of Dublin, Trinity College

in fulfillment of the requirements for the degree of

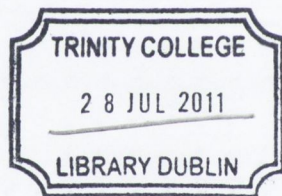
Doctor of Philosophy

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March 2009

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9215

DECLARATION

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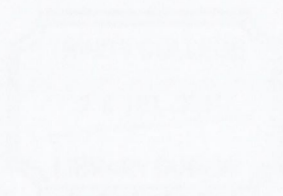
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SUMMARY

The present series of studies examined whether an oscillometric method (Arteriograph), which measures arterial stiffness indices, pulse wave velocity (PWV m/sec), and wave reflection as augmentation index [Alx %], is an accurate and reliable method when compared with two standard techniques using SphygmoCor and Complior in normal healthy subjects and untreated hypertensive patients. In addition of arterial stiffness, the determinants e.g. smoking status; stress (physical and mental) and environmental changes and the effect of therapeutic intervention on arterial stiffness were assessed with this technique.

By using Arteriograph, there was a significant correlation coefficients of PWV 0.85 and Alx 0.97 ($P < 0.0001$) in repeated measures in normal healthy subjects ($n=23$, chapter 3) and in untreated hypertensive patients ($n=40$, chapter 6) on same day by same operator, the correlation coefficients of PWV and Alx were ($r=0.95$ and $r=0.99$, $P < 0.0001$) respectively, and for two measurements performed a week apart were, PWV and Alx ($r=0.97$ and $r=0.96$, $P < 0.0001$) respectively. Both PWV and Alx were significantly correlated with age, height, heart rate, systolic BP, diastolic BP, pulse pressure (PP), mean arterial pressure (MAP), and Alx and PWV.

Small changes were detected in normal healthy subjects ($n=22$, chapter 4) with reactivity by mental arithmetic test (MAT), cold pressor test (CPT), and 30% maximal voluntary contraction (MVC), there was an increase in PWV 14%, 12% and 16% ($p < 0.0018$) and Alx 13%, 29% and 30% ($p < 0.04$) respectively. Values returned to baseline level five minutes following each stimulus.

In chapter 5, I first examined the effect of smoking using the standard SphygmoCor and Complior techniques in untreated hypertensive patients (554) were compared in a cross-sectional fashion PWV, Alx and transit time (T_R), in current smokers (CS, $n=150$), ex-smokers (ES, $n=136$), and nonsmokers (NS, $n=268$). ES were categorized into <1 year, >1 and <10 years, and >10 years of smoking cessation, PWV (Complior), Alx and T_R (SphygmoCor). CS and ES had significantly higher PWV and Alx compared with NS (PWV for CS: 10.7 ± 0.2 ; ES: 10.6 ± 0.2 ; NS: 9.9 ± 0.1 m/s; $P < 0.001$; Alx for CS: 31 ± 1 ; ES: 30 ± 1 ; NS: $27 \pm 0.8\%$; $P < 0.05$), whereas T_R was lower in CS and ES compared with NS (T_R for CS: 131 ± 1.0 ; ES: 135 ± 1 ; NS: 137 ± 0.8 m/s; $P < 0.0001$). There was a significant linear relationship between smoking status and PWV ($P < 0.001$), Alx ($P < 0.001$), and T_R ($P < 0.001$),

even after adjusting for age, sex, mean arterial pressure (MAP), heart rate (HR) and body mass index. In ES, duration of smoking cessation had a significant linear relationship with improvement in PWV ($P<0.001$), Alx ($P<0.001$), and T_R ($P<0.001$), with arterial stiffness parameters returning to non-significant levels after a decade of smoking cessation. Similarly, after establishing use of Arteriograph, subsequently cohort to determine if similar results were seen using this technique in untreated hypertensive patients ($n=254$).

Comparing the Arteriograph to SphygmoCor and Complior the determinants of Alx and PWV were studied using the oscillometric, tonometric and piezo-electronic techniques in untreated hypertensive patients ($n=254$). Arteriograph PWV and Alx were closely related with Complior ($r=0.60$, $P<0.001$), and SphygmoCor ($r=0.89$, $P<0.001$) respectively. Using stepwise regression analysis, the independent determinants of Arteriograph PWV were age, MAP, HR and gender ($R^2=0.44$ $P<0.0001$) and for Alx; age, weight, MAP, HR and gender ($R^2=0.65$, $P<0.0001$). The bias between the different techniques was determined by age and gender for PWV and age, body weight, gender, HR and MAP for Alx. Bland-Altman Plots showed that while the techniques were closely related, the limits of agreement were wide.

Respectively I evaluated the effects of antihypertensive drugs in hypertensive patients ($n=114$) before and 4 to 6 weeks following of treatment. There was significant fall in PWV with angiotensin-converting enzyme inhibitors ($n=26$), β -blocker ($n=40$) and calcium channel blockers ($n=16$), and with Alx angiotensin receptor blockers ($n=17$, $P<0.05$); although thiazide diuretics ($n=20$) were not significant.

My studies suggest that the Arteriograph can measure precisely and accurately with an acceptable reproducibility of Alx and PWV when compared with other standard techniques (SphygmoCor and Complior), and this Arteriograph can be reliably applied to future clinical as well as research settings.

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First and foremost, all my prays to Allah (Subhan-Ho-Wa-Taalla) the Almighty for His mercy and gave me the opportunity to complete my educational goal. There are many people who I would like to thank for all their support and guidance during my educational career. First I would like to express my most sincere gratitude to my supervisor Professor John Feely, who gave me an excellent opportunity to undertake this research. His knowledge, guidance with patience, and availability are acknowledged.

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DEDICATIONS

To My PhD Supervisor, the Late Professor John Feely

ABBREVIATION

ABPM	ambulatory blood pressure monitor
ACEi	angiotensin converting enzyme inhibitors
AER	albumin excretion rate
AGE	advanced glycation end product
AIx	augmentation index
ANOVA	analysis of variance
ARB	angiotensin receptor blocker
BB	beta blockers
BMI:	body mass index.
BP	blood pressure
BPM	beats / minute
BMI	body mass index
CAD	Coronary artery disease
CCB	calcium channel blocker
CHD	Coronary heart disease
CI	confidence interval
CVD	cardiovascular disease
DBP	diastolic blood pressure
FMD	Flow mediated dilatation
HDL	high density lipoprotein
HR	heart rate
IDDM	insulin dependent diabetes mellitus
LDL	low density lipoprotein
LVH	left ventricular hypertrophy
MAP	mean arterial pressure
MI	Myocardial infarction

NIDDM	non-insulin dependent diabetes mellitus
NO	nitric oxide
PP	pulse pressure
PWV	pulse wave velocity
RAAS	Renin angiotensin aldosterone system
SBP	systolic blood pressure
SD	standard deviation
SEM	standard error of the mean
T _R	time to return of the reflected pulse wave

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CHAPTER 1

LITERATURE REVIEW

1.1. ARTERIAL SYSTEM

1.1.1 Introduction to the arterial system

Arteries are muscular blood vessels that carry blood away from the heart (as opposed to veins, blood vessels carrying blood toward the heart). All arteries, with the exception of the pulmonary and umbilical arteries, carry oxygenated blood.

The circulatory system is extremely important for sustaining life. It's proper functioning is responsible for the delivery of oxygen and nutrients to all cells, as well as the removal of carbon dioxide and waste products, maintenance of optimum pH, and the mobility of the elements, proteins and cells of the immune system.

The arterial system is the higher-pressure portion of the circulatory system. Arterial pressure varies between the peak pressure during heart contraction, called the systolic pressure, and the minimum, or diastolic pressure between contractions, when the heart rests between cycles. This pressure variation within the artery produces the pulse, which is observable in any artery, and reflects heart activity.

1.1.2 Functions of the arterial system

There are two interrelated and distinguishable functions of the arterial system: the conduit and the cushion function. The conduit function is to maintain an adequate blood supply to the body tissues, which fulfill the required metabolic needs; and second, a cushioning function that can be understood by the difference in flow pattern entering the system, which is the pulsatile flow from the heart, and constant flow

required by the tissues at the end of vascular system. Therefore, the arteries must be capable of dampening the intermittent ventricular ejections to a more continuous peripheral blood flow. The stroke volume buffering is due to distension of preferentially large arteries with a concomitant volume increase (Windkessel effect), which increases cardiac performance (Nichols and O'Rourke, 2005). The forward pressure and flow waveforms of ascending aorta are identical during the phase of early ejection. The speed of the pressure wave is however much higher and its reflection at bifurcations and peripheral resistance sites is summed to the outgoing wave (*Figure 1.1*), in sharp contrast to the reflected flow wave that is inverted and causes a measured flow wave reduction.

Ageing exerts a marked effect on the arteries; Blood pressure slowly rises in response to normal ageing and along with reduction in pressure pulse amplification from central to peripheral arteries, results in almost equal central and peripheral systolic pressure in the elderly. In this setting isolated systolic hypertension is often found, resulting in increased pulse pressure, the pressure parameter most strongly associated with increased cardiovascular risk (Domanski *et al.*, 1999). Ventricular load increases due to elevated pulse pressure, over time leading to target organ damage, such as left ventricular hypertrophy (Khattar *et al.*, 1997) which together with a reduced diastolic blood pressure makes the myocardium more susceptible to ischemia. Arterial wall distensibility greatly influences systolic blood pressure, whereas end-diastolic pressure is determined by diastolic duration and rate of pressure fall, the latter in turn affected by peripheral resistance together with the arterial wall mechanical properties.

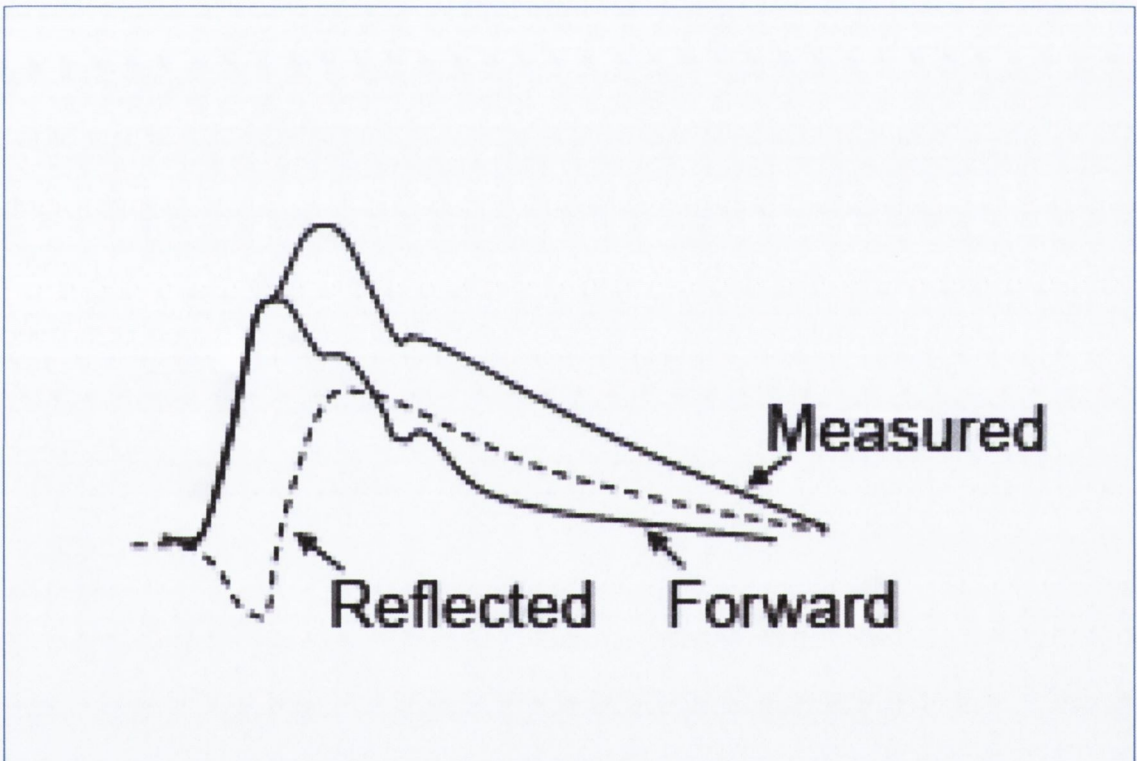


Figure 1.1: Schematic drawing of the aortic pressure wave in an elderly subject. The configuration of the measured wave is augmented by the reflected wave, which added to the forward pressure wave during late systole and diastole.

1.1.3 Structure of the arterial wall

The arterial wall is composed of predominantly elastic material such as collagens and elastic fibers, which together with smooth muscle cells, proteoglycans, fibronectin and fibrillin contribute to the mechanical properties of the arterial wall (Nichols and O'Rourke, 2005). The wall has three concentric zones; the tunica intima, media and adventitia (Figure 1.2). The inner layer of intima consists of the endothelium, followed by a thin layer of connective tissue, and finally the internal elastic lamina, which is the demarcation between intima and media. The media forms the dominating part of the wall and is also the determinant of mechanical properties. It consists of collagen fibers that run spirally between circularly arranged layers of smooth muscle cells and elastic lamellae, which are linked to each other by fibrillin-1 and type-VI collagen containing bundles of microfibrils. The rest of the extracellular matrix volume is to a large part filled up with highly viscous proteoglycans and glucoproteins, which cushion smooth muscle cells (SMC) within the media. The outer elastic lamina demarks the border zone to the adventitia, the outer shelf of the arterial wall that blends with the connective tissue consisting of predominantly collagen, nerves and small blood vessels. The dry weight of the arterial wall consists of about 50 percent elastin and collagen, whereas the remainder consists of smooth muscle cells and non-fibrous matrix. There are variations in the arterial wall structures within the arterial systems. Central elastic arteries like aorta and common carotid artery have a thicker intima and a media layer with much more lamellae of elastic fibers than muscular arteries, such as the femoral and radial artery where numerous layers of smooth muscle cells are the dominating component of the media.

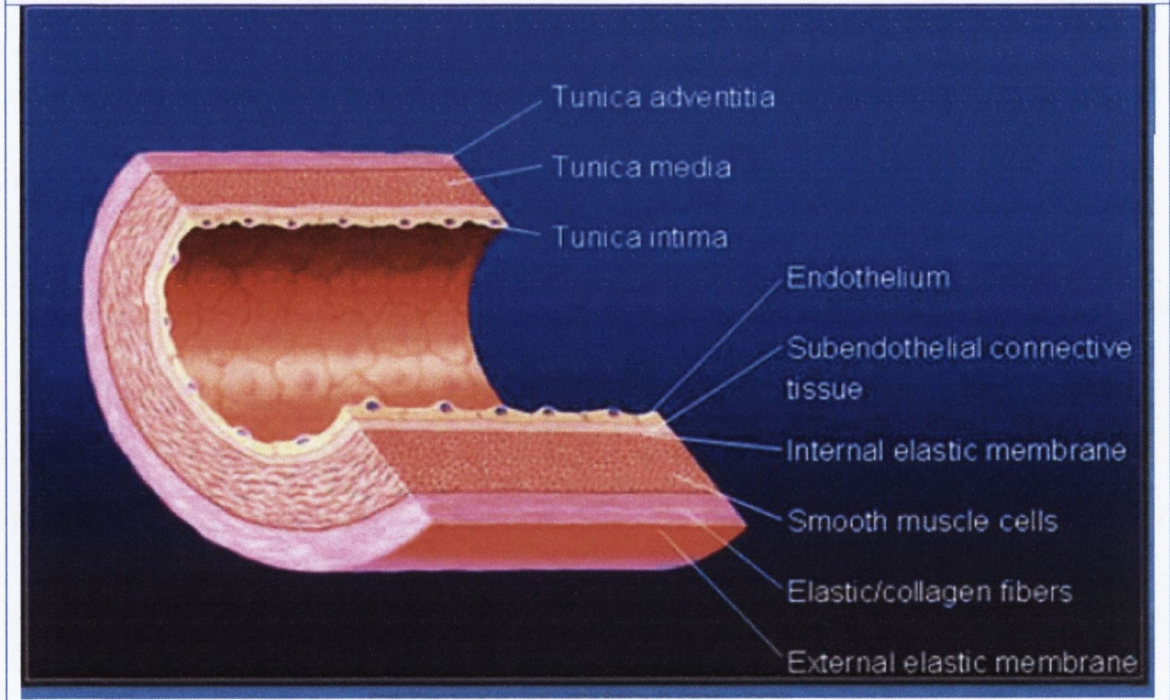
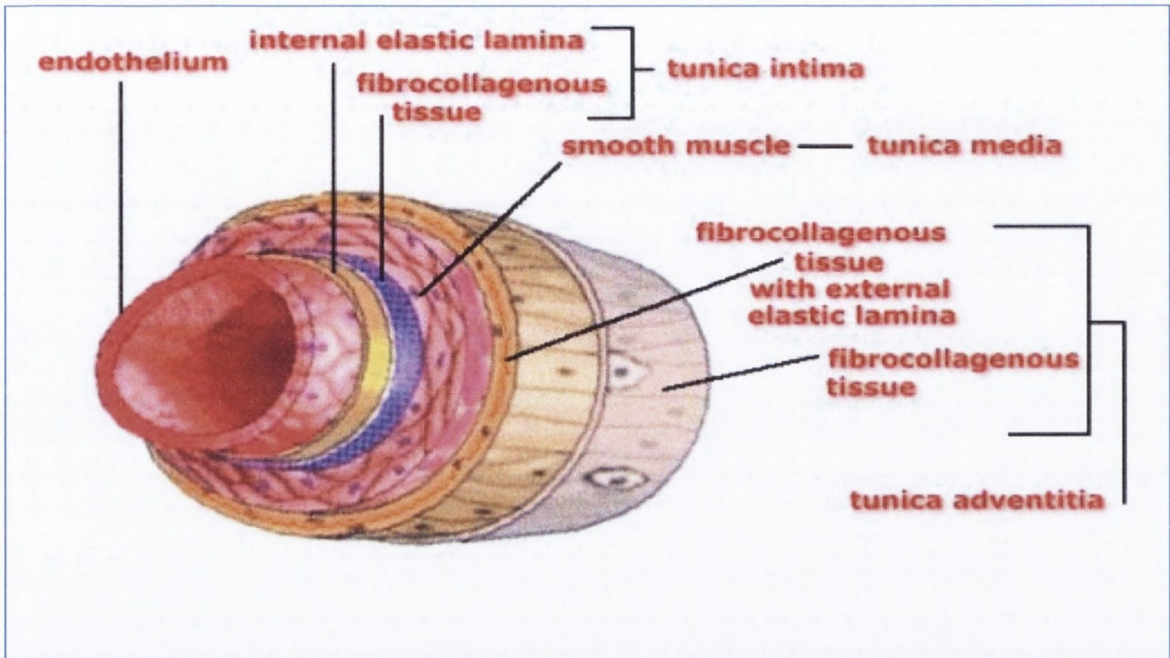


Figure 1.2: Schematic illustrations of the arterial wall layers (Anterior and lateral view).
Adopted from (Ross, 1993)

1.1.4 Mechanical properties of arteries

Blood vessel mechanical properties are strongly influenced by their location in the body and the elasticity of their walls (Fung, 1993). The wall's elasticity is altered by the concentration and structural arrangements of elastin and collagen fibers. Arteries can be organized into two groups: elastic and muscular groups. The elastic arteries are more distensible, larger in size, and positioned near the heart, while the muscular arteries are less distensible, smaller in size, and are located away from the heart, near the arterioles (Holzapfel and Ogden, 2003). The aorta, aorta branches, and the iliac are some examples of elastic arteries. While the coronary, the cerebral, the femoral, and the renal arteries are some examples of the muscular arteries.

Young (1773-1829), and other physicians and scientists like Moens and Kortweg defined much of the basic concepts about the relationship between elastic properties of arteries and the velocity of the pulse wave. More direct studies of the arterial wall properties were performed on excised isolated arteries first by (Fuchs, 1900), and later (Bergel, 1961), who found that the relative degree of arterial wall retraction differ within the arterial tree. The relation between the speed of the pressure wave, i.e. pulse wave velocity (PWV), and arterial wall elasticity was in 1878 described with the Moens-Kortweg equation. It was later modified (Bramwell and Hill, 1922) and may be written as: $PWV = \sqrt{1/\rho D}$, Where ρ . is the density of the blood, and D =distensibility. This means that the pulse wave travels faster in proportion to the decreasing distensibility of the vessel wall.

The arterial wall is able to distend under force and retract when the force is removed. The force per unit of area is named stress, which causes deformation of the wall material (Nichols and O'Rourke, 2005). The relative degree of deformation from the "unstressed" state is called strain. The ratio between stress and strain is used to calculate

the elastic modulus (i.e. the stretch force per unit of cross-sectional area required to elongate a strip of vessel 100%), which describes the stiffness in materials with linear stress-strain relationships. The arterial wall in man shows a non-linear relation between stress (pressure) and strain, making the calculation of incremental elastic modulus a better option than Young's elastic modulus, as it is difficult to define the unstressed state in vivo (*Figure 1.3*). Because of the difficulty in measuring the whole arterial wall thickness in vivo, Peterson (Peterson *et al.*, 1960) established the pressure strain elastic modulus (E_p), which relates pulse pressure to relative diameter change, but neglects wall thickness in the equation. The radial movement during the pulse wave propagation has been extensively studied (*Figure 1.4*). The longitudinal movement of the arterial wall has been considered to be negligible until recently when technical improvement in measurement has revealed a considerable longitudinal arterial wall motion in vivo (Cinthio *et al.*, 2006).

An important determinant of the passive mechanical properties of large arteries is the extra-cellular matrix, especially the relative amount of elastin and collagen (Nichols and O'Rourke, 2005). In the proximal aorta, elastin is the dominant component, whereas collagen and smooth muscle cells predominate at peripheral sites. As collagen is about 100 to 1000 times stiffer than elastin, a considerable stiffening of the arterial wall is seen in young subjects from proximal aorta to the peripheral muscular arteries. Several papers have reported that increased arterial stiffness is independently predictive of the risk of future cardiovascular events or all-cause mortality. A selection of such studies is compiled in Table 1.1, adopted and modified from (Blacher *et al.*, 1999b, Laurent *et al.*, 2001, Cruickshank *et al.*, 2002, Chirinos *et al.*, 2005, Blacher *et al.*, 1998, Weber *et al.*, 2005, Williams *et al.*, 2006).

Table 1.1: Longitudinal studies reporting an independent predictive value of arterial stiffness			
Method, site	Reference, year	Events	Cohort
PWV, Aorta	(Blacher <i>et al.</i> , 1999b) (Laurent <i>et al.</i> , 2001). (Cruickshank <i>et al.</i> , 2002). (William-Hansen <i>et al.</i> , 2006).	CV mortality CV mortality All cause mortality CV mortality	ESRD Hypertension IGT, DM-2 Gen population
Local CA dist	(Blacher <i>et al.</i> , 1998)	All cause mortality	ESRD
AP, Aorta	(Chirinos <i>et al.</i> , 2005)	CV event, mortality	CAD
PWV, Alx	(Weber <i>et al.</i> , 2005)	CV event, mortality	CAD
Alx	(London <i>et al.</i> , 2001)	All cause and CV, mortality	ESRD

Alx, augmentation index; AP, invasive aortic augmentation pressure; local CA dist, carotid artery distensibility; CAD, coronary artery disease; CV, cardiovascular; DM- 2, type 2 diabetes mellitus; ESDR, end stage renal disease; IGT, impaired glucose tolerance

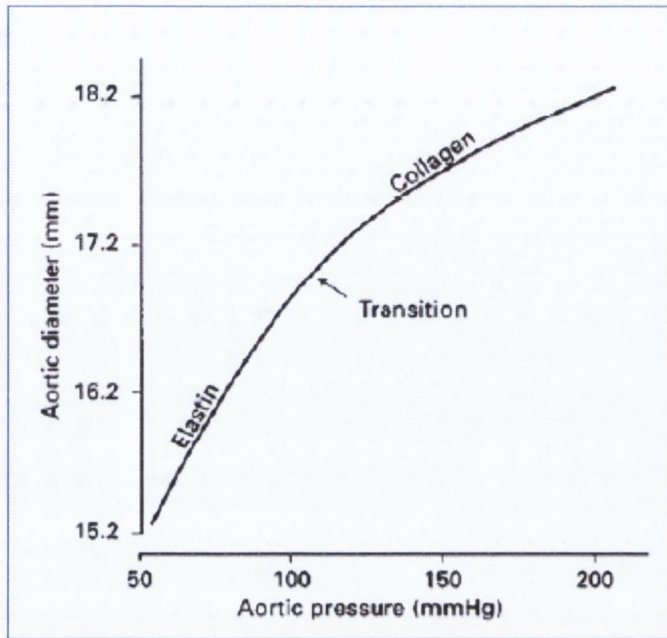


Figure 1.3: Pressure-diameter curve from abdominal aorta. The wall stiffens as the distending pressure increases (Länne *et al.*, 1992)

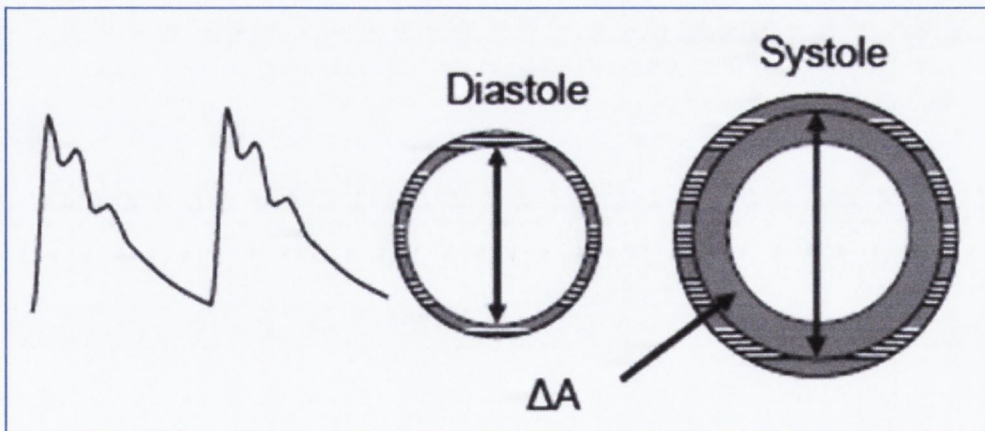


Figure 1.4: The arterial radial distension curve is obtained by recording the diameter change during the cardiac cycle. ΔA is the increase in cross-section area in response to the pressure pulse. Figure adopted and modified from (Laurent *et al.*, 2006) Functional regulation of arterial size and mechanics

Distending pressure is the most important factor that influences the mechanical wall properties since it functionally decreases wall distensibility, because of the non-linear configuration of the pressure-diameter curve. The functional role of heart rate *per se* has on arterial distensibility is less clear. In experimental studies in humans using pacing, central as well as peripheral distensibility has been found to decrease as heart rate exceeds 80 beats per minute (Giannattasio et al., 2003, Millasseau et al., 2005). This implies that a significant shortening of the diastolic time interval probably decreases "operating" arterial distensibility.

Several vasoregulatory substances are synthesized by the endothelium, among them nitric oxide (NO), considered to be the most important vasorelaxing factor with the ability to regulate lumen diameter of arterioles and muscular arteries. Impairment of endothelium dependent NO-mediated vasodilatation is an early marker of endothelial dysfunction that accompanies vascular diseases such as atherosclerosis (Bonetti *et al.*, 2003). Administration of an exogenous NO-donor such as nitroglycerin, increases arterial lumen, tensing the parallel collagen and elastin fibres, concomitantly as the reduced tension in smooth muscle has the opposite effect on distensibility (Bank et al., 1996, Bank and Kaiser, 1998). The effect of smooth muscle tone changes on arterial distensibility differed between earlier studies which showed distensibility in response to smooth muscle relaxation either increased (Bank *et al.*, 1999), unchanged (Bank *et al.*, 1995), or decreased (Peterson *et al.*, 1960). Nevertheless, several studies have shown that sympathetic activation by different kinds of stimuli as well as vasoconstrictor drugs reduce arterial distensibility in human muscular arteries (Bank et al., 1995, Failla et al., 1999, Sonesson et al., 1997), whereas sympathetic plexus anaesthesia increases distensibility (Failla *et al.*, 1999). A similar response is not found in elastic arteries, where sympathetic activation or administration of vasoactive drugs causes no pressure independent alteration of distensibility (Sonesson et al., 1997, Stewart et al., 2003). Whether basal endogenous NO production regulates arterial distensibility in humans is

still not proven, even if an experimental study suggests that local NO production is of importance (Schmitt *et al.*, 2005).

1.1.5 Endothelial Function

The endothelium is an autocrine-paracrine organ, which controls vascular tone and vascular structure. The endothelium produces multiple relaxing factors, with nitric oxide (NO) as the most important (Luscher, 1990). NO is produced and released under the influence of endothelial agonists, such as acetylcholine and bradykinin, acting on specific endothelial receptors, and by mechanical forces, such as shear stress (*Figure 1.5*). Healthy endothelium provides vascular tone and inhibits smooth muscle cell growth, blood platelet aggregation and the adhesion of white blood cells (Luscher, 1990) (*Figure 1.5*). By definition endothelial dysfunction is a functional and reversible alteration of endothelial cells, resulting from impairment in NO availability (Taddei and Salvetti, 2002) and is currently considered an early and major promoter for atherosclerosis and thrombosis (See *Figure 1.6* for sequences in progression of atherosclerosis).

In humans, endothelial function can be evaluated in different vascular districts, including the coronary and peripheral circulation. Intra-arterial infusions of endothelial agonists, such as acetylcholine, and local increases in shear stress have been used to study endothelium dependent vasodilatation.

