Tissue Doppler Echocardiography for the Characterisation of the Hypertensive Ventricle

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Abstract

This thesis used tissue Doppler echocardiography to characterise, in detail, left ventricular function in 1006 subjects and produced several novel findings.

Firstly, transmitral Doppler had limited ability to identify impaired diastolic function in a cohort at high risk of diastolic dysfunction. Further, the Valsalva maneouvre was found to be unreliable as a discriminator in subjects with an apparently normal transmitral Doppler flow profile.

Secondly, using tissue Doppler early mitral annular velocities (E'), subjects of African-Caribbean ethnicity were demonstrated to have more impaired diastolic function than populations of White European origin.

Thirdly, a correlation was demonstrated between fasting plasma glucose levels and left ventricular diastolic function, supporting the concept of a 'diabetic cardiomyopathy'.

Fourthly, the anti-hypertensive combination regimen atenolol+/- bendroflumethiazide was demonstrated to be associated with relatively adverse measures of diastolic function when compared to a regimen of amlodipine+/- perindopril.

Finally, a large cohort of hypertensive subjects underwent echocardiography and was followed for 4.2 years. The strongest predictor of future cardiac events proved to be the

subject's diastolic function, as measured using the ratio of the transmitral pulsed Doppler early filling velocity (E) to the tissue Doppler early mitral annular velocity (E').

Three of the chapters in this thesis formed the basis for publications in major cardiac journals, as described at the outset of this thesis. One of the aims of this body of research was to provide further support for the inclusion of Tissue Doppler echocardiography in standard clinical echocardiographic protocols. This is now the case, with most echo labs including TDE measures in their routine studies as a result of the large body of work now existing in the literature, of which this thesis forms one small component. The non-invasive assessment of left atrial filling pressure using the E/E' ratio is now routinely quoted in the average echocardiographic report and the data reported in this thesis add further focused support for that strategy.

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- Finally, my wife, Ellie, for her unending good grace and constant support.

Declaration

I declare that I am the author of this thesis. Unless otherwise stated, all references cited have been consulted by myself. The work contained within this thesis is my own and it has not previously been accepted for a higher degree.

Andrew Sharp

Aims

Hypertension is one of the principal causes of myocardial infarction, arrhythmia, heart failure and death. It has been estimated that there are currently 1 billion hypertensive subjects in the world, a number which is expected to rise to 1.5 billion by 2025 (Kearney 2005).

Despite this massive burden of disease and a long history of distinguished research in the field, the pathophysiology of hypertension and its' consequences on the myocardium remain incompletely understood. A better understanding of the physiological effects of hypertension on the heart might enable better protective strategies to be developed.

This thesis describes the use of tissue Doppler echocardiography to delineate the diastolic function of the hypertensive heart and to better understand the cardiac effects of ethnicity, diabetes, anti-hypertensive treatment regimens and statins.

Specific aims are:

1. To examine the role of tissue Doppler echocardiographic imaging in assessing diastolic function of the left ventricle in a hypertensive population.
2. To examine the impact of ethnicity on diastolic function.
3. To establish the relationship between plasma glucose levels and diastolic function.

4. To examine the effect of anti-hypertensive drug treatments on diastolic function.
5. To examine the effect of HMG-Co-acetyl reductase inhibitors (statins) on diastolic function.
6. To assess whether tissue Doppler measures of diastolic function can predict future cardiac events within a hypertensive population and whether these measures are superior to conventional echocardiographic risk markers in hypertension, such as left ventricular mass index.

Publications

1. Sharp AS, Tapp RJ, Thom SA, Francis DP, Hughes AD, Stanton AV, Zambanini A, O'Brien E, Chaturvedi N, Lyons S, Byrd S, Poulter NR, Sever PS, Mayet J; on behalf of the ASCOT Investigators. Tissue Doppler E/E' ratio is a powerful predictor of primary cardiac events in a hypertensive population: an ASCOT substudy.
European Heart Journal. 2010 Mar;31(6):747-52. Epub 2009 Nov 26.

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Chapter 1 General Introduction

The History of Hypertension

Whilst the modern understanding of hypertension begins in the 18th century, a rudimentary understanding of the concept of blood pressure has been documented for several millennia. In Ancient Egypt, references were made in texts to the concept of an 'excess of blood', with efforts made to drain this excess using leeches, venesection and cupping. Similar references can be found in texts from ancient China, Assyria, Greece and Rome (Ruskin 1956).

In 1733, Stephen Hales described what we now understand to be blood pressure when he took invasive physiological measurements of the great arteries through direct cannulation of the left and right sided circulations of a horse (Hales 1769). Over a hundred years later, the English doctor Richard Bright described the cardiovascular and renal consequences of hypertension in his seminal description of post-mortem studies performed at Guy's hospital in London. His description of a clustering of pathophysiological anatomical abnormalities in humans, including renal parenchymal disease, thickening of the aorta and thickening of the left ventricle, led to the eponymous syndrome of Bright's disease (Bright 1836). Whilst he was largely describing the consequences of essential hypertension, he perceived this clustering of pathologies to be a primarily renal phenomenon, rather than the poly-factorial disease we now know as hypertension.

In 1872, Gull and Sutton described generalised 'hyaline fibroid' abnormalities of the small vessels within the vascular tree, which seemed to occur in some humans whether or not they possessed the renal abnormalities characteristically associated with Bright's disease. They hypothesised that one underlying disease may lead to both the spectrum of pathological abnormalities described by Bright and the small vessel abnormalities they had observed on autopsy (Gull 1872). However, the nature of that underlying disease was not recognised until a practical, non-invasive, method for the measurement of human blood pressure was devised.

The invention in 1854 of the sphygmograph, by German physiologist Karl von Vierordt, enabled non-invasive measurements of the pressure required to stop blood flow in the radial artery . This was termed the 'blood pressure'. The design was improved shortly afterwards by Etienne-Jules Marey, rendering it portable and recordable through the use of pulse wave amplification and transcription via a stylet pen. The basis of the modern sphygmomanometer followed in the subsequent three decades, with adaptations on the original Marey design by Samuel von Basch, Scipione Riva-Rocci and Heinrich von Recklinghausen (Riva-Rocci 1896; Sinclair 1969; Freis 1990). These adaptations produced the first accurate and practical instrument for measuring systolic blood pressure.

The next important development in the field of hypertension came in 1905, when a Russian military surgeon named Nikolai Korotkoff published a description of the sounds that could be auscultated from a brachial artery when partially occluded by a Riva-Rocci

blood pressure cuff (Korotkoff 1905). This description of the method for measuring both systolic and diastolic human blood pressure is still taught to medical students to this day.

In 1913, the physiologist Janeway coined the phrase 'Essential Hypertension', to describe a systemic disorder leading to premature death from cardiac disease, renal disease and/or stroke (Janeway 1913). Shortly after, Liebbe described a case of hypertension caused by a phaeochromocytoma, with a subsequent paper describing resolution of normotension following surgical resection. Thus, we began to understand the concept of primary and secondary hypertension (Sinclair 1969).

Given the absence of oral drug treatments available at the time and the observed response of blood pressure to reduction in sympathetic drive following phaeochromocytoma resection, surgical sympathetic denervation became the principle accepted treatment for hypertension. In 1925, Rowntree and Adson described the first such successful invasive sympathectomy and, until the advent of oral pharmacotherapy for hypertension, this procedure was widely used (Freis 1990).

The first proven oral pharmacological treatment for hypertension was the oral diuretic chlorthiazide. In 1957, the first publication supporting its use in hypertension signalled the beginning of the end of the widespread use of the radical sympathectomy procedure (Freis 1957). In the 1960s, the drugs propanolol, a B-Blocker, and the centrally acting vasodilator methyldopa expanded available oral treatment options (Prichard 1964). More

modern agents, such as ACE inhibitors and calcium channel blockers, have subsequently shown at least similar efficacy to these older agents in reducing blood pressure and novel agents, acting via different pathways, continue to be devised, although interestingly most report broadly similar efficacies to those reported in the original chlorthiazide trial in 1957 (ALLHAT investigators 2002).

At around the time of the inception of oral anti-hypertensive therapy, epidemiological studies began to delineate in detail the natural history of hypertensive vascular disease. In 1948, in the town of Framingham, Massachusetts, in the North East of the United States of America, a population of over 5000 men and women between the ages of 30 and 62 years of age were extensively phenotyped and tracked throughout their adult life (Dawber 1951).

This seminal study quantified the cardiovascular risk associated with an elevated systolic and/or diastolic blood pressure, as well as establishing a series of associated risk factors which exacerbate the cardiovascular consequences of high blood pressure, such as an adverse lipid profile and body habitus (Wilson 1987). From this study, we also learned of the increased cardiovascular risk associated with adverse changes in left ventricular structure and function in hypertension (Levy 1990).

The pathophysiological consequences on the heart of hypertension

There is a vast amount of descriptive research describing the cardiac consequences of hypertension. Hypertension affects the myocardium both directly and indirectly, resulting in heart failure and/or myocardial infarction (Ho 1993; Haider 2003). The mechanisms involved include left ventricular hypertrophy, left ventricular diastolic dysfunction, left ventricular systolic dysfunction and ischaemic heart disease. The link between hypertension and coronary artery disease is well recognised (Collins 1990), but outwith the scope of this thesis and will not be discussed in detail. The first three mechanisms are now discussed in detail.

The cardiac consequences of hypertension: left ventricular hypertrophy

Left ventricular hypertrophy (LVH) occurs primarily as a result of two phenomena:

- Hypertrophy/hyperplasia of cardiac myocytes
- Fibrosis of the myocardium (Weber 1991).

This fibrotic component appears to be the more adverse of the two responses and may explain why myocardial thickening in response to high levels of physical exercise (which is predominantly due to the hypertrophy/hyperplasia response) appears to have fewer long-term adverse consequences than that seen with essential hypertension (Colan 1985; Lewis 1992)

Widespread physiological changes occur as a result of Essential Hypertension. Whilst it remains incompletely understood as to whether some, or all, of these changes are primary or secondary effects, it is broadly agreed that left ventricular hypertrophy is a consequence of hypertension, rather than a cause. The mechanism of LVH formation is complex, involving pressure overload of the left ventricle and neurohormonal modifications that likely accentuate the fibrotic response (Weber 1991). These neurohormonal changes in hypertension are important and may play a substantial role in the maintenance of hypertension and progression of subsequent cardiovascular disease.

The detection of left ventricular hypertrophy

When Richard Bright described left ventricular hypertrophy (LVH) in his original post-mortem series, he was of the opinion that this was secondary to renal disease. In 1852, George Johnson described the arterial wall thickening of the large systemic arteries that accompanied LVH (Ruskin 1956). Shortly after, Gull and Sutton described similar cardiac and arterial effects in the absence of renal disease, thus de-coupling LVH from advanced renal disease. In 1921, George Evans then described the link between protracted elevation of blood pressure levels and the post-mortem weight of the human heart (Evans 1921).

In the early 20th century, the only methods available for estimating cardiac mass pre-mortem were chest radiography and the electrocardiogram (ECG). In 1930, Blackford described the increased cardiac silhouette associated with LVH, though this proved to be

a relatively insensitive tool (Blackford 1930). Also in the earliest part of the twentieth century, Einthoven invented clinical electrocardiography. One of his earliest publications describes the pattern of ECG changes associated with a subsequent post-mortem diagnosis of cardiac hypertrophy (Einthoven 1906).

Several authors subsequently attempted to devise a reliable series of ECG criteria for establishing hypertrophy in live human subjects. One of the earliest methods was devised by Sokolow and Lyon, who published findings in a series of live hypertensives who they would expect to have LVH on post-mortem (Sokolow 1949). They described greater R wave height in the lateral ECG leads and greater S wave depth in the anterior ECG leads in hypertensive subjects than in norms, providing a specific, but still relatively insensitive method for the identification of LVH. Various formulae have improved on the low sensitivity of this method, but despite this, the ECG remains an imperfect tool for diagnosing LVH (Pewsner 2007).

In the 1980's, transthoracic echocardiography replaced the 12-lead ECG as the gold standard for evaluating cardiac mass (Reichek 1983; Levy 1987). However, ready accessibility of the ECG and the wealth of data describing the consequences of possessing ECG criteria for hypertensive LVH (and the response to treatment) mean the ECG continues to play a role in hypertensive disease management to this day.

Echocardiography for assessing left ventricular hypertrophy

Echocardiography is a much more sensitive and specific method of establishing left ventricular hypertrophy in hypertension than the 12-lead ECG (Woythaler 1983). The first cardiac ultrasound technology developed in the 1970s (M-Mode echo) took linear 'slices' through the left ventricle and used geometric models to calculate the left ventricular mass. These models were validated against post-mortem specimens by Devereux and Reichek in 1977 (Devereux 1977); however, it became apparent that the assumptions that underpinned the formulae used to calculate LV mass relied on a degree of intra-ventricular symmetry. If, for example, a patient had a previous myocardial infarction or exhibited an asymmetric hypertrophic pattern, then the calculations proved inaccurate (Woythaler 1983).

The reliability of M-mode measurements can be improved by the addition of volume calculations obtained from 2-dimensional (2D) echocardiography. By taking a cross sectional cut of the ventricle at the level of the papillary muscles and measuring the length of the ventricle in apical long axis views (Simpson's method), a reliable estimate of LV mass can be obtained. Whilst this calculation again relies on a degree of symmetry, it has good post-mortem correlation data with LV mass (Helak 1981).

Despite the advent of magnetic resonance imaging, which produces high quality images even in subjects with poor echocardiographic windows, echocardiography still remains the most widely used method for assessing left ventricular mass in the hypertensive

population, due to its longevity, accessibility, wide applicability and large prospective outcomes evidence base.

The clinical correlates of left ventricular hypertrophy in essential hypertension

The Framingham study examined the ability of the chest X-ray cardiothoracic ratio, the 12-lead ECG and the transthoracic echocardiogram to identify left ventricular hypertrophy. These measures were then used to predict clinical outcomes.

Because left ventricular hypertrophy without cavity enlargement leads to relatively minimal cardiac silhouette enlargement on CXR, this method showed a poor sensitivity for establishing LVH when compared to echo; however, when cardiac enlargement was present on the CXR, there was a proportionate increase in the frequency of cardiac events (Kannel 1992).

The 12-lead ECG also proved to have low sensitivity rates, but did strongly identify subjects at risk of cardiovascular events. Subjects with ECG LVH and repolarisation abnormalities in the Framingham study were shown to have a threefold risk of cardiac events when compared to matched subjects possessing a normal ECG (Kannel 1969; Kannel 1992) .

Later studies have demonstrated a direct relationship between echocardiographically derived LV mass (adjusted for body surface area as the left ventricular mass index (LVMI)) and the frequency of future cardiac events across both hypertensive and normal populations (Levy 1990, Verdecchia 1998). This risk has been shown to vary according to ventricular geometry (Koren 1991) . Thickened left ventricular walls increase the risk of cardiovascular events as does isolated left ventricular cavity dilatation. However, a combination of the two abnormalities imparts the highest risk (Koren 1991).

The cardiac consequences of hypertension: Left ventricular systolic dysfunction

Left ventricular systolic dysfunction has been demonstrated in various populations to be a powerful indicator of future cardiac events. In hypertensive heart disease, it likely represents the end of a continuum, beginning with asymptomatic diastolic dysfunction and ending in symptomatic systolic dysfunction (Yip 2002).

Systolic dysfunction in hypertension has several potential causes (Levy 1996). These include:

- The myocyte fibrotic process involved in hypertension
- Adverse remodelling, causing deterioration in the efficiency of the left ventricle
- Conduction disease, leading to left ventricular dyssynchrony
- Previous myocardial infarctions from ischaemic heart disease
- Hibernating myocardium, a phenomenon particularly common in patients with ischaemic heart disease.

There are many studies reporting the importance of left ventricular systolic dysfunction in the general population (Davies 2001; Redfield 2003; McDonagh 1997). These studies are, by definition, heterogeneous in their sampling and therefore must be cautiously extrapolated to the hypertensive population. Two large scale studies have, however, examined the prevalence and significance of left ventricular systolic impairment in the hypertension population.

In the HyperGEN study, left ventricular hypertrophy was shown to be a strong predictor of left ventricular systolic dysfunction (Devereux 2001); this finding was replicated in the PIUMA study (Progetto Ipertensione Umbria Monitoraggio Ambulatoriale; Verdecchia 2005). In HyperGEN, a study of 2086 subjects with essential hypertension, left ventricular ejection fraction was found to be normal (defined as >54% for this study) in 86% of patients. This study also included hypertensive patients with a history of myocardial infarction and/or symptomatic heart failure and therefore provided us with the first large scale cross-sectional assessment of the prevalence of left ventricular systolic impairment within the hypertensive population. However, no outcomes data are available on this cohort.

In the PIUMA study, 2384 asymptomatic subjects with hypertension underwent transthoracic echocardiography and were followed up for an average of 6.0 years. Within this asymptomatic population, 85 subjects were identified as having left

ventricular systolic impairment, defined for the purposes of this study as a left ventricular ejection fraction of less than 50%.

Whilst the number of patients within this cohort exhibiting LV systolic impairment was low, the authors were able to identify it as a strong marker for clinical events – specifically, admission to hospital with heart failure symptoms. For those with asymptomatic left ventricular systolic impairment, the hazard ratio for the future development of congestive heart failure was 9.99 (Confidence Intervals: 3.67-27.2). The numbers of patients developing either a new ischaemic cardiac event, or a stroke, were low in this study and therefore the authors were unable to demonstrate a link between left ventricular systolic impairment and these particular outcomes.

Similarly, there were insufficient number of deaths within the cohort to establish a relationship between left ventricular function and mortality. Given the relationship between left ventricular systolic impairment and outcomes in heart failure from any cause, and the relationship within the general population (Davies 2001), it seems likely that left ventricular systolic impairment in hypertension would be related to mortality outcomes in larger sample populations, particularly where patients are symptomatic.

The cardiac consequences of hypertension: Left ventricular diastolic dysfunction

Left ventricular diastolic dysfunction is one of the earliest adverse cardiac physiological changes in response to hypertension (Kitabatake 1982, Phillips, 1989). It is associated with heart failure even in the presence of normal left ventricular systolic function and may have significance when identified in the elderly (Sagie 2001).

The term diastole originates from the Greek term for expansion/filling of the heart. It encompasses the phase of the cardiac cycle that is not systole – i.e. the relaxation and filling phases and is defined as the time period beginning from the closure of the aortic valve to the closure of the mitral valve.

The four phases of diastole are as follows:

- Isovolumic relaxation phase. This is the time period that extends from aortic valve closure to mitral valve opening and creates a relatively negative pressure within the ventricle.
- Rapid filling phase. As pressure in the left ventricle is below that of the left atrium, the ventricle rapidly fills upon opening of the mitral valve. This component of diastole is a largely passive phenomenon and is responsible for 70-80% of filling in normal healthy subjects.
- Diastasis. This is the period of pressure equalisation between the left ventricle and left atrium.

- Atrial contraction. This contributes approximately 15% of the filling in normal individuals (Brutsaert 1985).

Diastolic dysfunction

Diastolic dysfunction is a complex phenomenon, representing a series of adaptations in the mechanisms of ventricular relaxation (Leite-Moreira 2006). The process of myocardial relaxation is an active one, incorporating the physics of volume shifts within chambers and the recoil of the myocardium into a "suction" force, which occurs shortly after systole. Derangement of calcium handling (for example in acute or chronic coronary ischaemia) can also alter myocardial contractile qualities in the early filling phase of diastole. Further, left ventricular electrical conduction defects may reduce ventricular efficiency, ultimately impairing ventricular relaxation.

The components that affect passive ventricular compliance include the degree of myocardial fibrosis and/or infiltration (e.g. in glycogen storage diseases), along with the effects of myocyte hypertrophy and the remodelling that occurs with chronic volume overload.

Extrinsic factors may also have a role in reducing ventricular compliance, such as diseases of the pericardium, dilated or pressure overloaded right ventricles and high intrathoracic pressure states, such as are achieved during ventilation. Abnormalities of

the mitral valve, particularly mitral stenosis, can also affect left ventricular filling markedly, as can heart rate, filling status and vasodilators.

Diastolic dysfunction can more readily described in terms of how it has been measured. The earliest accurate method of quantifying diastolic function was obtained through cardiac catheterisation (Hammermeister 1974, Gaasch 1975, Sanderson 1977). By placing a catheter within the left ventricle, pressure-volume curves could be created which allowed estimation of the dP/dt ratio; this represented the maximum rate of change in pressure within the ventricle and was taken as an index of compliance. However, dP/dt is, by definition, the peak rate of relaxation and therefore is only a snapshot of one time-point within diastole. This led to the development of the Tau index (Weisfeldt 1974), which used mathematical calculations to better represent the pressure changes observed from cardiac catheterisation throughout diastole. This measurement was not without its limitations, however, as it was shown to vary with age, heart rate and afterload (Karliner 1977; Raff 1981).