However, the most widely used non-invasive technique to measure endothelial function is flow-mediated dilation (FMD) of the brachial artery. It assesses with high-resolution ultrasound the endothelium dependent change in artery diameter induced by reactive

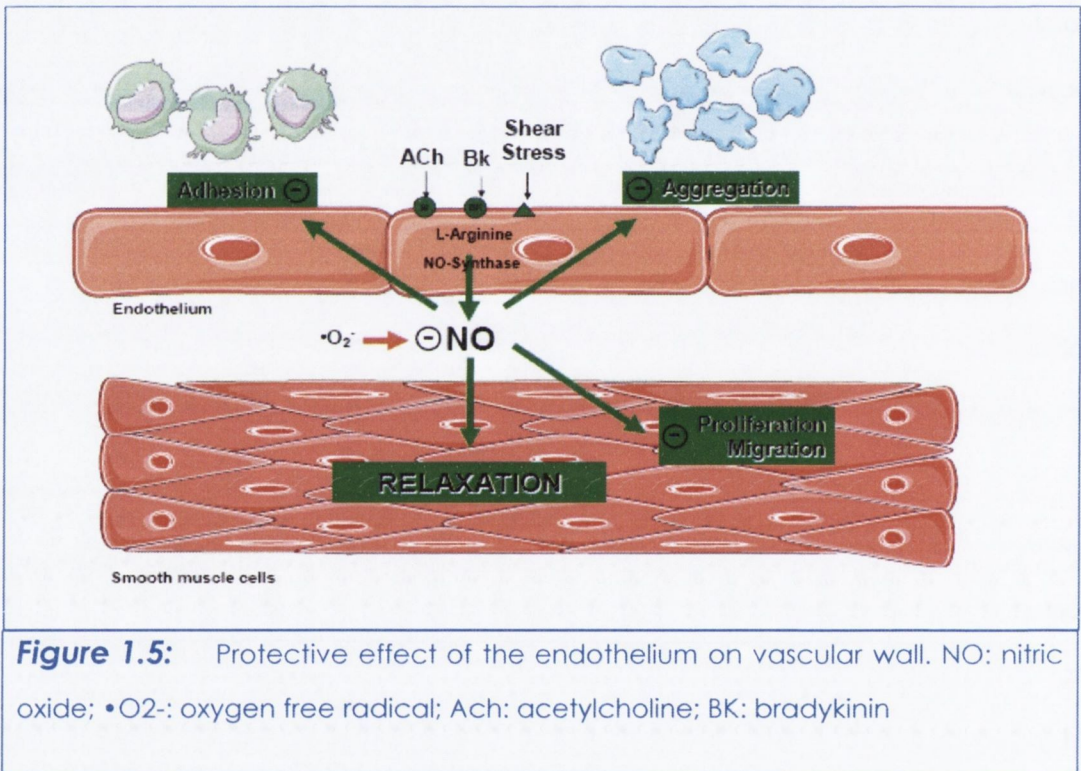
hyperemia and can be reported as the change in artery diameter after a period of induced ischemia compared to baseline artery diameter (François *et al.*, 2001).

Multiple studies already indicate that endothelial dysfunction is a characteristic of subjects with essential hypertension. However, endothelial dysfunction is not specific to essential hypertension but rather results from cardiovascular risk factors such as smoking, hypercholesterolemia, diabetes and aging (Taddei and Salvetti, 2002). Furthermore, the metabolic syndrome has been associated with a high risk of cardiovascular disease in hypertensive patients (Schillaci *et al.*, 2004) and it has been shown that the forearm response to acetylcholine is blunted in insulin resistant hypertensive patients in the presence of the metabolic syndrome as compared to hypertensive patients without the metabolic syndrome (Dell'Omo *et al.*, 2004). However, in clinically healthy older men, the metabolic syndrome is not associated with impaired endothelial function, measured as flow mediated dilation of the brachial artery (Wendelhag *et al.*, 2002).

CV risk factors are also accompanied by the presence of increased oxidative stress, defined as an excessive amount of oxidative substances relative to endogenous antioxidant capacity, which can inactivate NO (Taddei *et al.*, 1998, Landmesser and Harrison, 2001). Indeed, endothelium-dependent vasodilation to acetylcholine has shown to be significantly blunted in the forearm microcirculation of hypertensive patients (Taddei *et al.*, 1998) and this alteration is caused by increased vascular oxidative stress which reduces NO availability (Taddei *et al.*, 1998).

Endothelial dysfunction in hypertensive patients has been associated with target organ damage, such as increased intima-media thickening of the carotid arteries (Ghiadoni *et al.*, 1998). Moreover, several clinical trials have shown that endothelial dysfunction, evaluated in both coronary and peripheral circulation, is associated with increased incidence of cardiovascular events in high risk patients (Lerman and Zeiher, 2005).

Moreover, FMD is inversely associated with cardiovascular risk in low risk populations (Witte *et al.*, 2005). Thus, endothelial dysfunction is increasingly being recognized as an independent predictor of future cardiovascular events and strategies aimed to improve endothelial function may be effective in reducing cardiovascular risk of hypertensive patients (Modena *et al.*, 2002).



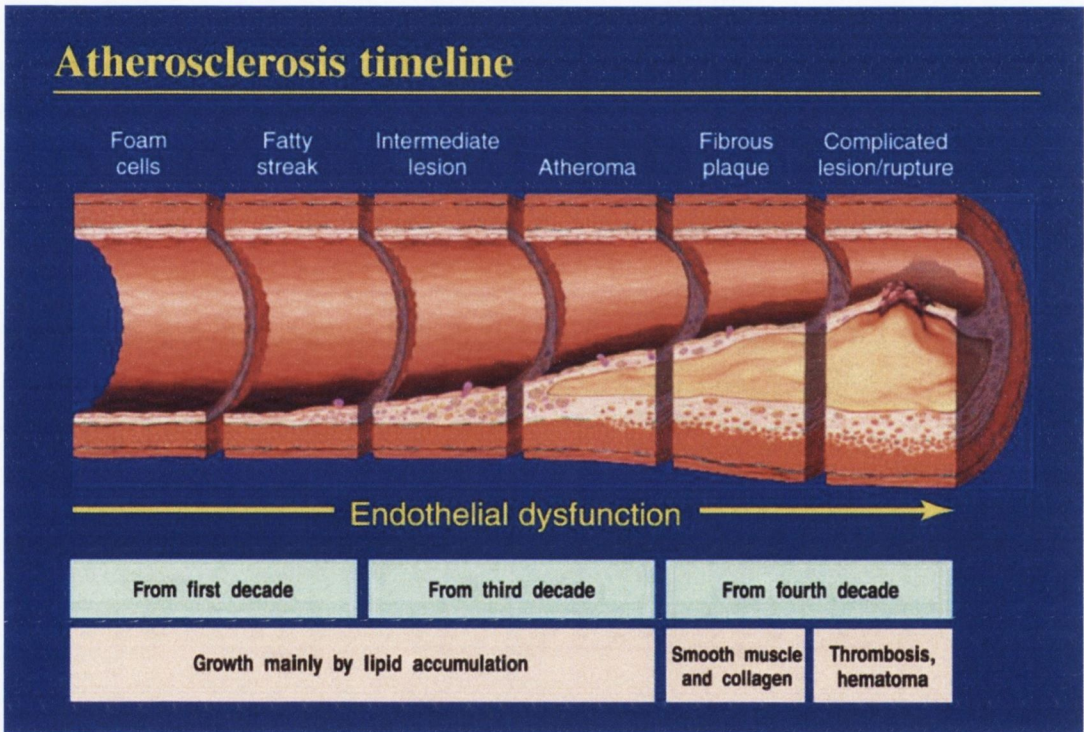


Figure 1.6: Sequences in progression of atherosclerosis; Adopted from (Pepine, 1998)

1.2. ARTERIAL STIFFNESS

1.2.1. Definitions of arterial stiffness

The definition of arterial stiffness and use of the term arterial stiffness; which characterizes the artery's ability to expand and contract during cardiac cycle (left ventricular contraction and relaxation), has been expressed by numbers of indices such as those in Table 1.2.

Table 1.2: Indices of arterial stiffness, Modified from (Gatzka et al., 1998, Nichols and O'Rourke, 1998, O'Rourke and Mancia, 1999)

INDEX	DEFINITIONS
Compliance	Arterial compliance is defined as absolute diameter or area or volume change for a given pressure step at fixed vessel length.
Distensibility	Relative diameter (or area) change for a pressure increment, $1/\text{elastic modulus}$).
Elasticity	Pressure step required for (theoretical) 100% stretch from resting diameter at fixed vessel length.
Pulse pressure	Difference between systolic and diastolic blood pressure
Augmentation index	Ratio of pressure augmentation caused by wave reflection to local pulse pressure.
Pulse wave velocity	Speed of travel of the pulse along an arterial segment.

Although these terms are interrelated, they are not synonymous. Compliance is defined as the change in volume for a given pressure change. In the arterial system compliance relates to the change in artery diameter caused by left ventricular ejection. Distensibility is used to define compliance relative to the initial volume or diameter of an artery. A decrease in the arterial elasticity results in reduced arterial compliance and distensibility. When pressure increases, a point is eventually reached with less distensibility occurring at higher pressures as a consequence of the elastic properties of the arterial media. At low pressures, elastin fibres take up pressure, whereas at higher pressures the more rigid collagen fibres absorb tension and compliance consequently decreases. Differences in arterial compliance therefore generally corrected for blood pressure. Arterial stiffness increases with the distending pressure (mean arterial pressure)(Oliver and Webb, 2003). Arterial compliance and distensibility are the terms used for quantification (Hughes *et al.*, 2004).

1.2.2. History of pulse wave measurement and arterial stiffness

Assessment of the pulse has been an important part of clinical examination for centuries. Many centuries before Christ, the Egyptians described the pulse wave as the "word" of the heart to the vessels. Later on, the Chinese started to analyse the pulse and the pulse amplitude as an index of health (Asmar, 1999). Today, the pulse is still used in diagnostics as a part of traditional Chinese medicine. In 130-200^{AD} Galen palpated the pulse and classified it in terms of strength, rate and rhythm, (Nichols and O'Rourke, 1998) (Nicholas and O'Rourke, 1998). The first sphygmograph, "the pulse writer", was introduced in the early 1860s by the Parisian physiologist Marey. Some years later Frederick Akbar Mohamed (1872 to 1884), described the normal radial pressure waveform and showed the difference between it and the carotid wave (Mahomed, 1872) and was the first to recognize the difference between pressure

waves in central and peripheral arteries. Mackenzie developed various types of sphygmographs and started to use them in clinical practices—(Mackenzie, 1902). In the late 1870s Mahomed described changes in arterial pulse (Sandra, 2006). However, these sphygmographs were difficult to use in daily clinical practice. Soon the sphygmographs were replaced by the cuff sphygmomanometer, to measure blood pressure, introduced by Riva-Rocci in 1896. In 1905 the Korotkov auscultatory method for recording SBP and DBP replaced in Riva-Rocci palpatory method and clinicians began to use the sphygmomanometer (Nabokov and Nevorotin, 1998), while sphygmographs were buried in oblivion for a long time. Sphygmographs permitting numerical expression of the arterial waveform were not available until the late 20th century when the studies of McDonald and Taylor (1959), originally from Harvey's own hospital (St Bartholomew's, London), led to the techniques described here for pulse wave analysis. Nicholas and O'Rourke in 1990 rediscovered the importance of the arterial waveform (Nichols and O'Rourke, 1998). Owing to the introduction of catheter-tip manometers by Murgu and Millar and practical high-fidelity applanation tonometers (Kelly et al., 1989c, O'Rourke and Gallagher, 1996), O'Rourke and colleagues developed the technique of pulse wave analysis (PWA), making non-invasive derivation of central pressure waveforms possible (O'Rourke and Gallagher, 1996). The recent development of non-invasive methods such as ultrasound, pressure-sensitive transducer, applanation tonometry and photoplethysmography for recording arterial flow or pressure waves has opened up a new chapter. Modern tonometer systems are piezo-electric and are far more accurate, reliable, and easy to use. While originally introduced clinically to measure intraocular pressure, they have been adapted for vascular use by Drzewiecki (Drzewiecki et al., 1983), Millar and others (Nichols and O'Rourke, 1998, O'Rourke et al., 2001). Non-invasive assessment of arterial function in epidemiological and therapeutic studies has been established, and has become an important tool as a therapeutic target as well as a basis in assessment of cardiovascular risk and arterial stiffness.

1.2.3. Basic concept of arterial stiffness

Advancing age is the major risk factor for vascular disease, and one of the main features of vascular ageing is a progressive increase in arterial stiffness, which can even occur without atherosclerosis (Lakatta and Levy, 2003).

The normal function of the elastic aorta is to transform the pulsatile blood flow from the heart into a less pulsatile flow in distal vessels and a non-pulsatile blood flow in capillaries (Hamilton *et al.*, 2007). This function depends on a normal arterial stiffness gradient, meaning that the central arteries are most elastic, and elasticity decreases gradually with increasing distance from the heart. The reflection of the initial blood pressure wave after each heartbeat may have beneficial effects depending partly on the timing of this reflected pressure wave. In healthy vasculature, the reflection waves limit the transmission of pulsatile energy to the microcirculation. Furthermore, when the reflected wave returns to the heart in diastole, it increases the diastolic pressure, thus increasing the perfusion of the myocardium of the left ventricle, which is perfused only during diastole. With increasing arterial stiffness, the velocity of the pulse wave is increased, and the reflected pulse wave returns earlier to the heart. When, eventually, the return of the pulse wave to the heart comes as early as during systole, it will augment the systolic blood pressure, whereas the diastolic blood pressure is reduced (Hamilton *et al.*, 2007). During age-related gradual loss of arterial compliance, the most pronounced increase in stiffness is seen in the proximal arteries, eventually resulting in a reversal of the arterial stiffness gradient, which facilitates for pulsatile energy to be transmitted to the microvasculature (Mitchell *et al.*, 2004).

1.2.4. Physiology of arterial stiffness

The endothelium releases several agents that affect vascular smooth muscle function. Endothelium-derived substances that relax the underlying smooth muscle include nitric oxide (NO), endothelium-derived hyperpolarizing factor, and prostacyclin (Furchgott and Zawadzki, 1980, Moncada et al., 1976). Contracting factors include endothelin-1, angiotensin II, thromboxane A₂ and prostaglandin H₂. NO is synthesized from L-arginine by NOS (Palmer et al., 1988). Several isoforms of nitric oxide synthase (NOS) have been identified, but only inducible and endothelial NOS are expressed in endothelial cells (Marletta, 1993). Endothelial NOS is responsible for the arterial tone at rest, and can be stimulated by several receptor-dependent agonists (acetylcholine (ACh), methacholine, carbachol, thrombin, bradykinin, substance P, and muscarinic agonists) and physical stimuli like shear stress. Activation of endothelial NOS is Ca²⁺-dependent (Boulanger and Vanhoutte, 1997), while inducible NOS is activated independent of Ca²⁺ during inflammation by cytokines (Vane et al., 1990). NO relaxes smooth-muscle cells through binding to guanylate cyclase and by increasing intracellular concentrations of cyclic guanosine monophosphate (Arnold et al., 1997). Vasodilator prostacyclin is formed from arachidonic acid in endothelial cell (Moncada et al., 1976). The same system produces also contracting factors like thromboxane A₂ and prostaglandin H₂ (Kato et al., 1990, Auch-Schwelk et al., 1990).

1.2.5. Pathophysiology of arterial stiffness

An increase in arterial stiffness causes a premature return of reflected waves in late systole, increasing central pulse pressure, and, thus, systolic blood pressure. Any rise in systolic blood pressure increases the load on the left ventricle, increasing myocardial oxygen demand. In addition, arterial stiffness is associated with left ventricular hypertrophy, a known risk factor for coronary events in normotensive and hypertensive

patient. The increase in central pulse pressure (PP) and the decrease in diastolic blood pressure may directly cause subendocardial ischemia (Ghiadoni *et al.*, 2009).

Indeed, the arterial pressure waveform is a composite of the forward pressure wave created by ventricular contraction, and a reflected wave. Waves are reflected from the periphery, mainly at branch points or sites of impedance mismatch (Nichols and O'Rourke, 1998). In elastic vessels, because pulse-wave velocity is low, the reflected wave tends to arrive back at the aortic root during diastole. In the case of stiff arteries, pulse-wave velocity rises, and the reflected wave arrives back at the central arteries earlier, adding to the forward wave, and augmenting the systolic pressure. This phenomenon can be quantified through the augmentation index. Apart from a high pulse-wave velocity, changes in reflection sites can also influence the augmentation index.

1.3. METHODS FOR ASSESSMENT OF ARTERIAL STIFFNESS AND ITS INDICES

1.3.1. Systolic blood pressure

The association between hypertension and a "hardening" of the pulse and apoplexy has been recognized for hundreds of years. The major problem in elderly people is isolated systolic hypertension (ISH), defined as a raised systolic blood pressure (BP) but normal diastolic BP. It affects around half of people aged over 60 years (Ramsay et al., 1999). Originally, because ISH was so common it was considered part of ageing and, like essential hypertension, benign. However, there is now compelling evidence from cross sectional, longitudinal, and randomized controlled trials that show that isolated systolic hypertension confers a substantial cardiovascular risk (Anon, 1991, Staessen et al., 1997). Despite this, it remains under-diagnosed and largely untreated (Coppola et al., 1997). The roots of this lie in a century of overreliance on the importance of diastolic pressure and largely unjustified concerns about the potential adverse consequences of treating systolic BP.

After the mercury sphygmomanometer was introduced, convention dictated that diastolic BP was a better determinant of cardiovascular risk than systolic BP. Systolic BP was thought to vary considerably throughout the day, and a high pressure was believed to reflect a "strong" left ventricle. This view was perpetuated by the reliance of life assurance companies on diastolic BP and the use of diastolic BP in the early studies of lowering blood pressure. The use of diastolic BP was further supported by the discovery that essential hypertension is characterized by increased peripheral vascular resistance and therefore raised mean arterial pressure, which more closely correlates with diastolic than systolic BP (Wilkinson, 2000). Evidence that systolic BP is equally, if not more, important than diastolic, particularly in people over 50, was largely ignored. Although the use of diastolic BP for risk prediction may be reasonably effective for

younger people and people with essential hypertension, data from cohort and intervention studies indicate that it is inappropriate for the over 50s, particularly those with isolated systolic hypertension (O'Rourke and Frohlich, 1999).

Borderline ISH was the most common form of untreated hypertension, with a prevalence of 18 percent among participants 65 years of age and older. Subjects with this type of hypertension were at substantially increased risk of progression to definite hypertension in both short-term and long-term analyses. Subjects with borderline ISH also had a greater risk of morbidity (long-term and short-term) and mortality (long-term) from cardiovascular disease than normotensive subjects. The increased risk of cardiovascular disease was even more striking when we compared subjects with borderline ISH with those with optimal blood pressure (Sagie et al., 1993).

In almost all populations, ageing is associated with a rise in systolic and fall in diastolic BP, and a widening of the pulse pressure (Franklin et al., 1997). This is due to arteriosclerosis (Bramwell and Hill, 1922). ISH could therefore be seen as something we might all develop given time—and, in contrast to essential hypertension, it is not associated with any appreciable change in peripheral resistance. Nevertheless, ISH is not a benign condition. Indeed, pulse pressure is a better predictor of cardiovascular events than systolic or diastolic BP alone in people aged over 50 (O'Rourke and Frohlich, 1999). Data from the Framingham study (based on 2000 men and women aged 50-79 at the onset of the study, none of whom had clinical evidence of coronary heart disease) indicate that for any given quarter of systolic BP, events are inversely related to diastolic BP (the lower the diastolic BP the higher the risk), showing, at least in the over 50s, that arterial stiffness is a key determinant of cardiovascular risk (Franklin et al., 1999).

Despite continued reluctance to accept ISH as a discrete pathological entity, the benefits of treatment are established (Anon, 1991, Staessen et al., 1997). The relative risk reduction of cardiovascular events in elderly people with ISH, reported in the latest Cochrane review, is similar to that in younger people (Mulrow et al., 2000). However, as elderly people are at much higher absolute risk of such events, they stand to benefit more from treatment than younger people (Mulrow et al., 2000). Indeed, the number needed to treat to prevent one stroke in people with ISH is around half that found in a study of mild hypertension (Mulrow et al., 2000, Anon, 1985). Moreover, elderly people tolerate antihypertensive drugs with few side effects (Mulrow et al., 2000). Yet patients with ISH remain under-recognized and undertreated (Coppola et al., 1997).

The World Health Organization and International Society of Hypertension guidelines for the management of hypertension emphasize the importance of arterial stiffness and pulse pressure as predictors of cardiovascular risk and call for further investigation of the prognostic relevance of other indices of arterial stiffness (Anon, 1999).

1.3.2. Pulse pressure

Pulse pressure is the difference between systolic and diastolic blood pressure and is the consequence of cardiac contraction and is strongly influenced by the properties of the arterial tree (Dart and Kingwell, 2001). As the pulse pressure is mainly determined by cardiac output, aortic and large artery stiffness, and pulse wave reflection, it constitutes a surrogate marker for arterial stiffness (Oskvig, 1999).

Whereas the systolic blood pressure tends to increase linearly with age in the western population, the diastolic blood pressure generally rises during adulthood and peaks at approximately 60 years of age and thereafter starts to decline due to arterial stiffening

(Sagie et al., 1993, Burt et al., 1995, Pearson et al., 1997). This naturally results in a rapidly increasing pulse pressure.

Pulse pressure is the most easily available measure of arterial stiffness since it can be assessed with a standard sphygmomanometer. Unfortunately, assessment of arterial stiffness by pulse pressure can be quite inaccurate. The brachial blood pressure is strongly determined by the phenomenon of pulse wave amplification from the aorta to the peripheral arteries. Due to pulse wave amplification, the peripheral systolic blood pressure and consequently the pulse pressure can differ markedly between central and peripheral arteries (Pauca et al., 1992). Pulse wave amplification decreases with age and is most prominent in the young (Franklin et al., 2005). Thus, the usefulness of brachial pulse pressure as a marker of arterial stiffness is poor in the young but increases substantially with age (Luoto et al., 2002).

Pulse pressure has been shown to be a powerful predictor of cardiovascular morbidity and mortality in a number of studies. The Framingham study demonstrated that in the elderly population pulse pressure is a stronger predictor of cardiovascular disease than systolic or diastolic pressure alone (Franklin, 1999b). Additionally, pulse pressure has been found to predict all-cause and cardiovascular mortality particularly in the elderly but also in the general population (Domanski et al., 1999, Domanski et al., 2001, Panagiotakos et al., 2005, Glynn et al., 2000). A meta analysis by Gasowski showed that in hypertensive patients pulse pressure, but not the mean arterial blood pressure, is associated with an increased risk of fatal coronary heart events (Schram et al., 2002). Conflicting results were provided by the Chicago Heart Association and Health Department Study, which failed to find a relationship between pulse pressure and subsequent mortality (Domanski et al., 2002). Another large study performed on African-Americans also challenged this view (Pastor-Barriuso et al., 2003).

There is however evidence that, in the young and middle-aged, diastolic pressure is a robust blood pressure index to predict coronary heart disease, as originally shown by the Framingham study (Nishizaka and Calhoun, 2006).

1.3.3. Pulse wave velocity

The speed at which the pressure wave generated by cardiac contraction travels from the aorta to the peripheral arteries is mainly determined by the artery wall stiffness and lumen diameter. Pulse wave velocity (PWV) can be calculated by measuring the time for the pulse to pass between two points with known distance (Figure 1.7). The measurement usually involves taking separate recordings from two sites and relating them to the R wave of a simultaneously recorded ECG. A variety of methods can be applied to register the pulse wave such as Doppler ultrasound, or applanation tonometry. Since the aorta is the major component of arterial stiffness, the carotid-femoral pulse wave velocity, which is a measure of aortic stiffness, and is the most commonly, used in the evaluation of regional stiffness.

Assessment of pulse wave velocity is relatively simple and the method has been widely applied and has been found to be both robust and reproducible. Studies show that pulse wave velocity is an independent predictor of cardiovascular disease and mortality in both hypertensive patients and in patients with end-stage renal disease (Blacher et al., 1999a, Boutouyrie et al., 2002, Laurent et al., 2001, Blacher et al., 1999b). Furthermore, aortic pulse wave velocity is a powerful independent predictor of mortality in diabetic and elderly population (Cruickshank *et al.*, 2002).

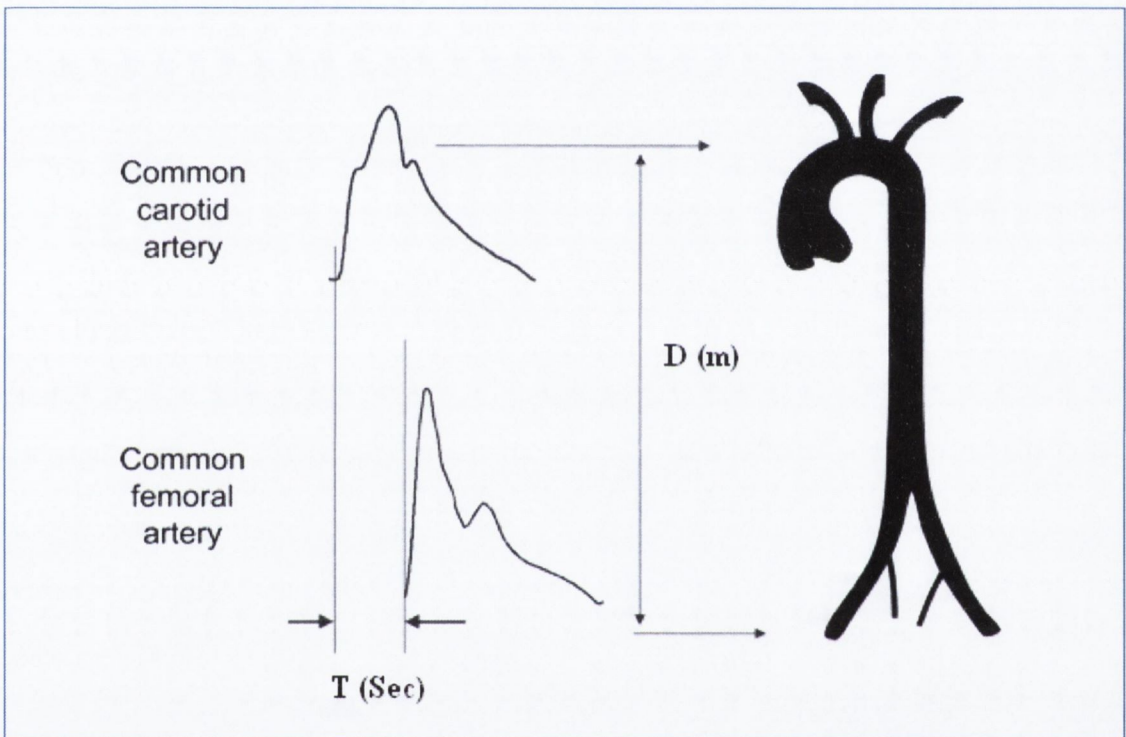


Figure 1.7: Measurement of carotid-femoral PWV with the foot-to-foot method.

The distance (D) covered by the waves is usually assimilated to the surface distance between the two recording sites (carotid artery and femoral artery), and T = Transit time. $PWV = D \text{ (meters)} / T \text{ (seconds)}$. Figure adopted (Laurent et al., 2006).

1.3.4. Pulse wave analysis

As the left cardiac ventricle contracts it creates a forward pressure wave that travels to the periphery throughout the arterial tree. When the forward wave reaches the branching points of arteries, regions of increased arterial stiffness, and high-resistance arterioles, a backward wave occurs as a consequence of wave reflection (O'Rourke and Gallagher, 1996, Latham et al., 1985, Nichols and O'Rourke, 2005). The reflected waves are superimposed on the wave that travels forward resulting in an arterial waveform that varies throughout the arterial tree.

Arterial stiffening increases the amplitude and the velocity of the reflected waves. In elastic vessels, the reflected wave tends to arrive back to the aorta during diastole and thereby augments diastolic pressure and improves coronary perfusion. As arterial stiffness and hence pulse wave velocity increases, the reflected wave returns to the aorta at an earlier phase of the cardiac cycle thereby augmenting the systolic pressure instead of the diastolic pressure. Consequently, arterial stiffening reduces coronary perfusion and increases cardiac oxygen consumption by augmenting cardiac afterload.

Since the arterial waveform varies throughout the arterial tree, the extent of wave reflection is assessed more accurately by analyzing the central pressure waveform than the peripheral waveforms. Although the reflected waves originate predominantly at the major branches of the aorta, stiffness of the smaller arteries and arterioles has a considerable influence on the central pressure waveform. Central pulse pressure augmentation may therefore provide a better marker of systemic arterial stiffness than single large artery measures, such as pulse wave velocity or aortic ultrasound.

Pulse wave analysis (PWA) is a non-invasive method to measure arterial stiffness (Pauca *et al.*, 2001). Applanation tonometry using a Millar transducer is employed to record pressures at the radial or the carotid artery, and a validated generalized transfer function based upon a comparison with intra-arterial pressures in patients undergoing cardiac surgery is then applied to generate the corresponding central waveform (Chen *et al.*, 1996). The augmentation index (Alx), which is a measure of systemic arterial stiffness can then be calculated as the difference between the first and second systolic peaks expressed as a percentage of the central pulse pressure (*Figure 1.8*). Satisfactory waveform recordings from the radial artery are typically obtained within a few minutes by a trained examiner.

The Alx has been associated with the presence and extent of coronary artery disease (Weber *et al.*, 2004). Increased arterial wave reflection is also a risk factor for cardiovascular events in patients with established coronary artery disease (Weber *et al.*, 2005). In renal failure patients, a high Alx has been established as an independent predictor of all-cause and cardiovascular mortality (Duprez and Cohn, 2007).

However, the use of pulse wave analysis to assess arterial stiffness is associated with some problems. Since the pulse wave reflection returns to the aorta at an earlier phase of the cardiac cycle when the heart rate is high, there is an inverse association between heart rate and Alx may need to be adjusted for (Wilkinson *et al.*, 2000a). The Alx is only in part determined by arterial stiffness as increases in peripheral wave reflectance may also be caused by increased peripheral vascular resistance and by the distending effect of an elevated blood pressure (Pauca *et al.*, 2001). Furthermore, it seems that the generalized transfer function may be inappropriate for the derivation of central waveforms in patients with diabetes thereby making the Alx less reliable measure of arterial stiffness in these patients (Hope *et al.*, 2004).

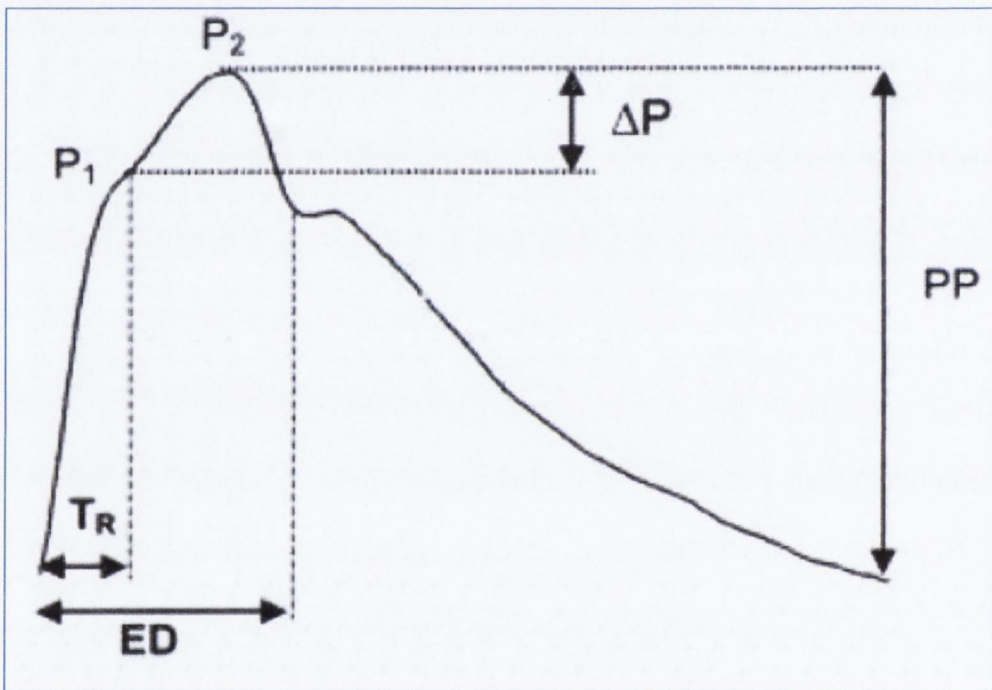


Figure 1.8: Graphic representation of augmentation index (Carotid pressure waveform is recorded by applanation tonometry). The height of the late systolic peak (P₁) above the inflection (P₂) defines the augmentation pressure (ΔP), and the ratio of augmentation pressure to pulse pressure (PP) defines the AIx (in percent, $AIx = \Delta P/PP \times 100$).

1.3.5. Oscillometric Method (Arteriograph)

Currently two systems are in common use to measure arterial stiffness, SphygmoCor (AtCor Medical) utilizes radial applanation tonometry and the application of a generalized transfer function to estimate wave reflection as augmentation index (Alx) and aortic pressures non-invasively. The Complior system (Artech Medical) simultaneously records pressure waves in the carotid and femoral arteries by using a piezo-electronic device and the PWV is calculated by dividing the distance between the two sites by the transit time between waves. The Arteriograph (TensioMed Ltd) is a recently developed computerized device using an oscillometric method to determine both PWV and Alx simultaneously.

The Arteriograph cuff with a sensitive sensor (very high fidelity oscillometric tonometers) is attached for detecting in a sufficiently good quality the weak signals appearing by the cuff. The cuff was applied on the left arm, oscillometric pressure curves (pulsatile pressure changes in the brachial artery) registered in the upper arm, are detected by plethysmography. Fluctuations in pulsatile pressure in the artery beneath an inflated pressure cuff induce periodic pressure changes in the inflated cuff. These periodic pressure oscillations provide an indirect measure for the pulsatile pressure changes in the artery beneath (Baulmann *et al.*, 2008). Arteriograph which measures blood pressure (BP [mmHg]) first and then immediately the cuff pressurized at least 35 mmHg more than the actual systolic blood pressure (while the brachial artery is completely closed), and Alx (%) and PWV (m/sec) measurements were detected. Both Alx and PWV, has recently been validated against the SphygmoCor and Complior, largely in a healthy population (Baulmann *et al.*, 2008), and for PWV in 64 longstanding hypertensive patients (Rajzer *et al.*, 2008). *Figure 1.8 and 1.9* shows the tracing for wave reflection analysis using SphygmoCor and Arteriograph.

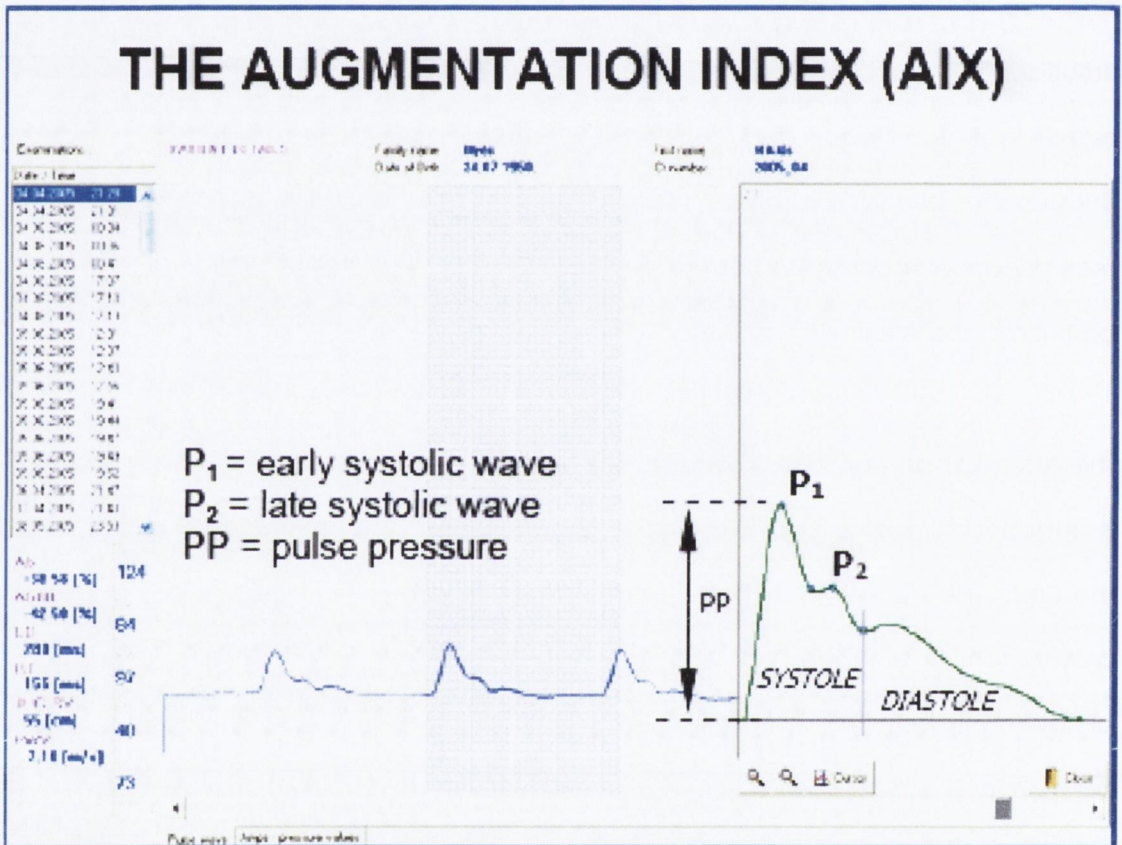


Figure 1.9: Graphic picture of augmentation index on Arteriograph (TensioMed) The height of the late systolic peak (P₁) above the inflection (P₂) defines the augmentation pressure, and the ratio of augmentation pressure to PP defines the AIX (in percent).

1.4. FACTORS THAT DETERMINING ARTERIAL STIFFNESS

1.4.1. Physiological factors

1.4.1.1. Age

Advancing age is the main cause of arterial stiffness and a major risk factor for vascular disease. Vascular changes progressively increase and arteries become less elastic with aging, stiffness can even occur without atherosclerosis (Lakatta and Levy, 2003). This ageing process is a consequences of stiffening of large arteries (Avolio *et al.*, 1985). Arterial stiffness increases both systolic blood pressure and pulse pressure (Sagie *et al.*, 1993). Kelly *et al.* determined arterial stiffness from the carotid, femoral and radial arteries using an applanation tonometer and analyzed arterial pressure waveforms in 1005 normal subjects aged 2 to 91 years (Kelly *et al.*, 1989a). Aging was associated with an increase in pulse amplitude, steepening of the diastolic decay and a decrease in the pressure of the diastolic wave (Kelly *et al.*, 1989a). Stiffening thus explains why diastolic pressure normally decreases and pulse pressure increases during aging. A decrease in diastolic pressure is observed from the age 50-59 years onwards (Franklin *et al.*, 1997, Burt *et al.*, 1995). In a recent analysis of the Framingham cohort, 75% of all hypertensive's (over 160/90 mmHg) were older than 50 years (Franklin, 1999a). This information has several important prognostic and therapeutic implications (*vide infra*). That aging stiffens arteries has also been demonstrated in several other studies using techniques such as ultrasound (Kawasaki *et al.*, 1987, Sonesson *et al.*, 1993), tonometry (Kelly *et al.*, 1989a, McVeigh *et al.*, 1999, Vaitkevicius *et al.*, 1993), magnetic resonance imaging (Mohiaddin *et al.*, 1993), arterial catheterization (McVeigh *et al.*, 1999), photoplethysmography (Bortolotto *et al.*, 2000, Takazawa *et al.*, 1998) and PWV (Avolio *et al.*, 1985, Vaitkevicius *et al.*, 1993). To what extent aging stiffens arteries independent

of other factors (vide infra) is unclear. However, stiffening does occur even in the absence of atherosclerosis (Avolio *et al.*, 1985).

1.4.1.2. Height

Height is inversely related to the risk of cardiovascular disease even after adjusting for known CHD risk factors (Cook *et al.*, 1994, Hebert *et al.*, 1993). An increased risk of CHD has been associated with short stature (Kannam *et al.*, 1994, Palmer *et al.*, 1990a). An increased risk has been observed in both men and women in *cross-sectional* (Palmer *et al.*, 1990a) and *follow-up* (Cook *et al.*, 1994, Hebert *et al.*, 1993, Kannam *et al.*, 1994, Rich-Edwards *et al.*, 1995) studies. Regarding the reasons underlying this association, short stature has been associated with reduced pulmonary function (Cook *et al.*, 1994), genetic factors (Cook *et al.*, 1994, Allebeck and Bergh, 1992), poor childhood nutrition (Palmer *et al.*, 1990b) and small diameter of coronary arteries (Fisher *et al.*, 1982). Short stature and arterial length increases the carotid Alx independent of mean arterial pressure (Smulyan *et al.*, 1998). Short stature also correlates with carotid artery compliance, heart rate and age (Smulyan *et al.*, 1998). Increased wave reflection in systole and increased ventricular load may therefore increase the risk of CHD in short individuals.

1.4.1.3. Gender

Cross-sectional Studies. Women have a higher Alx than men in all age groups and the difference increases with age (Hayward *et al.*, 2001a). In a group of elderly hypertensive patients matched for age, height and mean arterial pressure, there was no difference in systolic blood pressure between men and women, but diastolic blood pressure was lower and pulse pressure and the Alx higher in women. Women had a 5% smaller aortic arch and 11% smaller outflow area. Aortic stiffness index β and elastic

modulus (EP) were also higher in women. There were no differences in heart rate or stroke volume between men and women (Gatzka et al., 2001b, Yasmin and Brown, 1999). In an urban Chinese population aged 17 to 85 years there was an increase in aortic, arm or leg pulse wave velocities with age but there were no gender differences (Avolio et al., 1985). In another study, the aortic and carotid artery compliances were greater in young women than men and decreased rapidly with age so that after middle-age women had lower compliance than men (Laogun and Gosling, 1982).

lower blood pressure or the Alx values in a *follow-up study* of post-menopausal women (Hayward et al., 2001b). In women the systolic outflow time was longer and diastole was shorter than in men (Hayward et al., 2001a, Gatzka et al., 2001b). The greater aortic stiffness after menopause and the increase in the Alx may contribute to the greater age-associated increase in left ventricular mass and excess symptomatic heart failure in women than in men that are not explained by differences in brachial blood pressure (Hayward et al., 2001a, Marcus et al., 1994).

1.4.1.4. Heart rate

An increased heart rate (Kannel et al., 1987b) and low heart rate variability (Dekker et al., 2000) are associated with an increased risk of cardiovascular mortality and morbidity in *follow-up studies*. Increased heart rate in patients with type 2 diabetes is associated with impaired autonomic control of heart rate variation (Vinik et al., 2003). In a cross-sectional study, an increased heart rate was also associated with features of insulin resistance in non-diabetic subjects (Palatini et al., 1997). The Alx correlates inversely and linearly with heart rate. When heart rate was increased with a pacemaker by 10-beats/minute, the Alx decreased 5.6% (Wilkinson et al., 2000a). Ejection duration also shortened and peripheral systolic and diastolic blood pressures

increased, but central systolic blood pressure measured non-invasively from the brachial artery remained unchanged (Wilkinson *et al.*, 2000a). The Alx decreases because an increase in heart rate decreases the duration of systole and shifts the reflected wave to diastole. The lack of rise in central systolic blood pressure is explained by the decrease in wave pressure augmentation (Wilkinson *et al.*, 2000a). PWV has increased with heart rate in some (Lantelme *et al.*, 2002a) but not in all (Yasmin and Brown, 1999) *cross-sectional studies* and the results are conflicting also in studies where pacemakers were used to regulate heart rate (Wilkinson *et al.*, 2000a, Bush *et al.*, 1998). The increase in PWV may be due to the shortened time available for recoil, which results in vessel stiffening (Lantelme *et al.*, 2002b).

1.4.1.5. Hormonal state

Hormone replacement therapy (HRT) seems to have favorable effects on some aspects of vascular function. Several studies have reported improvements in *in-vivo* endothelial function, as measured from an increase in flow-mediated brachial artery diameter by ultrasound techniques (Bush *et al.*, 1998, Koh *et al.*, 1999). As those of insulin, the favorable vascular effects of estradiol may be mediated via increased synthesis of nitric oxide (Guetta *et al.*, 1997). Cross-sectional studies (Teede *et al.*, 1999, Hayward *et al.*, 1997, Waddell *et al.*, 1999), have also suggested that large artery stiffness, measured using Doppler techniques (Penotti *et al.*, 1996) or pulse wave analysis (Teede *et al.*, 1999, Hayward *et al.*, 1997), is greater in women using HRT than in nonusers. Withdrawal of HRT has been suggested to increase arterial stiffness (Waddell *et al.*, 1999, Rajkumar *et al.*, 1997), but no placebo-controlled studies have hitherto examined effects of estradiol or HRT on arterial stiffness.

1.4.1.6. *Physical activity*

Regarding arterial stiffness, available evidence indicates that physically active adults show attenuation in the age-related increase in arterial stiffness in comparison with their sedentary peers (Vaitkevicius et al., 1993, Tanaka et al., 1998). Furthermore, an observational study conducted in a cohort of men and women free of cardiovascular disease demonstrated an inverse association between habitual physical activity and arterial stiffness (Schmitz et al., 2001).

Studies have been conducted to evaluate the effect of high intensity exercise on arterial stiffness. A cross-sectional study showed that older men who performed endurance exercise had lower levels of aortic pulse wave velocity and augmentation index than their sedentary peers (Gates et al., 2003). A lower aortic pulse wave velocity was also demonstrated in individuals performing habitual endurance exercise as compared to recreationally active individuals (Vaitkevicius et al., 1993).

Intervention studies aimed to improve arterial stiffness showed that a moderate intensity and walking type of physical activity increased carotid arterial compliance in previously sedentary, but healthy middle-aged and older men (Tanaka et al., 2000). The main causes of arterial stiffening with age are a decrease in elastin and the proliferation of collagen fibers in the vascular wall, together with vasoconstrictor tone exerted by its smooth muscle cells (Lakatta and Levy, 2003). Physical activity seems to attenuate the age-related changes of the arterial wall. Explanations for the beneficial effect of physical activity on arterial stiffness could be a decrease in sympathetic tone and a possible improvement in endothelial function.

In summary, persons who are physically active maintain a more favorable cardiovascular risk profile. Regular physical activity not only lowers blood pressure levels

but also attenuates the age- related increase in arterial stiffness, which is increasingly being recognized as an independent cardiovascular risk factor. Arterial stiffness-related benefits of exercise are most likely to accrue if exercise prescription in young adults targets improvements in cardio-respiratory fitness (Boreham *et al.*, 2004). Furthermore, it is not known if physical activity exerts different effects on the vascular system in healthy subjects or in patients with hypertension.

1.4.1.7. Smoking

Smoking is an established risk factor for cardiovascular disease. An acute stiffening effect of cigarette smoking has been demonstrated in non-smokers as well as in smokers (Mahmud and Feely, 2003b). Passive smoking has also been associated with acutely increased aortic stiffening (Mahmud and Feely, 2004). Acute smoking increases blood pressure, heart rate, the Alx and PWV in *intervention studies* (Failla *et al.*, 1997, Mahmud and Feely, 2003b). Smokers have stiffer arteries than non-smokers measured using the Alx (Fennessy *et al.*, 2003, Mahmud and Feely, 2003b, Jatoi *et al.*, 2007), PWV (Levenson *et al.*, 1987), diastolic pulse contour analysis (McVeigh *et al.*, 1997) in *cross-sectional studies* (Kool *et al.*, 1993, Stefanadis *et al.*, 1997, Liang *et al.*, 2001). The vascular effects of smoking and hypertension may however be different. Both smoking and hypertension are associated with increased vascular stiffness indices and intima-media thickness (Howard *et al.*, 1998), but only hypertension is associated with increased central pulse pressure and lumento- wall ratio of carotid artery (Liang *et al.*, 2001).

1.4.2. Conditions / diseases and arterial stiffness

1.4.2.1. Arterial stiffness and hypercholesterolemia

Hypercholesterolemia or dyslipidemia have been found to have stiffer arteries than normocholesterolemic subjects in *cross-sectional studies*. Arterial stiffness is increased in hypercholesterolemia (Wilkinson *et al.*, 2002a), in adults the data are more conflicting. The Alx and central pulse pressure (Wilkinson *et al.*, 2002b) have been found to be higher in middle-aged, hyper-cholesterolemic patients than control subjects matched for peripheral blood pressure, height, age, smoking, weight and fasting glucose (Wilkinson *et al.*, 2002b). In young men oxidized but not total low density lipoprotein concentration was associated with increased stiffness measured with ultrasound and MRI (Toikka *et al.*, 1999). In other studies no associations were found between LDL or HDL cholesterol and arterial stiffness (Alagona *et al.*, 2003, Saba *et al.*, 1999).

1.4.2.2. Arterial stiffness and hypertension

Although large artery stiffening is a strongly age-related process, it is also markedly accelerated by the presence of hypertension (Bouthier *et al.*, 1985, Liu *et al.*, 1989) Benetos *et al* found that the age-induced pulse wave velocity progression was more than 3-times greater in poorly controlled hypertensive patients compared with well-controlled hypertensive patients (Benetos *et al.*, 2002). A study on 24-h blood pressure showed that impaired night time blood pressure decline is associated with increased arterial stiffness assessed by pulse wave analysis (Lekakis *et al.*, 2005). Another study showed that a determinant of hypertension, low birth weight is related to increased arterial stiffness (Lucas *et al.*, 1999). Interestingly, recent results demonstrating that aortic stiffness is an independent predictor of progression to hypertension in normotensive subjects suggest that lower arterial elasticity is related to the development of hypertension (Dernellis and Panaretou, 2005, Liao *et al.*, 1999).

1.4.2.3. Arterial stiffness and cardiovascular disease

Pulse pressure, a surrogate indirect measure of arterial stiffness (Laurent and Boutouyrie, 2007), has been shown to be a strong predictor of coronary heart disease independent of systolic, diastolic or mean arterial pressure (Madhavan *et al.*, 1994). In the Framingham Heart study, the hazard ratio for CAD increased as a function of pulse pressure regardless of systolic pressure (Franklin *et al.*, 1999) *Fig. 1.10*. The importance of pulse pressure for CAD risk at different ages was also studied in 3060 men and 3479 women in the same Framingham population (Franklin *et al.*, 2001). Age was found to strongly influence the predictive value of various components of blood pressure. Pulse pressure was the strongest predictor in subjects over 60 years (Franklin *et al.*, 2001) suggesting that age-related stiffening of the arteries is an important risk factor for CAD. In contrast, in individuals less than 50 years of age, diastolic blood pressure was the strongest predictor of CAD risk, whereas in individuals aged 50 to 59 years, all (systolic BP, diastolic BP and pulse pressure) components of blood pressure were comparable (Franklin *et al.*, 2001). The effects of pulse pressure vs. mean arterial pressure in predicting risk of CAD and cerebrovascular events in 2311 hypertensive subjects (mean age 51 years, 53% men) was studied using 24-hour ambulatory blood pressure measurements in the PIUMA study (Verdecchia *et al.*, 2001). Over a mean follow-up period of 4.7 years, 132 cardiac and 105 cerebrovascular events occurred. Pulse pressure, but not mean arterial pressure, was a major predictor of cardiac events after adjustment for age, sex, diabetes, serum cholesterol, and cigarette smoking (Verdecchia *et al.*, 2001). In contrast, mean arterial pressure was the major independent predictor of cerebrovascular events, whereas pulse pressure did not yield significance (Verdecchia *et al.*, 2001). These data demonstrate that an increase in the dynamic component of blood pressure i.e. pulse pressure or stiffness is indeed harmful for cardiac function, as might be predicted from the increase in afterload and

decrease in diastolic filling of coronary arteries that accompany an increase in wave reflection.

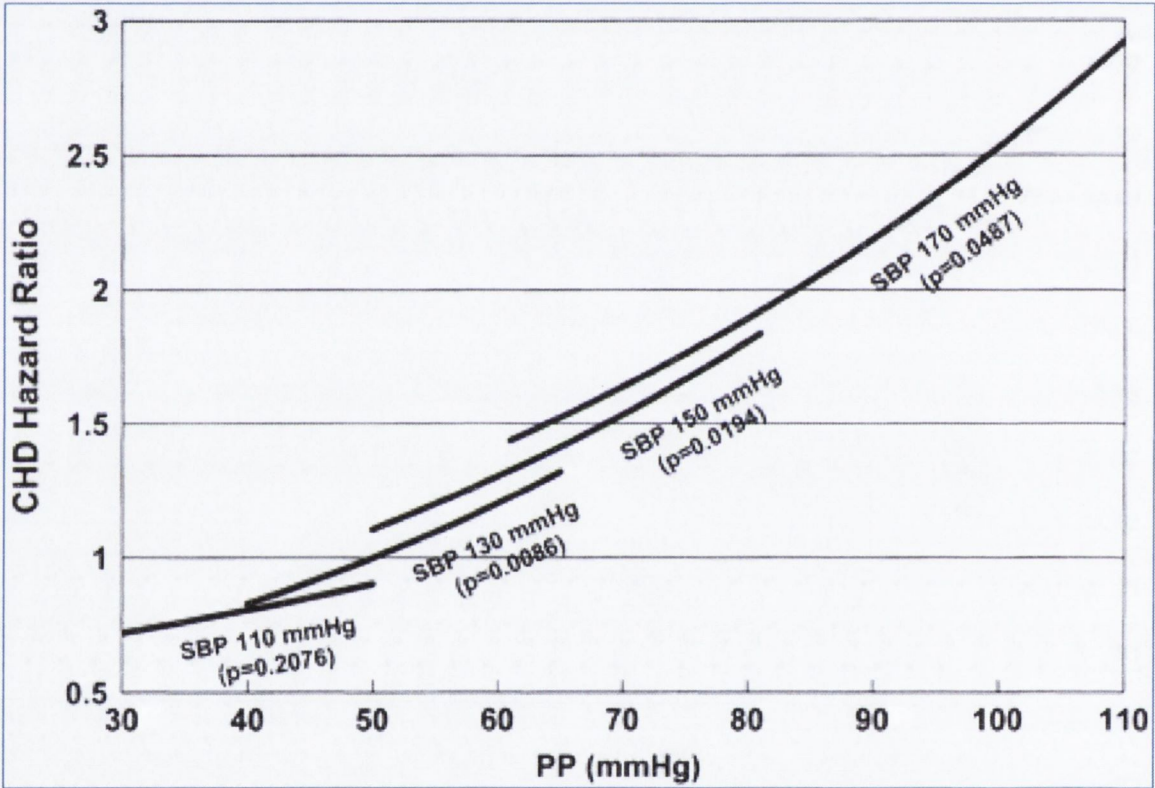


Figure 1.10: Independent influence of pulse pressure on CAD risk at different levels of systolic blood pressure. Adapted from (Franklin *et al.*, 1999).

1.4.2.4. Arterial stiffness and diabetes mellitus

The ARIC study was the first to implement measurements of carotid artery stiffness with the use of ultrasound in a large population survey comprising of 4701 white and black (19% black) subjects (Salomaa *et al.*, 1995). Of these subjects 5% had type 2 diabetes. In the entire study group, arterial stiffness increased with increasing concentrations of fasting glucose, independent of race or gender. The relationship between glucose and insulin and stiffness remained highly significant also after adjustment for age, smoking and total cholesterol. In all non-diabetic patients, fasting serum insulin was associated with arterial stiffness, again even after adjustment for age, smoking and total cholesterol. After further adjustment for BMI, triglycerides, HDL cholesterol, and hypertension status (49% of the black and 25% of the white subjects were hypertensive), glucose was significantly associated with stiffness in white and black female and insulin in white female and male participants. This cross-sectional study also found that hyperinsulinemia and hyperglycemia synergistically contributed to arterial stiffness, independent of artery wall thickness, in both men and women (Salomaa *et al.*, 1995).

In the study by Taniwaki *et al.* in Japanese subjects, arterial stiffness (aortic PWV) was measured in 271 diabetic patients and 285 healthy age-matched control subjects (Taniwaki *et al.*, 1999). Stiffness was significantly increased in diabetic patients compared to control subjects. In multiple regression analysis in diabetic patients, age and duration of diabetes were independently associated with arterial stiffness (Taniwaki *et al.*, 1999).

Type 1 diabetic patients have also been shown to have stiffer large arteries in studies (Ahlgren *et al.*, 1995, Berry *et al.*, 1999, Giannattasio *et al.*, 1999). Giannattasio *et al.* measured arterial stiffness in the abdominal aorta and in radial and common carotid artery using an arterial wall echo-tracking technique in 133 type 1 diabetic patients

(mean age 35 years) and in 70 age-matched control subjects (Giannattasio *et al.*, 1999). Diabetic patients were considered free of macrovascular disease, but 59% had microvascular complications. In the diabetic patients, regardless of the presence of complications, arterial stiffness was increased at all arterial sites when compared to control subjects (Giannattasio *et al.*, 1999). Brooks *et al.* measured Alx with the use of applanation tonometry and pulse wave analysis in 89 type 1 diabetic patients (age 34 years) and in 95 control subjects (Brooks *et al.*, 1999). Although there was no significant difference in the Alx between diabetic patients and control subjects, diabetes was (together with age, height and heart rate) an independent determinant of Alx. The lack of a significant difference in Alx might have been missed because heart rate was 10 beats/min higher in the diabetic patients than in the normal subjects (Brooks *et al.*, 1999). Wilkinson *et al.* also determined arterial stiffness using applanation tonometry and pulse wave analysis in 35 type 1 diabetic patients and 35 matched control subjects (Wilkinson *et al.*, 2000b). In this study, diabetic patients had a significantly higher Alx and PWV than the normal subjects, heart rate was 9 beats/min higher in the diabetic patients than in the normal subjects and it was not mentioned either it was corrected or not (Wilkinson *et al.*, 2000b). Intensive insulin therapy has been shown to slow arterial stiffening in type 1 diabetic patients (Jensen-Urstad *et al.*, 1996). Glycemic control as measured with HbA1c has not been reported to be correlated with arterial stiffness in non-diabetic subjects. At least theoretically, increases in blood glucose concentrations within the non-diabetic range could damage the arterial wall because of increased glycosylation of matrix proteins as in diabetic patients (Airaksinen *et al.*, 1993). In non-diabetic subjects, HbA1c has been reported to be correlated with thickening of arterial intima media (Vitelli *et al.*, 1997) and endothelium dependent vasodilatation (Vehkavaara *et al.*, 1999) suggesting that even small increases in blood glucose may be harmful to vascular function or may serve as markers of altered vascular function.

1.4.2.5. Arterial stiffness and other factors

The metabolic syndrome is closely related to hypertension and diabetes type 2. Metabolic syndrome is also associated with an increased acceleration of the pulse wave velocity with age (Safar *et al.*, 2006). In young individuals (Ferreira *et al.*, 2007), ultrasonically estimated carotid distensibility was associated with the metabolic syndrome (Ferreira *et al.*, 2005). Another feature of the metabolic syndrome, dyslipidaemia has also been associated with higher central pulse pressure and higher Alx (Wilkinson *et al.*, 2002b). Aortic and brachial pulse wave velocity, pulse wave reflection, and pulse pressure, relate to the levels of inflammation in healthy individuals, suggesting that inflammation may be involved in arterial stiffening (Yasmin *et al.*, 2004). High-sensitivity C-reactive protein, a marker of systemic inflammation, is independently related to pulse wave velocity, a marker of aortic stiffness, and Alx, a manifestation of wave reflection, in essential hypertension (Mahmud and Feely, 2005b). Increased arterial stiffness is a typical feature of subjects with renal insufficiency, from mild to moderate reduction of creatinine clearance to end-stage renal failure (Mourad *et al.*, 2001). In a study conducted in a large cohort of untreated subjects with normal or elevated blood pressure, Mourad *et al.* found a negative association between creatinine clearance calculated by the Cockcroft–Gault formula and the carotid–femoral pulse wave velocity (Mourad *et al.*, 2001). In patients with type 2 diabetes and treated hypertension, with a normal to elevated urinary albumin-to-creatinine ratio, creatinine clearance and carotid–femoral pulse wave velocity correlate inversely, independently of age (Smith *et al.*, 2005). Nutrition plays an important factor and has influences on blood pressure levels. Factors including salt, alcohol, calories and other nutrients all play a role (Anon, 2007). Moderate alcohol consumption has been associated with lower pulse wave velocity even after adjusting for blood pressure and other variables and there seems to be a J-shaped relationship between alcohol intake and pulse wave velocity (Sierksma *et al.*, 2004).

Mahmud and Feely found that the ingestion of a single alcoholic drink caused an immediate reduction of arterial stiffness and pressure, but long-term excessive use increased stiffness (Mahmud and Feely, 2002a). In an other study Mahmud and Feely, found both PWV and Alx has been increased 90 minutes after 150 mg of caffeine ingestion In healthy subjects (Mahmud and Feely, 2001).

1.5. TREATMENT OF ARTERIAL STIFFNESS

1.5.1. Life style modification

1.5.1.1. Sodium intake

Of all dietary factors, sodium intake probably has the most potent effect on arterial stiffness. Cross-sectional findings indicate that subjects who follow a low-sodium diet have more compliant arteries than age- and blood pressure-matched control subjects with higher sodium intake (Elliott et al., 1996, Avolio et al., 1986). Moderate dietary sodium restriction improved carotid Alx and ultrasonographically measured arterial compliance in postmenopausal women independent of changes in body weight, mean blood pressure, plasma volume, and heart rate indicating a direct effect of sodium restriction on arterial stiffness (Gates et al., 2004, Safar et al., 2000). Thus, high salt intake accelerates arterial aging, and both short-term and long-term sodium restriction decreases arterial stiffness independent of the effect on mean blood pressure (Et-taouil et al., 2001).

1.5.1.2. Weight loss

A number of intervention studies have investigated the short-term effects of weight loss on arterial stiffness and have shown that a reduction in body weight is associated with reductions in large artery stiffness (Ludvig et al., 2005, Toto-Moukouo et al., 1986, Balkestein et al., 1999, Wildman et al., 2005). But the effect of weight reduction on arterial stiffness may be merely an epiphenomenon of concomitant decreases in blood pressure (Tanaka and Safar, 2005) However, a recent intervention study showed that moderate weight loss reduces aortic pulse wave velocity in patients with type 2 diabetes independent of blood pressure (Barinas-Mitchell et al., 2006).

1.5.1.3. *Dietary modification*

Several dietary supplements seem to have an effect on arterial stiffness independent of their effect on body weight. Supplementation of n-3 polyunsaturated fatty acids found in fish oil improves systemic arterial compliance in dyslipidaemic subjects, most likely by lowering triglycerides and LDL concentrations (Nestel *et al.*, 2002). In an intervention study on healthy volunteers, administration of isoflavones that bind to human estrogen receptors reduced pulse wave velocity after six weeks (Teede *et al.*, 2003). Three weeks of folic acid supplementation increased systemic arterial compliance in a placebo-controlled study (Williams *et al.*, 2005).