Non-invasive assessment of diastolic function

M-Mode echocardiography

In 1973, Gibson described the use of M-mode echocardiography to measure the rate of relaxation of the septal and posterior walls during diastole (Gibson 1973). Measurements of left ventricular volumes using this technology allowed reproducible measurements of

left ventricular systolic and diastolic function to be made, which compared reasonably well with invasive measures (Gibson 1974; Kronik 1979).

Whilst this technology represented a significant step forward, there were difficulties with the technique. The technology was in its infancy at this stage and required a great deal of skill to obtain accurate images. Without the aid of 2-dimensional echocardiographic images to assist in beam orientation, it was difficult to be sure that the M-mode beam was cutting the septum and posterior walls at a perpendicular angle and therefore providing an accurate estimation of wall size and motion. It is now well recognised that even minor degrees of variation in the angle of the ultrasound beam with respect to the wall can create significant variations in the results obtained. Nevertheless, clinical correlation of abnormal flow patterns with outcomes was possible (Sanderson 1978).

Transmitral Doppler Ultrasound

Doppler ultrasound represented a significant step forward in echocardiographic technology and gave us a new, non-invasive, insight into diastolic function.

The Doppler effect was first described in 1842 by Christian Doppler, when he noticed changes in the colours of the stars as they moved towards or away from the Earth. He subsequently demonstrated that this 'Doppler shift' phenomenon occurred with sound waves as well as light (Weld 1986).

In 1880, it was discovered that controlled compression and expansion of crystalline substances could produce electric current. Conversely, applying an electric current to the crystals would produce compression and expansion waves which resulted in predictable wavelengths of sound being emitted. This is known as the piezo-electric effect (Weld 1986).

It was a further 70 years before experimentation demonstrated that the controlled emission of sound waves pointed at a moving source (in this case blood flow within the major vessels of an animal), followed by the detection of reflected waves, could be used to non-invasively estimate blood flow velocity. By pulsing the Doppler ultrasound, a transducer was produced that would both emit and receive the reflected signals, which could then be interpreted.

In 1976, it was demonstrated that Doppler could accurately measure pressure gradients across the mitral and aortic valves, with high degrees of correlation with invasive catheterisation measurements (Holen 1976). Shortly after, it was demonstrated that Doppler pressure gradients across stenosed aortic and mitral valves could accurately quantify disease severity, with good degrees of correlation (Hatle 1978; Holen 1979; Hatle 1980). Once this technology was combined with 2-dimensional echocardiographic imaging, to allow easy co-axial alignment of the beam to the cardiac long axis, it became readily possible to measure flow across the aortic and mitral valves with a high degree of accuracy.

The technology of Doppler ultrasound

The ability of moving objects to reflect a different frequency of wave to that which it receives is the basis of the Doppler effect. The speed of shift of the target object determines the difference between the frequency of sound wave emitted from the source and the frequency of the reflected wave. This is termed the degree of Doppler shift and is calculated by the following formula:

Δ frequency = [2(emitted frequency) x (target velocity) x cos(beam to target axis angulation)] / (velocity of sound in tissue)

If one is able to minimise the degree of ultrasound beam to target axis angulation, one knows the speed of sound in tissue (1,560 m/s) and one knows the emitting frequency of the ultrasound source, the target velocity can then calculated.

Pulsed and Continuous Wave Doppler

In pulsed Doppler echocardiography, a single ultrasound crystal emits and receives a reflected beam. By placing a sample volume over the area of interest, pulsed Doppler emissions interrogate a narrowly defined area of blood flow – typically, the default setting on a modern echocardiography machine would be a 5mm sample volume. Because the probe must emit and receive, there is a limit to the degree of frequency shift

that it can resolve, as there must be adequate time for the beam to return to the probe before a second beam can be emitted. This limit is known as the Nyquist limit and is equal to half the pulse repetition frequency. Beyond this limit, the Doppler signal begins to 'alias', giving overlapping signals; this means there is a limit to the velocity of blood flow that pulsed Doppler can measure.

Continuous wave Doppler resolves this issue, by continuously emitting and receiving an ultrasound beam via two separate crystals integrated into the same probe; this allows high blood velocities to be assessed. The disadvantage of continuous Doppler is that all blood flow along the path of the beam is incorporated into the received signal, rather than a selected area of interest, as with pulsed Doppler. In practical terms, this means, for example, that pulsed Doppler can selectively interrogate blood flow within the left ventricular outflow tract, whereas continuous Doppler will summate the signal from apex, mid-cavity, outflow tract, aortic valve and beyond, if aligned accordingly.

Transmitral Pulsed Doppler flow

When the sample volume of the pulsed Doppler probe is applied at the tips of the mitral valve leaflets in the apical 4 chamber view, we can align the probe to the angle of blood blow and quantify blood velocities at this point. The signal attained is adjusted in intensity through the gain setting and optimised for ease of interpretation through the range facility.

In early diastole, the passive filling phase is represented by an early filling ('E') wave. In normal subjects this is typically the larger of the two waves. The second wave represents atrial contraction ('A') and may be absent in atrial fibrillation, or appear absent during tachycardia, when the E and A waves merge due to the shortened time for diastole to take place.

Compare a normal E and A wave pattern to that of a subject who has mild diastolic dysfunction (Figure 1.1). Due to a higher end-diastolic pressure and/or resistance to passive filling, the component of blood flow during the passive phase – the E wave – is smaller than that contributed during the atrial contraction phase – the A wave. This phenomenon is known as E/A reversal and represents Class I (early) diastolic dysfunction. This is frequently seen in old age, irrespective of co-morbidities or symptoms (Rakowski 1996).

There is a significant weakness, however, in the reliability of transmitral Doppler assessment of blood flow for the assessment of left ventricular diastolic function. As left ventricular end-diastolic pressure rises and the E wave falls, the transmitted pressure in the left atrium at end-diastole gradually begins to rise. This means that in moderate diastolic dysfunction (Class II diastolic dysfunction), raised left atrial pressure increases the velocity of blood flow during the early passive filling phase again, producing an E wave signal that is greater in peak velocity than that seen in the atrial contraction phase.

This is shown in figure 1.1. Note how this E/A transmitral Doppler flow profile looks similar to that seen in a normal subject and yet the subject has diastolic dysfunction. This phenomenon is known as 'pseudonormalisation' and leads to inaccuracies in the assessment of diastolic function when the user relies on transmitral Doppler as the primary methodology of choice (Rakowski 1996).

As left ventricular pressure and left atrial pressure increase further, the profile may shift again, with a very large and rapid early filling phase (E wave). In such subjects, rapid equalisation of ventricular and atrial pressures may occur, meaning a small A wave component to filling. This profile is known as a restrictive transmitral Doppler profile and is shown in also shown figure 1.1. This profile is associated with an adverse clinical outcome and represents advanced left ventricular diastolic impairment (De Maria 1991).

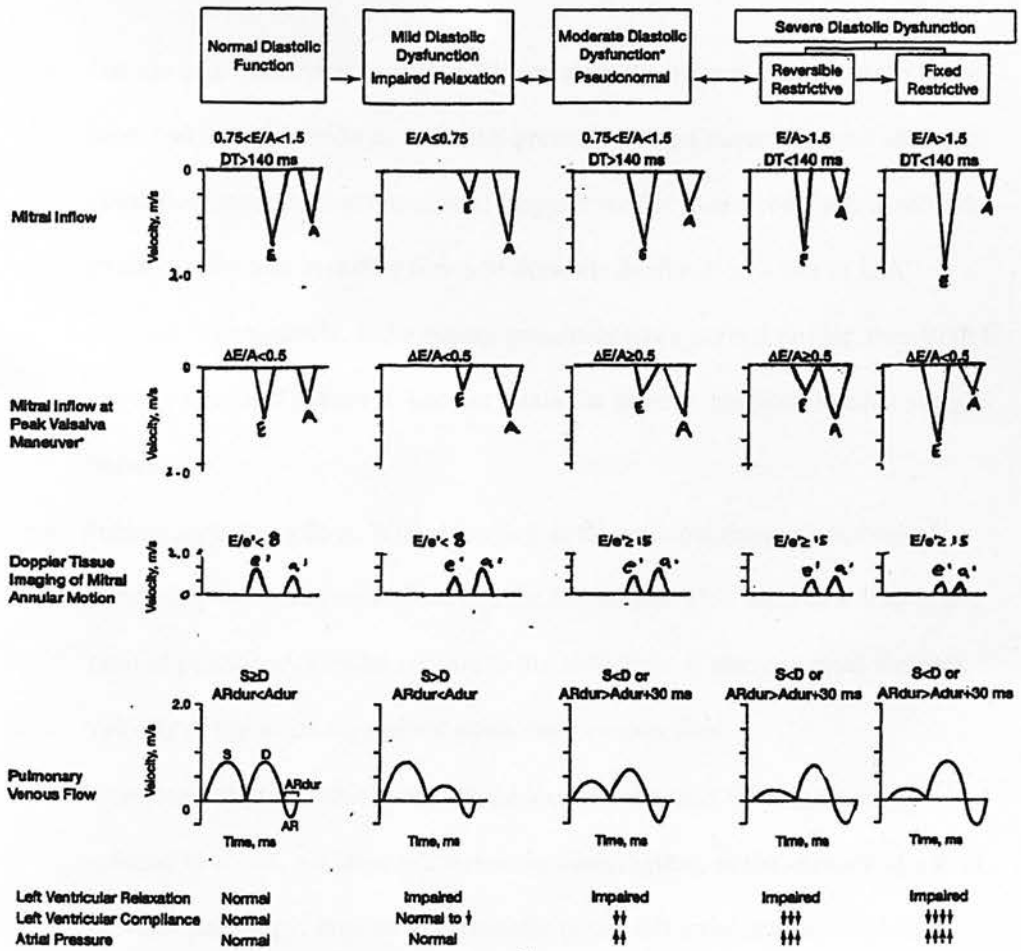


Figure 1.1. Doppler criteria for classification of diastolic function.

(Modified from JAMA 2003;289:194-202.)

Because of the problem of pseudonormalisation, further echocardiographic methods were developed to distinguish the normal from the pseudonormal transmitral Doppler profile; these improved the ability of Doppler ultrasound to accurately assess left ventricular diastolic function and include:

- The use of a Valsalva manoeuvre. This transiently reduces venous return to the heart and therefore reduces left atrial pressure. Such a move has been shown to cause the pseudonormal transmitral Doppler profile to revert for a few seconds to the profile seen in milder forms of diastolic dysfunction – that of E/A reversal. Alternatively, if the patient genuinely has a normal profile, then both E and A waves will reduce in size but retain the same proportions to each other as before.
- Pulmonary venous flow. With rising left atrial pressure, there is blunting of pulmonary venous systolic flow into the left atrium. This leads to a drop in the ratio of pulmonary venous systolic to diastolic flow. It also increases the peak velocity of the atrial regurgitant pulmonary venous flow.
- Measurement of left atrial size. Whilst less specific than the two measures referred to above, a dilated left atrium in sinus rhythm, in the absence of mitral valvular pathology, implies a chronically raised left atrial pressure and raises suspicion of a pseudonormal transmitral Doppler flow should the E/A ratio appear greater than one (Rakowski 1996). Left atrial volume is a modern, powerful predictor of outcomes in hypertension.

Despite these methods, distinguishing normal from pseudonormal is not straightforward and quantifying the degree of dysfunction remains difficult. Convention describes four major categories of diastolic dysfunction; however, such physiological properties tend

not to be categorical in nature – human subjects fluidly move from one to the next and can exist anywhere along a continuum. Passive early transmitral filling may be progressively falling or rising within one of these categorisations without yet changing 'Class', despite a significant shift in physiology. More quantitative methods were therefore required in the non-invasive assessment of diastolic function.

Tissue Doppler imaging.

One of the principle aims of echocardiography is to assess blood flow within the cardiac chambers. Echocardiography machines therefore apply filters to screen out low velocity, high amplitude signals (the cardiac walls) and focus on high velocity, low amplitude signals (the blood), with high gain settings to maximise the signal from blood. Over the last 15 years, greater interest has been taken in the information that could be obtained from these low velocity, low gain, high amplitude signals in the form of tissue Doppler echocardiography.

Spectral pulsed tissue Doppler

There are two ways in which tissue Doppler imaging can be displayed - via a spectral pulsed Doppler signal, or via colour Doppler signals. In order to minimise deviation in the angle of the beam from the target's perpendicular axis, images are acquired from the apical views at the level of the annulus. In these views, the apex will contract towards the base and with appropriate orientation of the Doppler sample volume, reproducible

estimates of long axis left ventricular function can be acquired, producing spectral waveforms similar to that seen in Figure 1.2.

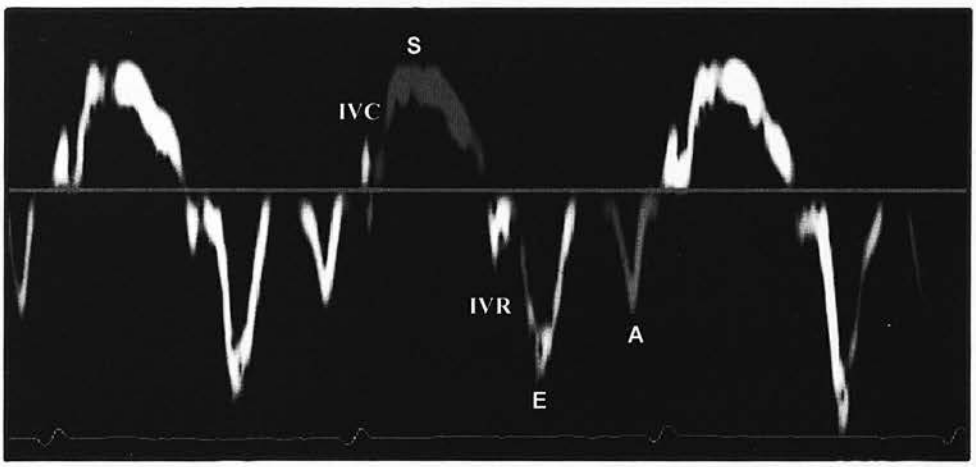


Figure 1.2. A sample pulse spectral tissue Doppler waveform.

The components are:

- E' wave – Early diastolic mitral annular velocity, corresponding to wall motion taking place during the passive phase of diastolic filling
- A' wave – Late diastolic mitral annular velocity, corresponding to the wall motion that occurs during atrial contraction
- S' wave – Systolic contraction of the ventricle
- IVC - Isovolumic contraction phase, corresponding to ventricular contraction occurring before the aortic valve opens
- IVR - Isovolumic relaxation phase, corresponding to ventricular relaxation occurring before the mitral valve opens

Tissue Doppler provides, effectively, a summation of the contractile qualities of the entire wall of myocardium attached to that aspect of the mitral valve, from annulus to apex. Typically, the anterior, posterior, inferior, septal and lateral walls of the left ventricle are assessed, along with the RV free wall in the apical four chamber view (Figure 1.3).

Reproducibility rates for tissue Doppler mitral annular velocities are much less dependent on blood volume than transmitral Doppler velocities are (which can vary by as much as 50% pre and post dialysis).

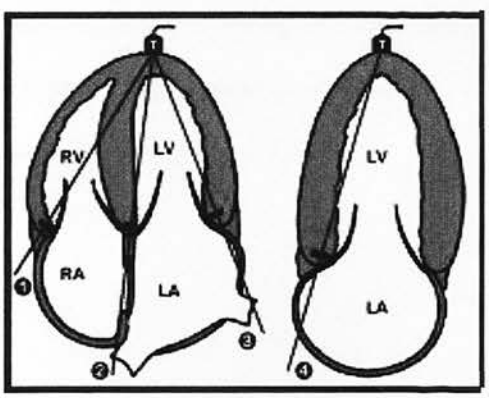


Figure 1.3. Beam orientation for assessing long-axis function of the heart using tissue Doppler.

Colour tissue Doppler

Whilst spectral Doppler is able to provide continuous high frame rates to examine the area of interest, it can only examine one segment of myocardium at a time. Colour tissue Doppler codes for mean long axis velocities across all visible myocardium in one simultaneously acquired 2-dimensional image, providing a colour coded guide to ventricular contraction and relaxation. With appropriate software, a region of interest can be interrogated post-acquisition, creating a spectral waveform similar to that seen in figure 1.2. This allows for quantification of the waveform components referred to above.

The frame rates of the post-acquisition spectral waveform images derived from colour Doppler source are lower than that acquired with online pulsed Spectral Doppler; however, colour mapping has the advantage of allowing immediate visual estimation of a broader range of wall motion and allows post-procedural interrogation of any acquired area of interest (Figure 1.4).

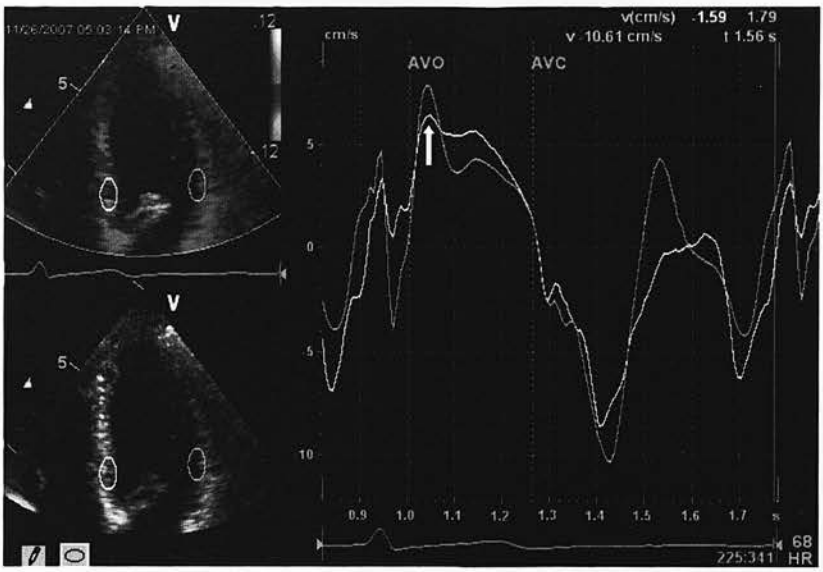


Figure 1.4. Colour tissue Doppler of the left ventricle in the apical four chamber view. Application of areas of interest during post-processing produces spectral Doppler waveforms, in this case to assess the degree of synchrony within the ventricle. Reproduced from Gorcsan J , Suffoletto M S Europace 2008;10:iii80-iii87

Development of tissue Doppler imaging as a research and clinical tool

Tissue Doppler imaging was first described by Isaaz in 1989. He studied wall motion in 17 normal subjects and 23 patients undergoing cardiac catheterization. Placing a sample volume just above the mitral valve over the LV posterior wall endocardium, he was able to record LV posterior wall motion. The Doppler signal obtained was similar to that of long-axis M-Mode echocardiography, with a significant correlation between the peak E' wave velocity on tissue Doppler echocardiography and M-mode peak diastolic

endocardial velocity ($r = 0.90$, $p<0.001$). A strong correlation was also demonstrated between peak S' wave velocity on tissue Doppler and M-mode peak systolic endocardial velocity ($r = 0.81$, p less than 0.001). The Tissue Doppler S' wave also proved to be a better marker of LV posterior wall motion on cine angiography than long-axis M-Mode (Isaaz 1989).

Subsequently, the peak tissue Doppler S' wave velocity was demonstrated by Gulati et al to correlate strongly with global LV function (Gulati 1996). In this study, tissue Doppler long axis velocities were compared to ejection fraction acquired by nuclear scanning; six mitral annular sites were sampled: the inferoseptal, lateral, anterior, anteroseptal and posterior walls, using the three standard apical 2-dimensional echocardiographic windows. The average peak mitral annular systolic velocity was 5.5 +/- 1.9 cm/s (range 2.4cm/s to 10.5cm/s), compared with a group mean ejection fraction of 49 +/- 18% (range 17 to 80%). A strong correlation was demonstrated between the two measures ($r = 0.86$; Figure 1.5).

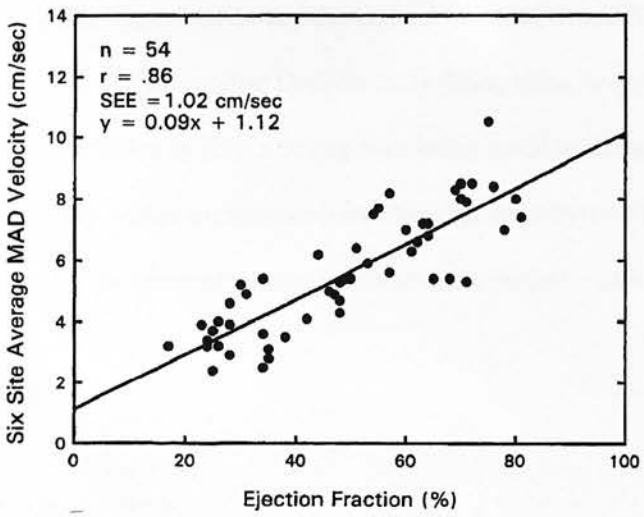


Figure 1.5. Plot of the correlation of the average of Peak mitral annular descent (MAD) velocity against ejection fraction by nuclear scanning.