The antioxidant vitamin C (ascorbic acid) has been reported to reduce pulse wave reflection acutely and after four weeks of oral administration (Wilkinson *et al.*, 1999, Mullan *et al.*, 2002). Similarly, vitamin E intake induced a substantial decrease in systemic arterial stiffness in middle-aged subjects (Mottram *et al.*, 1999). These results could however be a consequence of reduced peripheral resistance induced by antioxidative vitamins. Contrary to these results, a recent study involving both short-term and long-term administration of ascorbic acid did not show any effect on carotid arterial stiffness (Eskurza *et al.*, 2004).

1.5.1.4. *Smoking Cessation*

Cigarette smoking is a leading cause of atherosclerosis through damage to the endothelium and increased fibrinogen levels (Kannel *et al.*, 1987a, Zahler and Piselli, 1992). Over time, inhaled carbon monoxide from cigarette smoke damages the endothelium and accelerates the process of atherosclerosis (Goldstein and Niaura, 2000). Approximately 25% to 50% of the relation of cigarette smoking to the occurrence of CAD is attributable to the effect of smoking on increased fibrinogen levels in the

blood (Kannel *et al.*, 1987a). High fibrinogen levels enhance the tendency for thrombosis, leading to occlusive clinical events (Kannel *et al.*, 1987a). Smoking accelerates atherosclerosis and is associated with an increased risk of sudden death, angina, myocardial infarction (MI), peripheral vascular disease, and stroke (Benowitz and Gourlay, 1997, Ludvig *et al.*, 2005). Smoking cessation reduces the risk for recurrent coronary events to the level of healthy nonsmokers within 3 years (Kannel *et al.*, 1987a). Smoking cessation results in decreased fibrinogen levels, reduced oxidative damage from carbon monoxide, and also an increase in the ratio of high-density lipoprotein to low-density lipoprotein cholesterol (Goldstein and Niaura, 2000). These changes help reduce the risk of CAD and MI caused by atherosclerosis (Zahler and Piselli, 1992). Smoking cessation decreases mortality caused by atherosclerotic disease by 50% within 5 years in both men and women (Perkins and Dick, 1985).

1.5.2. Pharmacological (Anti-hypertensive) therapy

Structural and functional properties of the arterial wall are altered and morbidity and mortality associated with hypertension are related to arterial damage that may affect one or several organs. There are a number of strategies to reduce vascular stiffening. Several factors involve lifestyle issues, such as reducing body weight, exercise, lowering salt intake. Other strategies are pharmacological in nature, focusing on nitric oxide-dependent pathways, antioxidants, RAAS inhibitors, TGF- β inhibition, 3-hydroxy-3-methylglutaryl-coenzyme A-reductase inhibition, and AGE cross link breakers. Therefore, considering the potential implications of arterial assessment in the prevention of cardiovascular disease, evaluation of the arterial effects of antihypertensive agents is recommended (Asmar, 2001). The antihypertensive agents that are commonly used included diuretics, Alpha-blockers, Beta-blockers, Ca⁺ channel blockers, vasodilators, ACE inhibitors, angiotensin receptor blockers, and aldosterone antagonist. This section

intends to discuss whether these agents have an arterial effect independent of BP reduction.

An anti-hypertensive drug is one that reduces mean arterial pressure by decrease in systolic BP, diastolic BP and a resulting passive decrease in arterial stiffness. Antihypertensives are able to substantially reduce CVD risk in hypertensive's including the elderly (Staessen et al., 2000, Van Bortel et al., 2001). The target mechanisms are the decrease in ventricular ejection, the active decrease in arterial stiffness and the change in wave reflections (Van Bortel et al., 2001). It is even possible to target these mechanisms without influencing peripheral vascular resistance or mean arterial pressure, thus suggesting that certain medication do actually have an independent effect on arterial stiffness beyond blood pressure. However, not all anti-hypertensive drugs have such effects, thus well focus my scope on drugs with potential effects against arterial stiffness or wave reflections (Mitchell et al., 1997).

1.5.2.1. Angiotensin Converting Enzyme Inhibitors (ACEi)

Since the introduction of ACEi for the treatment of hypertension at the beginning of the 1980s, these drugs have been widely used in clinical practice to treat all stages of essential hypertension. Either as monotherapy or in combination with other antihypertensive drugs, in particular with diuretics, ACEi are effective and commonly used antihypertensive drugs (Lonn et al., 1994).

Numerous studies have been published to evaluate the effects of ACEi on arterial stiffness. Tsang et al (Tsang et al., 2006), in their study have shown that administration of quinapril causes a reduction in the augmentation index, an effect that was in part independent of BP reduction. A number of other studies have confirmed that other ACEi (Captopril, Perindopril, Ramipril, etc.) can reduce arterial stiffness as well

(Mallareddy et al., 2006, Cuspidi et al., 1997, Topouchian et al., 1998). In a study of 162 subjects with stage 1 and 2 essential hypertension who were either treatment-naïve or had not received any antihypertensive treatment for at least 6 months before the study. Results showed that Perindopril reduced mean BP and significant mean changes in PWV in addition were observed after two months of therapy. A further reduction in PWV was noted at six months. However, no correlation was seen regarding BP parameters at two months and further two months to six months changes in PWV, thus suggesting that a decrease of arterial stiffness independent of BP reduction. This signified the occurrence of a pressure-independent pharmacological remodelling of the arterial wall (Lacourciere et al., 2004).

ACEi improve large artery compliance. Benetos et al. reviewed the contribution polymorphisms of the ACEi insertion/deletion (I/D) genes on aortic stiffness regulation in hypertensive but not normotensive subjects. The presence of the ACEi allele is weakly associated with increased rigidity (Benetos et al., 1996a).

The HOPE study has shown beneficial effects of an ACEi on death, myocardial infarction, and stroke in over 9,000 high-risk patients (Anon, 1993). A meta-analysis determining the cardiovascular protection from blood pressure reduction suggested that blood pressure could have accounted for most – if not all – benefit seen in HOPE patients who were allocated the ACEi (Yusuf et al., 2000).

1.5.2.2. Angiotensin II receptor blockers (ARB)

ARB appear to be reno-protective independently of their blood pressure lowering effects (Klingbeil et al., 2003). ARB's are increasingly used in patients with hypertension; include those with concomitant clinical conditions such as heart failure and diabetic neuropathy. Because of their excellent tolerability and proved clinical efficacy, their

use is increasingly not confined to subjects with cough other contraindications to ACEi. Studies with ARB have proved they are more effective than beta-blockers in preventing stroke, and in inducing regression of left ventricular hypertrophy (Hollenberg, 2001). In addition to this a DETAIL study has shown that in those with type 2 diabetes and early nephropathy, ARBs provide long-term renoprotection similar to ACEi (Asai *et al.*, 2005). based on many of these findings, the 2003 European Society of Hypertension/European Society of Cardiology (ESH/ESC) hypertension guidelines (Barnett *et al.*, 2004), suggest that ARB are suitable for the initiation and maintenance of hypertensive therapy not dissimilarly from diuretics, beta-blockers, ACEi, and CCB. However the individual risk profile of the patient, including target-organ damage, cardiovascular or renal disease, or diabetes, should also influence the drug choice. The considerable evidence for the benefit of ARBs in patients with diabetic nephropathy has led to the American Diabetic Association guidelines recommending the use of ARB as first line therapy in patients with type 2 diabetes, hypertension, and microalbuminuria or clinical albuminuria (Nesbitt, 2004) .

The mechanisms underlying the effects of ARBs are likely to be similar to those of ACE inhibition, but may involve additional pathways. The presence of the angiotensin II type 1 receptor (AGTR1 A1166C) gene is a strong independent determinant of arterial rigidity and PWV, and more pronounced in the older subjects (Benetos *et al.*, 1996a). However, Sudhir *et al.* reported that angiotensin II-induced vascular smooth muscle proliferation in vitro was strongly enhanced by increased mechanical stretch and concluded that hypertension-associated mechanical or structural alterations may potentiate the AGTR1-mediated actions of angiotensin (Sydhir *et al.*, 1993).

To examine the effect of ARB on arterial stiffness, candesartan, improved tonic nitric oxide (NO) release and reduced vasoconstriction to endogenous endothelin 1 in the forearm of hypertensive patients after 12 months' therapy (Ghiadoni *et al.*, 2000). Topouchian *et al.* in TRANS study in patients at high cardiovascular risk found a

reduction in central and peripheral arterial stiffness after treatment with both Telmisartan (ARB) and Ramipril (ACEi), but it was more significant with ACEi (Topouchian *et al.*, 2007). Agata *et al.* Found PWV value in the ARB group was significantly lower than that in the control group, with long-term treatment (Agata *et al.*, 2004). Long-term treatment with valsartan improves vascular wall function and a haemodynamics in patients with essential hypertension. Okura, *et al.* showed in his study that Valsartan did not influence IMT; however, after 24 months, it caused a significant decrease in stiffness index beta compared to baseline. These results suggest that long-term treatment with valsartan improves vascular wall function and haemodynamics in patients with essential hypertension (Okura *et al.*, 2008).

1.5.2.3. Alpha-blockers and beta blockers

Both aortic compliance and endothelial dysfunction improved with doxazosin, an alpha-blockade in subjects with mild essential hypertension (Komai *et al.*, 2002, Dell'Omo *et al.*, 2005), whereas little or no significant effects on arterial stiffness were reported with prazosin (O'Rourke, 1992, Yıldız *et al.*, 2005).

Several studies have looked in-depth at the effect of beta blockers on arterial stiffness. Nebivolol, a relatively new vasodilating highly-selective β_1 adrenoceptor antagonist, stimulates NO production, which is an important regulator for arterial distensibility, may be beneficial in conditions of increased large artery stiffness, such as isolated systolic hypertension (McEniery *et al.*, 2004, Cockcroft, 2004). A recent study shows that the beta-blockers, atenolol and nebivolol, have a similar effect in reducing arterial stiffness in the large elastic aorta, largely secondary to BP reduction. Nebivolol, in contrast to Atenolol, has an effect on small muscular arteries, increasing PP amplification and reducing wave reflection, possibly because of increased levels of nitric oxide (NO). Such ancillary properties may impart important distinct hemodynamic effects, and

therefore beta-blockers cannot be regarded as a homogeneous group (Mahmud and Feely, 2008).

Studies that evaluate the effects of Atenolol in altering arterial wave reflections have yielded positive results (London et al., 2004, Chen et al., 1995, Pannier et al., 2001). However, controversies have arisen when comparing the effectiveness of atenolol against other antihypertensive medications such as perindopril/ indapamide. One study (Pannier et al., 2001) reported that atenolol is more effective than perindopril in reducing aortic PWV and improving arterial stiffness. The REASON project however found that perindopril/indapamide normalized SBP, pulse pressure, and arterial function to a greater extent than atenolol (Asmar et al., 2001, Cockcroft, 2005, de Luca et al., 2004). In the LIFE (Devereux et al., 2004) and ASCOT (Dahlof et al., 2005) studies, losartan- and amlodipine-based treatments, respectively, proved to be more effective than atenolol-based treatments for reducing CV events. β -blockers devoid of vasodilating properties are less effective for reducing central PP and AIX than vasodilating β -blockers (including celiprolol, dilevalol, and nebivolol), and other antihypertensive drugs (Laurent et al., 2006). All these studies thus concluded that β blockers have an effect against arterial stiffness, although the effectiveness is variable, depending on different types of β blocker.

1.5.2.4. Calcium channel blockers (CCB)

In a study looking at nineteen healthy volunteers who underwent maximal-effort upright ergometry tests after receiving verapamil or saline in a double-blind, randomized, crossover study, it was shown that baseline vascular stiffness, indexed by arterial PWV and augmentation index, declined with verapamil (Chen et al., 1999). Similarly, in another double-blind study to look at hypertensive subjects treated with verapamil, trandolapril, or their combination, showed that both compounds and more significantly

combination therapy, decreased mean blood pressure (Verapamil, $-10\% \pm 9$; 113 ± 8 to 101 ± 11 mmHg; Verapamil+Trandolapril, $-16\% \pm 9$; 118 ± 7 to 99 ± 11 mmHg), pulse pressures (Verapamil, aortic: 41 ± 11 to 37 ± 9 mmHg; Verapamil+Trandolapril, 44 ± 11 to 38 ± 10 mmHg), PWV (Verapamil, $-13\% \pm 6$ m/sec; Verapamil+Trandolapril, $-14\% \pm 10$ m/sec) and that changes in diameter, thickness, and stiffness were recorded (Van Bortel *et al.*, 2001). With verapamil, the diastolic diameter (mm) of the abdominal aorta reduced from 19.1 ± 2.8 to 17.8 ± 2.0 mm with a significant increase in stroke and changes in diameter ($P < 0.01$) and distensibility ($P < 0.01$, kPa^{-1} , 1.0 ± 0.4 to 1.4 ± 0.6).

Acute intravenous calcium channel blockers reduce vascular stiffening highlighting a role as a potentially therapeutic target for arterial stiffness, independent of anti-hypertensive effect. Baseline vascular stiffness, indexed by arterial PWV and augmentation index declined with calcium channel blockers ($-5.9\% \pm 2.1$ and $-31.7\% \pm 12.8$, respectively) (Topouchian *et al.*, 1999).

1.5.2.5. Diuretics

There is no effect of diuretics, like hydrochlorothiazide, on elastic properties of the arteries (Breithaupt-Grogler, 1996). In a randomised double blind study by Breithaupt-Grogler *et al.* (Benetos *et al.*, 1996b) subjects were allocated into 2 groups, Group 1 was given hydrochlorothiazide plus amiloride, Group 2 was given hydrochlorothiazide plus captopril. Both groups were investigated in terms of the arterial changes, in the common carotid artery and the terminal aorta, by evaluating the arterial stiffness and carotid wave reflections. Both groups showed a significant decrease in brachial BP, carotid diastolic diameter and an increase in aortic compliance and distensibility. However, wave reflections were modified in Group 2 but not in Group 1. Thus, captopril associated with hydrochlorothiazide resulted in a shift in arterial reflection wave from systole to diastole with no reduction in aortic diastolic dimension. For similar BP

reduction, the combination of hydrochlorothiazide and amiloride had no significant effect on the carotid reflection wave. Therefore, the study suggested that diuretics will only lower BP without significant alteration in aortic stiffness.

A study that compared the effect of losartan (50mg.day) to hydrochlorothiazide (12.5mg/day) on BP and arterial stiffness produced a significant and similar decrease in brachial BP, but only losartan induced a significant decrease in arterial wave reflection, increased pulse pressure amplification and reduced pulse wave velocity (Mahmud and Feely, 2002b). Smulyan et al. showed in his study that diuretics may increase forearm arterial compliance by lowering blood pressure without a demonstrable drug effect on arterial wall (Smulyan et al., 1984). Benetos et al. In a randomised study comparing hydrochlorothiazide (50mg) plus amiloride (5 mg) to hydrochlorothiazide (25mg) / captopril (50mg) combination, showed the ACEi-diuretics combination decreased arterial wave reflection despite similar BP reduction with two regimens (Benetos et al., 1996b). This may suggest a role for the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system activation secondary to salt and water depletion favouring arterial construction and increased arterial stiffness as a possible explanation why diuretics, that lower blood pressure favouring reduced stiffness, do not in practice reduce stiffness (Mahmud, 2007).

1.5.2.6. Other drugs

1.5.2.6.1 Vasodilators

Certain vasodilators showed favourable effects on arterial stiffness, e.g. glyceryl trinitrate (GTN), which can be attributed to effects on peripheral muscular arteries causing reduction in wave reflection (Pauca et al., 2005), whereas hydralazine have little or no significant effects (O'Rourke, 1992).

1.5.2.6.2. Aldosterone antagonist

Several *in vitro* investigations have indicated that aldosterone may act directly on large arterial vessels. Mineralocorticoid receptors predominate in the aorta and their distribution decreases with the size of the arteries, shown by Immuno-histochemical studies (Safar, 1988). In hypertensive subjects, increased aortic stiffness and plasma aldosterone levels are statistically associated in some (Roman *et al.*, 2000) but not all studies (Asmar, 2001). Safar and London, did not find a change in brachial artery stiffness after administration of spironolactone in hypertensive patients (Safar and London, 1994). Six weeks' treatment in an open study with the aldosterone antagonist, canrenone (50 mg) daily, did not alter PWV in a hypertensive population (Van Bortel *et al.*, 2001). However, in a randomized controlled one-month study in a hypertensive population, comparing spironolactone (50 mg) to bendrofluzide (2.5 mg), only spironolactone reduced PWV and Alx and, following adjustment for change in BP, the reductions remain significant (Mahmud and Feely, 2005a); hypertensive patients with the highest aldosterone-to-renin ratio (ARR) had the greatest fall in aortic BP and Alx% (Mahmud and Feely, 2005a). The more selective aldosterone antagonist, eplerenone, has also been shown in a comparative study to reduce aortic PWV in patients with systolic hypertension and wide PP (White *et al.*, 2003).

1.6. AIMS OF PRESENT STUDIES

The major objective of the present studies was to assess the utility of the Arteriograph and to examine arterial stiffness in healthy subjects and hypertensive patients in particular.

- I. The repeatability and interrelationship between repeated measures of arterial stiffness in young healthy subjects (I) and to determine the effects of stress (mental and physical) and environmental change on arterial stiffness using Arteriograph (TensioMed) in normal healthy subjects (II).
- II. As there was time between this study and the longer term objective of my research, I had the opportunity to assess the effect of smoking and smoking cessation on arterial stiffness first using the standard SphygmoCor and Complior techniques from an established data base and later on to generate similar data using Arteriograph in untreated hypertensive patients.
- III. To examine the validation of oscillometric Arteriograph (TensioMed) by comparing it to SphygmoCor (AtCor Medical, Version 8.0) and Complior (Artech Medical) in untreated hypertensive subjects referred for assessment of high blood pressure.
- IV. To evaluate the effects of five antihypertensive drug groups (ACEi, ARB, BB, CCB and Thiazide diuretics) on the change in arterial stiffness indices PWV and Alx in hypertensive subjects when treated for 4 to 6 weeks by using Arteriograph (TensioMed).

It is clear from my review that the measurement of arterial stiffness will become common place. It is now recommended in all hypertensive in the current European guidelines on hypertension (Authors/Task Force *et al.*, 2007) and is used in epidemiological, physiological and pharmacological studies. However the standard Complior and SphygmoCor techniques require considerable expertise and the patient to partially undress and are time consuming. The Arteriograph technique is akin to measuring blood pressure and both PWV and Alx are recorded simultaneously. It may represent such a practical method of recording stiffness.

CHAPTER 2

METHODS

2.1. GENERAL INFORMATION

2.1.1. Ethical Aspects

All studies described in this thesis were approved by the St James's Hospital and Federated Dublin Voluntary Hospital Research Ethics Committee. The nature of the study was first explained to the potential volunteers and an information leaflet was given to subjects. Patients were referred to the Hypertension Clinic at St James's Hospital for assessment of blood pressure. Volunteers were by and large medical students and they also gave informed consent.

2.1.2. Healthy young normotensive subjects (Study I [A and B] and StudyII)

Young normal healthy (medical students (Trinity College, University of Dublin) with normal blood pressure (systolic BP <140/ and diastolic BP < 90 [mm Hg] see *Table 2.1*). Subjects' characteristics (Study-I and II) are given in *Table 2.2*. None of them had any acute or chronic illness or history of hypertension.

Table 2.1 Blood pressure (BP) levels suggested by American Heart Association (Chobanian *et al.*, 2003), European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) (Authors/Task Force *et al.*, 2007).

Blood Pressure Category	Systolic BP (mm Hg)		Diastolic BP (mm Hg)
Normal	120 or lower	and	80 or lower
High or Hypertension	More than 140	or	More than 90

Table 2.2 Characteristics of normal healthy subjects and hypertensive patients

	Control Subjects			Hypertensive Patients			
Chapter	Three	Three	Four	Five		Six	Seven
Study	I-A	I-B	II	III	IV	V	VI
Number	60	22	23	554	254	254	114
Gender (M:F)%	45:55	39:61	36:64	44:56	56:44	56:44	64:36
Age (years)	20-39	18-30	18-30	18-80	17-85	17-85	23-70

Young healthy normotensive subjects (Study I-A) underwent for the assessment of arterial stiffness and repeated measurements were recorded on same day and by two different observers on two separate days (Study I-B) for to determine the reliability of the oscillometric device Arteriograph (TensioMed™). The effect of mental arithmetic stress (mental arithmetic test [MAT]), hand exercise (maximal voluntary contraction [MVC]) and cold pressor test (CPT) were examined in young healthy normotensive subjects (Study II).

2.1.3 Untreated hypertensive patients (Study III, IV, V and VI)

Untreated hypertensive patients with elevated blood pressure (BP >140/90 mmHg, see *Table-2.1*), were recruited from the hypertensive clinic at St. James's Hospital Dublin. Hypertension was confirmed by ambulatory blood pressure recording, with day time level >135/85 mmHg (*Table 2.3*). Patients' characteristics (Study III, IV, V and VI) are given in *Table 2.2*.

Untreated hypertensive patients underwent for the assessment of hypertension, and smoking status (Study-III and IV) recorded. I compared the Arteriograph to SphygmoCor and Complior and the determinants of Alx and PWV using the respective techniques in untreated subjects referred for assessment of high blood pressure (Study V).

In study VI patients were assessed for the hypertension and for the treatment effect (Single drug) of different five antihypertensive drugs. These including angiotensin converting enzyme inhibitors (ACEi), angiotensin II receptor inhibitors or blockers (ARB), β -blockers (BB), calcium channel blockers (CCB), and thiazide diuretics and subjects were studied on two separate visits (before and after four to six weeks treatment).

None of the healthy volunteers (Study I-A, I-B and II) and hypertensive patients (Study III, IV, V and at baseline in study VI) were on antihypertensive medication or any agents that influence blood pressure, such as oral contraceptives, steroids, or hormone replacement therapy. None of the patients had secondary hypertension, evidence of any vascular disease, cerebrovascular accident, coronary artery disease, valvular heart disorder, dysarthrias, diabetes, heart failure or any other significant medical conditions.

Exclusion criteria were blood pressure-lowering medication, secondary hypertension, unstable coronary artery disease, diabetes mellitus, malignant diseases, and pregnancy.

Table 2.3: Values suggested by the American Heart Association for daytime, nighttime, and 24-hour average blood pressure (Pickering *et al.*, 2005), European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) (Authors/Task Force *et al.*, 2007)

	Optimal	Normal	Abnormal
Daytime	<130/80 mm Hg	<135/85 mm Hg	>140/90 mm Hg
Nighttime	<115/65 mm Hg	<120/70 mm Hg	>125/75 mm Hg
24-hour	<125/75 mm Hg	<130/80 mm Hg	>135/85 mm Hg

2.2. METHODOLOGICAL CONSIDERATION IN MEASURING ARTERIAL STIFFNESS

2.2.1 Study protocol

All subjects' stiffness measurements were studied at similar time between 9:00 am to 5.00 pm. On first assessment day (study III and IV) and subsequently on two separate assessment days within 4 to 6 weeks (study V and VI), and five separate assessment days within 3 weeks [study I and II]. Prior to each visit subjects were asked to refrain from using alcohol or caffeine contained food and drinks for at least 24 hours, smoking and exercise for at least 30 minutes, but could otherwise maintain their usual lifestyle between assessment days. All testing procedures took place in a temperature controlled (22 °C) quiet room.

2.2.1.1 Maximal voluntary contraction

In study II the maximal voluntary contraction (MVC) was recorded; a calibrated hand grip dynamometer (HK51020; SUNCREA, Tokyo, Japan) was used to measure MVC (isometric hand grip exercise). To familiarise the subject and overcome a learning phenomena MVC was determined one day prior to the study, with the right hand by dynamometer, the arm positioned at right angles and the elbow by the side of the body. The handle of the dynamometer was adjusted according to subjects hand requirement. The base should rest on first metacarpal (heel of palm), while the handle should rest on middle of four fingers. The subjects squeezed the dynamometer with maximum isometric effort or MVC twice (each lasting 3-5 seconds) with 2 minutes time interval, the largest of which was used to determine the relative intensity of the MVC.

No other body movements were allowed and Valsalva maneuver was avoided to preventing from recruitment of accessory muscles.

On next visit on separate day after baseline measurement (Arteriograph) the subjects were asked to sustain handgrip on 30% strength of MVC for 3 minutes (throughout the duration of the Arteriograph readings). The handgrip pressure was released and two further measurements (Arteriograph) were recorded 5mins and 10mins post MVC respectively.

2.2.1.2 Cold pressor test

The cold pressor test (CPT) was performed in the young normal healthy subjects (In study II), this is a classical experiment often used to induce the sympathetic system activation there by producing arteriolar vasoconstriction and an increase in blood pressure. After the baseline measurements with Arteriograph the subjects were instructed to immerse their right hand in cold water at 3 to 4°C (constant temperature) for 3 minutes (throughout the duration of the Arteriograph readings). The hand was removed from the cold water and two further measurements (Arteriograph) were recorded 5mins and 10mins post CPT respectively.

2.2.1.3 Mental arithmetic test

The mental arithmetic test (MAT) was performed in the young normal healthy subjects (In study II). Mental and physical stress release catecholamine's, which is a major Pathophysiological mechanism, it may also contribute to endothelial dysfunction. Both catecholamine levels and endothelial function are regulators of arterial stiffness and wave reflections. After the baseline measurements with Arteriograph the subjects were

asked to subtract the number "13" respectively serially starting from "1079" while the mind distract methods like making noise for to distract the subjects and add mental stress for 3 minutes (throughout the duration of the Arteriograph readings). Then subjects were asked to relax and two further measurements (Arteriograph) were recorded 5mins and 10mins post MAT respectively.

2.2.2 Physiological Measurements

All subjects (study I, II, III, IV, V and VI) were assessed on first visit, their anthropometric measurements were recorded i.e. weight measurement to the nearest 1 kg, height to the nearest 1 cm (using a Stadiometer and a digital weight scale). Waist and hip circumferences were recorded in each patient at the largest circumference of the abdomen below the ribs (normally umbilical level) and hip circumference at level of throcanter major to the nearest 1 cm (using Tape measure). Body mass index (BMI) was calculated as body weight (kilograms) divided by height (meters squared), and the waist: hip ratio was calculated.

2.2.3 Blood sampling and analysis

Blood samples were obtained from patients (study III, IV, V and VI) attending the hypertension clinic, St. James' Hospital, Dublin. After an over night fasting, blood samples were taken in the morning at 9 to 10.30 A.M, from the antecubital vein. Blood samples were transported to the main laboratory immediately for standard haematological (full blood count) and biochemical (renal function test, glucose, cholesterol and lipids) indices by routine automated techniques within the hospital central laboratory. A single certified laboratory (Dept. of Chemical Pathology, St James's Hospital Dublin) was used for all analyses.

2.2.4 Blood pressure measurements

All subjects (study I, II, III, IV, V and VI) were rested in a supine position for 5 minutes in a quiet room at 22°C before the baseline hemodynamic measurements were obtained. Brachial blood pressure (BP) and heart rate were measured in the right arm by sphygmomanometer using an automated digital oscillometric sphygmomanometer (Omron, Model HEM 705-CP, Omron Corporation). This device has been validated and is approved by the British Hypertension Society. An appropriately sized cuff (cuff bladder encircling at least 80% of the arm) was used to ensure accuracy of measurement of the blood pressure readings.

2.2.5 Arteriograph (TensioMed™)

In study I, II, III, V and VI the Arteriograph cuff with a sensitive sensor (very high fidelity oscillometric tonometers) is attached for detecting in a sufficiently good quality the weak signals appearing by the cuff (*Figure 2.1*). The cuff was applied on the left arm, which measures blood pressure (BP [mmHg]) first and then immediately the cuff pressurized at least 35 mmHg more than the actual systolic blood pressure (while the brachial artery is completely closed), and Alx (%) and PWV (m/sec) measurements were detected. The system can detect the signals central pressure changes as early (direct) systolic wave (P1), late (reflected wave (P2) and diastolic wave (P3). These data are transferred for detailed analysis to the PC by a wireless connection.

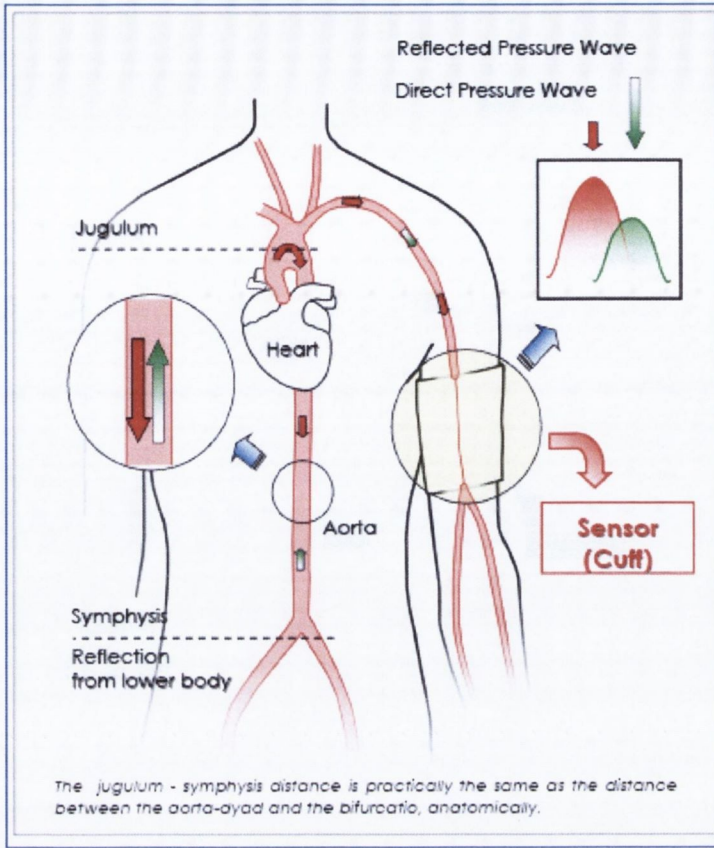


Figure 2.1: Above shows the measurement method and bottom one is the oscillometric Arteriograph (TensioMed™)

2.2.6 Complior [Artech Medical]

Carotid-femoral pulse wave velocity was measured (study III, IV, V and VI) using an automated system (Complior [Artech Medical]) using the foot-to-foot method. The carotid and femoral waveforms were acquired simultaneously with 2 pressure-sensitive (Carotid and Femoral) transducers (Figure 2.2) and pulse wave velocity was calculated by the system software. The distance between the 2 arterial sites (Carotid and Femoral) was measured on the body using a tape measure, and PWV was calculated as the distance divided by time (meters per second). At least 12 successive readings were used for analysis to cover complete respiratory cycle.

2.2.7 SphygmoCor (AtCor Medical, Version 8.0)

The aortic pressure waveforms were recorded non-invasively at the wrist (right arm) using radial applanation tonometry, utilising a previously validated transfer function relating radial to aortic pressure waveform within the system software of the SphygmoCor (SphygmoCor, AtCor Medical, Version 8.0 see figure 2.3) by a single operator (study III, IV, V and VI). Two measurements were derived from the aortic pressure waveform on each visit. The aortic Alx was calculated as the augmentation of the aortic systolic pressure by the reflected pulse wave expressed as a percentage of the aortic pulse pressure. The time to return of the reflected wave or transit time (T_R) was calculated as the time from the beginning of the derived aortic systolic pressure waveform to the inflection point. T_R can be used as a substitute for pulse wave velocity (a higher pulse wave velocity will result in a shorter transit time) (Weber T *et al.*, 2005). All measurements were subjected to quality control by the software and only high-quality recordings were included in the analyses.

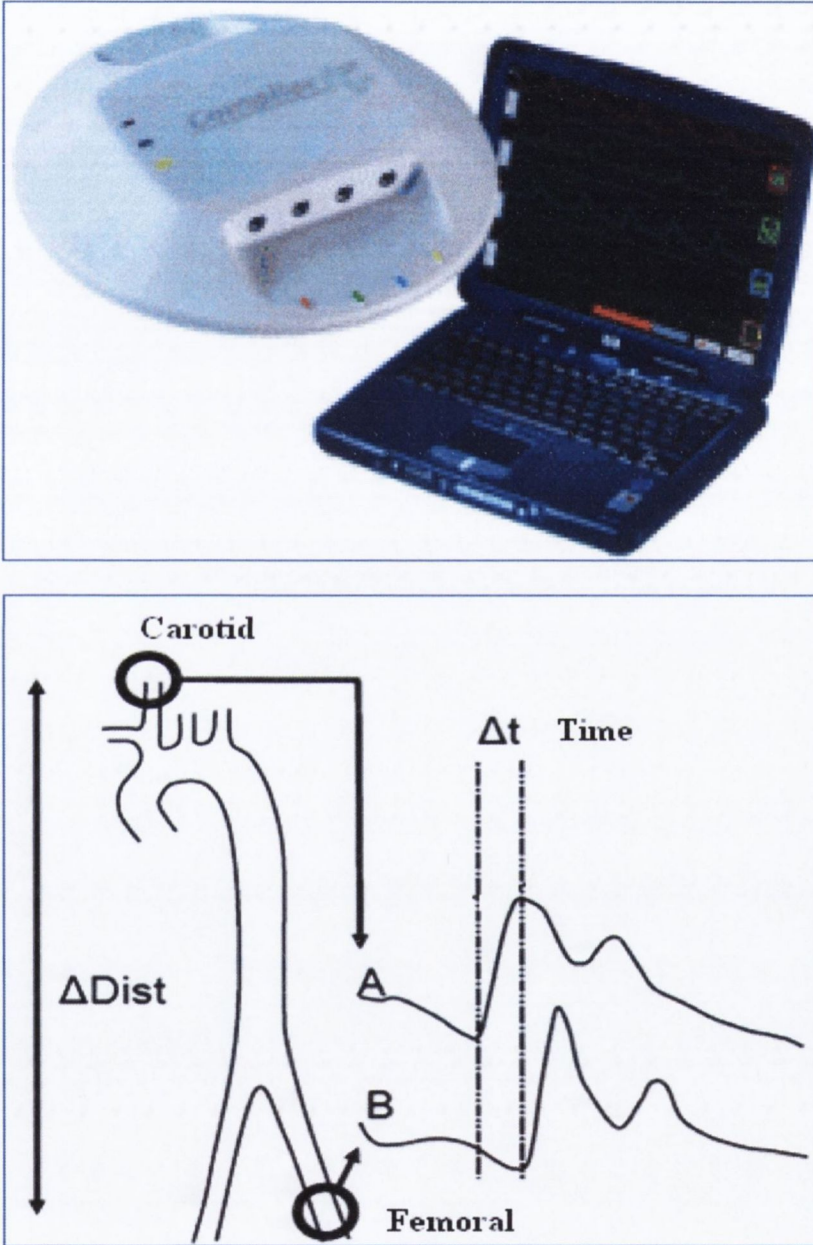


Figure 2.2: Complior (Artech Medical) and schematic drawing of the pulse wave velocity method.



Figure 2.3: SphygmoCor (SphygmoCor, AtCor Medical, Version 8.0)

2.2.8 Smoking Status

Smoking status was assessed through questionnaire. The subjects were asked four questions.

- (1) *If they had ever smoked, answer possibilities were "yes" or "no" If the subjects answered "no" to the first question, they were not asked the more questions. If they answered "yes" than*
- (2) *How often they smoked.*
- (3) *Were they still smoking, answer possibilities were "yes" or "no" If the subjects answered "yes" to the third question, they were not asked the more questions. If they answered "no" to 3rd question than*
- (4) *When they completely stopped smoking.*

Current smokers: As those who had smoked ≥ 1 cigarette per day for 1 year.

Nonsmokers: As those who had never smoked.

Former or ex-smokers: As those who had stopped smoking ≥ 1 month before examination.

For purposes of my smoking cessation study (study III) ex-smokers were categorized into 3 subgroups according to smoking cessation duration:

Those who quit cigarette smoking for ≤ 1 year

Those who quit cigarette smoking for between 1 and 10 years

Those who quit cigarette smoking for ≥ 10 years

2.3. STATISTICAL METHODS

In general, all data in studies I, II, III, IV, V and VI were analysed using JMP software (Version 7.0, SAS for Windows NC,USA) and in study III using SPSS (version 12.0). Results were expressed as mean standard deviation (Mean \pm SD) and / or standard error of mean (Mean \pm SEM) for continuous data or percentage (%) with 95% confidence intervals for categorized variables. $P < 0.05$ was considered statistically significant.

Comparisons between groups were performed using the unpaired t test and ANOVA for normally distributed variables, the Mann-Whitney and Kruskal-Wallis for non-normal variables (Study I, V and VI). Simple linear regression was used to examine univariate association; more complex association was analyzed by means of multiple regression analysis using forward stepwise regression.

The reproducibility of blood pressure and arterial stiffness indices was likewise calculated (MedCalc ® Version 9.3.9.0). Bivariate relationships were examined (Study II) using student t -test and ANOVA to test the significance among continuous variables. The differences observed between the average values of PWV and Alx (Arteriograph) measured by two observers, and correlations between two measurements on same day. Coefficients of variation in two visits and two observers' measures for PWV and Alx were observed. The relationship between parameters was analysed using correlation (Spearman Rho). Regression analysis of PWV and Alx were analysed separately by using the following determinants: age, body height, gender, and HR (forward stepwise regression). Regression coefficients and 95% confidence intervals are presented.

In study III, mean differences in PWV, Alx, and T_R between never smokers, ex-smokers, and current smokers and the effects of duration of smoking cessation were assessed using ANOVA with posthoc Bonferroni corrections for multiple comparisons. To

determine whether ex-smokers had arterial stiffness levels intermediate between never and current smokers, linear regression analysis was used to establish the presence of a linear trend. Three models were used: (1) adjusted for age and sex; (2) adjusted for age, sex, and mean arterial pressure; and (3) additional multivariate adjustment for the other major determinants of arterial stiffness, that is, heart rate and BMI (Study III and IV).

In study V, the difference observed between the average values of PWV (Arteriograph vs Complior) according to Bland-Altman (Bland and Altman, 1986) and the relationship between the values of Alx (Arteriograph vs SphygmoCor) in scatter plot, were likewise calculated (MedCalc ® Version 9.3.9.0) as an estimate of measurement error for the repeat measurements between two methods. For Alx we normalized the data to the standard deviation as values for Arteriograph are largely negative while those for SphygmoCor were positive. The relationship between parameters was analysed using correlation (Spearman Rho). Regression analysis of PWV and corrected Alx were analysed separately by using the following determinants: age, body height and weight, gender, heart rate and mean arterial pressure (forward stepwise regression). In addition, the differences between the standard and studied technique were calculated and regression analysis applied to obtain the determinants (as above) of any discrepant bias. Regression coefficients and 95% confidence intervals are presented.

All data in study VI was reported at baseline (visit 1) and follow up (visit 2). Paired sample student *t* test and repeated measures analysis of variance were used to determine the drugs effect. The difference observed between the average values of change in PWV (Arteriograph vs Complior) following treatment with major antihypertensive drug groups, according to Bland-Altman (Bland and Altman, 1986) and the relationship between the values of change in Alx (Arteriograph vs SphygmoCor) in scatter plot, following treatment with major antihypertensive drug groups, were likewise calculated (MedCalc ® Version 9.3.9.0) as an estimate of measurement error for the repeat measurements between two methods. The

relationship between the change in average values (pre and post treatment with all antihypertensive drugs group) of PWV (Arteriograph vs Complior) and Alx (Arteriograph vs SphygmoCor) in line plot, were likewise calculated (Microsoft Excel [2002]).

CHAPTER 3

REPRODUCIBILITY OF ARTERIAL STIFFNESS INDICES IN YOUNG HEALTHY NORMOTENSIVE SUBJECTS USING OSCILLOMETRIC DEVICE ARTERIOGRAPH

**REPRODUCIBILITY OF ARTERIAL STIFFNESS INDICES IN YOUNG HEALTHY
NORMOTENSIVE SUBJECTS USING OSCILLOMETRIC DEVICE ARTERIOGRAPH****3.1 BACKGROUND**

A number of techniques and devices have been developed for the assessment of arterial stiffness, some of which are more widely used in clinical settings. Arteriograph is one of the recently developed oscillometric device for the measurement of both indices PWV and AIx of arterial stiffness. The purpose of the present study was focused on the following points: (I) to determine; the reliability of the oscillometric device Arteriograph (TensioMed™) for the assessment of arterial stiffness, (II) the relationship between the repeated measures of arterial stiffness parameters by same operator on multiple visits on same subjects; and (III) the relationship among of the repeated measurements of arterial stiffness between two observers by using same oscillometric method (Arteriograph [TensioMed™]).

3.2 METHODS

Methods are described in chapter two.

3.3 RESULTS

The characteristics of young healthy normotensive blood pressure (< 140/90 mmHg) are given in *Table 3.1*. There were no significant differences between visit 1 to visit 5 (*Table 3.2*) and observer 1, observer 2. The correlation coefficients between two measurements on the same day by same operator was HR 0.97, systolic-BP 0.97, diastolic-BP 0.87, pulse pressure (PP) 0.89, mean arterial pressure (MAP) 0.94, PWV 0.85 and Alx 0.97 ($P < 0.0001$). The agreement between the two observer's measurements and their correlation coefficients of HR, systolic BP, diastolic BP, pulse pressure (PP), mean arterial pressure (MAP), PWV and Alx are shown in *Table 3.3*.

The coefficients of variation in two visits for PWV and Alx were 1.1 5m/sec and 1.04% respectively; and the coefficients of variation in two observers for PWV and Alx were 1.03 m/sec and 1.17% respectively.

Determinants of arterial stiffness indices PWV was significantly correlated with BMI only in univariate analysis in stepwise regression analysis of PWV the independent determinants were gender and height ($R^2 = 0.18$ p 0.083 [*Table 3.4*]). Alx was significantly correlated with age, height, BMI and PWV in univariate analysis (*Table 3.5*), in stepwise regression analysis of Alx the independent determinants were age, height and HR ($R^2 = 0.46$ $p < 0.0001$).

Table 3.1 - Characteristics of the young healthy normotensive subjects (n=60)

Note: All the data in Table 1 are expressed as mean \pm SD or %.

Abbreviations: SD= Standard deviation, % = percent, A = Arteriograph and † = Sphygmomanometer.

Variables	Mean \pm SD or (%)
Age (years)	23 \pm 3
Sex (m/f) %	45:55
Height (cm)	172 \pm 8
Weight (kg)	69 \pm 12
Body Mass Index (kg/m ²)	19.94 \pm 8.9
Non-Smoker/Ex-Smoker/ Smoker (%)	82: 05: 13
Alcohol consumption (No/Yes) %	25:75
Alcohol Units/week	6.1 \pm 8.3
Heart Rate (min ⁻¹) †	68 \pm 12
Systolic Blood Pressure (mmHg) †	117 \pm 12
Diastolic Blood Pressure (mmHg) †	66 \pm 6
Pulse Pressure (mmHg) †	50 \pm 9
Mean Arterial Pressure (mmHg) †	83 \pm 7
Pulse Wave Velocity (m/sec) A	6.5 \pm 0.8
Augmentation Index (%) A	-63 \pm 17

Table 3.2: Haemodynamic and arterial stiffness indices reproducibility measured by same observer on 5 different visits on separate days (n = 22, Mean \pm SD). † = Sphygmomanometer

Variables	Visit -1	Visit-2	Visit-3	Visit-4	Visit-5
Heart Rate (min ⁻¹) †	66 \pm 10	67 \pm 12	67 \pm 10	70 \pm 12	69 \pm 12
Systolic Blood Pressure (mmHg) †	112 \pm 11	114 \pm 11	112 \pm 11	115 \pm 12	112 \pm 11
Diastolic Blood Pressure (mmHg) †	66 \pm 6	65 \pm 5	64 \pm 6	67 \pm 5	64 \pm 5
Pulse Pressure (mmHg) †	46 \pm 9	49 \pm 9	47 \pm 10	48 \pm 9	48 \pm 10
Mean Arterial Pressure (mmHg) †	82 \pm 6	81 \pm 6	80 \pm 7	83 \pm 7	80 \pm 6
Pulse Wave Velocity (m/sec, Arteriograph)	6.59 \pm 0.8	6.78 \pm 0.8	6.7 \pm 0.8	6.83 \pm 1.1	6.85 \pm 0.8
Augmentation Index (%.Arteriograph)	-74.2 \pm 13	-73.6 \pm 12	-74.85 \pm 13	-75 \pm 13	-75.1 \pm 11

Table 3.3: Haemodynamic and arterial stiffness indices reproducibility measured by two observers (n = 22)**Abbreviations:** SD = Standard deviation, R = correlation coefficient,

Variables	Observer-1 Mean±SD	Observer-2 Mean±SD	R	P Value
Heart Rate (min ⁻¹) †	68±12	69±12	0.923562	<0.0001
Systolic Blood Pressure (mmHg) †	114±11	113±9	0.958718	<0.0001
Diastolic Blood Pressure (mmHg) †	65±5	65±5	0.817643	<0.0001
Pulse Pressure (mmHg) †	49±8	48±8	0.94958	<0.0001
Mean Arterial Pressure (mmHg) †	81±7	81±6	0.88744	<0.0001
Pulse Wave Velocity (m/sec, Arteriograph)	6.94±0.92	6.89±0.85	0.932807	<0.0001
Augmentation Index (% , Arteriograph)	-74.9±13.4	-74.7±13.2	0.9656	<0.0001

Table 3.4: Stepwise regression analysis of Alx (%) and PWV (m/sec) by Arteriograph

Model for PWV (m/sec) R ² = 0.18, P< 0.0083 (Arteriograph)				
Variables	r ²	β	SE	P Value
Gender [Female]	0.10	-0.37	0.11	0.002
Height (cm)	0.08	-0.03	0.01	0.04
Model for Alx (%) R ² = 0.46, P< 0.0001 (Arteriograph)				
Variables	r ²	β	SE	P Value
Age (years)	0.29	2.49	0.62	0.0002
Height (cm)	0.12	-0.82	0.24	0.001
Heart Rate (min ⁻¹)	0.04	-0.27	0.15	0.07

Table 3.5: Univariate analysis of Alx (%) and PWV (m/sec) by Arteriograph

* = P < 0.001, ** = P < 0.01, *** = P < 0.05, NS= P Not Significant,

Variable	Alx (%) Correlation Coefficient Arteriograph	PWV (m/sec) Correlation Coefficient Arteriograph
Age (years)	0.47*	0.07 NS
Height (cm)	-0.36***	-0.04 NS
Weight (kg)	-0.2 NS	0.15 NS
BMI (kg/m ²)	0.05***	-0.28***
Heart Rate (min ⁻¹) †	-0.25 NS	0.10 NS
Systolic Blood Pressure (mmHg) †	0.11 NS	0.05 NS
Diastolic Blood Pressure (mmHg) †	0.05 NS	0.22 NS
Pulse Pressure (mmHg) †	-0.17 NS	-0.07 NS
Mean Arterial Pressure (mmHg) †	-0.05 NS	0.16 NS
Pulse Wave Velocity (m/sec, Arteriograph)	-0.03 NS	-----
Augmentation Index (% , Arteriograph)	-----	-0.03 NS

3.4 DISCUSSION

The main focus of this study was to compare the indices of arterial stiffness measured by same single operator on multiple separate visits and measured by two different observers in a young healthy normotensive population.

This study in a young healthy normotensive population demonstrates that PWV and Alx measurement values obtained by oscillometric (Arteriograph) were significantly correlated each other on 2 separate visits on the same day (PWV $r=0.85$ and Alx $r=0.97$, $P<0.0001$) and two different observers on same visit (PWV $r=0.93$ and Alx $r=0.97$, $P<0.0001$). Augmentation index correlated with BMI and negatively correlated with height, although pulse wave velocity negatively correlated with BMI only.

The determination of PWV is clinically validated, i.e. via indirect parameters and general consensus. Nevertheless, there has so far for PWV been no invasive and thus no direct validation either for SphygmoCor or Complior, whereas the determination of the augmentation index with SphygmoCor is validated invasively (Pauca *et al.*, 2001). The oscillometric method first measures the blood pressure by detecting the pulsation of the brachial artery, which is caused by the heart, as the pressure oscillation in the cuff. When the cuff around the upper arm is fully inflated, blood flow stops but pulsation of artery continue and cause oscillation of the pressure in the cuff. As the pressure in the cuff decreased slowly, the amplitude of the pressure oscillation in the cuff gradually increases and eventually reaches to a peak. Further decrease of cuff pressure causes the oscillation amplitude to decrease. Cuff pressure when the oscillation reach the peak, is taken as the mean arterial pressure (MAP) (Naidu *et al.*, 2005). The Arteriograph cuff is inflated again 35 mmHg more than the actual reading of blood pressure and determines the PWV by measuring the time elapsed between the first wave ejected from the left ventricle, and its reflection from the bifurcation as the

second systolic wave, consequently no opposite direction of the propagation modifies the measure values. The measured distance between the sternal notch to the upper edge of the pubic bone is anatomically roughly equal to aortic root-bifurcation distance (Baulmann *et al.*, 2008). In principle, the Alx can be obtained calculating the pressure difference between the first pulse wave (P1), indicated by the heart systole and the second pulse wave (P2), appearing from the reflection of P1, divided by the pulse pressure.

The independent determinants of Alx PWV after stepwise regression analysis are given in Table 5, it must be remembered this was a somewhat homogenous group of students with small numbers, little age span and possibly a diurnal variability effect. Fantin *et al.* shows in his study Alx was independently related in a wide range with age (13.1–90.3 years) (Nürnberg *et al.*, 2002). The Alx increased with age up to 55 years and tended to plateau thereafter (Fantin *et al.*, 2007). In subjects with and without cardiovascular disease, augmentation index was significantly correlated in healthy subjects with heart rate and height (Nürnberg *et al.*, 2002). During incremental pacing from 60 to 110 beats min^{-1} , Wilkinson *et al.* observed a significant and linear reduction in Alx (Wilkinson *et al.*, 2000a). This confirms the results obtained by Stefanadis *et al.*, who employed invasive ventricular pacing in younger subjects undergoing cardiac catheterization (Stefanadis, 1998). My study is in agreement with Wilkinson *et al.* and Stefanadis *et al.* they reported an inverse relationship between heart rate and Alx. Tomiyama *et al.* shows in his study PWV was lower in females than in males until age 60. Height was significantly and positively correlated with pulse wave velocity in young teen age study (Cheung *et al.*, 2002).

The results in this study show little variability and good correlations between the measurements of two independent observers and measurement of one and the same observer at different time points. Two independent observers on the same visit correlations

are better than the correlations on two different visits by same single operator. A more formal validation study in healthy volunteers has recently been published by Baulmann (Baulmann *et al.*, 2008). PWV values obtained by the Arteriograph device may vary by 0.05 m/sec on same day measurement by two observers and 0.18 m/sec on two separate visits on different days. The former is similar to that seen for PWV 0.18 m/sec in Baulmann study (Baulmann *et al.*, 2008).

3.5 CONCLUSION

The oscillometric method is therefore suitable to determine arterial stiffness and wave reflection. The study shows acceptable results those measured by single operator on different visits and as well as by two independent observers. This means that Arteriograph can measure precisely and accurately, with an acceptable reproducibility and can be reliably applicable in future clinical as well as research studies.

CHAPTER 4

**THE ACUTE EFFECTS OF MENTAL ARITHMETIC, COLD
PRESSOR AND MAXIMAL VOLUNTARY CONTRACTION ON
ARTERIAL STIFFNESS IN YOUNG HEALTHY NORMOTENSIVE
SUBJECTS**

THE ACUTE EFFECTS OF MENTAL ARITHMETIC, COLD PRESSOR AND MAXIMAL VOLUNTARY CONTRACTION ON ARTERIAL STIFFNESS IN YOUNG HEALTHY NORMOTENSIVE SUBJECTS

4.1 BACKGROUND

Human daily life is regularly subjected to mental stress, physical activity and environmental temperature adjustment. It is well known that mental stress, dynamic exercise and cold pressor all increase blood pressure (BP, mmHg) but their effects on arterial stiffness are not well described. It is of importance to not alone document said effects but also to see if a device can pick up relatively rapid changes in haemodynamics.

The aim of this study was to determine the effects of 30% maximal voluntary contraction (dynamic exercise of hand [MVC), cold pressor test (CPT) and mental arithmetic test (MAT) on arterial stiffness using Arteriograph (TensioMed).

I examined the effects of MVC, CPT and MAT in same control healthy subjects for arterial stiffness changes in a group of healthy volunteers.

4.2 METHODS

Methods are described in chapter two.

4.3 RESULTS

4.3.1 Reactivity during Maximal Voluntary Contraction

The baseline maximum voluntary contraction (MVC) was 34.8 ± 4 and 30% of MVC was estimated 10.44 ± 2.8 . There were significant increases from baseline during MVC testing in heart rate (HR/min), systolic BP, diastolic BP and mean arterial pressure (MAP, $P < 0.01$). Pulse wave velocity (PWV, m/sec) were significantly increased 6.7 ± 0.8 to 7.8 ± 0.8 ($P < 0.01$), and augmentation index (AIx), -73.2 ± 12.4 to -51 ± 24.8 , (P was not significant), the values 5 minutes following and 10 minutes following of MVC were almost similar to as baseline as shown Table 4.1, Figure 4.1 A and B.

4.3.2 Reactivity during Cold Pressor Test

CPT reactivity was examined after baseline measurements, significant differences were seen between baseline and during CPT in HR, systolic BP, diastolic BP and MAP ($P < 0.001$). PWV were significantly increased 6.75 ± 0.8 to 7.56 ± 0.9 , ($P = 0.002$) and augmentation index (AIx), -72.9 ± 12.3 to -51.4 ± 19.6 ($P < 0.001$). The values of 5 minutes following and 10 minutes following of CPT were almost similar as baseline see Table 4.1, Figure 4.1 A and 4.1 B.

4.3.3. Reactivity during Mental Arithmetic Test

Significant changes were observed between the baseline and during MAT in HR, systolic BP, diastolic BP and MAP ($P < 0.05$). PWV were significantly increased 6.78 ± 0.9 to 7.75 ± 1.6 , ($P = 0.014$) and augmentation index (AIx), -74.6 ± 13.4 to -64.6 ± 14.6 ($P = 0.019$), The

values of 5 minutes following and 10 minutes following of MAT were almost similar to as baseline see *Table 4.1, Figure 4.1 A and B.*

Overall reactivity effect of MVC, CPT and MAT was significant in separate measures in all three methods for Alx and PWV.

Table 4.1: Haemodynamic measurements in normal healthy subjects and maximal voluntary contraction (30%), cold pressor test and mental arithmetic test (n=23, Mean±SD)

Variables	Mean Baseline	During	After 5 Min	After 10 Min
<i>Maximal voluntary contraction (30%)</i>				
Heart Rate (min ⁻¹)	70±12	77±12	67±11	68±12
Systolic BP (mmHg)	112±10	125±14	113±10	116±12
Diastolic BP (mmHg)	65±6	75±6	66±6	66±6
Pulse Pressure (mmHg)	47±8	51±9	46±7	50±11
Mean Arterial Pressure (mmHg)	81±7	87±20	82±7	82±7
<i>Cold pressor test (CPT)</i>				
Heart Rate (min ⁻¹)	67±12	78±13	66±11	65±10
Systolic BP (mmHg)	113±10	132±19	115±11	116±10
Diastolic BP (mmHg)	65±5	80±8	68±8	67±7
Pulse Pressure (mmHg)	48±8	52±12	47±7	48±8
Mean Arterial Pressure (mmHg)	81±6	96±9	83±9	83±7
<i>Mental arithmetic test (MAT)</i>				
Heart Rate (min ⁻¹)	70±12	89±17	69±11	68±10
Systolic BP (mmHg)	116±12	134±17	117±11	116±10
Diastolic BP (mmHg)	67±6	77±8	67±6	67±6
Pulse Pressure (mmHg)	49±9	58±13	50±9	49±9
Mean Arterial Pressure (mmHg)	83±8	96±10	83±7	83±6

4.4 DISCUSSION

This study demonstrated an acute response to mental stress, cold pressor test and maximal voluntary contraction not alone on HR and blood pressure but also on Alx and PWV. The influence of mental stress on heart rate (HR) and spectral measures of heart rate variability (HRV) has been well documented, and it has been reported that various types of mental stresses performed in laboratory conditions increase HR and decrease HRV (Vuksanovic and Gal, 2007).

Increases in blood pressure in my study were similar to those previously published Yoshida *et al.* (Yoshida *et al.*, 1999). Mental stress may increase the blood pressure and heart rate through the changes in serum catecholamine and/or neurally mediated vasoconstriction. The increases of systolic and diastolic blood pressure during isometric exercise were of almost the same magnitude as those during the cold pressor test (Peikert and Smolander, 1991).

While the above results are not unexpected the major purpose of these studies was to examine the response in terms of aortic stiffness (PWV) and wave reflection (Alx). There was an increase in augmentation index of 13%, 29% and 30% during MAT, CPT and MVC respectively. The increase in the augmentation index may be due to change in blood flow or an increase in peripheral resistance which contributes to enhancing the impact of the backward pressure (Lavleche *et al.*, 1998). Mental stress had a lower effect on augmentation index than CPT and MVC. Alx is in part determined by BP and heart rate; other determinants such as height, weight and age are fixed in this study. There was a similar degree of activation of blood pressure, which increases Alx; there was a greater rise in heart rate +19 vs +7 and +11/min. Increased heart rate decreases Alx by approximately 4% for 10 /min increment (Wilkinson *et al.*, 2000a) and this may be one

reason for the linear reduction seen with mental stress. Pulse wave velocity was increased by approximately 15% during MAT, CPT and MVC. Also it is well known that during CPT, MAT and MVC, the blood pressure becomes high, as the blood pressure increases, the pulse wave velocity will increase, which could affect the enhancement of the reflected wave (Kyung-Won and Kwang-Sup, 2002, Vlachopoulos et al., 2006, Sharman et al., 2005).

After correction for systolic blood pressure and diastolic blood pressure both Alx and PWV were not significant independently, which shows changes in Alx and PWV are associated with change in blood pressure in this study.

4.5 CONCLUSION

I found evidence of an acute effect of mental stress, cold pressor and maximal voluntary contraction on arterial stiffness indices pulse wave velocity and augmentation index but they are dependent on blood pressure changes. This emphasizes the importance of room temperature and relaxation of both muscles and mind when measuring arterial stiffness.

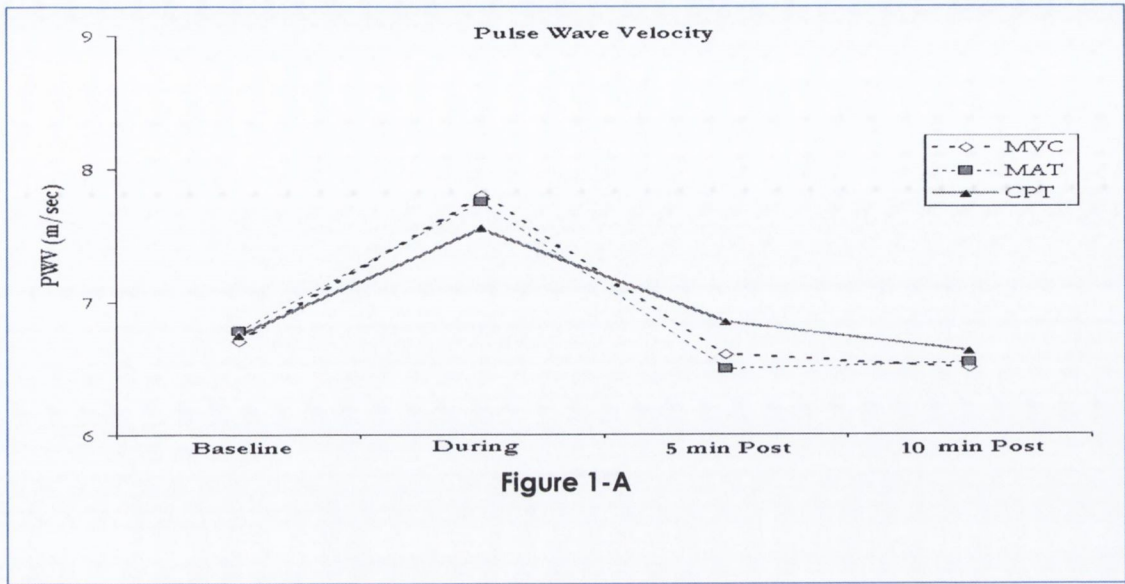


Figure 1-A

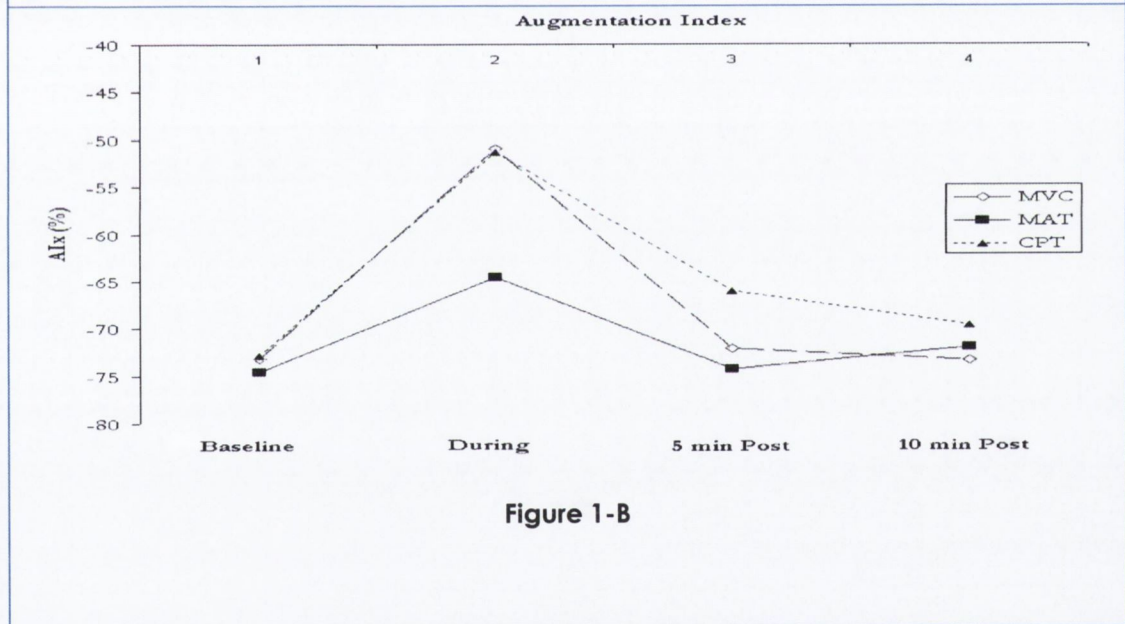


Figure 1-B

Figure 4.1: Changes in measurements of Pulse Wave Velocity (A), Augmentation Index (B) with Maximal Voluntary Contraction, Mental Arithmetic Test and Cold Pressor baseline, during and after 5 and 10 minutes.