Gulati VK et al. 1996.

A six-site peak mitral annular systolic velocity average of greater than 5.4 cm/s was 88% sensitive and 97% specific for identifying an ejection fraction >50%, with the strongest correlation obtained between velocities from the apical 4-chamber inferoseptal and lateral walls and the LV ejection fraction ($r = 0.85$).

High degrees of correlation were demonstrated between tissue Doppler E' velocity and invasive assessments of left ventricular diastolic function. Additionally, the relative pre-load independence of Tissue Doppler measurements were confirmed before and after saline loading and/or nitroglycerin, (Sohn 1997).

In 1997, Nagueh advanced the clinical utility of tissue Doppler. He demonstrated that by dividing the transmitral Doppler early filling velocity (E) by the mitral annular tissue Doppler velocity (E'), a strong correlation could be demonstrated with the pulmonary capillary wedge pressure acquired through catheterisation; thus, the E/E' ratio provides us with an apparently reliable non-invasive estimate of left ventricular filling pressure (Figure 1.6).

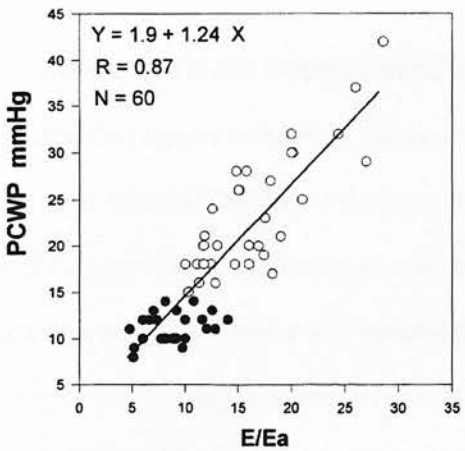


Figure 1.6. Relationship of Pulmonary Capillary wedge pressure on catheterisation with E (TMD)/Ea (TDE) ratio. Adapted From Nagueh, 1997JACC Vol. 30, No. 6 November 15, 1997:1527–33

These findings have since been replicated (Ommen 2000) and validated in patients with hypertrophic cardiomyopathy, sinus tachycardia and atrial fibrillation, amongst others (Nagueh 1998; Nagueh 1999).

Applications continued to expand, with Tissue Doppler echocardiography developing a role in the assessment of patients for biventricular pacemakers (Whinett 2005), the assessment of athlete's heart (Vinereanu 2005) and in assessing suitability for valvular heart surgery (Bauer 2008). Tissue Doppler imaging has now become part of the standard transthoracic echocardiographic assessment for many echo labs around the world.

Limitations of Tissue Doppler imaging

Whilst tissue Doppler technology has several useful aspects, it also suffers from several limitations. Spectral Doppler of the myocardium at the level of the mitral annulus effectively provides a summation of long axis along the wall of a ventricle. This means if one area of the ventricular wall subtending that portion of the mitral annulus is infarcted (low velocity) and another area is healthy (normal velocity), then the sum velocity assessed at the annulus will be an intermediate value that does not distinguish the above scenario from a uniform cardiomyopathic process that causes general deterioration in wall motion (Stoylen 2003).

Strain rate imaging solves this issue by referencing one point of wall motion against an adjacent one, rather than assessing the effect of a whole wall. However, without anatomical landmarks, selective sampling of, for example, the mid-cavity septal wall is prone to reproducibility issues (Urheim 2000). This can be partially solved by assessing at points of fixed anatomical structures (e.g. adjacent to the base of the papillary

muscle). Strain imaging, however, requires more post-processing than tissue Doppler, in order to acquire clinically useful information, making it a less flexible clinical tool.

Whilst Sohn convincingly demonstrated that under normal physiological shifts in pre-load, tissue Doppler remains reliable, some small variations in findings can be demonstrated in circumstances of extreme volume shift (Graham 2003, Drighil 2008).

Pulsed spectral Doppler allows for high frame rates and accuracy; however, all desired waveforms must be acquired at one sitting. This issue can be solved by acquiring long axis colour tissue Doppler images for post-processing, but as mentioned, this provides lower frame-rate areas of interest and requires specialist software to readily analyse.

There are several anatomical issues that can limit the accuracy of pulsed spectral Doppler waveform imaging. Mitral annular disease or heavy calcification can reduce reliability and reproducibility, as can off-axis hearts – the apex must lie under the probe in an orthodox position to acquire meaningful data. Echocardiographic windows need not be high quality for tissue Doppler and this is one of its strengths; however, there must be a sufficiently good and on-axis view to ensure that the Doppler cursor is lined up from probe to apex to annulus. Tachycardia can also inhibit the quality of the data acquired, through fusion of the E' wave and A' wave.

It has been reported that variations in left ventricular ejection fraction can affect what are considered to be 'normal ranges' for tissue Doppler values, such as E' or the E/E' ratio. The cut-off value for normal filling pressures may be lower for patients with a normal ejection fraction when compared to patients with impaired ventricular function. This remains a source of discussion and can contribute to confusion within labs and between labs (Firstenberg 2000; Yamamoto 1997).

Despite these limitations, tissue Doppler remains an important clinical and research tool. This manuscript describes a body of research intended to develop the role of tissue Doppler echocardiography in the assessment of the hypertensive ventricle.

Chapter 2

Methodology

Patient Population

This thesis examines a cohort of hypertensive subjects enrolled within the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT).

ASCOT was a multicentre, prospective, randomised controlled trial in 19 257 patients with hypertension, aged between 40–79 years with at least three other cardiovascular risk factors. Patients were assigned either amlodipine 5–10 mg, adding perindopril 4–8 mg as required (amlodipine-based regimen; n=9639) or atenolol 50–100 mg, adding bendroflumethiazide 1·25–2·5 mg and potassium as required (atenolol-based regimen; n=9618). The primary endpoint of the parent study was non-fatal myocardial infarction (including silent myocardial infaction) and fatal CHD (Sever 2001).

Within ASCOT, detailed cardiovascular phenotypic data were collected on a subset of 1006 participants recruited from two centres (St Mary's Hospital, London and the Adapt Centre, Beaumont Hospital, Dublin). This phenotyping process included the collection of serum samples, urinalysis, 12-lead electrocardiography, transthoracic echocardiography, 24-hour blood pressure recordings (Stanton 2001). These subjects form the basis of this thesis.

ASCOT inclusion criteria

There were strict entrance criteria for ASCOT. At screening and randomisation, all subjects were required to be aged between 40 and 80 years old, with untreated

hypertension (systolic BP maintained at ≥160mmHg and/or a diastolic BP ≥100mmHg OR at randomisation) or treated hypertension (with a systolic BP maintained at ≥140mmHg and/or a diastolic BP at ≥90mmHg on ≥1 drug).

They were also required to possess three or more of the following risk factors for a future cardiovascular event:

(i) Pre-existing LVH on echocardiography according to ASE criteria (Lang 2005)

OR

Pre-existing LVH on ECG by Cornell voltage duration product or Sokolow Lyon criteria:

- Cornell voltage criteria: S wave magnitude in lead V3+ R wave magnitude in aVL >2.6 mV, with 0.6 mV added in women. Cornell voltage product = Cornell voltage criteria with 0.6 mV added in women x mean QRS duration >240 mV ms.
- Sokolow Lyon criteria (S wave magnitude in V_1 + R wave magnitude in V_5 or V_6 (whichever is larger) ≥ 35 mm or R wave magnitude in aVL of ≥ 11 mm).

N.B. These prior diagnoses of LVH were required to have been made in routine clinical practice before being screened for the study and did not take into account any investigations conducted as part of the study.

(ii) Any of the following other ECG abnormalities: left ventricular strain pattern, abnormal Q waves, left bundle branch block, ST-T changes compatible with IHD.

(iii) Type II diabetes mellitus (non-insulin dependent) by WHO criteria:

- A fasting venous plasma glucose concentration of 7mmol/l or more
- A 2-hour value of 11mmol/l or more following a 75g load.

(iv) Peripheral vascular disease according to the Edinburgh claudication questionnaire (see Appendix 1).

(v) Past history of cerebrovascular event(s) including TIA's ≥three months previously.

(vi) Male sex.

(vii) Age ≥55 years.

(vii) Microalbuminuria/Proteinuria.

(viii) Smoking (i.e. regular smoker within the last year of ≥ 20 cigs/week).

(ix) Plasma total/HDL cholesterol ratio ≥6.

(x) A history of a coronary artery disease event occurring in a first degree relative before the age of 55 (males) or 60 years (women).

ASCOT exclusion criteria

Any contraindications to, or previous history of, major intolerance to dihydropyridine calcium channel blockers (CCBs), ACE inhibitors, beta-blockers, thiazide diuretics, doxazosin, or statins.

(i) A history of secondary hypertension.

(ii) Malignant hypertension.

(iii) Previous myocardial infarction or currently treated angina pectoris.

(iv) Stroke, transient ischemic attacks, or cerebrovascular surgery <3 months before study onset.

(v) Patients requiring CCBs, ACE-Is, beta-blockers or diuretics for concomitant diseases or conditions.

(vi) Fasting serum triglycerides >4.5 mmol/l.

(vii) Patients requiring other drugs which are also prescribed for hypertension (eg alpha-blockers for prostatism).

(viii) Second or third-degree A-V block.

(ix) Clinical congestive heart failure (NYHA II-IV).

(x) Uncontrolled arrhythmias.

(xi) Concomitant clinically important haematological, gastrointestinal, hepatic (liver function test [ALT] >3x upper normal level), renal (se creatinine >200 μmol/l), or other disease which, in the opinion of the investigator, will interfere with the treatment or the patient's ability to complete the study.

(xii) A history of alcoholism, drug abuse, psychosis, antagonistic personality, poor motivation or other emotional or intellectual problems that are likely to invalidate informed consent, or limit the ability of the subject to comply with the protocol requirements.

(xiii) Participation in any other studies involving investigational or marketed products within one month prior to entry into this study or concomitantly with this study.

(xiv) Pregnant or lactating women and those of child bearing potential (i.e. pre-menopausal without appropriate contraception.

Blood pressure measurement protocol

Whilst historically, the mercury sphygmomanometer is the most accurate measure of cuff blood pressure in skilled hands, the potential for observer error and bias within a multicentre trial could have been significant (O'Brien 1991). At the onset of this trial, there was also a strong suggestion that mercury devices would be banned from general use on the grounds of health and safety legislation. An alternative method for measurement was therefore required. Aneroid sphygmomanometers had been used in previous studies as alternatives to the mercury sphygmomanometer but these become inaccurate with use and are also subject to observer error and bias (O'Brien 1990; Conroy 1993; O'Brien 1995).

Many automated devices were available on the market but most of those that have been validated have been inaccurate. However, the OMRON HEM 705-CP has fulfilled the requirements for accuracy of the protocols of the Association for the Advancement of Medical Instrumentation (AAMI) and the British Hypertension Society (BHS). Moreover these devices provide hard copies of the blood pressure measurements and pulse rate, together with the time and date of recording. This device was therefore chosen for the ASCOT trial (O'Brien 1996).

Protocol for using the OMRON HEM 705-CP

Before use

- The device was calibrated for accuracy at yearly intervals.
- The time and date was set at the time of each measurement and hard copy of the results produced.

The patient

- Patients should have neither smoked nor consumed alcohol in the 30 minutes before blood pressure measurement.
- Patients should rest for 5 minutes in the sitting position before measurement.
- Patients should not talk during blood pressure measurement

The cuff

- Arm circumference (AC) was measured midway between the shoulder tip and the olecranon process.

- If AC <32cm the standard cuff was used; if AC ≥32cm the large cuff was used.
- The cuff is designed for blood pressure measurement in the left arm but if this was not possible, the green strip on the lower boundary of the cuff was always positioned over the brachial artery. The same arm was used for each subject throughout the study.
- The cuff was placed on skin or over a shirt or blouse sleeve.
- The arm was supported at heart level.

The measurement

- The device was switched on by pressing the SPHYG/CLOCK button and waiting until the display shows a '0'.
- A range for blood pressure was selected using the PRESSURE VALVE PRESET switch. In the AUTO mode the cuff inflates to about 170mmHg. If pressures above this level were anticipated the switch was changed to 240mmHg.
- The mean of the last two readings was used for the purpose of analysis and recorded in an electronic records form.

Phlebotomy protocol

Two serum samples were taken at the time of randomisation and at the time of echocardiography. These were spun and frozen at -80 degrees centigrade within appropriate storage medium for future batch analysis.

Electrocardiography protocol

Standard 12 lead electrocardiograms were recorded at a paper speed of 25mm/s and standardisation of 1mV/cm. The criteria for the diagnosis of silent myocardial infarction were the appearance of major Q or QS waves during the course of the study.
ECGs were read and coded blindly according to the 'Minnesota code 1982' classification by two trained laboratory technicians (independent of each other) at the Clinical Experimental Research Laboratory, Department of Medicine, Östra University Hospital (ECG Corelab).

Echocardiography protocol

Echocardiography was performed using an ATL HDI 5000 ultrasound machine equipped with a standard multi-frequency transducer 12 months after initiation of treatment within ASCOT. The following echocardiographic views were obtained: parasternal left ventricular long and short axis; right ventricular inflow and outflow views; parasternal short axis; apical two, three, four and five chamber views.

Reproducibility data

A two-dimensional echocardiographic study was performed by one of two echocardiographers according to site, using a standard examination protocol. Between echocardiographer variability of echocardiographic measurements was assessed before commencement of the study and subsequently at regular intervals during the study. Using the Bland-Altman method, the standard deviation of difference of individual

variables (e.g. septal thickness in diastole, E wave, E' wave etc.) was less than 7.5% of the mean value, demonstrating that our reproducibility measurements were in keeping with other studies (Palmieri 2003). Appendix 1 contains detailed samples from the extensive reproducibility data acquired for this study.

To further confirm that any differences in the methods of data acquisition from our two centres could not have affected our outcomes analysis, we constructed a separate multivariate model containing centre of origin of the subject, patient demographic factors, co-morbidities and outcomes. Centre of assessment did not prove to be a predictor of outcomes on univariate or multivariate analysis, suggesting it did not significantly influence outcomes.

Left ventricular wall measurements

Left ventricular septal wall thickness, posterior wall thickness and cavity size were measured from the left ventricular parasternal long axis view using two-dimensionally guided M-mode echocardiography. Particular attention was paid to obtaining a precise cross-sectional "on-axis" image of the left ventricular tips of the mitral valve leaflets. Where M-mode was not possible, measurements were made from 2-dimensional echocardiographic images.

Measurements of left ventricular septum, posterior wall and cavity dimensions were made at end diastole using the leading edge to leading edge technique as recommended by the American Society of Echocardiography (Lang 2005). Three consecutive cardiac

cycles were measured and average values obtained. Images were also stored digitally for offline verification.

Systolic measurements of left ventricular internal diameter and posterior wall thickness were made using the leading edge to leading edge method. These were measured online from still M-mode images in the parasternal long axis images.

Left ventricular mass was calculated using the cubed formula with an appropriate validation factor, as recommended by the American Society of Echocardiography.

- LV mass = 0.8 [1.04(IVSd+LVIDd+PWTd)3 – (LVIDd)3] + 0.6 g

  IVSd = Intraventricular Septal Thickness in diastole

  LVIDd = Left Ventricular diameter in diastole

  PWTd = Posterior Wall Thickness in diastole.

This figure was then divided by the patient's body surface area, which was derived from an algorithm based on the patient's height and weight (DuBois and DuBois 1916), to give a value for left ventricular mass index (Levy 1987). This method for deriving an M-mode derived left ventricular mass has been previously validated against post-mortem measured left ventricular mass (Devereux and Reichek 1977).

Relative wall thickness gives an indication of the ratio of wall thickness to cavity size. This was calculated using the following formula, where all measurements are made using the leading edge to leading edge technique:

- Relative wall thickness = 2*PWT/LVID

LVID = Left ventricular internal diameter in diastole

PWT = Posterior wall thickness in diastole

Measurements of left atrial size were made from M-mode imaging, taken from the parasternal long axis at the level of the aortic valve. The left atrial dimension was measured at end-ventricular systole and included the thickness of the posterior wall of the aorta but not the thickness of the posterior atrial wall, as recommended by the American Society of Echocardiography (Lang 2005). Three consecutive cardiac cycles were measured and average values obtained.

Transmitral Doppler echocardiography

Transmitral pulsed Doppler flow was measured from the apical four chamber view. It was assessed using a 5 mm sample volume placed at the tips of the mitral leaflets in passive end-expiration. To obtain optimal velocity recordings, the beam angle was as close to zero as possible compared to the angle of blood flow. Sweep speed was set at 100mm/second and gain settings were minimalised to reduce artefact.

A standardised loop of ten cardiac cycles was downloaded to computer for off-line analysis of the early filling phase (E wave), the late filling phase (A wave) and E wave deceleration time.

The Valsalva maneouvre was performed on all subjects at the St Mary's site according to standard methods and a further loop of 10 cardiac cycles of transmitral Doppler flow was downloaded to computer for off-line analysis.

Tissue Doppler echocardiography

Spectral pulsed Doppler tracings were obtained from the apical four, two and three chamber views, with the 5 mm sample volume placed over the myocardium on the septal, lateral and inferior walls at the level of the mitral annulus. This was followed by measurements from the free wall of the right ventricle at the level of the tricuspid annulus. Using minimalized gain settings, a series of ten cardiac cycles was recorded. These were then downloaded for off-line analysis, with measurements made of systolic velocity (S' wave), early diastolic velocity (E' wave) and late diastolic velocity (A' wave) at each location. A composite average value for the S' wave, E' wave and A' wave velocity was then made for each wall.

Analysis of all transmitral and tissue Doppler images was performed using the HDI Lab package by a single researcher (AS), who was blinded to all patient details.

Clinical endpoint coding methodology

This thesis incorporates a prospective study of clinical outcomes, as related to tissue Doppler derived measures of diastolic function. Specifically, we analysed the relationship of diastolic function to future cardiac events and death. The methodologies used to code clinical events were rigorous and are now detailed.

Endpoint adjudication

Endpoints were adjudicated by the ASCOT endpoints committee, whose members were: Ulf Dahlstrom, Linkoping, Sweden; Frej Fyhrquist, Helsinki, Finland; Kjell Midtbo, Oslo, Norway and Harry Hemingway (Chair), London, England.

All the documentation for each potential endpoint was reviewed independently by two of the Endpoint Committee members. In the event of disagreement, a third member the Endpoint Committee would adjudicate. Final classification required agreement between two members of the Endpoint Committee.

Information available to panel members:

All available relevant documents were provided by investigators for review by the Endpoint Committee (EPC). The EPC needed to see the evidence on which diagnoses are based, rather than the opinion of the physician responsible for the patient. Thus the EPC required photocopies of ECGs or exercise ECGs (rather than their reports), actual values of biochemical markers of myocardial necrosis, and so on. This documentation typically included:

- medical records detailing specific symptoms and signs
- hospital discharge summaries
- procedure reports
- operation reports
- copies of resting electrocardiograms (must have the date recorded)

- copies of exercise electrocardiograms
- laboratory test results
- autopsy reports
- death certificates

Definitions of specific clinical endpoints and methods of categorisation.

Endpoint 1. Fatal CHD

The Endpoint Committee members reviewed all the documentation provided for all deaths. The final determination of the cause of death was made by the Endpoint Committee and thus the Endpoint Committee could over-ride what was written on the death certificate. Fatal CHD was defined as definite + probable + possible CHD death, regardless of timing or mechanism of death.

Definite CHD death was one in which there is post-mortem evidence of myocardial infarction (macroscopically visible new or old infarction or recent occlusion of coronary artery), or coronary artery disease (chronic occlusion of coronary artery or stenosis more than 50 %) and the absence of another cause of death.

Probable CHD death is one in which there is ante-mortem evidence of definite CHD (i.e. MI, unstable angina or chronic stable angina) and in the absence of another cause of death.

Possible CHD death is where the underlying cause of death on the death certificate is the only documented clinical evidence of CHD.

Endpoint 2. Symptomatic non-fatal myocardial infarction

Any symptomatic myocardial infarction (MI) occurring in this trial (first and recurrent) was coded using the definition of the MONICA study (Tunstall-Pedoe, Circulation 1994) and in line with European Society of Cardiology and American College of Cardiology 2000 recommendations. The definition of myocardial infarction is made on the evidence, in dated records, of biochemical markers of myocardial necrosis, symptoms and electrocardiographic changes.

Biochemical markers of myocardial necrosis

The table contained in Appendix 1 outlines the MONICA definitions of biochemical markers of myocardial infarction and the European Society of Cardiology / American College of Cardiology 2000 consensus redefinition of myocardial infarction that were used for the purposes of MI adjudication in this study.