CHAPTER 5

IMPACT OF SMOKING ON ARTERIAL STIFFNESS AND AORTIC

WAVE REFLECTION IN HYPERTENSION AS DETERMINED

USING COMPLIOR, SPHYGMOCOR AND ARTERIOGRAPH

DEVICES

SMOKING STATUS, COMPLIOR AND SPHYGMOCOR

5.1 BACKGROUND

I examined the effect of smoking using the standard SphygmoCor and Complior techniques for Alx and PWV measurements. Cigarette smoking is one of the most important avoidable causes of cardiovascular diseases worldwide (Teo *et al.*, 2006), and arterial stiffness may be one of the underlying pathophysiological mechanisms. Chronic cigarette smoking has been shown to be associated with increased arterial stiffness (Li *et al.*, 2006, Mahmud and Feely, 2003b) and increases immediately after smoking 1 cigarette (Mahmud and Feely, 2003b). The standard measurement of arterial stiffness, aortic pulse wave velocity (PWV) in conjunction with augmentation index (Alx), an estimate of aortic wave reflection, provide a comprehensive assessment of arterial stiffness (Laurent *et al.*, 2006). There is evidence that both PWV (Boutouyrie *et al.*, 2002, Laurent *et al.*, 2001) and Alx (London *et al.*, 2001) are independent predictors of cardiovascular events.

Smoking cessation is an important lifestyle measure for the prevention of cardiovascular disease. Patients with myocardial infarction may experience as much as a 50% reduction in risk of re-infarction, sudden cardiac death and total mortality if they quit smoking (Lee *et al.*, 2001). However, the speed and magnitude of risk reduction when a smoker quits is debatable, with studies quoting 3-20 years of smoking cessation associated with significant risk reductions in CHD (van den Berkmortel *et al.*, 2004).

Whether long-term smoking cessation is associated with a reduction in arterial stiffness compared to chronic smokers is not known (Boutouyrie *et al.*, 2002). In this chapter I compared in a cross-sectional fashion PWV, Alx and T_R in non-smokers, ex-smokers and current smokers.

5.2 METHODS

Methods are described in chapter two.

5.3 RESULTS

5.3.1 Patient characteristics

The clinical characteristics of the study population are shown in Table 5.1. The ex-smokers were 5 years older than nonsmokers ($P<0.01$) and 3 years older than current smokers, although this did not reach statistical significance ($P=0.09$). Although there was no difference in sex distribution for smokers, significantly more males quit smoking than females ($P<0.001$) as shown in Table 5.1. There was no significant difference in BMI among the 3 groups, although there was a trend for higher waist circumference and waist: hip ratio in ex-smokers. Total cholesterol was raised in smokers ($P<0.05$), and plasma creatinine ($P<0.05$) was higher in ex-smokers (Table 5.2).

Table 5.1: Clinical characteristics of the Hypertensive patients' population (n=554, mean \pm SEM).

Variables	Current-Smokers n=150 (27.08%)	Ex-Smokers n=136(24.55%)	Non-Smokers n=268 (48.38%)	p value
Age (years)	48.39 \pm 1	50.66 \pm 1	45.99 \pm 0.8	0.003
Sex, male: female	78:72	89:47	126:142	0.001
Height (cm)	169.13 \pm 0.84	170.62 \pm 0.88	168.52 \pm 0.63	NS
Weight (kg)	81.34 \pm 1.45	84.29 \pm 1.52	81.79 \pm 1.08	NS
Body Mass Index (kg/m ²)	28.16 \pm 0.4	28.98 \pm 0.42	28.73 \pm 0.3	NS
Waist (cm)	95.85 \pm 1.45	96.95 \pm 1.36	92.92 \pm 1.04	0.04
Hip (cm)	104.81 \pm 1.18	105.43 \pm 1.09	105.62 \pm 0.83	NS
W/H Ratio	0.91 \pm 0.01	0.92 \pm 0.01	0.89 \pm 0.01	0.01

Table 5.2: Biochemistry of the Hypertensive patients' population (n=554, mean \pm SEM).

Variables	Current-Smokers	Ex-Smokers	Non-Smokers	p value
Total Cholesterol (mmol/ l)	5.36 \pm 0.08	5.19 \pm 0.08	5.07 \pm 0.06	0.02
HDL Cholesterol (mmol/ l)	1.34 \pm 0.03	1.33 \pm 0.03	1.34 \pm 0.02	NS
Triglycerides (mmol/ l)	1.92 \pm 0.11	1.97 \pm 0.11	1.43 \pm 0.08	0.001
Fasting Glucose (mmol/ l)	5.3 \pm 0.06	5.31 \pm 0.06	5.23 \pm 0.04	NS
Serum Creatinine (mmol/ l)	86.12 \pm 1.09	89.57 \pm 1.13	86.86 \pm 0.83	0.02

3.2 Smoking status and arterial stiffness

Brachial and aortic systolic BP and pulse pressure were significantly lower in non-smokers compared with both smokers and ex-smokers ($P < 0.05$) as shown in Table 5.3. For PWV, Alx, and T_R there was a direct linear relationship between smoking status and arterial stiffness, with ex-smokers having levels intermediate between current smokers and nonsmokers (Table 5.1). To study whether there was an independent relationship between arterial stiffness and smoking status, we constructed linear regression models for PWV, Alx, and T_R . We took PWV as the dependent variable and the independent variables included in the first model were age and sex; age, sex, and mean arterial pressure in the second model; and age, sex, mean arterial pressure, heart rate, and BMI in the third model, even after adding other factors such as waist: hip ratio ($P = 0.029$), triglycerides ($P = 0.007$), and total cholesterol ($P = 0.01$) in a multiple regression model. There was a significant linear relationship between PWV and smoking status (Table 5.4). Similar models with Alx and T_R as the dependent variables showed significant linear relationships with smoking status (Table 5.4).

Table 5.3: Haemodynamic and arterial stiffness measurements of the Hypertensive patients' population(n=554, mean \pm SEM)

Variables	Current-Smokers	Ex-Smokers	Non-Smokers	p value
Heart Rate (bpm)	71.55 \pm 0.96	70.17 \pm 1.02	70.44 \pm 0.73	NS
Brachial SBP (mmHg)	158.16 \pm 1.83	159.98 \pm 1.93	154.43 \pm 1.38	0.05
Brachial DBP (mmHg)	92.78 \pm 0.93	94.53 \pm 0.98	92.69 \pm 0.70	NS
Brachial Pulse Pressure (mmHg)	65.38 \pm 1.42	65.45 \pm 1.5	61.74 \pm 1.07	0.05
Mean Arterial Pressure (mmHg)	112.03 \pm 1.28	113.48 \pm 1.15	110.34 \pm 0.8	NS
Aortic SBP (mmHg)	146.27 \pm 1.82	148.87 \pm 1.91	142.86 \pm 1.37	0.02
Aortic DBP (mmHg)	94.26 \pm 0.95	95.79 \pm 0.99	94.08 \pm 0.71	NS
Aortic Pulse Pressure	52.13 \pm 1.41	53.08 \pm 1.41	48.78 \pm 0.97	0.02
Augmentation Index (AIx, %)	30.60 \pm 0.1	30.07 \pm 1.02	27.33 \pm 0.84	0.03
Pulse Wave Velocity, (PWV) m/s	10.72 \pm 1.93	10.6 \pm 0.2	9.95 \pm 0.12	0.001
Transit Time (T _R), ms	130.96 \pm 1.05	134.63 \pm 1.03	136.83 \pm 0.82	0.0001

Table 5.4: Pulse wave velocity (PWV) top and augmentation index (bottom) in never, ex- and current smokers (mean, 95% C.I.). Three models were used (i) adjusting for age and gender (ii) adjusting for age, gender and mean arterial pressure and (iii) additional multivariate adjustment for mean heart rate and body mass index (BMI)

	Never Smoker (n=150)	Ex-smoker (n=136)	Current Smoker (n=268)	P ANOVA	P Linear trend
Pulse Wave Velocity (PWV) m/sec					
Model 1	10.26 (10.01-10.52)	10.46 (10.11-10.80)	10.89 (10.53-11.24)	0.021	0.006
Model 2	10.31 (10.08-10.55)	10.41 (10.09-10.73)	10.87 (10.54-11.19)	0.023	0.008
Model 3	10.34 (10.11-10.57)	10.40 (10.09-10.71)	10.86 (10.53-11.18)	0.033	0.017
Augmentation Index (Aix)					
Model 1	27.7 (26.4-29.0)	29.7 (27.9-31.6)	30.3 (28.6-32.0)	0.041	0.015
Model 2	27.8 (26.6-29.1)	29.6 (27.8-31.3)	30.3 (28.6-31.9)	0.049	0.016
Model 3	27.7 (26.6-28.7)	29.4 (28.0-30.9)	30.7 (29.3-32.1)	0.003	0.001
Transit Time (TIR)					
Model 1	136.61 (135.2-137.9)	134.55 (132.6-136.5)	131.06 (129.2-132.9)	0.001	0.001
Model 2	136.53 (135.2-137.9)	134.68 (132.8-136.6)	131.08 (129.3-132.8)	0.001	0.001
Model 3	136.55 (135.2-137.9)	134.75 (132.8-136.6)	131.5 (129.72-133.3)	0.001	0.001

Model 1: Adjusted for age, gender, and mean arterial pressure

Model 2: Adjusted for age, gender, mean arterial pressure and mean heart rate

Model 3: Adjusted for age, gender, mean arterial pressure, mean heart rate
and BMI

5.3.3 Smoking cessation status and arterial stiffness

Data on duration of smoking cessation were available for 122 ex-smokers, and the demographics categorized according to smoking cessation status are given in Table 5.5. Ex-smokers of <1-year duration had arterial stiffness similar to current smokers, ex-smokers of 1 to 10 years in duration had intermediate levels, and arterial stiffness in ex-smokers of >10 years duration was not significantly different to that of never smokers (Figure 5.1). Linear regression models showed a significant direct relationship between the duration of smoking cessation and PWV ($P<0.001$), A1x ($P<0.001$), and T_R ($P<0.001$) in ex-smokers, after adjusting for age, sex, BMI, and mean arterial pressure.

**TABLE 5.5: Clinical Characteristics of Ex-Smokers Categorized According to Smoking Cessation Status
(Mean ± SEM, n=122)**

Parameters	<1 y (n=22)	>1 and <10 y (n=40)	>10 y (n=60)	P Value
Age (Years)	47±3	49±2	55±1	0.01
Sex, male/female	9/13	16/24	15/45	0.19
BMI, kg/m ²	28±0.8	28±0.6	30±0.5	0.12
Waist: hip ratio	0.90±0.02	0.90±0.02	0.94±0.01	0.14
Systolic BP, mm Hg	156±5	157±3	164±3	0.15
Diastolic BP, mm Hg	92±2	94±2	96±1	0.23
Total cholesterol, mmol/L	5±0.2	5.2±0.2	5.3±0.1	0.84
Triglycerides, mmol/L	2±0.4	1.9±0.2	2±0.2	0.96
High-density lipoprotein cholesterol, mmol/L	1.40±0.07	1.3±0.05	1.3±0.04	0.50
Glucose, mmol/L	5.3±0.09	5.2±0.07	5.4±0.07	0.16

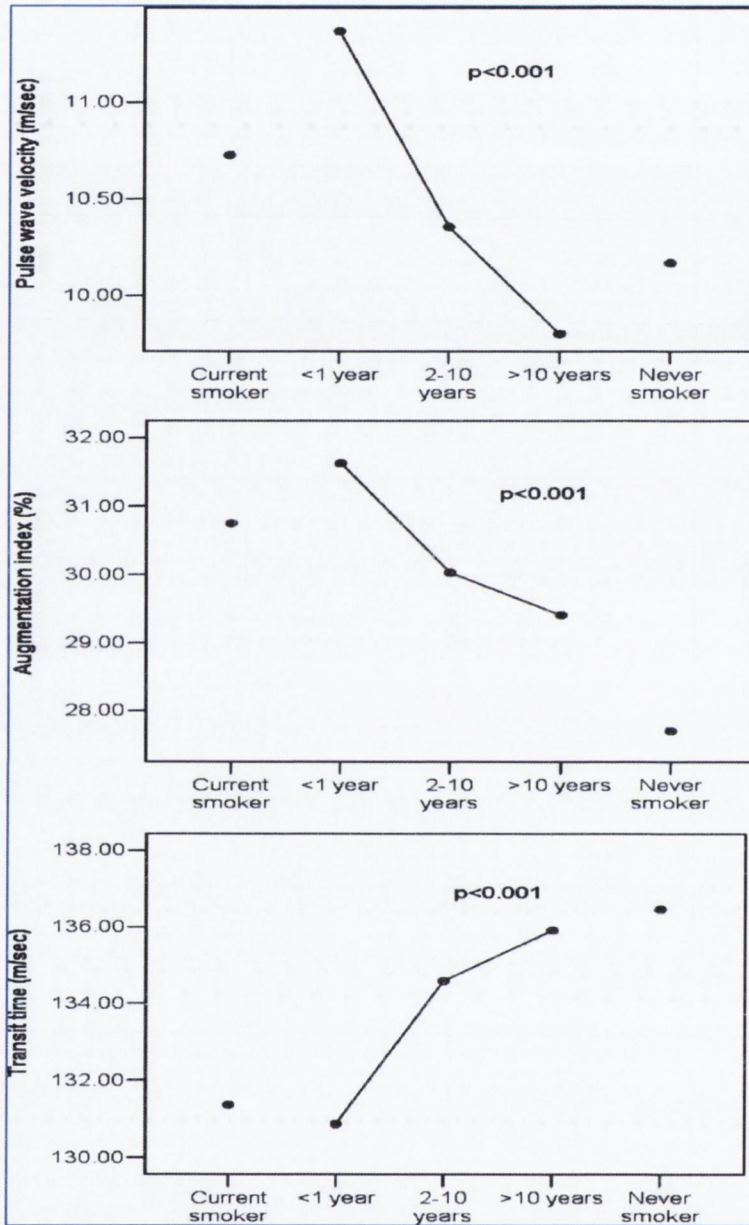


Figure 5.1: Aortic stiffness pulse wave velocity (PWV, top panel), augmentation index (AIx, middle), and transit time (T_R , lower panel) plotted against duration of smoking cessation (estimated marginal means after adjustment for age, sex, mean arterial pressure, mean heart rate, and BMI). Values for current and never smokers included for comparison.

5.4 DISCUSSION

This study confirms in a large, untreated homogenous population that there is a significant linear relationship between smoking status and PWV, Alx, and T_R , even when adjusted for covariates including age, sex, mean arterial pressure, and BMI (Table 5.4). Further analysis that included duration of smoking cessation showed that reversal of the deleterious effects of smoking on arterial stiffness is likely to take >10 years to achieve levels of stiffness similar to that of never smokers (Figure 5.1).

Because arterial stiffness is an independent predictor of events in hypertensive patients, (Boutouyrie et al., 2002, Laurent et al., 2001, London et al., 2001) it may be one of the mechanisms for smoking-related vascular disease in such patients. Although chronic smoking has been shown to be associated with increased stiffness in healthy subjects, (Li et al., 2006, Mahmud and Feely, 2003b) data on the effects of smoking on arterial stiffness are lacking in an untreated hypertensive population. To the best of my knowledge, this is the first study to show that, in untreated hypertensive patients, a population characterized by already stiff vessels, chronic smoking further increases arterial stiffness. I also think that, as the increased aortic stiffness is independent of BP, the effects of hypertension and smoking on the vascular wall may be cumulative.

Although effects of smoking cessation on BP are well documented, (Lee et al., 2001) those on arterial stiffness parameters are variable. A comparative longitudinal study over 2 years (van den Berkortel et al., 2004) did not show any effect of smoking cessation on carotid parameters, including intimal thickness, stiffness, or distensibility in people who stopped smoking compared with smokers and nonsmokers. This was a relatively small (n=33) study, and the nonsmoking group included ex-smokers. In a recently published longitudinal study of 4 weeks of smoking cessation, Alx was reduced but not to the level of the nonsmokers, (Nirandeeep et al., 2006) whereas PWV was

unchanged in this short-term study. However, my findings are in agreement with the recent study by Li et al, (Li et al., 2006) where, in healthy younger normotensive subjects, again in a somewhat older group, smoking cessation was associated with a significant reduction in arterial stiffness and systemic vascular resistance. Also, in agreement, they showed a reduction in arterial stiffness in former smokers to the level of nonsmokers after 10 years of smoking cessation. However, using radial pressure waveform analysis, they did not find any difference in large artery stiffness. It is possible that the measure, PWV, used in my study is more specific for a large artery (aorta), or perhaps the presence of hypertension in an older population amplified the effects of smoking on large artery stiffness (Li et al., 2006). The mechanisms involved in amelioration in arterial stiffness with smoking cessation may include lipid-soluble smoke particles, (Zhang et al., 2006) endothelial dysfunction, (Celermajer et al., 1993) or vascular inflammation, (Mahmud and Feely, 2005b) because smoking cessation leads to reduction in levels of inflammatory markers (Bakhrui and Erlinger, 2005). BP does not appear to be involved, because ex-smokers have higher BP levels than current smokers in my study, as seen previously, (Lee et al., 2001) and when adjusted for both age and BP, the association with arterial stiffness remained significant in my study (Table 5.4). Although it may take more than a decade to reverse these vascular changes, and the effect is relatively small, smoking cessation may help reduce cardiovascular events through amelioration in arterial stiffening even in long-term hypertensive smokers. My results are in agreement with published data reporting cardiovascular risk reduction with smoking cessation for periods ranging from 3 to 20 years (Critchley and Capewell, 2003).

There are certain caveats in this study. Using a cross-sectional design, I can only observe an association between vascular parameters and smoking status and cannot establish a causal relationship. Although I have adjusted for all of the major confounders in the analysis, the presence of unknown confounders cannot be ignored. For example, people who successfully quit smoking may be different from those who continue in different ways, including age, sex, psychosocial characteristics, and other factors. I

have adjusted for age and sex in my analysis, because ex-smokers were older and predominantly male. A further limitation may be the misclassification of smoking status, because patients who continued to smoke may claim to have quit smoking, as I do not have data on biochemical markers of smoking in this study. However, self-reporting has been shown to be quite accurate when compared with biochemical evidence of tobacco inhalation. (Patrick *et al.*, 1994) Despite these caveats, I believe that this my shows not only that smoking-induced arterial stiffness is increased in hypertensive smokers compared with nonsmokers but suggests also that these vascular changes are reversible, although it may take more than a decade for values to revert to that of never smokers. This highlights the importance of avoidance of smoking and the great need to promote smoking cessation in hypertensive patients who continue to smoke, as reduction in arterial stiffness may still be possible. However, these results need to be confirmed in a prospective longitudinal study.

5.5 CONCLUSION

I have shown in a large, untreated hypertensive population that chronic cigarette smoking is associated with increased aortic stiffness and wave reflection, which are reversible with smoking cessation, although it may take more than a decade to see levels of nonsmokers. The high aortic stiffness and wave reflection seen with chronic smoking may be one of the underlying mechanisms for the increased cardiovascular events observed in hypertensive patients. Therefore, considering the independent prognostic usefulness of arterial stiffness in the hypertensive population (Boutouyrie *et al.*, 2002, Laurent *et al.*, 2001, London *et al.*, 2001, Williams *et al.*, 2006), its assessment may not only identify hypertensive patients at higher cardiovascular risk but may also be used to monitor arterial health in those who quit smoking.

SMOKING STATUS A COMPARISON OF ARTERIOGRAPH VS SPHYGMOCOR AND COMPLIOR

5.6 BACKGROUND

Recently, an oscillometric measurement device (Arteriograph) represents a relatively simple method to detect small changes in arterial properties. Arteriograph can simultaneously measure the pulse wave velocity (PWV) and augmentation index (AIx). PWV and AIx can provide information about the level of arterial stiffness in hypertensive patients with smoking status. Firstly I examined the effect of cigarette smoking using the standard SphygmoCor (AtCor Medical) and Complior (Artech |Medical) techniques (Study –III). Similarly when I here established the use of Arteriograph (TensioMed), I studied a subsequent cohort to determine if similar results were seen using this technique. The present study also evaluated the accuracy of PWV and AIx measurements obtained by this device in untreated hypertensive patients with smoking status.

5.7 METHODS

Methods are described in chapter two.

5.8 RESULTS

5.8.1 Patient characteristics

The clinical characteristics of the untreated hypertensive patients in this study are shown in Table 5.6. The ex-smokers were 7 years older than nonsmokers ($P<0.0023$) and 1 years older than current smokers. 44.35% were females, although there was no difference in sex distribution for smokers, significantly more males quit smoking than females ($P=0.05$) as shown in Table 5.6. There was no significant difference in BMI among current smokers (CS), ex-smokers (ES) and non-smokers (NS).

5.8.2 Smoking status and arterial stiffness

There were no statistically significant differences in systolic BP, diastolic BP and pulse pressure, in all three groups as shown in Table 5.7. For PWV, Alx, and T_R were not statistically significant when assessed with Arteriograph. To study whether there was an independent relationship between arterial stiffness and smoking status, I constructed linear regression models for PWV, Alx and T_R (Arteriograph). I took PWV (Arteriograph and Complior) and Alx (Arteriograph and SphygmoCor) as the dependent variable and the independent variables included age, gender and mean arterial pressure. Smoking status was only significant for augmentation index measured by both Arteriograph and SphygmoCor methods in stepwise analysis when corrected with age, gender, MAP, and smoking [Nil and Smoker] $P=0.02$. Although it was not significant with ex-smoker status nor it was significant for pulse wave velocity measured by both Arteriograph and Complior.

Table 5.6: Clinical characteristics of the Hypertensive patients' using Arteriograph (n=254, mean ± SEM)

Variables	Current-Smokers n=59 (23.2)	Ex-Smokers n=70(27.6%)	Non-Smokers n=125 (49.2%)	p value
Age (years)	45.46 ± 1.82	52.82 ± 1.66	46.20 ± 1.25	0.0023
Sex, male: female	36:23	45:25	60:65	0.05
Height (cm)	169.46 ± 1.47	171.78 ± 1.35	169.43 ± 1	NS
Weight (kg)	79.30 ± 2.39	86.18 ± 2.2	84.87 ± 1.63	NS
Body Mass Index (kg/m ²)	27.47 ± 0.68	29.12 ± 0.62	28.86 ± 0.46	NS

Table 5.7: Haemodynamic and arterial stiffness measurements of the Hypertensive patients' (n=254, mean \pm SEM)

Variables	Current-Smokers	Ex-Smokers	Non-Smokers	p value
Heart Rate (min ⁻¹)	71.42 \pm 1.67	68.54 \pm 1.53	70.57 \pm 1.14	NS
Systolic BP(mmHg)	150.30 \pm 2.52	151.30 \pm 2.33	150.94 \pm 1.73	NS
Diastolic BP(mmHg)	89.77 \pm 1.46	90.29 \pm 1.34	90.55 \pm 1	NS
Pulse Pressure (mmHg)	60.53 \pm 1.87	60.73 \pm 1.73	60.40 \pm 1.28	NS
Mean Arterial Pressure (mmHg)	111 \pm 1.85	111.38 \pm 1.69	111.34 \pm 1.27	NS
Augmentation Index (Aix, %) Arteriograph	-0.30 \pm 4.20	4 \pm 3.73	-4.09 \pm 2.85	NS
Augmentation Index (Aix, %) SphygmoCor	27.77 \pm 1.85	29.37 \pm 1.65	25.47 \pm 1.26	NS
Pulse Wave Velocity,(PWV) m/s Arteriograph	9.65 \pm 0.32	9.91 \pm 0.29	9.68 \pm 0.21	NS
Pulse Wave Velocity,(PWV) m/s Complior	10.05 \pm 0.28	10.50 \pm 0.25	10.60 \pm 0.18	NS
Transit Time (T _R), ms Arteriograph	109.93 \pm 4.06	111.74 \pm 3.76	114.93 \pm 2.8	NS
Transit Time (T _R), ms SphygmoCor	130.88 \pm 1.75	133.89 \pm 1.58	136.25 \pm 1.18	0.04

5.9 DISCUSSION

The present study was designed to evaluate the effects of smoking on arterial stiffness using Arteriograph. As I have already shown in my previous study (Impact of smoking and arterial stiffness in hypertensive population) smoking status was statistically significant for arterial stiffness indices PWV, Alx and T_R when measured by SphygmoCor and Complior. Alx was the only significant when measured by Arteriograph after correction with age gender and MAP, but PWV and T_R were not significant. There may be a number of reasons why no effect was seen in this study, the most likely is one of number. There was a larger number of subjects in my previous study than studied by Arteriograph one (554 vs 254). A second reason is the age difference in two studies as current smokers (48.39 ± 1 vs 45.66 ± 1.8) ex-smokers (50.66 ± 1 vs 52.66 ± 1.7) and non-smokers (45.99 ± 0.8 vs 46.20 ± 1.3) respectively SphygmoCor and Complior vs Arteriograph, also systolic BP and PWV were higher in the previous study suggesting in some what stiffness arteries.

In this study ex-smokers were older than current smokers and non-smokers like study III. Although there was significant age differences between these two studies (Study III and IV) as discussed above. Current smokers were 1 year younger than the non-smokers and ex-smokers 7 years older than non-smokers (Table 5.6).

There were similarities in the measured values of arterial stiffness indices pulse wave velocity values measured by Complior and augmentation index values measured by SphygmoCor in both studies (study III and IV).

5.10 CONCLUSION

In comparative terms smoking status and arterial stiffness values measured in both studies (Study III and IV), were only significant for Alx using Arteriograph and SphygmoCor but it was not for the PWV using Arteriograph and Complior. Following correction for other variables an effect, albeit small of smoking status was seen on Alx using both Arteriograph and SphygmoCor. The failure to see effects observed in the larger study (Study III) suggests the study was underpowered.

CHAPTER 6

ASSESSMENT OF ARTERIAL STIFFNESS: COMPARISON OF OSCILLOMETRIC (ARTERIOGRAPH), PIEZO-ELECTRONIC (COMPLIOR) AND TONOMETRIC (SPHYGMOCOR) TECHNIQUES

ASSESSMENT OF ARTERIAL STIFFNESS: COMPARISON OF OSCILLOMETRIC (ARTERIOGRAPH), TONOMETRIC (SPHYGMOCOR) AND PIEZO-ELECTRONIC (COMPLIOR) TECHNIQUES

6.1 BACKGROUND

Two systems are in current use to measure arterial stiffness; Complior system (Artech Medical, France) simultaneously records pressure waves in the carotid and femoral arteries by using a piezo-electronic device and the PWV is calculated by dividing the distance between the two sites by the transit time between waves. The SphygmoCor (Version 8.1, AtCor Medical, Sydney, Australia) utilizes radial applanation tonometry and the application of a generalized transfer function to measure wave reflection as augmentation index (Alx) and aortic pressures non-invasively. The Arteriograph (TensioMed Ltd) is a recently developed computerized device using an oscillometric method to determine PWV and Alx; and has been described in chapter one. The Arteriograph which yields a simultaneous measure of brachial blood pressure (BP), PWV and Alx, has recently been validated against the Complior and SphygmoCor largely in a healthy population (Baulmann *et al.*, 2008), and for PWV in 64 patients with longstanding hypertension (Rajzer *et al.*, 2008). We compared the PWV and Alx measures obtained using the Arteriograph with those generated by the Complior and SphygmoCor and explored the determinants of these measures using the respective techniques in a large population of untreated subjects referred for assessment of high blood pressure (BP). In addition we used a statistical method for assessing agreement

between two methods of clinical measurement as described by Bland and Altman (Bland and Altman, 1986).

6.2 METHODS

Methods are described in chapter two.

65.3 RESULTS

The characteristics of the untreated hypertensive patients are shown in *Table 6.1*. The correlation coefficients between two consecutive measurements using the Arteriograph on the same day by same operator were; systolic BP($r=0.92$), diastolic BP ($r=0.95$), pulse pressure (PP) ($r=0.88$), mean arterial pressure (MAP) ($r=0.96$), PWV ($r=0.95$) and Alx ($r=0.99$) (all $P<0.0001$). The correlation coefficients for two measurements performed a week apart were, systolic BP($r=0.89$), diastolic BP ($r=0.75$), PP ($r=0.83$), MAP ($r=0.85$), PWV ($r=0.97$) and Alx ($r=0.96$) (all $P <0.0001$). The coefficients of variation of two measurements of PWV and Alx performed on the same day were 0.08 m/sec and 0.55 % and 0.1 m/sec and 0.77% performed a week later.

The values of BP measurements by Arteriograph, and digital oscillometric sphygmomanometer (Omron, Model HEM 705-CP, Omron Corporation) were correlated; for systolic BP ($r=0.81$, $P<0.001$), diastolic BP ($r=0.79$, $P<0.001$) and pulse pressure ($r=0.67$, $P<0.001$). The difference observed between the average values of systolic and diastolic BP (mmHg) for both methods (Arteriograph and sphygmomanometer) using Bland Altman analysis is presented in [Figs 1 A and B].