ECG adjudication

The classification of the acute ECGs was based on the Minnesota coding system (Zhang 2010) and divided into definite or probable ECG evidence of MI:

Definite ECG evidence of MI

- Development in serial records of a q wave

  AND/OR

- Evolution of an injury current which lasts more than one day

- ST segment elevation lasting more than one day (is present on consecutive records of different dates)

AND

- T wave progression on three or more records with an abnormality present on consecutive records of different dates.

Probable ECG evidence of MI

Evolution of repolarisation changes

- Major ST depression present in one ECG and no major ST depression in another ECG.
- ST elevation present in one ECG and absent in another ECG.
- Major T wave inversion present in one ECG and absent in another ECG

Overall endpoint coding of definite symptomatic myocardial infarction

This coding will be made on the basis of combinations of symptoms, ECG and biochemical abnormalities and may take any one of three forms:

- Definite ECG evidence of acute MI or a definite autopsy diagnosis of acute MI.
- Symptoms consistent with acute MI, probable changes of ECG MI and abnormal biochemical markers
- Typical symptoms, abnormal ECG and abnormal biochemical markers.

Endpoint 3. Silent myocardial infarction

Silent myocardial infarction was diagnosed when there was appearance on the 12-lead ECG of new major Q or QS waves without a history of symptomatic myocardial infarction.

All study ECGs (at randomisation, at 2 year follow up and at study completion) were read and coded blind to all clinical information according to the 'Minnesota code 1982' classification by two trained laboratory technicians (independent of each other) at the Clinical Experimental Research Laboratory, Department of Medicine, Östra University Hospital (ECG Core Centre).

A comparison was then made between ECGs taken at:

- 2 year follow up vs. randomisation
- Study end vs. 2 year follow up
- Study end vs. randomisation (in absence of 2 year ECG)

and a list was generated (by computer) of all study participants with a Q or QS code present at one time point but absent on the previous study ECG. These ECG pairs in which the later ECG has a new Q or QS code were then visually compared by two independent coders. The purpose of the third step was to exclude obvious errors from further consideration. However, major new Q or QS items occurring in the same set of leads in the immediately preceding ECG, were included among those ECG pairs for step four.

Finally, the Endpoint Committee then reviewed the sources of information for each case of potential silent MI, which were all available ECGs (minimum of 2) and all the participant's previous endpoints (e.g. details of previous angina, unstable angina).
The Endpoint Committee members then judged the significance of new Q waves on the extent to which:

- There was significant serial change (rather than marginal changes that occur within the limits of recording variation)
- Changes were found in a new territory and not found in the immediately preceding ECG
- Changes were present in at least two contiguous leads

The date of the event of a silent MI was defined as the midpoint between the two ECGs between which the significant serial change was shown to have occurred.

Endpoint 4. All cause mortality

This was defined as death from any cause.

Endpoint 5. Cardiovascular death

This was defined as any death in which the underlying cause of death agreed by the Endpoint Committee lay within "Chapter VII Diseases of the Circulatory System, ICD 1975 revision" ICD9 codes 390 – 459 (ICD 10 I00-I99)

- CHD
- Stroke

- Other heart and vascular disease (ICD 9 390-405, 415-429, 440-459) (ICD 10 all remaining I codes).

Endpoint 6. Fatal and non-fatal new onset heart failure

The definition of new onset heart failure required three components:

- *≥ 2 New Symptoms / Signs / Response to treatment present in the following list:*
  - dyspnoea at rest or ordinary exertion, night cough, or orthopnoea
  - sinus tachycardia
  - pulmonary rhales
  - third heart sound
  - bilateral ankle oedema
  - hepatomegaly
  - raised jugular venous pressure
  - diuresis and relief of symptoms with loop diuretic
- *≥ 1 abnormality on one or more of the following investigations:*
  - Chest x-ray findings consistent with heart failure:
    - acute pulmonary oedema
    - congestion with interlobar lines (considered due to heart failure)

- cardiothoracic ratio >0.5, or report of enlarged heart

o Impaired left ventricular systolic function

- LV ejection fraction is ≤ 40% or if there is a statement of mild, moderate or severe LV systolic impairment on echocardiography, nuclear scanning or left ventricular angiography
- Raised left ventricular end diastolic pressure on left ventricular angiography

- *Statement of a diagnosis of heart failure by the attending physician*

Endpoint 7. Coronary revascularisation procedures

Revascularisation procedures were defined as:

- Coronary angioplasty
- Coronary atherectomy
- Coronary stent implantation
- Coronary artery bypass graft (CABG) surgery

For study participants undergoing primary angioplasty for acute myocardial infarction, both events were coded, but for the purposes of combined endpoint analysis, only one event was considered within any given model.

Endpoint 8. Unstable angina

It was recognised that the distinction between unstable angina and possible acute MI can be difficult. For patients with multiple CHD events occurring during a 28 day period, only the most serious one was coded (MI > unstable angina > chronic stable angina).

A case of definite unstable angina was defined as satisfying each of the following conditions:

- Admission to hospital with chest pain, but not an acute MI on this admission
- Angina symptoms which are new, or severe or increasing

  The Braunwald definitions were used to define the angina (Braunwald 1989):

  - New onset angina is that starting within two months of the admission.
  - Severe or frequent angina ≥ 3 episodes per day.
- One or more positive investigatory evidence of ischaemia
  - Transient ST-T wave changes during pain.
  - Positive exercise ECG or thallium scan.
  - Angiographic evidence of coronary artery disease within 6 months of the episode.

Endpoint 9. New diagnosis of chronic stable angina

Chronic stable angina was defined as the combination of typical chest pain and a test abnormality

- ≥ 2 Features of typical chest pain:
    - Quality: ache, burning, discomfort, squeezing, heaviness or feeling of pressure
    - Duration: several minutes, but less than 20 min
    - Location: central sternum, precordium
    - Precipitation: exercise or emotional upset
    - Relief: by rest or nitro-glycerine
    - In the opinion of the attending physician the chest pain / discomfort was due to myocardial ischaemia
- ≥ 1 Test abnormality:
    - Coronary angiography showing >50% stenosis in left main stem or at least one stenosis of at least 70% in another major artery.
    - Positive exercise ECG (≥ 1mm ST depression). In all cases the ECG tracings will be reviewed by EPC members.
    - Positive thallium scintigraphy.

- Abnormal resting ECG showing ischaemic Q, ST or T wave changes.

Endpoint 10. Life threatening arrhythmia

This was defined as one of:

- Ventricular fibrillation
- Sustained ventricular tachycardia requiring urgent cardioversion (DC shock)
- Complete (third degree) heart block.

Statistical analysis

Statistical analysis was performed using SPSS v14.0 for Windows (SPSS Inc., 2005, Chicago, IL). All continuous variables were tested for normality and log transformed where skewed distributions were demonstrated. These are presented as mean ± standard deviation. Categorical demographic factors are presented as number (n) followed by percentages (%). Demographic and echocardiographic factors were analysed between groups using t-test or ANOVA for continuous variables and chi-square for categorical variables where appropriate.

Several multivariate statistical strategies were used within this manuscript according to the requirements of the dataset under interrogation. These methodologies will be described within the methods section of each subsequent chapter within the manuscript.

Ethics

All centres cleared this study with their local ethics committee. All patients who were consented for this study did so with informed consent. Sample patient information sheets are contained in Appendix 1. This study was designed and enacted according to the principles of the Declaration of Helsinki.

Chapter 3.

Establishing the prevalence of a pseudonormal transmitral Doppler flow profile within a hypertensive population

Abstract

Background

Patients with hypertension are at significant risk of diastolic dysfunction. The routine echocardiographic assessment of diastolic function relies on Transmitral Doppler (TMD) flow, with an early (E) to late (A) diastolic flow ratio of < 1 considered abnormal. As diastolic dysfunction advances, however, this ratio may falsely normalize, producing a pseudonormal picture. Distinguishing normal from pseudonormal has historically proved difficult, with the Valsalva maneouvre recommended as a potential discriminator between normal and pseudonormal transmitral Doppler flow profiles.

Studies have suggested that the early diastolic Tissue Doppler Echocardiography mitral annular velocity (TDE E') can reliably quantify diastolic function, with a lateral wall TDE E' wave velocity of < 12.5cm/s suggestive of abnormal function and less than 8cm/s suggestive of significant dysfunction.

This study took a cohort of hypertensives with three other cardiovascular risk factors, and performed echocardiography with TDE to assess the frequency of pseudonormalisation in patients at high risk of diastolic impairment and to assess the ability of the Valsalva maneouvre as a discriminator.

Methods

Standard 2D, Doppler and Tissue Doppler echocardiography was performed on 1006 patients at two sites as part of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). Doppler images were acquired from the apical four chamber view with patients in the left lateral position, and Tissue Doppler velocities were obtained from the lateral wall at the level of the mitral annulus. None of the patients had evidence of a regional wall abnormality, or a history suggestive of previous myocardial infarction. Patients at the St Mary's site then underwent the Valsalva maneouvre when the TMD E/A ratio was >1, to look for pseudonormalisation.

Results

The cohort was predominantly male (77%), with a 21% incidence of diabetes and 25% incidence of smoking. Mean age was 62 years.

Of the 1006 patients enrolled, 989 subjects underwent echocardiography; 235 (23.8%) had an apparently normal TMD E/A ratio of greater than 1. Within those, 63/235 had a TDE E' velocity of >12.5cm/s, suggestive of normal diastolic function (6.3% of the whole cohort).

At one site, 163 of the 579 subjects scanned performed a Valsalva maneouvre as per protocol. Of these, 26/163 subjects reverted transiently to an E/A ratio of <1, suggesting a pseudonormal transmitral flow profile. This suggested normal function in the

remaining 137/163. However, 98 out of these 137 had TDE E' velocities < 12.5cm/s of the lateral mitral valve annulus, suggestive of diastolic dysfunction. Furthermore, 32 of these 137 subjects (23%) had a TDE velocity of <8cm/s, suggestive of significant diastolic dysfunction.

Conclusion

In this large population of patients with hypertension, we demonstrate that a significant proportion have an apparently transmitral Doppler flow profile with a TMD E/A ratio>1 (24%). Tissue Doppler lateral wall mitral annular velocities suggest that three quarters of these were in fact pseudonormal. The Valsalva maneouvre appeared unreliable as a discriminator of normal diastolic function.

Background

Hypertension is amongst the principal causes of heart failure in the developed world and its incidence has been shown to increase with age (Haider 2003; Aurigemma 2001). A large proportion of heart failure patients with a clinical diagnosis of congestive cardiac failure have preserved left ventricular function (Owan 2006); typically these patients are labelled as having diastolic dysfunction as the cause of their heart failure. Diastolic dysfunction has been implicated in up to 40% of hospital admissions in the elderly with heart failure and as populations are living to a greater age, it is therefore increasingly important that we are able to accurately identify diastolic dysfunction where present (Owan 2006).

Prior to the wide availability of echocardiography, cardiac catheterisation was the main method used for assessing diastolic function; however, in view of the cost and risks involved, it is impractical as a routine tool. Echocardiography allows accurate estimation of LV systolic function and by using transmitral pulsed Doppler technology, an estimation of diastolic function can be acquired (Marabotti 1989).

Transmitral Doppler flow assessment does, however, have limitations. As diastolic dysfunction becomes more advanced, the end diastolic pressure rises in the left ventricle. This increase propagates to the atria, producing a rise in atrial pressure which begins to increase the rate of passive transmitral filling once again. We therefore have a situation whereby if a patient has, for example, an E/A ratio of 0.7, and this number

rises to 0.9 on follow-up echocardiography, it is unclear whether this is due to an improvement in the patient's diastolic function, or a deterioration caused by higher left atrial pressures. Eventually patients can fully "normalize" their transmitral flow pattern, despite progressive diastolic dysfunction. This process is known as pseudonormalisation, and various adjuncts to standard echocardiographic technique have been proposed to tackle this problem (Wijbenga 1999; Naqvi 2003).

Tissue Doppler echocardiography (TDE) has been established as an additional method of assessing diastolic function. The first diastolic component of the tissue Doppler waveform, the E' mitral annular velocity, has been shown to be exhibit a strong and linear correlation with invasive measures of diastolic function on catheterisation (Nagueh 1997; Garcia 1998; Ommen 2000; Dokainish 2004).

We studied a large population of hypertensive subjects who might be expected to have some degree of left diastolic impairment, with the aim of establishing what proportion of subjects exhibited a normal transmitral Doppler profile. We then aimed to establish, through tissue Doppler imaging, what proportion of those apparently normal subjects had a pseudonormal transmitral blood flow pattern and test the reliability of the Valsalva maneouvre as a discriminator between normal and abnormal physiology.

Methods

Detailed cardiovascular phenotypic data were purposefully collected on a subset of 1006 participants recruited from two centers (579 subjects from St Mary's Hospital, London and 427 subjects from the Adapt Centre, Beaumont Hospital, Dublin) as part of the Hypertension Associated Cardiovascular Disease (HACVD) sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT; Stanton 2001). This study describes data collected from 989 of those subjects in whom appropriate data was obtained.

Patients underwent echocardiography after one year of treatment at one of two sites: St Mary's Hospital, London, or Beaumont Hospital, Dublin. Patients were scanned in the left lateral decubitus position with an ATL 5000 cardiac ultrasound machine. Doppler Images were obtained with a multi-array phased 3.75MHz probe, and images were downloaded to computer for off-line analysis using the HDILab (1998) commercially available programme.

Transmitral Doppler flow profiles were obtained by placing a 5mm sample volume at the tips of the mitral leaflets in the apical four chamber window with ten loops of optimized image recorded. The E wave velocity, A wave velocity and the E wave deceleration time were measured from three consecutive traces and then averaged. Patients at one site (St Mary's), who were demonstrated to have a transmitral Doppler E/A ratio >1, were then asked to perform a Valsalva maneouvre during repeat transmitral flow assessment.

Tissue Doppler echocardiography was also obtained in the apical four chamber window, with the sample volume placed at a point which was equidistant between the epicardium and endocardium at the level of the mitral annulus over the lateral wall (Figure 3.1). Ten loops of spectral trace were downloaded for off-line analysis (Figure 3.2). Three loops were selected, with the E wave, A wave, S wave and E wave deceleration time measured. A mean value for each of component of the waveform was recorded.

Statistical analysis

Statistical analysis was performed with the SPSS version 14.0 statistical package, using the student's t-test to assess continuous variables and chi-square to assess categorical variables. P values <0.05 were taken to be significant.

Results

Demographic data are contained in Table 3.1. The cohort was predominantly male (77%), with a 21% incidence of diabetes and 25% incidence of smoking. Mean age was 62 years.

Of 989 subjects who were studied at both sites, 235 patients (23.8%) had an apparently normal TMD E/A ratio of greater than 1. The remaining 754 subjects (76.2%) exhibited an E/A ratio of less than 1, suggestive of mild diastolic dysfunction.

Within the group exhibiting a normal TMD profile (E/A>1), 63/235 patients had a TDE E' velocity of >12.5cm/s (Dumesnil 2002), suggestive of normal diastolic function (6.3% of whole cohort; 26.8% of those with E/A ratio>1).

At the St Mary's site, 163/579 subjects studied there had a normal transmitral Doppler flow pattern and therefore performed a Valsalva maneouvre. Twenty six of these 163 subjects reverted transiently to an E/A ratio of <1, suggesting a pseudonormal transmitral flow profile. This implied that the remaining 137 of 163 subjects (84%) had normal diastolic function.

However, 98 of those 137 subjects with apparently normal diastolic function by the TMD/Valsalva method, had TDE E wave velocities < 12.5cm/s, suggestive of diastolic dysfunction. Furthermore, 32 of the 137 subjects (23%) who exhibited apparently normal diastolic function using the TMD/Valsalva method had a TDE velocity of <8cm/s, suggesting significant diastolic dysfunction.

Discussion

In a large cohort of patients with hypertension and three cardiovascular risk factors, one might expect a degree of diastolic dysfunction to be present in most subjects (Sagie 1993; Lee 1997; Shapiro 1984; Boyer 2003; Schillaci 2002). Using transmitral Doppler echocardiography, we found that 23.8% of this high-risk cohort had an E/A ratio >1, suggestive of normal diastolic function or a pseudonormal transmitral flow profile.

Using a normal range cut-off for the TDE mitral annular velocity of the lateral wall of the left ventricle of 12.5cm/s, this proportion dropped to 6.3%. Within a sub-section of this cohort, we tested the Valsalva maneouvre as a discriminating measure; this method appeared to be unreliable, suggesting normal diastolic function in 32 subjects who, using tissue Doppler, had apparently significant diastolic impairment (TDE lateral wall E' velocity of <8cm/s).

When clinicians see an apparently normal transmitral Doppler profile in patients at significant risk of diastolic function they are frequently sceptical of the appearance of normality. Our data suggest they are correct to suspect a pseudonormal profile. In this large cohort of 989 subjects, nearly a quarter of subjects had a normal transmitral Doppler profile, but this fell to 6% of subjects once tissue Doppler velocities had been taken into account.

The Valsalva maneouvre appeared a poor discriminator of diastolic function in our cohort, suggesting normality in 32 subjects with apparently advanced diastolic dysfunction. This lack of reliability reflects one of three possibilities. Firstly, our operator was performing the maneouvre inadequately; secondly the maneouvre itself is a poor method of identifying a pseudonormal TMD picture; thirdly, our cut-off used for a "normal" TDE velocity was incorrect. With respect to the first two points, our operator was an experienced clinician and echocardiographer, who was highly motivated and under no time pressure during scanning. If he was not performing the

maneouvre correctly, then the technique itself would seem to be a sub-optimal one on the grounds of reproducibility. The limitations of the Valsalva maneouvre have been documented by other authors and these data appear to support those concerns (Wijbenga 1999).

With respect to the normal range for TDE, the velocity of 12.5cm/s has been suggested by other authors as a cut-off point based on invasive cardiac catheterisation studies (Dumesnil 2002). The present study suggests that either the Valsalva maneouvre is unreliable or this cut-off is unreliable. Given the wide range of values detected within unselected populations who undergo tissue Doppler studies, it may be that an absolute cut-off value is indeed inappropriate (Garcia 1998).

However, a significant proportion of our subjects who demonstrated a normal transmitral Doppler flow profile and a normal Valsalva maneouvre had a TDE lateral wall E' mitral annular velocity of less than 8cm/s. These are TDE velocities similar to those seen in patients with cardiomyopathies, the very elderly and in subjects with systolic wall motion abnormalities, all of which are known to be associated with significant diastolic impairment (Jarnert 2000; Yu 2002; Hillis 2003;McMahon 2004; Diller 2009). This suggests that even though an absolute cut-off value for tissue Doppler E' velocities may not be as reliable as purported, the Valsalva maneouvre is likely to be the more unreliable of the two.

TDE has been demonstrated to be a highly reproducible method of assessing myocardial function and is much less dependent on pre-load status (Yalcin 2002; Graham 2003) than transmitral Doppler flow profiles, which can vary substantially with physiological shifts in preload (Sohn 1997). It also appears be linearly proportionate to invasive measures of diastolic function (Isaaz 1989; Nagueh 1997; Ommen 2000). It would therefore seem sensible that we rely on a single methodology which behaves predictably, rather than on two (TMD and Valsalva) which may behave unpredictably. On this basis, tissue Doppler assessment of the lateral wall mitral annular velocity is recommended as part of the routine assessment of diastolic function on echocardiography.

Conclusion

In a large population of patients with hypertension, we demonstrate that a significant proportion have an apparently transmitral Doppler flow profile (24%). Tissue Doppler lateral wall mitral annular velocities suggest that three quarters of these were in fact pseudonormal. The Valsalva maneouvre appeared unreliable as an alternate discriminator.

Table 3.1. Demographics.

	Mean	SD
Age	62.25	8.01
Creatinine	98.20	16.60
Body Mass Index (BMI)	29.04	4.72
	n	%
Male	760	76.85
Diabetes mellitus	211	21.33
Current Smoker	246	24.87
Peripheral vascular disease	55	5.56
Cerebrovascular events including TIA	91	9.20
Microalbuminuria/Proteinuria	572	57.84
Plasma total/HDL cholesterol ratio >=6	220	22.24

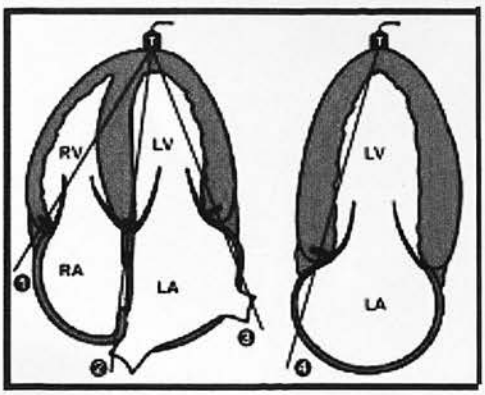


Figure 3.1: Pulsed wave tissue Doppler imaging sampling. The spectral trace is produced by placing the Doppler sample volume over a region of myocardium, and if placed at the level of the mitral and/or tricuspid annuli in the apical four chamber window, reproducibility of technique can be assured. In the figure: 1. RV free wall. 2. LV septal wall 3. LV lateral wall 4. LV Inferior wall.