Difference observed between the average values of PWV using both methods with Bland Altman analysis is presented in Fig 1 C. PWV measured by Complior and Arteriograph were positively correlated ($r=0.60$, $P<0.0001$) as shown in Fig 2 A. PWV measured by Arteriograph and Complior was significantly correlated with age; $r=0.56$ vs. 0.50 ($p<0.001$); height $r=-0.32$ vs. -0.19 ($p<0.01$); HR $r=0.21$ vs. 0.19 ($p<0.05$); systolic BP $r=0.40$ vs. 0.45 ($p<0.001$); diastolic BP $r=0.33$ vs. 0.31 ($p<0.001$) and MAP $r=0.45$ vs. 0.44 ($p<0.001$) respectively and in the case of Arteriograph body weight was also significant $r=-0.14$ ($p<0.05$). In stepwise regression analysis of PWV (Arteriograph) the independent determinants were age, gender, heart rate and MAP ($R^2 = 0.44$ $p<0.0001$) and for PWV (Complior) age, HR and MAP ($R^2 = 0.37$ $p<0.0001$), although gender was not significant (Table 6.2).

Differences observed between the average values of Alx (%) of both methods using Bland Altman analysis is presented in Fig 1 D. The two methods of measuring Alx (SphygmoCor as compared to Arteriograph) correlated significantly with each other ($r=0.89$, $P<0.001$), as shown in Fig 2 B. The Alx measured by Arteriograph and SphygmoCor was significantly correlated with age $r=0.60$ vs. 0.58 ($p<0.001$); height $r=-0.45$ vs. -0.44 ($p<0.01$); body weight $r=-0.45$ vs. -0.41 ($p<0.001$); HR $r=-0.15$ vs. -0.19 ($p<0.05$); systolic BP $r=0.35$ vs. 0.29 ($p<0.001$); diastolic BP $r=0.32$ vs. 0.32 ($p<0.001$) and MAP $r=0.44$ vs. 0.44 ($p<0.001$) respectively. In stepwise regression analysis, the independent determinants of Alx were; (Arteriograph) age, body weight, MAP, HR and gender ($R^2 = 0.65$ $p<0.0001$ [Table 6.3]), and (SphygmoCor), age, body weight, MAP, HR, height and gender ($R^2 = 0.54$ $p<0.0001$, [Table 6.3]). A regression model was constructed to analyse the determinants of the residual difference between the different techniques; Arteriograph vs. Complior for PWV and Arteriograph vs. SphygmoCor for corrected Alx (Table 6.4). For PWV, the bias between PWV measured with the Arteriograph and the Complior increased in young male subjects. For Alx, the difference between the Arteriograph and SphygmoCor measurements was determined by age, body weight,

gender, HR and MAP (Table 6.4); the bias decreased with body weight and HR and increased with age, female gender and MAP.

Table 6.1: Clinical characteristics of untreated hypertensive patients (n=254; mean±SD).

Variable	Untreated hypertensive patients (n=254)
Age (years)	48 ±14
Sex (m/f) %	56:44
Height (cm)	170 ±11
Weight (kg)	84 ±18
Body Mass Index (kg/m ²)	28.93 ±6.7
Non-Smoker/Ex-Smoker/ Smoker (%)	50/27/23
Heart Rate (min ⁻¹)	69±13
Systolic BP(mmHg)	146±20
Diastolic BP(mmHg)	90±10
Pulse Pressure (mmHg)	56±46
Mean Arterial Pressure (mmHg)	109±13
Pulse Wave Velocity (m/sec) [Arteriograph]	9.73±2.1
Pulse Wave Velocity (m/sec) [Complior]	10.45±1.86
Augmentation Index (%) [Arteriograph]	-0.65±30.7
Augmentation Index (%) [SphygmoCor]	27.26±13.6

Table 6.2: Stepwise regression analysis of corrected PWV (m/sec) and determinants measures by Arteriograph and Complior (n=254).* = $P < 0.001$, ** = $P < 0.01$, *** = $P < 0.05$, NS = P Not Significant.Model for PWV $R^2 = 0.44$, $P < 0.0001$ (Arteriograph)

Variables	r^2	β	SE	P Value
Age (years)	0.32	0.04	0.004	<0.0001*
Gender [Female]	0.05	0.22	0.054	<0.0001*
Heart Rate (min^{-1})	0.01	0.01	0.004	0.044***
Mean Arterial Pressure (mmHg)	0.07	0.02	0.005	<0.0001*

Model for PWV $R^2 = 0.37$, $P < 0.0001$ (Complior)

Variables	r^2	β	SE	P Value
Age (years)	0.25	0.03	0.004	<0.0001*
Gender [Female]	0	0.08	0.056	0.15 NS
Heart Rate (min^{-1})	0.017	0.01	0.005	0.025***
Mean Arterial Pressure (mmHg)	0.10	0.024	0.005	0.0001*

Table 6.3: Stepwise regression analysis of corrected AIx (%) and determinants measures by Arteriograph and SphygmoCor (n=254).

* = $P < 0.001$, ** = $P < 0.01$, *** = $P < 0.05$, NS= P Not Significant.

Model for AIx (%) $R^2= 0.65$, $P < 0.0001$ (Arteriograph)

Variables	r^2	β	SE	P Value
Age (years)	0.36	0.033	0.0029	<0.0001*
Weight (kg)	0.14	-0.02	0.0026	<0.0001*
Height (cm)	0.005	-0.009	0.0046	0.06 NS
Gender [Female]	0.034	0.21	0.049	<0.0001*
Heart Rate (min ⁻¹)	0.077	-0.024	0.0033	<0.0001*
Mean Arterial Pressure (mmHg)	0.038	0.025	0.0035	<0.0001*

Model for AIx (%) $R^2= 0.54$, $P < 0.0001$ (SphygmoCor)

Variables	r^2	β	SE	P Value
Age (years)	0.34	0.032	0.003	<0.0001*
Weight (kg)	0.11	-0.014	0.005	0.047***
Height (cm)	0.04	-0.015	0.005	0.0057**
Gender [Female]	0.01	0.11	0.057	0.047***
Heart Rate (min ⁻¹)	0.02	-0.017	0.004	<0.0001*
Mean Arterial Pressure (mmHg)	0.03	0.017	0.004	<0.0001*

Table 6.4: Stepwise regression analysis of PWV (m/sec) and Alx (%), n=254).* = $P < 0.001$, ** = $P < 0.01$, *** = $P < 0.05$, NS = P Not Significant.**Model for PWV(m/sec) $R^2= 0.07$, $P= 0.0064$ (Arteriograph and Complior)**

Variables	r^2	β	SE	P Value
Age (years)	0.024	-0.02	0.009	0.023***
Gender [Female]	0.038	-0.34	0.13	0.008**
Heart Rate (min ⁻¹)	0.004	-0.009	0.01	0.37 NS
Mean Arterial Pressure (mmHg)	0.0002	0.002	0.01	0.86 NS

Model for Alx (%) $R^2= 0.58$, $P < 0.0001$ (Arteriograph and SphygmoCor)

Variables	r^2	β	SE	P Value
Age (years)	0.29	0.56	0.064	<0.0001*
Weight (kg)	0.12	-0.30	0.06	<0.0001*
Height (cm)	0.0008	-0.065	0.10	0.51 NS
Gender [Female]	0.058	4.92	1.07	<0.0001*
Heart Rate (min ⁻¹)	0.073	-0.54	0.07	<0.0001*
Mean Arterial Pressure (mmHg)	0.039	0.54	0.08	<0.0001*

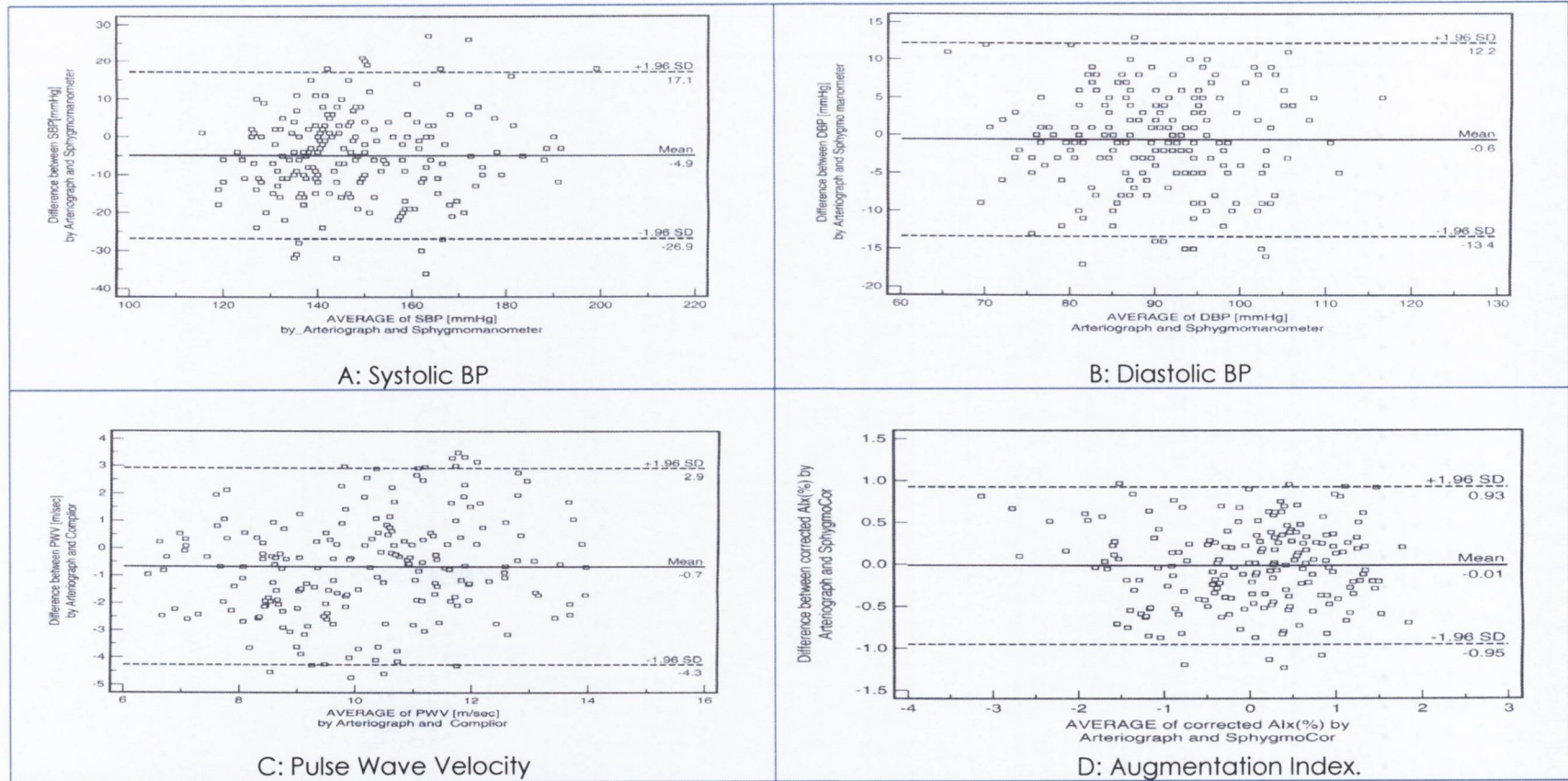
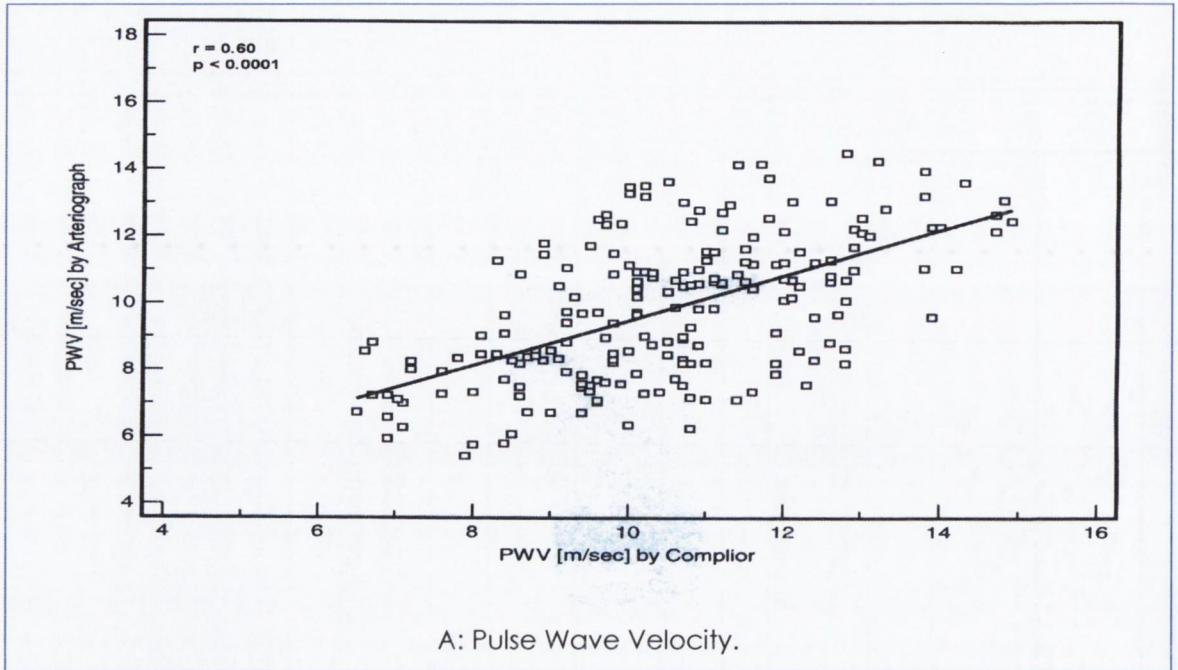
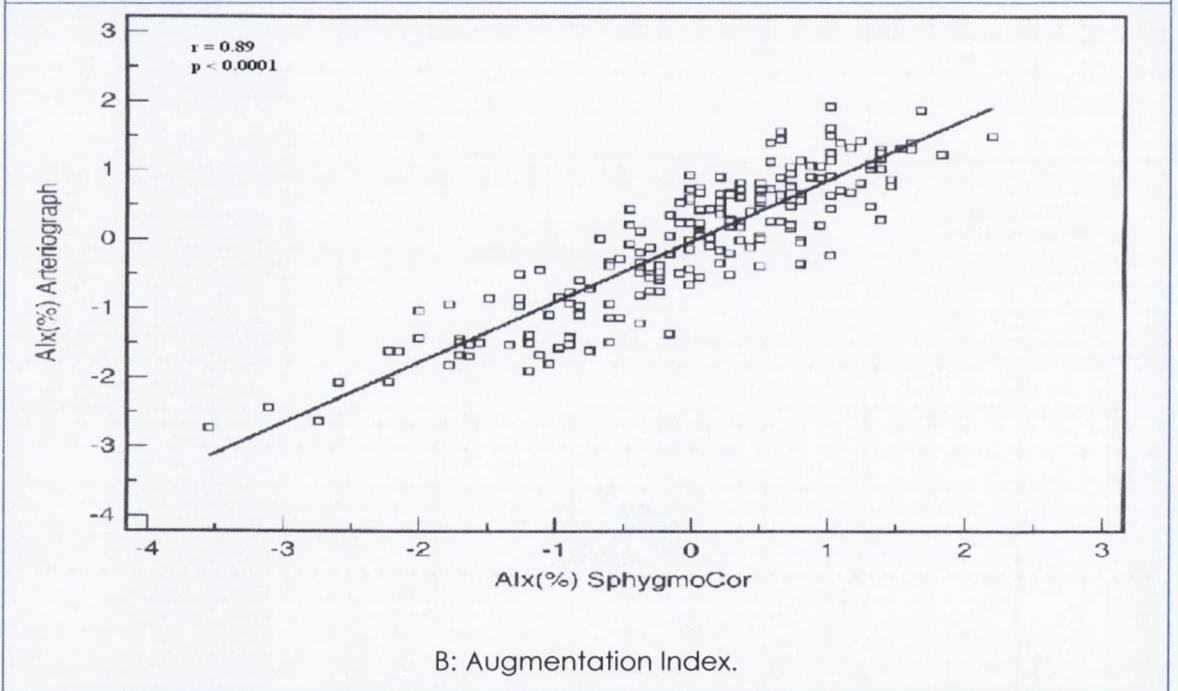


Figure 6.1: Bland Altman analysis of the difference in values obtained by comparative techniques vs the average of the techniques A: and B systolic blood pressure (SBP) and diastolic blood pressure (DBP) by Arteriograph and Omron Sphygmomanometer, C pulse wave velocity (PWV) by Arteriograph and Complior, D augmentation index (AIx) by Arteriograph and SphygmoCor.



A: Pulse Wave Velocity.



B: Augmentation Index.

Figure 6.2: Comparison of values for A: pulse wave velocity (m/sec) obtained with Arteriograph and Complior, and B: values for corrected augmentation Index (%) obtained with Arteriograph and SphygmoCor (untreated hypertensive patients, n=254).

6.4 DISCUSSION

The aim of this study was to compare the indices of arterial stiffness measured by Arteriograph to those measured by Complior and SphygmoCor in an untreated hypertensive population; to study whether the determinants of PWV and Alx were similar with the different techniques and finally to explore the determinants of the discrepancies observed between the different techniques. .

The present study shows that that PWV and Alx measurements obtained with the Arteriograph were significantly correlated with piezo-electronic PWV (Complior $r=0.60$) and tonometric Alx(SphygmoCor $r=0.89$) and extends similar observations by Baulmann *et al.*, (Baulmann *et al.*, 2008) in a largely normotensive or treated hypertensive population with correlations of 0.69 and 0.92 respectively, and by Rajzer *et al.*, (Rajzer *et al.*, 2008) for PWV by Arteriograph and Complior ($r=0.36$) in 64 patients with long standing hypertension. The lower correlation coefficient for measurements observed in the present study may in part be expected as one move from a normotensive to a hypertensive population with a wider age and BP range. Similarly our study examined a wider range of values for PWV; 5.4 to 14.5 compared with 5.8 to 11.3 m/sec in the study by Baulmann *et al.* (Baulmann *et al.*, 2008) but similar to that of Rajzer *et al.*, (Rajzer *et al.*, 2008). Covering a wider range of values is of importance as the major purpose of such measurements in clinical practice is to stratify risk in patients with medical conditions, such as hypertension, renal failure etc, where PWV values > 12 m/sec are used to influence therapy and determine prognosis. Both Baulman *et al.*, (Baulmann *et al.*, 2008) and Rajzer *et al.*, (Rajzer *et al.*, 2008) reported a similar divergence for PWV determined by Arteriograph and Complior and indeed also with SphygmoCor.

While the strength of relationship between values by Arteriograph and SphygmoCor / Complior is reassuring the correlation coefficient does not measure agreement and is

misleading when comparing two techniques (Bland and Altman, 1986). A more appropriate statistical approach is to estimate the 95% confidence interval for the ability of one method to predict another. By applying Bland Altman analysis [Figure 6.2], it is clear that the extent of variation for all parameters is greater than one would accept and is outside the published reproducibility of the individual techniques employed and greater than the standard deviation of both PWV and Alx in our population. For PWV a value obtained by one technique may vary by +/- 3.6m/sec and by +/- 17% for Alx to that determined by the other technique. The former is similar to that seen in Baulmann's and Rajzer's studies, (Baulmann et al., 2008, Rajzer et al., 2008) although such data were not reported for Alx. Alx examined by the Arteriograph has the lower values compared those measured by SphygmoCor. Infact Arteriograph Cuff examines at brachial artery (left arm) and SphygmoCor at radial artery (right wrist), these changes might be due to two different sites, apparently which shows type II statistical error. Such a magnitude of difference implies that studies using one technique cannot utilize the others for follow up.

A second objective of our study was to compare the determinants of PWV and Alx as recorded by the different techniques, although our findings relate only to a cohort of untreated hypertensives. A number of physiological factors influence augmentation index and PWV, including age (Kelly et al., 1989a) body height (Smulyan et al., 1998), heart rate (Gatzka et al., 2001a), systolic and diastolic BP (Kelly et al., 2001), MAP (Wilkinson et al., 2001) and pulse pressure (Wilkinson et al., 2002b). The present study shows that age, mean arterial pressure and heart rate are the main determinants of PWV regardless of which technique is employed with gender only a significant determinant for PWV measured using the Arteriograph. For Alx, age, body weight, gender, heart rate and mean arterial pressure were independent determinants with both techniques with body height only significant for the SphygmoCor. Exploring the determinants of the differences between the different techniques showed that for PWV, the bias between Arteriograph and the Complior increased with young age and male

gender. For Alx; age, female gender and BP increased whereas body weight and heart rate decreased the discrepancy between the two techniques. For PWV, the greater bias in young males may reflect the high prevalence of abdominal obesity in these subjects which may influence the distance measured to calculate PWV; however, more studies are needed to investigate this observation. The greater bias observed between Alx measured with the Arteriograph and SphygmoCor with older age, female gender and higher BP may suggest differences in identification of the inflection point at high levels of Alx; how higher body weight and heart rate may improve bias is not entirely clear. More studies are needed to explore these interesting data in different populations as our results apply only to hypertensive patients.

6.5 CONCLUSION

In comparative terms the Arteriograph is easier to apply as it measures BP, PWV and Alx simultaneously. The precise placement of the brachial cuff is less critical in contrast to the placing of sensors over the carotid and femoral artery or tonometers over the radial (or carotid) artery. The low correlation observed between the BP values measured using a standard sphygmomanometer and the Arteriograph however, suggests that the latter cannot be used to reliably measure BP on its own. It has less variation and similar reproducibility as the other two techniques and the determinants for both Alx and PWV are generally similar for the different methods. As there is poor agreement between PWV and Alx measured with the different devices, these techniques cannot be used interchangeably. Furthermore, as the Complior method for measuring PWV has been validated against the gold-standard manual method¹⁹, a poor agreement with the Arteriograph method suggests that the latter is not a suitable method for assessing PWV in clinical practice.

To conclude, the Arteriograph is an operator-independent, reproducible oscillometric method for the estimation of arterial stiffness and wave reflection in hypertensive patients and as shown previously, in normotensive populations. While the Arteriograph, SphygmoCor and Complior are not interchangeable, the Arteriograph cannot be considered the 'gold standard' technique pending prospective outcome studies.

CHAPTER 7

EFFECT OF ANTIHYPERTENSIVE DRUGS ON ARTERIAL STIFFNESS IN HYPERTENSIVE PATIENTS

EFFECTS OF ANTIHYPERTENSIVE DRUGS ON ARTERIAL STIFFNESS IN HYPERTENSIVE PATIENTS

7.1 BACKGROUND

Arterial stiffness has been traditionally associated with hypertension. Indeed most of the early epidemiological studies have concentration on the increased risk of coronary artery disease (CAD), stroke and renal failure associated with increased stiffness (Laurent *et al.*, 2006). Consequently early pharmacological studies have examine the effects of antihypertensive agents on arterial stiffness (Mahmud and Feely, 2003a). The demonstration that stiffness associated risk is independent of BP and the results of studies in chronic renal failure (Blacher *et al.*, 1999b) and in hypertension (Williams *et al.*, 2006) where reduction of stiffness independent of BP change was associated with survival advantage gives an added impetus to this field. These studies suggest that antihypertensive agents that reduce stiffness, independently of the expected reduction associated with blood pressure may be advantageous. I have reviewed the effects of antihypertensive agents on indices of stiffness, PWV and Alx in chapter one. Here I evaluated the effect of the following five different classes of drugs on arterial stiffness in a hypertensive population using an Arteriograph (TensioMed™).

1. Treatment effect with angiotensin-converting enzyme Inhibitors (ACEi).
2. Treatment effect with angiotensin receptor antagonists or blockers (ARB).
3. Treatment effect with a β -blocker (BB).

4. Treatment effect with a calcium channel blocker (CCB).
5. Treatment effect with thiazide diuretics.

To examine the sensitivity of Arteriograph, I compared the post treatment changes of PWV and Alx measures detected by Arteriograph vs Complior (PWV), SphygmoCor (Alx).

7.2 METHODS

Methods are given in chapter two.

7.3 RESULTS

One hundred fourteen untreated hypertensive patients were recruited from the Hypertension clinic at St James's Hospital Dublin-8, patients' aged 23 to 78 [Mean \pm SD, 49 \pm 13] years, and 32% were women. Clinical characteristics of the patient population are given in *Table 7.1*. Patients were evaluated before and 4 to 6 weeks after treatment with a single antihypertensive drugs; angiotensin-converting enzyme inhibitors (ACEi, n=26), angiotensin receptor antagonists or blockers (ARB, n=17), β -blocker (BB, n=40), calcium channel blocker (CCB, n=16), and thiazide diuretics (n=15), as shown in *Table 7.2*.

Table 7.1: Clinical characteristics of the patient population (n=114, Mean±SD)

Variables	ACEI	ARB	BB	CCB	Diuretics
n=	26	17	40	16	15
Age, (Years)	49±13	49±14	45±10	51±14	57±13
Sex (Male: Female)	19:7	11:6	29:11	10:6	8:7
Height (cm)	173± 10	171±9	174±10	170±9	168±11
Weight (kg)	87±16	78±15	88±15	75±15	86±20
BMI (kg/m ²)	29.1±4.3	27±5.3	29.1±4.5	25.6±4	30.2±4.5
Smoker: Ex-Smoker: Non Smoker	8:8:10	3:7:7	9:11:20	4:4:8	2:6:7

Table 7.2: Antihypertensive drugs and dose used in the study (Mean±SD)

Antihypertensive class	Specific drug name	Daily Dose
Angiotensin converting enzyme inhibitor (ACEi, n=26)	Enalapril (n=19)	10 mg
	Lisinopril (n=7)	10 mg
	Perindopril (1)	5 mg
Angiotensin receptor antagonists (ARB, n=17)	Olmesartan (n=10)	20 mg
	Valsartan (n=3)	160 mg
	Candestran, (n=4)	8 mg
Calcium channel blockers (CCB, n=16)	Amlodipine (n=16)	5 mg
Beta blockers (BB, n=40)	Nebivolol (40)	5 mg
Diurectics (DIU, n =15)	Bendroflumethazide, (n=15)	2.5 mg

7.3.1 Angiotensin converting enzyme inhibitors

Patients treated with Angiotensin converting enzyme inhibitors (ACEi) showed a significant reduction in systolic blood pressure (BP, mmHg), diastolic BP (mmHg) and mean arterial pressure (mmHg). Pulse wave velocity (m/sec) and Augmentation index (%) those measured by Arteriograph were significantly reduced with ACEi, as shown in Table 7.3.

Table 7.3: Effect of angiotensin-converting enzyme inhibitors on haemodynamic and arterial stiffness measurement (Mean±SD)

* = $p < 0.001$, ** = $p < 0.01$, **** = $p < 0.05$, NS = $p < \text{Not significant}$.

Variables	Pre-Treatment Mean±SD	Post-Treatment Mean±SD	P Value
Heart Rate (min ⁻¹) †	69±9	72±12	0.27 NS
Systolic BP(mmHg) †	156±17	143±14	0.003**
Diastolic BP(mmHg) †	93±8	83±10	0.0005**
Pulse Pressure (mmHg) †	67±16	61±12	0.16 NS
Mean Arterial Pressure (mmHg) †	115±11	103±11	0.0003*
Pulse Wave Velocity (m/sec) [A]	10.7±2.6	9.4±2.3	0.05***
Pulse Wave Velocity (m/sec) [C]	12.25±2.4	10.59±2.2	0.014***
Augmentation Index (%) [A]	5.68±31.5	-11.38±28	0.044***
Augmentation Index (%) [S]	28.9±11	22.2±15.3	0.08 NS

† = Sphygmomanometer and A = Arteriograph, C = Complior and S = SphygmoCor

7.3.2 Angiotensin receptor blockers

Treatment with angiotensin receptor blockers (ARB) showed a significantly reduction in systolic BP (mmHg), diastolic BP (mmHg), mean arterial pressure (mmHg), augmentation index (%; Arteriograph); although pulse wave velocity (m/sec, Arteriograph), reduced with treatment but *p* value was not significant (Table 7.4).

Table 7.4: Effect of angiotensin receptor blockers on haemodynamic and arterial stiffness measurement (Mean±SD)

* = $p < 0.001$, ** = $p < 0.01$, **** = $p < 0.05$, NS = $p < \text{Not significant}$.

Variables	Pre-Treatment Mean±SD	Post-Treatment Mean±SD	P Value
Heart Rate (min ⁻¹) †	67±10	70±9	0.37 NS
Systolic BP(mmHg) †	160±19	137±14	0.0005*
Diastolic BP(mmHg) †	94±11	81±12	0.0027**
Pulse Pressure (mmHg) †	65±16	56±13	0.09 NS
Mean Arterial Pressure (mmHg) †	117±14	99±14	0.0006*
Pulse Wave Velocity (m/sec) [A]	10.12±2.34	9±2.6	0.19 NS
Pulse Wave Velocity (m/sec) [C]	11.54±2.3	10.51±1.9	0.17 NS
Augmentation Index (%) [A]	8.83±34	-16.9±38.2	0.046***
Augmentation Index (%) [S]	28.1±15.1	20.35±15.8	0.15 NS

†= Sphygmomanometer and A= Arteriograph, C= Complior and S=SphygmoCor

7.3.3 β -blockers

Treatment with β -blockers (BB) showed a significant reduction in systolic BP (mmHg), diastolic BP (mmHg), and mean arterial pressure (mmHg). There was significant improvement in pulse wave velocity (m/sec, Arteriograph) although augmentation index (%; Arteriograph) shown some reduction but p value was not significant (Table 7.5).

Table 7.5: Effect of β -blockers on haemodynamic and arterial stiffness measurement (Mean \pm SD)

* = $p < 0.001$, ** = $p < 0.01$, **** = $p < 0.05$, NS = $p < \text{Not significant}$.

Variables	Pre-Treatment Mean \pm SD	Post-Treatment Mean \pm SD	P Value
Heart Rate (min ⁻¹) †	71 \pm 11	63 \pm 8	0.0006*
Systolic BP(mmHg) †	152 \pm 15	137 \pm 15	< 0.0001*
Diastolic BP(mmHg) †	94 \pm 8	82 \pm 8	< 0.0001*
Pulse Pressure (mmHg) †	58 \pm 12	55 \pm 11	0.29 NS
Mean Arterial Pressure (mmHg) †	113 \pm 12	100 \pm 12	< 0.0001*
Pulse Wave Velocity (m/sec) [A]	10.49 \pm 2.4	9 \pm 1.9	0.0028*
Pulse Wave Velocity (m/sec) [C]	11.27 \pm 1.9	9.97 \pm 1.6	0.0014**
Augmentation Index (%) [A]	-3.51 \pm 26.4	-9.15 \pm 23.9	0.32 NS
Augmentation Index (%) [S]	27.85 \pm 11.65	25.23 \pm 13.8	0.36 NS

†= Sphygmomanometer and A= Arteriograph, C= Complior and S=SphygmoCor

7.3.4 Calcium channel blockers

Treatment with calcium channel blockers (CCB), showed a significant reduction in diastolic BP (mmHg) and mean arterial pressure (mmHg), although there was good reduction in systolic BP (mmHg, Omron), augmentation index (%) and pulse wave velocity (m/sec) measured by using Arteriograph but *p* value was not significant (Table 7.6).