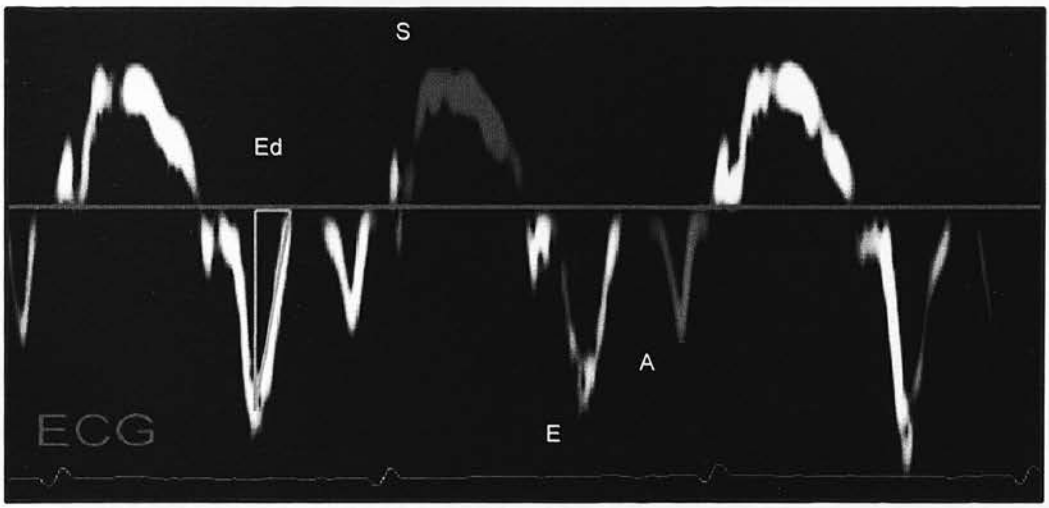


Figure 3.2: A typical TDE waveform. S: Systolic wave, E: Early relaxation, A: Late relaxation; Ed: E wave deceleration time.

Chapter 4.

Ethnicity and left ventricular diastolic function in hypertension

Abstract

Background

African-Caribbeans (AC) are known to have a higher prevalence of heart failure than white Europeans (WE) but it is unclear whether this is a result of known risk factors. Tissue Doppler technology now allows accurate quantification of diastolic function, which is recognized as an important factor in the development of heart failure.

Methods

Participants from a single centre participating in the ASCOT study, comprising subjects with hypertension but no evidence of heart failure, were studied. Left ventricular structure and function were measured in 509 subjects using conventional and tissue Doppler echocardiography. Diastolic function was assessed using the tissue Doppler early diastolic velocity E' (averaged from three LV segments) and the ratio of this and the transmitral early filling velocity E (E/E').

Results

In AC subjects, mean E' was significantly lower (7.7 vs. 8.6 cm/s, p=0.003) and mean E/E' significantly higher (8.85 vs. 7.93, p=0.003). After adjustment for confounding variables - age, sex, systolic BP, pulse pressure, cholesterol, smoking, ejection fraction, LVMI and diabetes - the effect of AC ethnicity on diastolic function remained highly

significant (E': 7.52 vs. 8.51; $p<0.001$. E/E': 8.89 vs. 7.93; $p=0.003$. AC vs.WE for both comparisons).

Conclusion

Diastolic function is significantly worse in hypertensive subjects of African-Caribbean origin than in white Europeans. This difference in diastolic performance is not due to known confounding variables.

Introduction

People of Black African descent in the Western world (African Americans in the US and African Caribbeans in the UK) have a greater risk of heart failure than comparator populations of white European origin (Alexander 1999). It is not known whether this cardiac dysfunction occurs because of an ethnic difference in myocardial susceptibility, or because of an increased prevalence in African-Caribbeans of factors that contribute to ventricular dysfunction, such as left ventricular hypertrophy (LVH), type 2 diabetes, obesity and high blood pressure (Alexander 1995; East 2004; Dries, 1999). In addition, interpretation of comparator studies performed in subjects with established end-organ failure is made more difficult by possible differential effects of complex treatment regimes. What is required is a study performed at an earlier stage of disease, with stratification or adjustment for risk factors.

The earliest cardiac consequence of hypertension is diastolic dysfunction, which is part of a continuum of ventricular impairment ending in systolic heart failure. However, until relatively recently it has been difficult to reliably quantify diastolic dysfunction.

The advent of tissue Doppler technology has provided a solution to some of the problems associated with traditional Doppler echocardiography. Rather than interpreting patterns of blood flow, it measures myocardial velocities directly and is more reproducible than historically used echocardiographic methods for assessing diastolic function, such as the Valsalva maneouvre or pulmonary vein flow. It provides measures

that are less affected by volume status or vasodilator drug therapy than conventional techniques (Nagueh 1997;Sohn 1997; Arandra 1998) and when combined with the transmitral early filling wave (E) to form a ratio (E/E'), provides an estimate of left atrial filling pressures (Nagueh 1997; De Boeck 2003, Ommen 2003).

In this study, tissue Doppler was used to investigate whether diastolic function differs between hypertensive individuals of African-Caribbean origin and white Europeans, and whether any differences observed could be explained by potential confounding variables.

Methods

The population, methods and response rate for the Anglo–Scandinavian Cardiac Outcomes Trial (ASCOT study) can be found in detail elsewhere (Sever 2001). In brief, ASCOT was a clinical trial of antihypertensive therapy (amlodipine ± perindopril vs. atenolol ± bendroflumethiazide) in 19,342 men and women aged 40 - 79 years with hypertension. Detailed cardiovascular phenotypic data were collected on a subset of 579 participants recruited at a single centre in North West London. Subjects were asked to categorize their own ethnicity, and 509 declared themselves to be of white European or African-Caribbean origin. The remaining 70 participants were of Oriental, South Asian or mixed ethnicities and were excluded from this sub-study on the grounds of insufficient numbers within each of those ethnic groups to allow analysis.

All subjects within the study were hypertensive (either untreated hypertension: systolic BP ≥160mmHg and/or diastolic BP ≥100mmHg at both screening and randomization; or treated hypertension: systolic BP ≥140mmHg and/or diastolic BP ≥90mmHg at randomization) with at least three of the following cardiovascular risk factors: previously identified LVH on echo or ECG (identified prior to enrolment in ASCOT through previous investigations), other previously identified specific ECG abnormalities, type 2 diabetes mellitus, peripheral vascular disease, previous transient ischaemic attack or stroke, male sex, age ≥ 55, microalbuminuria/proteinuria, smoking, plasma total cholesterol/HDL ratio ≥ 6, family history of ischaemic heart disease in a first degree relative (male relative <55 years old at the time, female <60 years old). Diabetes was diagnosed on the basis of fasting plasma glucose ≥ 7.0mmol/l or previous diagnosis of diabetes mellitus. Demographic data shown is that at baseline for categorical variables and that at the time of echocardiography for continuous data. Those with pre-existing ischaemic heart disease or heart failure were excluded (Sever 2001).

Echocardiography

Following a one year period of standardized anti-hypertensive therapy according to the ASCOT study protocol, all patients underwent echocardiography using an ATL HDI 5000 ultrasound machine equipped with a 7-4MHz broadband linear array transducer. All scans were performed by one of two experienced echocardiographers, with the patient semi-recumbent in the left lateral position. LV measurements were performed using M-Mode from the parasternal long axis according to the American Society of

Echocardiography guidelines (Lang 2005). Where on axis M-Mode measures were not possible, measurements were made from 2D. Left ventricular mass was calculated according to the Devereux formula (Devereux 1994). This was then indexed for body surface area to give the LV mass index (LVMI). Relative wall thickness (RWT) was calculated according to the standard formula of RWT = (2*Posterior wall thickness in diastole)/left ventricular internal diameter in diastole). Midwall fractional shortening was calculated using formulae detailed elsewhere (De Simone 1994).

Transmitral Doppler flow velocity was measured using a 5 mm sample volume placed at the tips of the mitral leaflets in passive end-expiration. A standardised loop of ten cardiac cycles was downloaded to computer for off-line analysis of the early filling phase (E wave) and the late filling phase (A wave). Tissue Doppler was performed in the apical four chamber view, with the 5 mm sample volume placed over the myocardium on the septum at the level of the mitral annulus. Using minimalized gain settings, a series of ten cardiac cycles was recorded. These were then downloaded for off-line analysis, with measurements made of: systolic motion (S' wave), early diastolic motion (E' wave) and late diastolic motion (A' wave). The E' wave velocity from the septal, lateral and inferior walls were averaged and the ratio of the transmitral E wave to E' velocity (E/E' ratio) calculated.

Analysis was performed using the HDI Lab software by a single researcher (AS), who was masked to all patient details, and each value represents the mean of three

measurements taken from three, consecutive, representative cardiac cycles. Inter-observer reproducibility data was acquired for the two echocardiographers involved at the single site used in this study, and showed a variation for all echocardiographic parameters of less than 7.5%. This is within acceptable limits as per previous studies (Palmieri 2003).

Statistical Analysis

Statistical analysis was performed using SPSS v14.0 for Windows (SPSS Inc., 2005, Chicago, IL). Data are presented as mean ± SD or median (interquartile range) for skewed data. Skewed data were normalized by $\log_{10}$transformation prior to statistical analysis. Continuous data were analysed according to ethnicity using one-way ANOVA, while categorical data were analysed using chi-square tests.

Multivariate analysis

Three multivariate models (ANCOVA), applied separately to each of the two measures of diastolic function, were built to allow tiered analysis of the effect of ethnicity on cardiac function.

- Model 1 adjusted for age and gender only.
- Model 2 adjusted for age, gender, systolic blood pressure and diabetes.
- Model 3 adjusted for age, gender, systolic blood pressure, pulse pressure, diabetes, smoking, total cholesterol, ejection fraction and LVMI.

Further analyses were undertaken separately to assess whether any measures of LV geometry other than LVMI would better explain ethnic differences in diastolic function (RWT, RWT/height^2, LVM/height$^{2.7}$) when incorporated into Model 3. Additionally, substituting mid-wall fractional shortening for ejection fraction within an alternate Model 3 was performed.

Models were also constructed incorporating drug-treatment arm (calcium channel blocker vs. B-blocker) into model 3. As almost all of the subjects were receiving combination therapy (CCB+ACE or BB and diuretic), a separate analysis for ACE inhibitors and diuretics was not undertaken. Marginal means are quoted, representing the mean values for both E' and for E/E' after adjustment for covariates. A p value of <0.05 was considered statistically significant.

Results

The overall patient characteristics are shown in Table 4.1 and are comparable to the population from the ASCOT parent study (Dahlof 2005). There were several differences between the African-Caribbean and white European groups; white Europeans were older, had lower diastolic blood pressure, higher total cholesterol and higher triglycerides. African-Caribbeans had a higher prevalence of diabetes.

Left ventricular geometry

Whilst LVMI did not differ between groups (Table 4.2) African-Caribbeans had a significantly greater mean relative wall thickness. These ethnic differences in ventricular structure were due to the greater ventricular internal diameters in white Europeans on the one hand, and thicker ventricular walls in African-Caribbeans on the other.

Diastolic function in the African-Caribbeans and white Europeans

Tissue Doppler echocardiography revealed clear differences between the two ethnic groups (Table 4.3). African-Caribbeans had lower mean E' velocity and higher E/E' values than white Europeans. Transmitral Doppler flow did not differ by ethnicity.

Multivariate analysis for relationship between E' and ethnicity (Table 4.4)

African-Caribbeans had a significantly lower mean E' velocity than White Europeans, which remained statistically significant after adjustment for age, diabetes, systolic blood pressure, pulse pressure, smoking, cholesterol and structural LV differences (7.52 vs. 8.51 cm/s respectively, $p<0.001$). Additional models substituting other measures of LV geometry for LVMI (RWT, RWT/height^2 and LVM/height$^{2.7}$) were also performed, but these did not significantly alter the findings. Using midwall fractional shortening as an alternate measure of LV function to ejection fraction also did not significantly alter the findings (data not shown).

Multivariate analysis for relationship between E/E' and ethnicity (Table 4.4)

African Caribbeans had a significantly higher mean E/E' ratio than White Europeans, which similarly remained statistically significant after adjustment for risk factors and confounders (8.89 vs. 7.93 respectively, p=0.003). Additional models substituting other measures of LV structure and function for LVMI (RWT, RWT/height^2 and LVM.height$^{2.7}$) or ejection fraction (mid-wall fractional shortening) were again performed, but these did not significantly alter the findings (data not shown).

Since an imbalance in known diabetes prevalence by ethnicity could have affected our observations through some unforeseen interaction, all models were re-run after excluding all subjects with diabetes. The magnitude of the ethnic differences seen in both diastolic parameters was essentially unchanged and remained statistically significant (E' 8.59 vs. 7.60; p=0.003 and E/E' 7.87 vs. 8.77; p=0.021). Anti-hypertensive treatment arm (calcium channel blocker ± ACE-inhibitor versus β-blocker ± diuretic) was added to the model as an additional variable and also did not significantly change the findings (data not shown).

Figure 4.1. demonstrates the magnitude of the ethnic difference in these markers of diastolic function in comparison with that of each decade of ageing.

Discussion

This study shows that African-Caribbeans in our study had a greater degree of diastolic impairment than white Europeans, either measured directly through reduced E' or through implied higher left atrial filling pressures, as represented by an increased E/E' ratio. This ethnic effect was large, and using regression coefficients was calculated to be equivalent to 18 years of ageing.

African-Caribbeans have a significantly greater risk of heart failure, renal failure and stroke compared with white Europeans (Hagstrom 1971; Keil 1984; McClellan 1984; Solberg 1972). In these later stages of disease, the adverse risk factor profile in African Caribbeans, such as a greater prevalence of diabetes and hypertension, accounts for some of the excess risk. However, a considerable amount remains unexplained. In the US, it is thought that inequitable access to healthcare, and therefore poorer treatment for risk factors, may also contribute to this excess risk. However, we have shown that this excess risk persists even at quite early stages of disease, independent of other major risk factors, in a healthcare and clinical trial setting where care provision is equitable. This implies that other explanations for this excess risk must be sought.

Diastolic function has been compared between ethnicities in two previous studies, with equivocal results. One study of 24 subjects found a lower transmitral E/A ratio in African-Caribbeans (Rittoo 1990). Another study investigating 29 African-Caribbeans and 29 white Europeans found no such difference in transmitral E/A ratio, but did find a

longer isovolumic relaxation time in the African-Caribbeans (Mayet 1994). Since neither study employed tissue Doppler, these conflicting findings may be attributable to the limitations of conventional Doppler in terms of its ability to distinguish normal function from 'pseudonormalisation'. Left atrial diameters were mildly increased in both ethnic groups, but were not significantly different. This is consistent with the left ventricular mass measurements which were also elevated to a similar degree in both groups. Neither of these two structural parameters were able to detect differences between these groups at this stage of the disease process. In contrast, tissue Doppler provided unambiguous evidence of early impaired diastolic function in the African-Caribbean group.

Age affects diastolic function regardless of the method of measurement (Henein 2002). In the present study, the age difference between the groups was small and ethnic differences in diastolic function remained after statistical adjustment for age. Differences in blood pressure might also contribute to differences in diastolic function, as African-Caribbeans are not only known to have an increased prevalence of hypertension, but also an increased severity (Chaturvedi 1993). However, in the present study (by design) blood pressure differences in Europeans and African-Caribbeans were small and statistical adjustment for blood pressure had little impact on the ethnic difference in diastolic function.

LVH is another cause of diastolic dysfunction. Previous studies exploring ethnic differences in LV structure have generally found an increased relative wall thickness in people of black African descent (Hinderliter 1992), sometimes in association with an increase in LV mass (Dunn 2004; Kizer 2004; Chaturvedi 1994) although not consistently (Lee 1992). In our study, LVMI was similar in both ethnic groups, probably as a result of previously identified LVH being one of several entry criteria for the study and the close similarity in blood pressure (again as a consequence of the entry criteria). Statistical adjustment for differences in LVMI did not attenuate ethnic differences in diastolic function nor did other methods of adjusting for LV geometry. It seems unlikely, therefore, that LV geometry fully explains the more impaired diastolic function seen in African-Caribbeans.

Diabetes mellitus is known to be more common in the African-Caribbean population and may be associated with diastolic dysfunction (Vinereanu 2003). However, in the present study, excluding subjects with a diagnosis of diabetes from the analysis did not alter the strong association between ethnicity and diastolic function.

Inequality of access to healthcare and treatments has also been suggested as a factor mediating poorer outcomes in some ethnic groups (Alexander 1990; Alexander 1995). From the onset of randomisation within the ASCOT study, all hypertension treatment decisions were strictly protocol-based, being uniformly managed throughout the trial in a 'treat to target' fashion. Also, prior to entry into the trial, all subjects within this sub-

study received their general medical care and hypertension treatment through the United Kingdom national health service, which provides "free at the point of delivery" access to health care for all. Prescription medicines are given free to those of low incomes and heavily subsidised for others. Recruitment also took place through local primary care facilities in a small geographical area, with large numbers from the same practices. These factors limit the effects of differences in access and the proposed inequalities of care seen in other health care systems. This is an important difference from several prior studies looking at the consequences of hypertension in different ethnic groups and is a strength of the present study.

A study of this nature can identify ethnic differences in disease patterns, but cannot establish underlying mechanisms. Our observations do exclude previously proposed mechanisms, such as those related to co-morbidity burden or LV structural differences. However, there remain several possible reasons for the observation of greater diastolic dysfunction in African-Caribbeans. Firstly, it may represent an increased susceptibility to myocardial impairment in response to the same degree of hypertension for reasons unknown. Secondly, ethnic differences in rennin (Chrysant 1979), aldosterone (Grim 2005) and salt sensitivity (Sealey 1998) are well established, but the relationship of these factors to diastolic dysfunction remains unclear and therefore we did not adjust for these. Such factors might have a direct bearing on myocardial function. If such factors were to affect our results simply through plasma volume status, this might have an impact on left atrial filling pressure, but less so on the tissue Doppler E' velocity, as this is a direct

myocardial velocity measurement and is relatively independent of preload variation (Arandra 1998; Graham 2003; Yalcin 2002). Finally, as these findings are entirely in African-Caribbean subjects, further work is also required to examine other African populations, or those with African heritage such as the African-American population.

Clinical implications

The relatively impaired diastolic function found in this study suggests that the greater burden of heart failure seen in the African-Caribbean hypertensive population may begin to develop from an earlier stage than previously thought, in this case well before symptoms have developed. Tissue Doppler E' velocities are a measure of early diastolic relaxation and have been shown to progressively deteriorate with increasing diastolic dysfunction and be closely related to symptoms of heart failure in those with preserved systolic function (Yu 2002). The E/E' ratio is also a marker of left atrial filling pressure, and a higher ratio has been shown to be an adverse prognostic marker in cardiovascular disease (Hillis 2004).

In summary, African-Caribbeans with treated hypertension have a greater degree of myocardial dysfunction than their white European counterparts despite good blood pressure control. This difference could not be accounted for by known confounding variables and may partly explain the increased burden of heart failure in the African-Caribbean population.

Tables and figures.

Table 4.1. Patient Characteristics

	White European n=449		African-Caribbean n=60		p value
Continuous variables	Mean	S.D.	Mean	S.D.	
Age (years)	64.3	± 7.6	61.8	± 6.8	0.018
Systolic Blood pressure (mmHg)	140.4	± 13.5	143.3	± 14.3	0.123
Diastolic Blood pressure (mmHg)	79.9	± 8.1	82.4	± 7.0	0.024
Pulse Pressure (mm Hg)	60.5	± 12.0	60.9	± 12.7	0.807
Heart Rate (bpm)	62.7	± 12.1	62.8	± 11.0	0.936
Body Surface area (m^2)	2.0	± 0.2	2.0	± 0.2	0.977
BMI (kg/m^2)	28.7	± 4.4	29.0	± 3.9	0.594
Total Cholesterol (mmol/L)	5.9	± 1.0	5.4	± 1.2	0.002
Triglycerides (mmol/L)*	1.5 (1.1 - 2.0)		1.2 (0.8 - 1.7)		<0.001
Categorical variables	n	%	n	%	
Males n (%)	390	(87)	51	(85)	0.691
Prior known ECG or echo LVH	106	(23)	21	(35)	0.055
Diabetes Mellitus	85	(19)	23	(38)	0.001
Cerebrovascular disease	35	(8)	6	(10)	0.556
Microalbuminuria	243	(54)	39	(65)	0.111
Smoker	104	(23)	14	(23)	0.977
Calcium channel blocker based treatment	227	(51)	26	(43)	0.268
ACE inhibitor treatment					
Metabolic syndrome	163	(36)	23	(38)	0.759

*Data quoted are median (interquartile range).

Table 4.2. Left ventricular structural data

	White European	African-Caribbean	p value
Interventricular septum, diastole (cm)	1.25 ± 0.20	1.32 ± 0.21	0.100
LV internal dimension, diastole (cm)	4.81 ± 0.53	4.67 ± 0.51	0.059
Posterior wall thickness, diastole (cm)	1.16 ± 0.17	1.24 ± 0.18	0.003
Interventricular septum, systole (cm)	1.61 ± 0.23	1.75 ± 0.26	<0.001
LV internal dimension, systole (cm)	3.30 ± 0.57	3.00 ± 0.55	<0.001
Posterior wall thickness, systole (cm)	1.56 ± 0.20	1.68 ± 0.25	<0.001
LV ejection fraction (%)	66.28 ± 13.16	71.08 ± 14.62	0.010
LVMI (g/m^2)	114.02 ± 27.93	118.44 ± 26.69	0.250
Relative wall thickness	0.51 ± 0.09	0.55 ± 0.11	<0.001
Left atrial size (cm)	4.34 ± 0.62	4.16 ± 0.50	0.081

All values are mean ± standard deviation

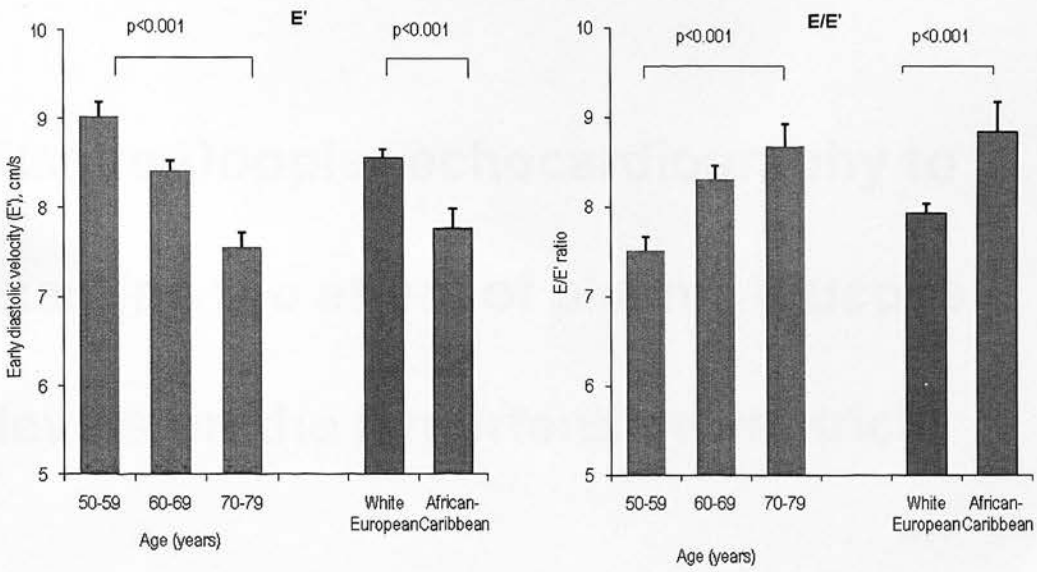
Table 4.3. Conventional and tissue Doppler echocardiographic data

	White European	African-Caribbean	p value
Transmitral Doppler			
E wave (cm/s)	63.41 ± 13.99	63.15 ± 14.78	0.895
A wave (cm/s)	73.85 ± 15.84	74.51 ± 16.53	0.762
E/A ratio	0.89 ± 0.25	0.87 ± 0.22	0.623
E wave deceleration time (ms)	201.40 ± 5.20	199.00 ± 4.60	0.685
Tissue Doppler (cm/s)			
Mean early diastolic velocity (E') cm/s	8.57 ± 1.84	7.77 ± 1.63	0.003
Mean E/E' ratio	7.93 ± 2.07	8.85 ± 2.39	0.003

Table 4.4. Multivariate analysis: Adjusted values for E' and for E/E' according to ethnicity.

Model 1	Factors included: Age, Gender					
	White-European	CI	**African-Caribbean**	CI	**Difference**	**p value**
Adjusted E'	8.60	8.34 ± 8.86	7.59	7.10 ± 8.08	-1.01	<0.001
Adjusted E/E'	7.92	7.61 ± 8.24	9.00	8.40 ± 9.60	1.08	0.001
Model 2	Factors included: Age, Gender, Diabetes, Systolic BP					
	White-European	CI	**African-Caribbean**	CI	**Difference**	**p value**
Adjusted E'	8.53	8.25 ± 8.81	7.63	4.00 ± 8.13	-0.90	0.001
Adjusted E/E'	7.94	7.61 ± 8.28	8.86	8.27 ± 9.45	0.92	0.003
Model 3	Factors included: Age, Gender, Diabetes, Systolic BP, EF,HR, LVMI					
	White-European	CI	**African-Caribbean**	CI	**Difference**	**p value**
Adjusted E'	8.49	8.20 ± 8.78	7.53	7.03 ± 8.03	-0.96	<0.001
Adjusted E/E'	7.93	7.67 ± 8.20	8.80	8.23 ± 9.36	0.87	0.006

Figure 4.1. Relationship of age group and ethnicity to diastolic function, as measured by E' and E/E'



Chapter 5.

Tissue Doppler echocardiography to examine the effect of plasma glucose levels on the hypertensive ventricle

Abstract

Background

It remains unclear whether type II diabetes mellitus has a significant effect on diastolic function in the hypertensive population. Preliminary studies have supported this concept but more data is required. This study examines the association of left ventricular diastolic function with fasting glucose levels, in order to determine if fasting plasma glucose (FPG) is an independent predictor of diastolic dysfunction and to explore possible mechanisms for this.

Methods

The Anglo–Scandinavian Cardiac Outcomes Trial (ASCOT study) of antihypertensive therapy collected substantial cardiovascular phenotypic data on a subset of 1006 participants recruited from two centres (St Mary's Hospital, London and the Adapt Centre, Dublin). Echocardiography was performed (using both conventional and tissue Doppler echocardiography (TDE)) and diastolic function was assessed using the ratio between early transmitral flow and mitral annular velocity during early diastole (E/E').

Results

The E/E ratio (average of inferior, lateral and septal E/E') was significantly higher among those with diabetes mellitus compared to those with normal glucose levels (means (SD), 8.3 (2.2) v 7.8 (2.1), p=0.017). Linear regression identified FPG as an

independent factor associated with a higher E/E ratio, after adjustment for other known risk factors. The standardised B-coefficient indicated the strength of association between the E/E ratio and FPG after adjustment for age, gender, creatinine, body mass index and heart rate (β= 0.098; p=0.007).

Conclusions

This is the first large study to assess the association of left ventricular diastolic function with FPG (across the spectrum of glucose values) using a combination of TDE and conventional echocardiography. FPG was independently associated with left ventricular diastolic function. This association was significant and similar to the association between diastolic function and systolic BP, suggesting FPG is an important factor in the development of diastolic dysfunction, independent of vascular effects.

Background

It is suspected that diabetes mellitus can lead to a specific cardiomyopathy, thought perhaps to be mediated through impairment of diastolic function, but as yet there are no clear data to establish a definitive link. Subjects with diabetes frequently have concomitant hypertension and/or coronary disease as alternative explanations for their symptoms of breathlessness and whilst these may play a major role, it would be valuable to examine the diastolic properties of a hypertensive ventricle to establish the effect glucose levels have (Boyer 2003).

There are data in the literature showing that even when there is no evidence of other known cardiac disease, people with diabetes may have systolic left ventricular dysfunction (Kosmala 2004; Vinereanu 2003; Fang 2003). It has been proposed that these abnormalities occur early in the course of diabetes. This has been difficult to elicit, largely because of the limitations of conventional echocardiography (Rakowski 1996; Appleton 1988; Wijbenga 1999). The recent development of Tissue Doppler echocardiography (TDE), which measures myocardial velocities directly, rather than through blood pool velocities, has overcome many of limitations associated with transmitral Doppler flow assessment of diastolic function. When the two are used together, echocardiography is able to distinguish normal from pseudonormal filling patterns, estimate left ventricular filling pressures and distinguish the latter stages of ventricular dysfunction (Naqvi 2003; Dokainish 2004).

Few studies have assessed the association of diabetes with diastolic function using TDE, all have used relatively small populations and no studies to date have assessed diastolic function across the spectrum of glucose values (Vinereanu 2003). The Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT study) collected substantial cardiovascular phenotypic data on a subset of 1006 participants at baseline, including both conventional echocardiography and TDE. This provides an ideal setting to determine if fasting plasma glucose (FPG) is an independent predictor of diastolic dysfunction and to explore possible mechanisms for this.

Methods

The population, methods and response rate for the Anglo – Scandinavian Cardiac Outcomes Trial (ASCOT study) are found in detail elsewhere (Sever 2001). In brief, the ASCOT study is a clinical trial of antihypertensive therapy in 19,342 men and women aged 40 - 80 years, with hypertension. Substantial cardiovascular phenotypic data were collected on a subset of 1006 participants recruited from two centres (St Mary's Hospital, London and the Adapt Centre, Dublin). Participants had no previous history of: myocardial infarction, currently treated angina, cerebrovascular event within the past three months, fasting triglycerides >4.5mmol/l, heart failure, uncontrolled arrhythmias, or any clinical important haematological or biochemical abnormality on routine screening. All patients were hypertensive (untreated hypertension (systolic BP ≥160mmHg and/or diastolic BP ≥100mmHg at both screening and randomisation); or treated hypertension (systolic BP ≥140mmHg and/or diastolic BP ≥90mmHg at

randomisation) with at least three other cardiovascular risk factors. Diabetes classification was based on plasma glucose results, using the 1999 WHO Diabetes classification (World Health Organisation 1999). Diabetes was diagnosed on the basis of FPG of ≥7.0mmol/l or previous diagnosis of diabetes mellitus. Impaired fasting glucose (IFG) was defined on the basis of FPG of ≥6.1mmol/l & <7.0mmol/l. Oral glucose tolerance testing was not performed and therefore diagnostic categorisation was based only on FPG as follows: normal glucose levels (FPG<6.1mmol/L); impaired glucose tolerance (FPG of 6.1-7.0mmol/L) and DM (FPG>7.0mmol/L).

Echocardiography was performed using ATL HDI 5000, utilizing 3.5 megahertz and 2.5 megahertz transducers as well as a 1.9 megahertz Doppler probe. Echocardiograms were performed by one sonographer at each site. M-mode left ventricular measurements of septal and posterior wall thicknesses (LVPW) and left ventricular internal diameter (LVID) were made at end diastole from the parasternal window using the ASE criteria (Lang 2005). Three consecutive cycles were measured and averaged.

Left ventricular mass was calculated using the formula:
$LVMI=0.8*(1.04[(IVS+LVID+LVPW)^3-LVID^3])+0.6g$ (Devereux 1986). Diastolic function was assessed using the ratio between early transmitral flow and mitral annular velocity during early diastole (E/E') as a non-invasive surrogate for filling pressure (Nagueh 1997).

Plasma glucose and serum total cholesterol samples were taken at the time of randomisation and at the time of echocardiography; these were spun and frozen at -80 degrees centigrade for future batch analysis. Height and weight were measured in light clothing by a trained observer. Body mass index (BMI) was calculated as weight (kg) / height (m^2). Information on a history of diabetes was obtained by interview.

The study was approved by the ethics committee of Imperial College, London. Written informed consent for the study was obtained from all participants.

Statistical Methods

The data analysis was performed with SPSS Version 14.0 for Windows (SPSS Inc., 1999, Chicago, IL). Descriptive information for each of the variables was derived and distribution assessed. Fasting plasma glucose, CRP and BNP values were log transformed (to correct for skewness) and are presented as geometric mean x/÷ anti-logged standard deviation. Univariate associations were assessed using t-tests for metric variables and chi-square tests for categorical variables. Linear regression modelling was used to assess the association of FPG with diastolic function, adjusted for known risk factors for diastolic dysfunction. Standardised betas were used, as they allow you to compare the strength of associations between risk factors and disease directly.

Results

Of the 1006 patients enrolled, 796 participants (79% of the population sampled) provided data suitable for the purposes of this sub-study. There was no significant difference in age between those with and without complete data (p=0.443), however incomplete data was more common among women (24.3%) than men (17.3%, p=0.021). The study population was predominately of White/European origin (86.1%), with 4.6% of the population being South Asian, 5.5% of the population being African-Caribbean and 3.8% of the population mixed or other. The mean age (% male) of participants with normal glucose levels (NGL) was 62 years (82%), with IFG was 63 years (81%) and with diabetes mellitus (DM) was 63 years (73%).

The characteristics of the population, according to glucose categorisation, are shown in table 5.1. The inferior E/E ratio was significantly higher among those with DM compared to those with normal glucose tolerance ((NGT) (means (SD), 8.5 (2.4) v 7.6 (2.5), p<0.001). The lateral E/E ratio was significantly higher among those with DM compared to those with NGL 7.4 (2.4) v 6.9 (2.3), p=0.011). No significant association was evident between the septal E/E ratio and glucose categorisation. The mean E/E ratio (average of inferior, lateral and septal E/E') was significantly higher among those with DM compared to those with NGL (means (SD), 8.3 (2.2) v 7.8 (2.1), p=0.017).

Correlation coefficients between clinical and echocardiographic measures are shown in table 5.2. Inferior and lateral E/E' correlated significantly with age, FPG and systolic BP

(table 5.2). In contrast, septal E/E' and right ventricular S wave correlated significantly with age, creatinine and heart rate. Linear regression modelling was used to assess the association of FPG with measures of diastolic function, using four levels of adjustment (table 5.3). Model 1 – unadjusted, model 2 adjusted for age and gender, model 3 adjusted for age, gender, BMI, creatinine, systolic BP and heart rate. Linear regression identified FPG as an independent factor associated with higher inferior and lateral E/E ratios (after adjustment for age, gender, creatinine, BMI and heart rate). The standardised beta coefficients for FPG were similar to those for systolic BP and multivariate adjustment for confounding had little impact on the B coefficient for FPG. Standardised B coefficients between the E/E ratio (average of the inferior, lateral and septal E/E') and FPG and systolic BP were 0.098 (p=0.007) and 0.095 (p=0.007) respectively (model 3). The same trend was observed when data for men and women were analysed separately (data not shown).

Logistic regression modelling was used to assess the association between E/E ratio ≥8 and glucose tolerance status (NGL vs. DM (IFG was excluded as there were insufficient numbers for multivariate analyses). Among those with diabetes the increased risk of having an E/E ratio ≥8 compared to those with NGL remained after adjustment for age, gender, creatinine, BMI, systolic BP and heart rate (odds ratio for diabetes 1.76 [95%CI 1.22-2.53]). Figure 1 shows the frequency of E/E ratio ≥8 separately for inferior, lateral and septal measures, by glucose categorisation.

Discussion

This is the first large study to assess the association of left ventricular diastolic function with fasting plasma glucose (across the spectrum of glucose values) using a combination of TDE and conventional echocardiography. The incorporation of TDE into this study is important, as previous studies using conventional echocardiography would have underestimated the strength of the association between glycaemia and diastolic dysfunction due to pseudonormal filling patterns(1). Pseudonormal filling patterns occur in the intermediate stage of diastolic dysfunction when left ventricular relaxation and compliance are reduced and filling pressure is increased to maintain cardiac output (Garcia 1998). The present study showed a significant, positive association between FPG and transmitral E/E' waves (inferior and lateral, table 5.3) after adjustment for key risk factors (age, gender, creatinine, systolic BP, BMI and heart rate). This association was strong and similar to the association between diastolic function and systolic BP, suggesting FPG is an important factor in the development of diastolic dysfunction, independent of vascular effects. In addition, diabetes was associated with an increased risk of having an E/E ratio ≥ 8 compared to those with NGL. This is a significant finding. Raised filling pressures, as assessed by the E/E' ratio, indicate diastolic dysfunction, which is an early indicator of myocardial disease, preceding the development of overt left ventricular dysfunction(Vinereanu 2003) and is a powerful predictor of cardiac mortality (Wang 2003) and cardiac events (Sharp 2010).

The results from the present study support the findings of the only previous study to assess left ventricular function and glucose metabolism (across the continuum of values, those with glucose values in the diabetic range were excluded) using the combination of TDE and conventional echocardiography (Holzmann 2002). The study showed a strong association between FPG and diastolic function, however due to the small sample size (n=35) only very simple correlation measures were possible. Other studies using a combination of TDE and conventional echocardiography have all used relatively small populations (n<100) and either adopted a case control design (diabetes, no diabetes) or limited the study to people with diabetes (Vinereanu 2003).

Previous research using conventional echocardiography has been divided over the role of glycaemia in the development of diastolic dysfunction (Chaturvedi 2001; Sanchez-Barriga 2001; Lee 1997; Celentano 1995). There are several reasons for this. Many of the studies were very small and underpowered to detect associations either in univariate or multivariate analyses. Studies inconsistently adjusted for confounding, used various inclusion and exclusion criteria and most limited their population to people with diabetes. Very few studies (using conventional echocardiography) have assessed the association of glycaemia with diastolic function across the spectrum of glucose values but none have used the linear mechanism of assessment that is tissue Doppler imaging (Chaturvedi 2001; Rutter 2003).

In the present study multivariate adjustment for confounding had little impact on the B coefficient for FPG. This suggests the association between glucose and diastolic dysfunction is distinct to other vascular effects. The likely possible causes of a diabetic cardiomyopathy are many, and include alterations in oxidative stress levels as a response to hyperglycaemia, increased expression of Transforming Growth Factor β (TGF-β), as well as increased formation of advanced glycated end-products (AGEs; Singh 2001; Young 2002).

The present study has several limitations. It had a cross-sectional design and therefore some of the estimates for risk factors may have been subject to survival bias, as diastolic dysfunction is a strong predictor of mortality. In addition, the study was unable to adjust for the use of hypoglycaemic medication. These study limitations would certainly have led to an underestimation of the association between FPG and diastolic dysfunction. The study population was predominately male.

Conclusion

This is the first large study to use tissue Doppler echocardiography to assess the association of FPG (across the spectrum of glucose values) with diastolic dysfunction.

FPG is independently associated with left ventricular diastolic function, an association which was independent of co-morbidities. The strength of association was similar to that seen between systolic blood pressure and diastolic function, emphasising the potential

importance of this link. The mechanism by which diabetes might lead to diastolic dysfunction and an increase in left atrial filling pressures is unclear and further studies are required. Given the recognised progression to heart failure seen with progressive diastolic dysfunction, a greater understanding of the mechanism of diabetic left ventricular impairment is required to see if pharmacotherapy targeted at this process can reduce the chances of developing heart failure in the future.

Table 5.1. Clinical and echocardiographic characteristics of the population, according to glucose tolerance category

FPG Category (mmol/L)	<6.1	6.1-7.0	>7.0	
N	555	59	182	
Age (years)	62 ± 8	63 ± 8	63 ± 8	0.386
Male (%)	82	81	73*	0.028
BMI (kg/m²)	28.5 ± 4.3	29.8 ± 4.9*	29.9 ± 5.0*	<0.001
Fasting plasma glucose (mmol/l) †	5.1 ± 1.1	6.3 ± 1.0	7.9 ± 1.4	<0.001
Cholesterol (mmol/l)	6.0 ± 1.1	5.9 ± 1.1	5.7 ± 1.0*	0.004
Creatinine (mmol/l)	98.4 ± 16.8	97.4 ± 14.5	94.3 ± 15.7	0.096
BNP (pg/ml) †	25.0 ± 2.5	24.3 ± 2.6	25.4 ± 2.7	0.952
Systolic BP (mmHg)	153 ± 20	154 ± 21	152 ± 21	0.785
Diastolic BP (mmHg)	89 ± 11	89 ± 10	85 ± 10*	<0.001
Heart rate (bpm)	69 ± 13	75 ± 16*	75 ± 15*	<0.001
Left ventricular mass index (g/m²)	122.4 ± 31.7	113.4 ± 28.9	121.3 ± 34.5	0.157
Ejection fraction (%)	66 ± 12	63 ± 12	68 ± 12	0.035
Transmitral E wave / A wave ratio	0.89 ± 0.24	0.87 ± 0.23	0.89 ± 0.32	0.896
Transmitral E wave / inferior E wave	7.6 ± 2.5	7.8 ± 2.5	8.5 ± 2.4*	<0.001
Transmitral E wave / lateral E wave	6.9 ± 2.3	6.6 ± 1.7	7.4 ± 2.4*	0.011
Transmitral E wave / septal E wave	8.8 ± 2.7	9.1 ± 2.9	9.0 ± 3.2	0.590
Right ventricular S wave velocity	13.4 ± 4.0	13.2 ± 3.6	13.1 ± 4.6	0.683

Data given as mean ± S.D., †geometric mean x/÷ SD.