Table 7.6: Effects of calcium channel blocker on haemodynamic and arterial stiffness measurement (Mean±SD)

* = $p < 0.001$, ** = $p < 0.01$, **** = $p < 0.05$, NS = $p < \text{Not significant}$.

Variables	Pre-Treatment Mean±SD	Post-Treatment Mean±SD	P Value
Heart Rate (min ⁻¹) †	64±9	67±9	0.39 NS
Systolic BP(mmHg) †	155±18	142±19	0.08 NS
Diastolic BP(mmHg) †	92±10	83±7	0.016****
Pulse Pressure (mmHg) †	64±19	59±20	0.51 NS
Mean Arterial Pressure (mmHg) †	115±11	104±9	0.007**
Pulse Wave Velocity (m/sec) [A]	11±3.1	9.5±2.7	0.18 NS
Pulse Wave Velocity (m/sec) [C]	12.3±1.8	10.9±1.9	0.05****
Augmentation Index (%) [A]	18.8±24.2	0.9±32.8	0.09 NS
Augmentation Index (%) [S]	33.6±7.6	28.7±9.2	0.12 NS

†= Sphygmomanometer and A= Arteriograph, C= Complior and S=SphygmoCor

7.3.5 Thiazide diuretics

Treatment with thiazide diuretics showed significant reduction in systolic BP (mmHg), diastolic BP (mmHg) and mean arterial pressure (mmHg), although there was good reduction in the values of augmentation index (%) and a poor reduction in the values of pulse wave velocity (m/sec) measured by using Arteriograph but *p* value was not significant (Table 7.7).

Table 7.7: Effect of thiazide diuretics on haemodynamic and arterial stiffness measurement (Mean±SD)

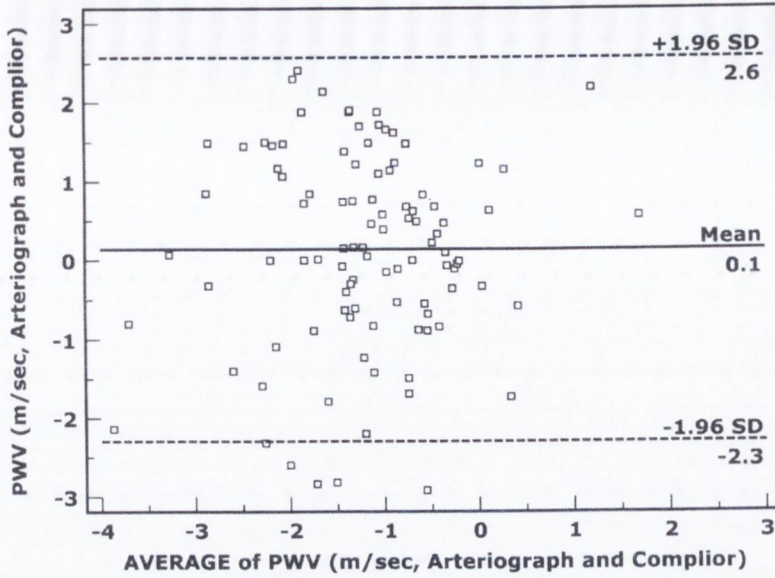
* = *p* < 0.001, ** = *p* < 0.01, **** = *p* < 0.05, NS = *p* < Not significant.

Variables	Pre-Treatment Mean±SD	Post-Treatment Mean±SD	P Value
Heart Rate (min ⁻¹) †	69±9	70±11	0.94 NS
Systolic BP(mmHg) †	168±17	152±19	0.03***
Diastolic BP(mmHg) †	99±13	89±11	0.04***
Pulse Pressure (mmHg) †	68±16	62±19	0.37 NS
Mean Arterial Pressure (mmHg) †	120±19	108±16	0.06 NS
Pulse Wave Velocity (m/sec) [A]	11.6±2.7	10.2±2.7	0.15 NS
Pulse Wave Velocity (m/sec) [C]	11.8±2.6	11.7±2.8	0.97 NS
Augmentation Index (%) [A]	13.4±28.1	4.6±26.48	0.39 NS
Augmentation Index (%) [S]	34.1±10.5	30.1±10.5	0.31 NS

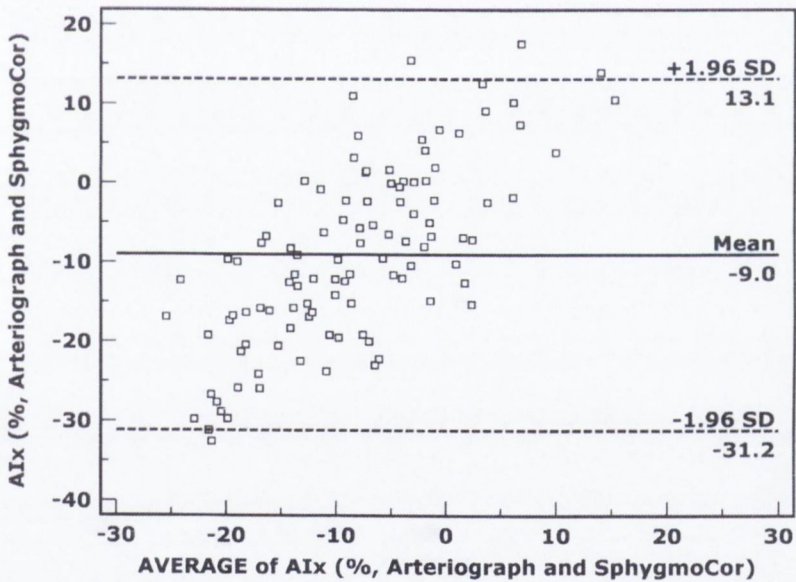
†= Sphygmomanometer and A= Arteriograph, C= Complior and S=SphygmoCor

Difference observed between the change (Following treatment with antihypertensive drug groups) in average values of PWV (m/sec) using both methods (Arteriograph and Complior) with Bland Altman analysis is presented in *Fig 7.1-A*. PWV measured by Arteriograph and Complior were positively correlated ($r=0.36$, $p < 0.0001$) as shown in *Fig 7.2-A*.

Difference observed between the change (Following treatment with antihypertensive drug groups) in average values of Alx (%) using both methods (Arteriograph and SphygmoCor) with Bland Altman analysis is presented in *Fig 7.1-B*. Alx (%) measured by Arteriograph and SphygmoCor were positively correlated ($r=0.53$, $p < 0.0001$) as shown in *Fig 7.2-B*.

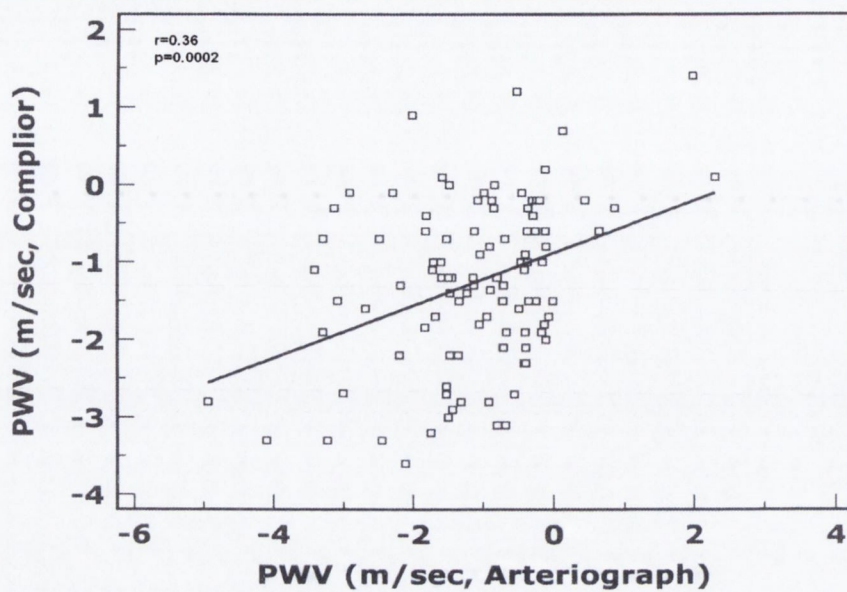


A. Pulse wave velocity (m/sec)

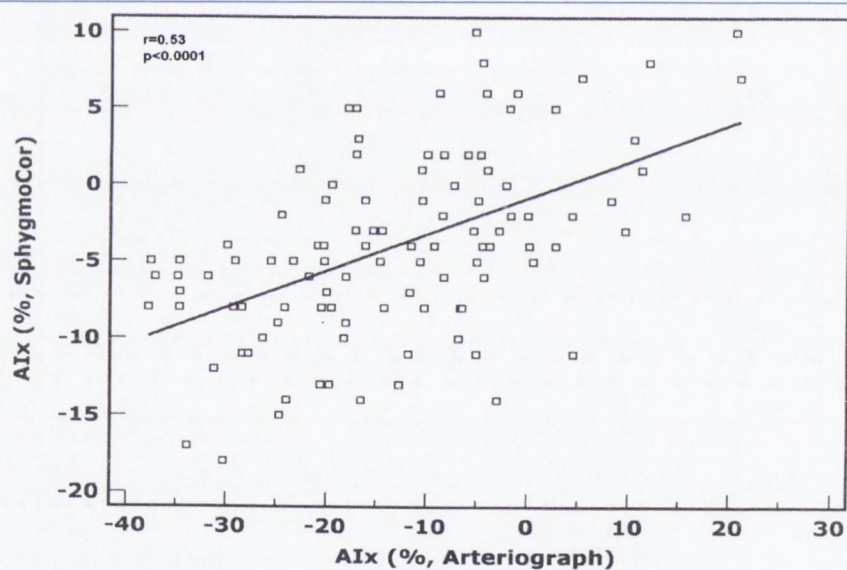


B. Augmentation Index (%)

Figure 7.1: Bland Altman analysis of the difference in values obtained by comparative techniques vs the average of the technique A change in pulse wave velocity (m/sec, Arteriograph and Complior) and B. change in augmentation index (% , Arteriograph and SphygmoCor), following treatment with major antihypertensive drugs (ACEi, ARB, BB, CCB and Diuretics).



A. Pulse wave velocity (m/sec)



B. Augmentation Index (%)

Figure 7.2: Comparison of values for A. change in pulse wave velocity (m/sec) obtained with Arteriograph and Complior and B. change in augmentation index (%) obtained by Arteriograph and SphygmoCor, following treatment with major antihypertensive drugs (ACEi, ARB, BB, CCB and Diuretics).

7.4 DISCUSSION

The purpose of this study was to determine the oscillometric Arteriograph (TensioMed^{TD}) might be useful for assessing the effects of antihypertensive drugs on PWV and wave reflections.

Several mechanisms may be involved in producing reductions in arterial stiffness with a given treatment, reduction in arterial stiffness depends on the treatment duration; acute or short-term and long-term or chronic treatments. For example, after acute administration of an antihypertensive drug, improvement of arterial stiffness is principally related to functional or mechanical mechanisms such as reduction of distension pressure, reduction of smooth muscle tone, enhancement of endothelial functions, whereas after long-term chronic treatment, additional mechanisms can be involved, e.g., changes in the arterial geometry and structure, reduction in degree of fibrosis, increase in elastin/collagen ratio, remodeling of the arterial wall (Asmar, 1999, Laurent et al., 2002).

Several pharmacological studies have evaluated the effects of antihypertensive drugs on arterial stiffness (as shown in *Table 7.8*), shows the effects on PWV of different antihypertensive drug classes, administered double blind, either short-term (<28 days) or long-term (≥28 days) (Asmar, 1999, Asmar et al., 2002, Rajzer et al., 2003, White et al., 2003). Angiotensin converting enzyme inhibitors work by suppressing the enzyme action that causes the conversion of Angiotensin-I to Angiotensin-II. Angiotensin-II is a strong vasoconstrictor causing an increase in blood pressure. Thus the ACEI is a vasodilator – by blocking the action of Angiotensin-II it causes vasodilatation. There is extensive literature on the effect of ACEI on arterial stiffness as summarized in *Table 7.8*. ACEI have been shown favorable effect on arterial stiffness, when compared with other

antihypertensive drugs. During long term treatment, improvements in PWV and Alx have been found with ACE inhibitors (Topouchian *et al.*, 2007). My study is generally in agreement with this previous research, as it showed a significant effect of treatment on PWV with short term treatment. Reduction of augmentation index in my findings is also in agreement with other study trials (Mahmud and Feely, 2002b, Deary *et al.*, 2002, Dart *et al.*, 2001, Morgan *et al.*, 2004).

Angiotensin-II receptor antagonists (or blockers) are a newer class of antihypertensive agents. These drugs are selective for angiotensin II (type 1 receptor); unlike angiotensin-converting enzyme inhibitors, they do not inhibit bradykinin metabolism or enhance prostaglandin synthesis (Messerli *et al.*, 1996). Angiotensin-II receptor antagonists are well tolerated, cough occurs much less often with these agents than with angiotensin-converting enzyme inhibitors, and they do not adversely affect lipid profiles or cause rebound hypertension after discontinuation. Klingbeil *et al.* found in his study a greater reduction in augmentation index (-22 +/- 11) with valsartan than with hydrochlorothiazide (-3 +/- 11) and placebo (0 +/- 13) (Klingbeil *et al.*, 2002). The X-CELLENT Study observed a reduction in augmentation index with four weeks treatment with Candesartan. An other crossover study with AT1 receptor antagonists Losartan in patients with essential hypertension, there was a significant reduction in PWV and Alx (Mahmud and Feely, 2002b). Reduction in Alx with valsartan treatment has been reported in *Table 7.8* adopted from Mahmud *et.al* review (Mahmud, 2007). My results show significantly reduction in systolic and diastolic BP as well a reduction in Alx (by Arteriograph) although the effect on PWV did not achieve significant. There was a great reduction in the values of PWV and Alx those measured by Complior and SphygmoCor but statistical not significant, the reason may be Arteriograph is better in sensitivity than Complior and SphygmoCor. An acute functional change in vascular smooth muscle relaxation, reduction in endothelial dysfunction, arterial wall thickness, collagen content and reversal of smooth muscle cell hypertrophy have been proposed as mechanisms of the improvement in PWV by ARB (Levy *et al.*, 1991, Barra *et al.*, 1997,

Isobe et al., 2002). However, it is less likely that treatment with ARB for only 4 weeks would improve the structure of the vasculature. Short-term treatment of ARB may improve PWV mainly through functional mechanisms such as vascular smooth muscle relaxation and reduction in endothelial dysfunction, because BP was also decreased after ARB treatment.

β -blockers have exhibited variable results according to the particular drugs used (Topouchian et al., 2007). Most studies have shown that β -blockers reduce PWV (Mahmud, 2007). Nebivolol decrease Alx (Mahmud, 2007), Kelly et al. showed that vasodilating β -blockers may have a beneficial effect on wave reflection (Kelly et al., 1989b) . In general the data suggests that most but not all beta blockers may have a favorable effect on arterial stiffness. My study shows statistically significant reduction in PWV by both methods (Arteriograph and Complior) but there was no reduction in Alx when measured by both methods (SphygmoCor and Arteriograph). Alx was seen significant with only SphygmoCor ($p=0.026$) when corrected heart rate but it was not for the Arteriograph method, my study is an agreement with Mahmud et.al study.

At the aortic level, all calcium channel blockers have shown a significant reduction in arterial stiffness, in parallel with BP reduction, but at the peripheral level, arterial stiffness reduction was less evident (Topouchian et al., 2007). Calcium channel blockers (CCBs) block the influx of calcium into the cells of the myocardium and the vasculature. Lower levels of calcium within these cells results in less stimulation and therefore, less contraction, less contraction within the myocardium reduces myocardial contractility, automaticity, and conduction velocity. Less contraction within the vasculature leads to dilatation of the blood vessels leading to decreased total peripheral resistance (Holler, 2008). Calcium channel blockers selectivity inhibit the passage of calcium ions through specific ion channels of the cell membrane in muscle cells of the heart and arteries, causing the vascular smooth muscle to relax and hence producing a decrease in peripheral resistance and a fall in blood pressure. My

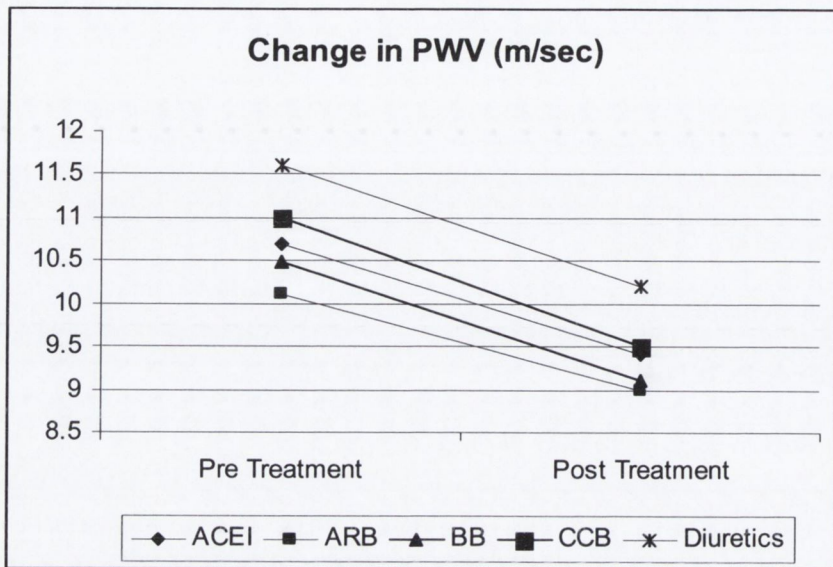
study result shows significant reduction in diastolic blood pressure only, PWV significantly reduced with Arteriograph method but not with Complior. Although there was good reduction in Alx (18.8 ± 24.2 vs 0.9 ± 32.8 , mean \pm SD, $p=0.09$) but statistically not significant when measured with Arteriograph).

As hypertensive complications mainly affect the conduit arteries and the sodium contributes to arterial stiffness, when sodium intake is high, bradykinin blockade produces more carotid hypertrophy, and when sodium intake is normal, less aortic collagen accumulates because of AT₁-receptor blockade (Safar *et al.*, 2000). It is suggested that diuretics may have blood pressure independent effects on the large arteries. However, the reality has been the converse; the effects of diuretic compounds on the vascular wall in hypertensive subjects have been disappointing (Mahmud and Feely, 2003a). Diuretics have shown no significant effects on arterial stiffness (Topouchian *et al.*, 2007). Mahmud *et al.* showed, in randomized control trial comparing losartan to hydrochlorothiazide in mild to moderate hypertension, that the latter had no effect on PWV or arterial wave reflection (Mahmud and Feely, 2002b). Patient treated with thiazide diuretics in our study showed statistically significant reduction in blood pressure but did not show significant reduction in the PWV as well as in Alx, which is in keeping with the literature *Table 7.8*.

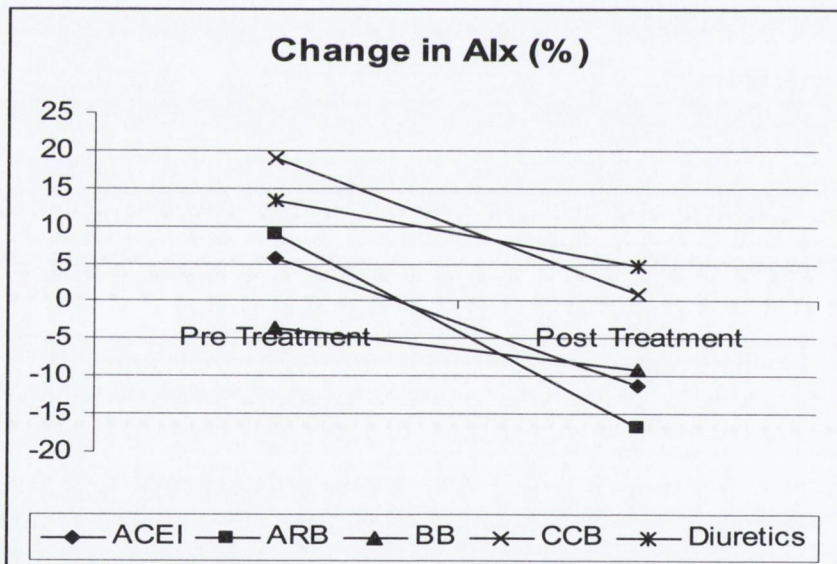
Over all there was greater reduction in the arterial stiffness indices PWV and Alx, following treatment with five antihypertensive drugs groups (ACEi, ARB, CCB, BB, and diuretics) as shown in *Figure 7.3-A and B*. There was also a positive but poor correlation between the values of PWV ($r=0.36$, $p=0.0002$) measure by using Arteriograph vs Complior (*Figure 7.1-A*) and values of Alx ($r=0.53$, $p<0.0001$) measured by using Arteriograph vs SphygmoCor (*Figure 7.1-B*).

Table 7.8: The effect of the major anti-hypertensive drug groups on arterial stiffness as measured by PWV and wave reflection

	PWV	AIx (%)
ACE Inhibitors		
Enalapril (Morgan <i>et al.</i> , 2004)		↓
Lisinopril (Asmar <i>et al.</i> , 1992)	↓	
Perindopril (Lacourciere <i>et al.</i> , 2004, Pannier <i>et al.</i> , 2001)	↓	↓
Angiotensin receptor blockers		
Olmesartan (Mediavilla García <i>et al.</i> , 2007)	↓	
Valsartan (Mahmud and Feely, 2000, Klingbeil <i>et al.</i> , 2002)	↓	↓
Candesartan (Sprat <i>et al.</i> , 2001)	↓	
β-blockers		
Nebivolol (Mahmud and Feely, 2008)	↓	↓
Calcium channel blockers		
Amlodipine (Matsui <i>et al.</i> , 2005)	↔	↓
Diuretics		
Bendroflumethiazide (Mahmud, 2007, Mahmud and Feely, 2002b)	↔	↔
↔ = no change; ↓ = decreased; ↑ = increased.		



A. Pulse wave velocity (m/sec, Arteriograph)



B. Augmentation Index (% , Arteriograph)

Figure 7.3: A. Change in PWV (m/sec) and B. change Alx (%) obtained by Arteriograph following treatment with major antihypertensive drugs (ACEi, ARB, BB, CCB and Diuretics).

7.5 CONCLUSION

Reduction of arterial stiffness indices PWV and wave reflection with antihypertensive drugs will likely have a significant impact on morbidity and mortality of hypertensive subjects (Mulrow *et al.*, 1998). As we live longer, arterial stiffness is emerging as an endemic problem and in light of the recent CAFE study (Williams *et al.* 2006), which is well established as a therapeutic target in its own right. This study gives some guideline that ACEi may be suitable treatment for preventing increased arterial stiffness than other antihypertensive drugs, as both PWV and Alx reduced with ACEi treatment as shown in my results, but it has not proved to be the ideal 'de-stiffening' agents. Therefore, we need a clearer understanding of the 'root causes' of arterial stiffness to develop specifically targeted therapeutic interventions to reduce stiffness and wave reflection beyond reduction in cuff pressures. There is poor agreement between PWV and Alx measured with different devices, these techniques can not be used interchangeable.

CONCLUSION

CONCLUSIONS

The aim of the studies described in this thesis was to examine arterial stiffness and its determinants using a novel oscillometric Arteriograph device and to compare it with other established techniques and devices (piezo-electronic Complior and tonometric SphygmoCor).

A variety of methods, studies design, and subjects which differed quite substantially were employed in the six studies of this thesis. The common denominator of these six studies has been arterial stiffness, which has been examined from several aspects. The first study established reproducibility of arterial stiffness indices in young healthy normotensive subjects using the oscillometric device Arteriograph.

The second study looked at its ability to record rapid changes in stiffness parameters, the acute effects of mental arithmetic, cold pressor, and maximal voluntary contraction on arterial stiffness in young healthy subjects. An important lesson here for any study of arterial stiffness is the need to minimise any form of "stress" and obtain recordings in a controlled environment.

The third study on hypertensive patients has been focused on impact of smoking and smoking cessation on arterial stiffness and aortic wave reflection as determined using Complior and SphygmoCor devices, and similarly in fourth study when I here established the use of Arteriograph, I studied a subsequent cohort to determine if similar results were seen using this technique. Results here were not consistent, while AIx performed by Arteriograph were consistent with SphygmoCor data this was not the case compared to Complior PWV. Perhaps my fourth study was underpowered.

In fifth study, a major portion of the work undertaken involved a comparison of the three commonly used techniques (oscillometric, piezo-electric and tonometric) in a large hypertensive population comparing the Arteriograph to SphygmoCor and Complior, the determinants of pulse wave velocity (PWV) and augmentation index (AIx) by each technique (study V). Finally, in the last study (study six) I evaluated whether Arteriograph can detect changes in arterial stiffness brought about by using therapeutic agents (anti-hypertensive drugs).

MAIN FINDINGS

- I. Oscillometric device Arteriograph (TensioMed™) can measure precisely and accurately, with an acceptable reproducibility and can be reliably applicable in future clinical as well as research studies.
- II. Mental stress, cold pressor and maximal voluntary contraction has been found with an increase arterial stiffness indices pulse wave velocity and augmentation index in normal healthy subjects.
- III. Chronic cigarette smoking is associated with increased aortic stiffness and wave reflection, which are reversible with smoking cessation, although it may take more than a decade to see levels of nonsmokers. The high aortic stiffness and wave reflection seen with chronic smoking may be one of the underlying mechanisms for the increased cardiovascular events observed in hypertensive patients. Therefore, considering the independent prognostic usefulness of arterial stiffness in the hypertensive population, its assessment may not only identify hypertensive patients at higher cardiovascular risk but may also be used to monitor arterial health in those who quit smoking.

IV. Arteriograph (TensioMed™) is simple and easier to apply as it measures BP, PWV and Alx simultaneously. The precise placement of the brachial cuff is less critical in contrast to the placing of sensors over the carotid and femoral artery or tonometers over the radial (or carotid) artery. It has less variation and similar reproducibility than the other two techniques but to date there is no outcome data associated with its use (Baulmann *et al.*, 2008), as the determinants for both Alx and PWV are almost identical using the different techniques, I believe they are reflecting the same vascular properties. I have shown a strong relationship with values obtained for BP, PWV and Alx by standard techniques but also that the techniques are not interchangeable.

V. I obtained three notable findings from this study; first treatment with ACEI and BB; both drugs reduced PWV. Second the augmentation index was reduced with ACEI and ARB, both PWV and Alx in accordance with a reduction in BP in hypertensive subjects. Third and final one, separate two techniques for the each measurement of PWV and Alx show some similarity but not in all measurements it may be due to the different sensitivity of the devices.

IMPLICATIONS AND SIGNIFICANCE

My studies confirm that the use of Arteriograph in normal healthy subjects and untreated hypertensive patients has good reproducibility. This also shows Arteriograph is very sensitive to acute changes in physiological condition (mental arithmetic, cold pressor and maximal voluntary contraction) and arterial stiffness in young healthy subjects.

Early epidemiological studies shown that smoking is a major risk factor, not only for lung disease and cancer, but also for heart attack, stroke and heart failure, and increased aortic stiffness and wave reflection. My study on smoking status also confirm that the smoking is associated with arterial stiffness, but this is the first study in hypertensive population which shows that quitting smoking is a very important step in improving cardio-vascular health. Long-term smoking cessation can heal arterial walls and reverse the risks of developing cardiac and vascular problems. Although it may take more than a decade to see levels of nonsmokers, the high aortic stiffness and wave reflection seen with chronic smoking may be one of the underlying mechanisms for the increased cardiovascular events observed in hypertensive patients. Therefore, considering the independent prognostic usefulness of arterial stiffness in the hypertensive population, its assessment may not only identify hypertensive patients at higher cardiovascular risk but may also be used to monitor arterial health in those who quit smoking.

In my Arteriograph study with smoking status shows less sensitivity in chronic smokers, the disadvantage of this study is the lower numbers of the patients in this study, this suggest long term study with larger number of subjects may be needed for future studies. Arteriograph comparison in hypertension patients shows very close to SphygmoCor but less for Complior.

In comparative terms the Arteriograph is easier to apply as it measures BP, PWV and Alx simultaneously. Brachial cuff is less critical in contrast to the placing of sensors over the carotid and femoral artery or tonometers over the radial (or carotid) artery. It has less variation and similar reproducibility as the other two techniques and the determinants for both Alx and PWV are generally similar for the different methods. Arteriograph has low correlations for BP values those compared with standard sphygmomanometer however suggests that the latter cannot be used to reliably measure BP on its own. As

there is poor agreement between BP and PWV measured with the different devices, these techniques cannot be used interchangeably, the choice of the 'gold standard' technique deserves a prospective outcome study.

I also studied the antihypertensive drug effect on arterial stiffness using Arteriograph, most findings are consistent with literature but not with all the drugs, the reason may be lower numbers of the patients in my study, a larger number patients and longer duration of study may be needed for future studies.

FUTURE PROSPECTUS

High blood pressure is a powerful modifiable cardiovascular risk factor that acts on the arterial wall and is responsible in part for various cardiovascular events, such as heart failure, ischemic heart disease and cerebrovascular accidents (Cohn, 1998). Structural and functional properties of the arterial wall are altered in hypertension even at the early stages of the disease (Duprez *et al.*, 2004).

Morbidity and mortality associated with hypertension are primarily related to arterial damage and may affect one or several organs. Considering the potential implications of arterial assessment in the prevention of cardiovascular disease, and evaluation of the arterial effects of antihypertensive treatment in longitudinal studies are recommended.

Continuing noninvasive technique will be used to assess the function and structure of the arterial wall in subjects with hypertension or even subjects at risk of developing hypertension. The effect of antihypertensive therapy on the functional and structural

vessel wall abnormality can then be monitored during therapeutic intervention in short term and longer duration of treatment. Thus, a single antihypertensive drug regimen that does not favorably affect the arterial structure or function in a given patient would be replaced by another drug regimen and / or combined with another antihypertensive drug regimen and / or lipid lowering drug can be added that might be more effective. The correction of the arterial wall abnormality could then serve as a guide to therapeutic efficacy rather than the absolute level of blood pressure, which now serves as the surrogate marker. How the blood pressure changes and the arterial wall changes might relate needs to be intensively studied.

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2. Mahmud A, Jerrard-Dunne P, **Jatoi NA** and Feely J. (2007) Response to Selection Bias, Confounding, or Information Bias? *Hypertension*. [Journal of American Heart Association] 28;
3. **Jatoi NA**, Bennett K, Mahmud A and Feely J. (2009) Assessment of arterial stiffness in hypertension: comparison of oscillometric (Arteriograph), tonometric (SphygmoCor), and piezo-electronic (Complior) techniques. *J Hypertens* (In Press).
4. **Jatoi NA**, Bennett K, Mahmud A and Feely J. (2009) Acute effects of Mental Arithmetic test, cold presser test and maximal voluntary contraction (handgrip exercise) on arterial stiffness in young healthy normotensive subjects. (In preparation).
5. **Jatoi NA**, Mahmud A and Feely J. (2009) Effect of antihypertensive agents and arterial stiffness in hypertensive population. (In preparation).