Table 5.2 Correlation coefficients between echocardiographic and clinical measures

	Inf E/E'	Lat E/E'	Sep E/E'	Mean E/E'	LVMI	LVM	RV S wave
Age	0.154**	0.157**	0.096**	0.159**	-0.025	-0.108**	0.103**
Fasting plasma glucose (mmol/l)†	0.100**	0.075*	0.044	0.085*	-0.030	-0.011	0.002
BMI (kg/m²)	-0.002	-0.024	-0.023	-0.019	0.157**	0.318**	0.033
Cholesterol (mmol/l)	0.104**	0.040	-0.033	0.040	-0.002	-0.047	-0.056
Creatinine	0.057	0.016	0.123**	0.083*	-0.056	0.002	0.115**
Systolic BP (mmHg)	0.117**	0.125**	0.031	0.104**	0.171**	0.120**	0.003
Diastolic BP (mmHg)	-0.040	-0.062	-0.055	-0.062	0.086*	0.111**	0.004
Heart rate (bpm)	-0.056	-0.052	-0.111**	-0.090*	-0.092**	-0.085*	-0.038

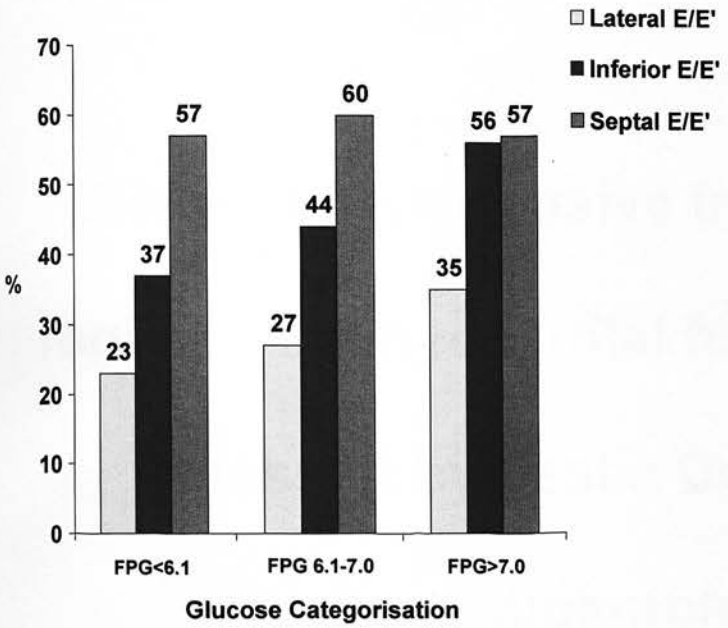
Inf E/E' – transmitral E wave / inferior E wave; Lat E/E' – transmitral E wave / lateral E wave; Sep E/E' – transmitral E wave / septal E wave; LVMI – left ventricular mass index.; LVI – left ventricular mass. RV S wave - Right ventricular S wave velocity * p value <0.05, ** p value <0.01. † values have been log transformed

Table 5.3 Beta coefficients and p values for fasting plasma glucose and systolic BP

	Model 1*		Model 2*		Model 3*	
	β	p value	β	p value	β	p value
Inferior E/E'						
Fasting plasma glucose†	0.100	0.005	0.096	0.007	0.102	0.005
Systolic blood pressure	0.107	0.002	0.095	0.006	0.107	0.002
Lateral E/E'						
Fasting plasma glucose†	0.082	0.014	0.074	0.036	0.078	0.029
Systolic blood pressure	0.131	<0.001	0.107	0.002	0.114	0.001
Septal E/E'						
Fasting plasma glucose†	0.046	0.205	0.047	0.188	0.068	0.064
Systolic blood pressure	0.035	0.315	0.028	0.430	0.027	0.449
Mean E/E'						
Fasting plasma glucose†	0.090	0.012	0.085	0.017	0.098	0.007
Systolic blood pressure	0.108	0.002	0.088	0.011	0.095	0.007
LVMI						
Fasting plasma glucose†	-0.029	0.445	-0.027	0.477	-0.051	0.168
Systolic blood pressure	0.264	0.055	0.177	<0.001	0.187	<0.001
RV S wave						
Fasting plasma glucose†	-0.004	0.919	0.006	0.858	-0.001	0.979
Systolic blood pressure	0.025	0.485	0.024	0.479	-0.001	0.988

*Model 1 – unadjusted; model 2 – adjusted for age and gender; model 3 - age, gender, systolic BP, creatinine, BMI and heart rate. † values have been log transformed. B are standardized. Inf E/E' – transmitral E wave / inferior E wave; Lat E/E' – transmitral E wave / lateral E wave; Sep E/E' – transmitral E wave / septal E wave; Mean E/E' – average of inferior, Lateral and septal E/E'; LVMI – left ventricular mass index.; RV S wave - right ventricular S wave.

Figure 5.1. Frequency of E/E'≥8 according to glucose categorisation.



Chapter 6.

Effect of hypertensive treatment regimen on myocardial function as assessed by tissue Doppler echocardiography

Abstract

Background

Different anti-hypertensive therapies may vary in their effect on left ventricular diastolic function. The Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) randomized patients to β-blocker (atenolol) + diuretic (bendroflumethiazide-K)-based therapy (BB/D) or calcium antagonist (amlodipine) + ACE inhibitor (perindopril)-based therapy (CA/ACEI). We compared left ventricular diastolic function in the two treatment groups.

Methods

The HACVD sub-study of ASCOT collected detailed cardiovascular phenotypic data on a subset of 1006 participants recruited from two centres (St Mary's Hospital, London and Beaumont Hospital, Dublin). Conventional and Tissue Doppler Echocardiography and measurement of plasma BNP were performed to assess left ventricular diastolic function.

Results

Treated blood pressure (BP) was similar in both treatment groups (systolic BP - BB/D = 137(17), CA/ACEI = 136(15); $p = 0.2$), but heart rate was significantly lower in BB/D group (BB/D = 58(10), CA/ACEI = 73(11); $p < 0.001$). Ejection fraction did not differ between groups (BB/D = 69.5 (11.3) %, CA/ACEI = 69.2 (12.1) %, $p = 0.8$), but early diastolic mitral annular velocity (E'), a measure of diastolic relaxation, was significantly

lower in BB/D group (BB/D = 7.9(1.8), CA/ACEI = 8.8 (2.0); p <0.001). E/E' (a measure of left ventricular filling pressure) and BNP were also significantly higher in BB/D group. Differences in E', BNP and E/E' remained significant after adjustment for age and sex. Further adjustment for SBP, LVMI and heart rate had no impact on differences in mean E' (p<0.001) or BNP (p<0.001), but differences in E/E' were attenuated (p=0.703).

Conclusions

BB/D based therapy is associated with poorer diastolic function compared with CA/ACEI therapy. Differences in the effect of anti-hypertensive therapy on diastolic function could influence the risk of heart failure and other cardiovascular events in hypertensive patients with preserved systolic function.

Background

Heart failure is a common consequence of hypertension (Haider 2003) and in many patients is related to impaired left ventricular systolic function. However, heart failure is also commonly associated with diastolic dysfunction and apparently preserved systolic function. This accounts for approximately one third to a half of heart failure cases (Carson 2005; Owan 2006) and most of these patients have a history of hypertension (Haider 2003), often with left ventricular hypertrophy and remodelling (Shapiro 1984; Marabotii 1989; Kapuku 1993; Sagie 1993; Muller-Brunotte 2006).

While many studies have focused on the effectiveness of hypertension treatment on reducing cardiac hypertrophy, less is known about the impact of treatment on LV diastolic function (Muller-Brunotte 2006; Solomon 2007). Previous studies addressing the impact of different antihypertensive agents on left ventricular diastolic function have largely used traditional echocardiographic approaches to assessing transmitral filling and isovolumic relaxation.

These conventional assessments have limitations as measures of left ventricular diastolic function as firstly; they are load dependent, which makes it difficult to separate alterations in loading conditions from intrinsic changes in left ventricular diastolic function with treatment. Secondly, they can undergo 'pseudonormalization', where the ratio of the early to atrial transmitral peak velocity (E/A ratio) paradoxically increases with progressive diastolic impairment.

Tissue Doppler Echocardiography (TDE) offers improved assessment of diastolic function (Yu 2007). TDE measurements of myocardial velocities are significantly less load dependent than traditional echocardiographic measurements; do not show 'pseudonormalization' and recently have been shown to be independent predictors of cardiovascular events in patient populations with existing cardiac disease (Hillis 2004; McMahon 2004; Okura 2006). Few studies to date have used TDE to assess diastolic function in relations to different antihypertensive agents.

The Anglo–Scandinavian Cardiac Outcomes Trial (ASCOT) was a large multicentre randomized clinical trial that compared the effect of a beta blocker (atenolol) + diuretic (bendroflumethiazide-K)-based therapy (BB/D) with calcium antagonist (amlodipine) + angiotensin converting enzyme (perindopril)-based therapy (CA/ACEI) on non-fatal myocardial infarction (MI) and fatal coronary heart disease (Dahlof 2005). This study showed that CA/ACEI-based therapy was superior to BB/D-based therapy on all major CV end points and all-cause mortality. As part of a substudy of ASCOT, extensive data on LV diastolic function was collected using conventional Doppler and TDE. These data were used to compare the effects of CA/ACEI –based therapy with BB/D- based therapy on LV diastolic function in a large group of well controlled hypertensive subjects.

Methods

The population, methods and response rate for ASCOT are found in detail elsewhere (Sever 2001). In brief, ASCOT was a clinical trial of antihypertensive therapy in 19,342

men and women aged 40 - 79 years, with hypertension. All patients were randomized to either a standard antihypertensive regimen (BB/D) or to a more contemporary regimen (CA/ACEI), and patients with a non-fasting cholesterol level of ≤6.5 mmol/L were further randomized to either atorvastatin 10 mg or placebo. The criteria for inclusion were hypertension (either untreated hypertension: systolic BP ≥160mmHg and/or diastolic BP ≥100mmHg at both screening and randomization; or treated hypertension: systolic BP ≥140mmHg and/or diastolic BP ≥90mmHg at randomization) with any three of the following cardiovascular risk factors: LVH on echo or ECG, type 2 diabetes, peripheral vascular disease, previous TIA/stroke, male sex, age > 55, microalbuminuria / proteinuria, current smoker, plasma total cholesterol/HDL ratio > 6, family history of ischaemic heart disease (IHD) in a first degree relative (male relative <55 years old at the time, female <60yrs old).

Detailed cardiovascular phenotypic data were collected on a subset of 1006 participants recruited from two ASCOT centres (St Mary's Hospital, London and the Adapt Centre, Beaumont Hospital, Dublin) as previously described (Stanton 2001). Echocardiography was performed using an ATL HDI 5000 ultrasound machine equipped with a standard multi-frequency transducer 12 months after initiation of treatment. All scans were performed by experienced echo-cardiographers with the patients semi-recumbent in the left lateral position. LV measurements were performed using M-Mode from the parasternal long axis according to the American Society of Echocardiography conventions (Lang 2005); LV mass was calculated according to the formula:

LV mass = 0.8 [1.04(IVSd+LVIDd+PWTd)3 – (LVIDd)3] + 0.6 g (Devereux 1986) where IVSd = Intraventricular Septal Thickness in diastole, LVIDd = Left Ventricular diameter in diastole, PWTd = Posterior Wall Thickness in diastole. This was then indexed for body surface area to give the LV mass index (LVMI).

Transmitral Doppler was assessed using a 5 mm sample volume placed at the tips of the mitral leaflets in passive end-expiration. A standardised loop of ten cardiac cycles was downloaded to computer for off-line analysis of the early filling phase (E wave) and the late filling phase (A wave). TDE was performed in the apical four, two and three chamber views, with the 5 mm sample volume placed over the myocardium on the septal, lateral and inferior walls at the level of the mitral annulus and the free wall of the right ventricle at the level of the tricuspid annulus. Using minimalised gain settings, a series of ten cardiac cycles were recorded. These were then downloaded for off-line analysis, with measurements made of systolic velocity (S' wave), early diastolic velocity (E' wave) and late diastolic velocity (A' wave) at each location and these were averaged. Analysis was performed using the HDI Lab package by a single researcher, who was blinded to all patient details, and each value represents the mean of three measurements taken from three, consecutive, representative cardiac cycles.

Blood pressure was measured after resting in a seated position for 5 minutes, using an Omron HEM 705-CP semiautomatic oscillometric recorder. Height and weight were

measured in light clothing by a trained observer. Body mass index (BMI) was calculated as weight (kg) / height (m^2). Information on history of diabetes was obtained by interview. Plasma glucose and serum total cholesterol were measured using standard enzymatic methods on a Roche / Hitachi 921 (Roche Diagnostics, Basel, Switzerland) automated analyzer.

The study was approved by the respective local hospital ethics committees (St Mary's Hospital, London and Beaumont Hospital, Dublin). Written informed consent for the study was obtained from all participants.

Statistical Methods

Data analysis was performed with SPSS Version 14.0 for Windows (SPSS Inc., 2006, Chicago, IL). Data are presented as means (SD) or marginal means ± standard error (SE) for ANCOVA. Statistical comparisons were made using a Student's t-test for metric variables and a chi-squared test for categorical variables. ANCOVA was used to assess the impact of covariates on the effect the two therapies (BB/D vs. CA/ACEI) on LV diastolic function. $P < 0.05$ was considered statistically significant.

Results

The population characteristics are shown in table 6.1. Baseline demographic and clinical characteristics of the two treatment groups were similar. There were no significant

differences in age, BP, BMI, lipid profile or eligibility risk factors between the treatment groups at baseline.

After 12 months of treatment, systolic BP was similar in both treatment groups (table 6.1), but predictably heart rate was significantly lower in BB/D group, however ejection fraction did not differ between groups. LVMI tended to be lower in people treated with CA/ACEI, although this did not quite achieve statistical significance ($p = 0.089$). Treatment with CA/ACEI was associated with higher early diastolic mitral annular velocity (E'), lower plasma BNP, lower E/E', a smaller atrial diameter and a shorter E wave deceleration time (table 6.2), whereas E/A ratio was higher in people randomized to BB/D.

After adjustment for age and sex the marginal mean for E' remained significantly lower among those treated with atenolol compared to those treated with amlodipine ($p<0.001$ adjusted for age and sex; table 6.2) and the group difference remained highly significant ($p<0.001$) after further adjustment for systolic BP and LVMI. Further adjustment for heart rate, a factor directly related to hypertension treatment did not weaken the association ($p<0.001$). Adjustment for the same covariates also did not significantly affect the differences in BNP between treatment regimens (table 6.2).

Differences in E/E' and E/A ratio remained significant after adjustment for age and sex, systolic BP or LVMI (table 6.2), but further adjustment for heart rate attenuated the

difference in E/E' and E/A between the treatment groups (p=0.703 and p = 0.139 respectively). No significant differences in LVMI were observed after statistical adjustment for the same variables (table 6.2).

Discussion

This is the first large randomised clinical trial to compare the effect of newer versus older anti-hypertensive medications on LV diastolic function using TDE. In the present study after 12 months of intensive therapy those treated with CA/ACEI had evidence of better LV diastolic function compared to those treated with BB/D based therapy (i.e. higher E', lower plasma BNP, lower E/E', smaller atrial diameter and shorter E wave deceleration time). These effects were independent of other factors associated with diastolic dysfunction, including the BP lowering effect of the drug and LVMI. In the case of E/E', differences between the two treatment groups could be accounted for by differences in heart rate, but this did not explain differences in E', BNP or atrial diameter. The difference in E/A ratio, a widely used indicator of diastolic function was discordant with other more sensitive measures of diastolic function, in that unadjusted E/A was higher in the atenolol group. This difference was also accounted for by differences in heart rate.

A previous study also observed an improvement in the E/A ratio after 48 weeks of treatment with atenolol which was highly correlated with the reduction in heart rate (Muller-Brunotte 2006). We therefore suggest that E/A ratio is not a particularly useful

indicator of diastolic function when heart rate differs between treatments. Differences in diastolic function seen in this study could be a consequence of differences in mechanisms of action of the drugs: amlodipine, perindopril and bendroflumethiazide reduce blood pressure principally by reducing peripheral resistance, while atenolol has negative cardiac inotropic and chronotropic effects (Williams 2004).

As far as we are aware, no previous large randomized clinical trials have evaluated the effect of antihypertensive treatment on LV diastolic function using TDE. A small number of studies with limited follow-up have examined the impact of monotherapy with antihypertensive agents on LV diastolic function and have shown improvements in measures of LV diastolic function (Onose 2001; Di Bello 2004; Tanaka 2004). Two small studies that have used TDE to compare the effectiveness of antihypertensive treatments on LV diastolic function have yielded conflicting results (Muller-Brunotte 2006; Solomon 2007). In a recent study of 134 people by Muller-Brunotte et al, irbesartan, an angiotensin (AT_1) receptor blocker was shown to provided greater improvement in E/E' compared with atenolol among those with and without hypertensive LV hypertrophy (Muller-Brunotte 2006). In contrast, a study by Solomon et al. of 186 people with evidence of diastolic dysfunction, valsartan (an AT_1 receptor blocker) was shown to be no more effective than standard treatment in improving LV diastolic function over 38 weeks (Solomon 2007). Our findings demonstrate clear benefits in terms of diastolic function in those randomized to CA/ACEI compared with BB/D.

Among those aged 65 years and over with evidence of diastolic dysfunction, approximately 15% will develop heart failure within 5 years (Aurigemma 2001). Effective treatment could potential delay or reduce the number of people developing LV diastolic dysfunction and later progression to heart failure. Previous research has suggested that anti-hypertensive medications vary in their ability to maintain or improve LV diastolic function and filling pressure (Kamp 2003; Moller 2004; Muller-Brunotte 2006). In particular, many studies have focused on effectiveness of hypertension treatments on LVM, however due to the serious limitations of the study designs and methodologies conclusions from these studies have been viewed with great caution (Diez 2001). In general, studies have used small samples sizes (Schiffrin 2002; Kamp 2003; Fountoulaki 2005; Ilgenli 2006; Muller-Brunotte 2006) and have been underpowered to detect a difference between therapies, with the majority of study durations ranging from just weeks to 6 months, with very few studies extending to a year of follow-up. Secondly, conventional echocardiography alone, which is used in the majority of published studies to date, has limitations as a means of assessing left ventricular diastolic function (Garcia 1998; Boyer 2003).

The present study has several limitations. The majority of participants were male, elderly and of White European ethnicity, our observations should therefore not be extrapolated to other groups. Measures of LV diastolic function were not recorded at baseline and therefore we cannot comment on how treatment changed diastolic function

from the pre-treatment state. However since most participants had been receiving treatment for hypertension prior to commencement of the study, changes with respect to baseline would be difficult to interpret. With regard to the comparison of treatment regimens, the lack of baseline data is not a major problem since this was a randomised study and potential confounders at baseline would be expected to be balanced by randomization; the similarity in BP, lipid profile and other demographic characteristics between the treatment arms, suggests that randomization was successful in this regard.

Conclusions

In summary, this prospective randomized study in hypertensive individuals showed that individuals receiving treatment with CA/ACEI based therapy have better diastolic function than those treated with BB/D therapy. Treatment-related differences in diastolic function were independent of blood pressure reduction or other factors that are known to affect diastolic function.

Table 6.1 Characteristics of the population

	Atenolol			Amlodipine			p value
Baseline characteristics	**Mean**		**S.D.**	**Mean**		**S.D.**	
Age (years)	62.1	±	7.9	62.4	±	7.8	0.542
Systolic blood pressure (mmHg)	159.9	±	17.5	159.9	±	18.7	0.965
Diastolic blood pressure (mmHg)	92.9	±	9.7	92.3	±	9.6	0.349
Heart Rate (bpm)	71.1	±	12.0	70.9	±	12.5	0.766
Body Surface area (m^2)	2.0	±	0.2	1.9	±	0.2	0.720
BMI (kg/m^2)	28.2	±	4.5	28.8	±	4.6	0.971
Total Cholesterol (mmol/L)	5.8	±	1.0	5.8	±	1.0	0.932
Triglycerides (mmol/L)*	1.6	(1.1-2.1)		1.5	(1.1-2.1)		0.714
	%			**%**			
Eligibility Risk Factors							
Age ≥ 55 years	85			85			0.870
Male	81			79			0.456
Peripheral arterial disease	6			6			0.986
Cerebrovascular risk factor	9			9			0.884
Prior known ECG or echo LVH	4			3			0.557
Diabetes Mellitus	20			22			0.516
Smoker	21			24			0.257
Coronary risk factor	20			20			0.753
Hemodynamic factors at year 1							
Systolic blood pressure (mmHg)	137.8	±	17.4	136.2	±	14.6	0.167
Diastolic blood pressure (mmHg)	81.6	±	9.3	80.1	±	8.6	0.012
Heart Rate (bpm)	57.8	±	10.0	72.8	±	11.4	<0.001
LV Structural Measures							
Interventricular septum, diastole (cm)	1.27	±	0.23	1.26	±	0.23	0.170
LV internal dimension, diastole (cm)	4.92	±	0.55	4.84	±	0.60	0.046
Posterior wall thickness, diastole (cm)	1.17	±	0.18	1.18	±	0.18	0.761
Interventricular septum, systole (cm)	1.65	±	0.25	1.63	±	0.27	0.352
LV internal dimension, systole (cm)	3.27	±	0.55	3.22	±	0.56	0.180
Posterior wall thickness, systole (cm)	1.58	±	0.25	1.59	±	0.23	0.510
LV ejection fraction (%)	69.48	±	11.32	69.21	±	12.19	0.759
LVMI (g/m^2)	122.66	±	30.92	118.80	±	31.56	0.089
Relative wall thickness	0.51	±	0.10	0.51	±	0.10	0.412
Left atrial size (cm)*	4.25	±	0.59	4.14	±	0.64	0.022

Table 6.2. Multivariate analysis: Echocardiographic measures by treatment group.

		Atenolol	Amlodipine	p value
Left ventricular mass index				
Model 1	Adjusted for age and sex	121.02 ± 1.81	117.25 ± 1.80	0.096
Model 2	Adjusted for model 1 and SBP	119.92 ± 1.78	116.77 ± 1.80	0.155
Model 3	Adjusted for model 1, SBP and HR	118.26 ± 1.92	118.67 ± 1.94	0.879
E/A Ratio				
Model 1	Adjusted for age and sex	0.90 ± 0.02	0.85 ± 0.02	0.007
Model 2	Adjusted for model 1 and LVMI	0.90 ± 0.02	0.85 ± 0.02	0.004
Model 3	Adjusted for model 1 and SBP	0.91 ± 0.02	0.85 ± 0.02	0.004
Model 4	Adjusted for model 1, LVMI, SBP and HR	0.87 ± 0.02	0.90 ± 0.02	0.139
Mean early diastolic velocity (E') cm/s				
Model 1	Adjusted for age and sex	7.76 ± 0.11	8.59 ± 0.10	<0.001
Model 2	Adjusted for model 1 and LVMI	7.78 ± 0.11	8.54 ± 0.11	<0.001
Model 3	Adjusted for model 1 and SBP	7.80 ± 0.11	8.62 ± 0.11	<0.001
Model 4	Adjusted for model 1, LVMI, SBP and HR	7.72 ± 0.12	8.64 ± 0.12	<0.001
Mean E/E' ratio				
Model 1	Adjusted for age and sex	8.32 ± 0.13	8.00 ± 0.13	0.043
Model 2	Adjusted for model 1 and LVMI	8.32 ± 0.13	8.03 ± 0.13	0.060
Model 3	Adjusted for model 1 and SBP	8.31 ± 0.13	8.00 ± 0.13	0.031
Model 4	Adjusted for model 1, LVMI, SBP and HR	8.20 ± 0.14	8.12 ± 0.14	0.703
BNP pg/ml				
Model 1	Adjusted for age and sex	35.80 ± 1.05	19.26 ± 1.05	<0.001
Model 2	Adjusted for model 1 and LVMI	35.69 ± 1.05	19.57 ± 1.05	<0.001
Model 3	Adjusted for model 1 and SBP	35.87 ± 1.05	19.36 ± 1.05	<0.001
Model 4	Adjusted for model 1, LVMI, SBP and HR	32.52 ± 1.05	22.02 ± 1.05	<0.001

All values are mean ± standard error

Chapter 7.

Assessing the effect of statins on the hypertensive ventricle with traditional and tissue Doppler echocardiographic parameters.

Abstract

Background

It is thought that statin therapy may affect left ventricular (LV) mass or diastolic function. We assessed this in a double-blind, prospective, randomized, placebo-controlled trial.

Methods

This was a sub-study of ASCOT-LLA, which randomized hypertensive subjects with total cholesterol levels of ≤251mg/dl (6.5 mmol/L) to either Atorvastatin 10mg or placebo in a factorial design. Blood pressure was treated according to the ASCOT protocol. After a mean follow-up period of 1.4 years, echocardiography was performed on 422 subjects and cardiac structure and function were analyzed. Results are presented as mean (95% confidence interval (CI)).

Results

Conventional and tissue Doppler measures of diastolic function did not differ between groups (Transmitral flow E/A ratio: Atorvastatin 0.90 (CI 0.85-0.95) vs. Placebo 0.88 (CI 0.85-0.95) cm/s, p=0.42); nor did tissue Doppler E' mitral annular velocity (Atorvastatin 8.4 (CI 8.1-8.6) vs. placebo 8.3 (CI 8.1-8.7) cm/s, p=0.87) and tissue Doppler E/E' (Atorvastatin 7.8 (CI 7.6-8.1) vs. placebo 7.8 (CI 7.5-8.1) cm/s, p=0.85).

Similarly, LV mass index (Atorvastatin: 119.3 (CI 115.3-124.0) vs. placebo: 121.4 (CI 116.7-126.1) g/m^2; p=0.59) and relative wall thickness (0.50 vs. 0.50; p=0.98) did not differ according to statin treatment.

Conclusions

LV structure or function did not differ after 1.4 yrs of randomized treatment with atorvastatin 10mg vs. placebo in well controlled hypertensive patients.

Background

There is a great deal of interest in the pleiotropic effects of statins, with potentially beneficial effects on endothelial function, vascular smooth muscle proliferation, myocardial fibrosis and on blood pressure control proposed (Laufs 2003; Krysiak 2003; Shishehbor 2003; Prasad 2003; Mehta 2003; Kwak 2003). Some small studies in animals have suggested that statins may also have beneficial effects on left ventricular (LV) mass and diastolic function, irrespective of concomitant blood pressure or anti-hypertensive therapy (Patel 2001; Indolfi 2002; Luo 2002; Oi 1999). Furthermore, a possible benefit of statins on the prognosis of subjects with diastolic heart failure has been proposed (Fukuta 2005). Randomised clinical trials into the impact of statins on diastolic function have therefore been recommended (Zile 2005).

The Anglo-Scandinavian Cardiac Outcomes Trial – Lipid Lowering Arm (ASCOT-LLA) was a multi-centre randomised, prospective double-blind, placebo-controlled trial of atorvastatin versus placebo in hypertensive subjects. Echocardiography was performed in sub-study of ASCOT-LLA which allowed us to compare the effect of atorvastatin treatment vs. placebo on LV structure and function.

Methods

ASCOT was a clinical trial of antihypertensive therapy (amlodipine ± perindopril vs. atenolol ± bendroflumethiazide) in 19,257 subjects aged 40 - 79 years with hypertension(Sever 2001). Of these, 10,305 subjects who had a total cholesterol level

≤251mg/dl (6.5mmol/L) were randomized in a factorial design in ASCOT-LLA to receive either Atorvastatin 10mg once daily, or placebo. Detailed cardiovascular phenotypic data were collected on 1006 ASCOT patients as part of the pre-specified Hypertension Associated Cardiovascular Disease (HACVD) sub-study of ASCOT (Stanton 2001). Within this sub-study, 422 subjects were also participants within ASCOT-LLA and form the basis of this study.

All subjects in the study were hypertensive (either untreated hypertension: systolic BP ≥160mmHg and/or diastolic BP ≥100mmHg at both screening and randomization; or treated hypertension: systolic BP ≥140mmHg and/or diastolic BP ≥90mmHg at randomization) with at least three other cardiovascular risk factors and were treated to a target blood pressure of 140/90 (or 130/80 for those with diabetes) according to the ASCOT protocol (Sever 2001).

Double-blinded treatment with anti-hypertensive therapy plus either atorvastatin or placebo was also commenced at randomization in eligible patients and patients underwent echocardiography after a mean treatment period of 1.4 years.

Scanning was performed by experienced echocardiographers, using ATL HDI 5000 ultrasound machines. Two-dimensional echocardiography was performed in the left lateral position using a standard multi-frequency probe, with detailed LV measurements made from M-Mode in the parasternal long axis according to the American Society of

Echocardiography convention (Lang 2005). If the technical quality of the M-mode images was sub-optimal, measurements were made from the two-dimensional images.

LV mass was calculated according to the formula:

$$\text{LV mass} = 0.8\,[1.04\,(\text{IVSd}+\text{LVIDd}+\text{PWTd})^3 - (\text{LVIDd})^3] + 0.6\ \text{g}$$

where IVSd = Intraventricular Septal Thickness in diastole, LVIDd = LV Diameter in diastole, PWTd = Posterior Wall Thickness in diastole

This was then divided by body surface area to give LV mass index (LVMI) (Devereux 1986). Relative wall thickness was calculated by the formula: RWT=2*PWTd/LVIDd (Lang 2005).

Pulsed Doppler echocardiography was performed using a 5mm sample volume placed at the tips of the mitral leaflets parallel to inflow during diastole; tissue Doppler measurements were sampled at the level of the mitral annulus over the septal, lateral and inferior walls. Each individual measurement represents the mean of three consecutive representative cardiac cycles. The average value of the septal, lateral and inferior wall early diastolic annular velocity (E') was then taken and combined with the transmitral early filling blood flow velocity (E) to give the E/E' ratio, a non-invasive estimate of left atrial filling pressure.

Analysis was performed offline by a single, blinded researcher using HDILab software. Quality control of echocardiography was performed regularly and the within and between observer variability was found to be less than 7.5% of the standard deviation of the mean value, in keeping with prior accepted limits of reproducibility (Palmieri 2003).

This study was approved by local and regional ethics committees; all subjects entered following acquisition of informed consent and the study was conducted in accordance with the principles of the Declaration of Helsinki on clinical research.

Statistical Analysis

Data analysis was performed using SPSS Version 14.0 for Windows (SPSS Inc., 1989, Chicago, IL). Descriptive information for each of the variables was derived and distribution assessed. Baseline data are presented as mean ± SD or percentages and demographic data were compared using Student's t-tests for continuous variables and chi-square tests for categorical variables. Given the factorial nature of the study, interactions between antihypertensive and lipid lowering treatments were tested at the outset, and the main effect (i.e. all lipid lowering treatment vs. all placebo) only analyzed if the interaction term was not significant (as advocated by McAlister et al (McAlister 2003)). Subsequently ANOVA was used to assess the unadjusted association of lipids with the measures of diastolic function. ANCOVA was then used to build three multivariate models, adjusting in turn four echocardiographic parameters (LVMI, LVM/height$^{2.7}$, E' and E/E') for demographic and clinical factors thought *a priori* to be

potential confounders on the basis of the literature. Model 1 adjusted for age and gender, model 2 for age, gender, systolic blood pressure at the time of echocardiography and diabetes. Model 3 adjusted for age, gender, systolic blood pressure at the time of echocardiography, diabetes, antihypertensive regimen and systolic blood pressure at enrolment into ASCOT. A p-value of <0.05 was considered statistically significant.

Results

The baseline population characteristics at randomization are shown in table 7.1. There were no significant differences between the treatment groups, consistent with randomization being effective. The mean age of the population was 62 years and 83% were male. Echocardiographic findings in the two groups after an average of 1.4 years of treatment are shown in tables 7.2 & 7.3. There were no significant differences in measures of LV structure between the treatment groups (Table 7.2). Left ventricular mass index (LVMI) for the atorvastatin group was 119.3 (CI 115.3-124.0) g/m^2 and for the placebo group was 121.4 (CI 116.7-126.1) g/m^2; p=0.59 for difference between the groups. Similarly LV mass indexed for $height^{2.7}$ did not differ significantly between groups (table 7.2). Relative wall thickness (RWT) also did not differ significantly between treatment groups (table 7.2).

Conventional Doppler and tissue Doppler echocardiography measures were also similar between the treatment groups (table 7.3). The mean transmitral E/A ratio was 0.90 (CI 0.85-0.95) cm/s in the atorvastatin group and 0.88 (CI 0.85-0.95) cm/s in the placebo

group (p=0.42). Tissue Doppler E' mitral annular velocity also did differ between groups: Atorvastatin 8.4 (CI 8.1-8.6) cm/s vs. placebo 8.3 (CI 8.1-8.7) cm/s; p=0.87. The tissue Doppler E/E' ratios were the same, irrespective of randomization arm: Atorvastatin 7.8 (CI 7.6-8.1) vs. placebo 7.8 (CI 7.5-8.1) cm/s, p=0.85).

Further analysis of LV structure and function, according to pre-treatment levels of total cholesterol, also showed no significant differences (Figure 7.1). LVMI and measures of LV diastolic function (tissue Doppler E', E/E', transmitral E/A ratio and transmitral E wave deceleration time) were then each compared using linear regression to assess whether the magnitude of the lipid-lowering had any effect on diastolic function or LV mass. No significant relationships were found, before, or after adjustment for covariates.

Finally, multivariate models were built to examine the relationship of LVMI, LVM/height$^{2.7}$, transmitral Doppler and tissue Doppler parameters in relation to atorvastatin treatment after adjustment for potential confounding factors (age, gender, systolic blood pressure at enrollment, diabetes, anti-hypertensive regimen and systolic blood pressure at the time of echocardiography). After partial or full adjustment, no significance differences were seen in echocardiographic indices between atorvastatin and placebo arms (data not shown).

Discussion

Echocardiography used in a prospective randomised, double-blind, placebo-controlled clinical trial failed to show any difference in LV diastolic function or mass in well controlled hypertensive patients randomised to either atorvastatin, or placebo for an average of 1.4 years. This finding does not support some previous small studies in animals and humans suggesting that statins may have specific beneficial effects on cardiac structure and diastolic function(Patel 2001; Indolfi 2002; Su 2000; Nishikawa 2004).

Statins and diastolic function

Animal studies have previously suggested a possible improvement in diastolic function with statin use. Furthermore, an association has been reported between statin use and improved mortality in diastolic heart failure, leading to calls for a randomised trial examining the effect of statins on diastolic function (Fukuta 2005; Zile 2005).

Few treatments have been shown to be beneficial in diastolic heart failure in the past. This might be due to the gradual nature of the proposed aetiology behind this process, such as sub-endocardial ischemic injury or myocyte fibrosis. As a result, any proposed interventions designed to prevent or treat diastolic heart failure may require a prolonged period of follow-up in order to establish clinical efficacy in terms of hard endpoint data, something currently lacking in the literature. Previously tried interventions have also

tended to focus on subjects with symptomatic diastolic heart failure and as a result may be instituted too late in the disease process as to have any discernable benefit.

All subjects in the current study were hypertensive but free from heart failure. Therefore, this study was well suited to examining strategies which might improve or prevent deterioration in LV function. Traditional measures of diastolic function based on transmitral Doppler echocardiography were used, along with more sensitive tissue Doppler measures, but no significant difference was found between the placebo and atorvastatin groups using either approach. Overall, the confidence limits of the estimated differences between groups are not consistent with any major effects of atorvastatin on diastolic function

Statins and LV mass

Two relatively small unblinded and non-randomized studies in humans have suggested that statins may reduce LV mass. The larger of the two, which was an observational study in a diverse group of patients attending for cardiac catheterisation, found a lower LVMI in 62 patients taking statins than in 127 patients selected as controls (Nishikawa 2004). The other study was an open parallel group study of 40 hypertensive individuals, with 20 patients receiving pravastatin 10mg (up-titrated to 20mg if target lipids nor achieved) and a further 20 patients not receiving a statin; both groups received anti-hypertensive therapy. Subjects receiving pravastatin had a lower LVMI after 6 months of treatment when compared to those on dietary control alone (Su 2000).

In the present study, there was no detectable statistically significant difference in LVMI in 422 patients randomised to either atorvastatin or placebo. The size of the reduction in total cholesterol seen with atorvastatin in this study was similar to that seen in the above mentioned pravastatin study (46 mg per dl /1.2mmol per litre) suggesting that inadequate dosage is unlikely to account for the divergent results between these two studies.

One conceivable explanation for the negative findings seen in the present study is that baseline total cholesterol levels of our subjects were not sufficiently high to achieve potentially benefit effects on cardiac structure. If this explanation were correct, then one might expect to see differences in LVMI between those in the uppermost tertile of baseline cholesterol levels, compared to those in the lowermost tertile, but we did not (Figure 7.1). We also examined the effect on LV mass according to the degree of lipid lowering achieved; there was no correlation and indexed LVM to height$^{2.7}$ to account for the prevalence of obesity within the population; this did not change the findings.

Study limitations

There were several important limitations to this study. Firstly, this study used only one type of statin. We therefore cannot categorically rule out a statin specific effect, though this seems unlikely. Next, we cannot exclude the possibility that higher doses of statin might influence LV structure or function; however, the dose used in this study was

effective in reducing serum cholesterol and was shown in the parent ASCOT study to achieve a 36% reduction in cardiovascular events in a similar population (Sever 2003).

The lack of echocardiography at baseline prevents us from assessing the effect of statin on progression of LV mass or diastolic function, but since this was a prospective randomized double blind study it is unlikely that unexpected differences at baseline between groups can account for our failure to observe a difference between the atorvastatin and placebo arms after an average of 1.4 years of treatment.

For ethical reasons, those with a total cholesterol > 251mg/dl (6.5mmol/L) were deemed to be unsuitable for randomization and therefore were not included in this study. By excluding those with very abnormal cholesterol levels (who arguably might have most to gain from lipid-lowering), this study may have biased the findings towards the null hypothesis. However, a third of cases randomized to atorvastatin or placebo had a total cholesterol of greater than 224mg/dl (5.8mmol/L), and the mean cholesterol for the cohort was 208mg/dl (5.4mmol/L), so we could not consider this a cohort with below-average lipid levels.

The duration of follow-up (average 1.4 years) may also have been insufficient to demonstrate any benefits in LVMI or diastolic function. On the basis of prior animal and human studies, this duration would seem adequate (most showing benefit in less than one year) and we were using very sensitive measures on echocardiography. Furthermore,

the effect on cardiovascular outcomes of this dose of atorvastatin became apparent in the parent ASCOT-LLA study within the first year, though of course different mechanisms of action may exist between risk reduction and modification of ventricular structure or function.

Finally, if the effect of statins on LV mass or diastolic function were mediated through changes in blood pressure these could be obscured by the optimal blood pressure lowering strategies used in ASCOT which would minimize blood pressure differences between placebo and atorvastatin-treated groups. At present, evidence that statins lower blood pressure is inconsistent, but a recent meta-analysis indicated that statins might lower systolic blood pressure by less than 2mmHg (Strazzullo 2007); if this estimate is correct, then the effects on LVM are likely to be modest and unlikely to be detectable in our study.

Conclusion

When a population of well treated hypertensives was randomised in a double-blind, placebo controlled fashion to either atorvastatin or placebo, no differences were found in LV mass or diastolic function between the two groups after an average of 1.4 years follow-up.

Tables

Table 7.1. Baseline Patient Characteristics

	Placebo		Atorvastatin		p value*
Continuous variables	Mean	S.D.	Mean	S.D.	
Age (years)	62.4 ±	8.0	61.8 ±	7.8	>0.2
Systolic Blood pressure (mmHg)	158.7 ±	16.9	157.5 ±	17.0	>0.2
Diastolic Blood pressure (mmHg)	92.7 ±	9.1	92.3 ±	8.9	>0.2
Heart Rate (bpm)	70.2 ±	12.2	70.2 ±	11.8	>0.2
Body Surface area (m^2)	2.0 ±	0.2	2.0 ±	0.2	>0.2
BMI (kg/m^2)	28.9 ±	4.6	29.1 ±	4.7	>0.2
Total Cholesterol (mg/dl)	208.0 ±	30.9	208.0 ±	30.9	>0.2
HDL (mg/dl)	51.0 ±	14.3	49.0 ±	13.5	0.17
LDL (mg/dl)	133.6 ±	29.0	132.0 ±	27.8	>0.2
Glucose (mmol/L)	5.6 ±	1.6	5.6 ±	1.5	>0.2
Creatinine (mmol/L)	99.3 ±	16.5	98.1 ±	15.3	>0.2
Categorical variables	n	%	n	%	
Males	170	83	182	84	>0.2
Prior known ECG or echo LVH	45	22	46	21	>0.2
Diabetes Mellitus	30	15	33	15	>0.2
Smoker	54	27	52	24	>0.2

P values were calculated using a Student's t-test for continuous variables or a Chi^2 test for categorical variables.

Table 7.2. Left ventricular structural data

	Placebo			Atorvastatin			
	Mean	CI		Mean	CI		p value
Interventricular septum in diastole (cm)	1.27	1.24	- 1.30	1.26	1.23	- 1.29	>0.2
Left ventricular internal diameter in diastole (cm)	4.91	4.83	- 4.99	4.93	4.86	- 5.03	>0.2
Posterior wall thickness in diastole (cm)	1.17	1.14	- 1.19	1.18	1.15	- 1.20	>0.2
Interventricular septum in systole (cm)	1.66	1.62	- 1.70	1.62	1.58	- 1.65	0.10
Left ventricular internal diameter in systole (cm)	3.31	3.22	- 3.39	3.26	3.19	- 3.33	0.10
Posterior wall thickness in systole (cm)	1.57	1.53	- 1.60	1.58	1.54	- 1.62	>0.2
Left ventricular ejection fraction (%)	68.47	67.67	- 69.27	69.30	68.52	- 70.10	>0.2
LVMI (g/m^2)	121.41	116.75	- 126.07	119.31	115.35	- 123.97	>0.2
Relative wall thickness	0.50	0.49	- 0.52	0.50	0.49	- 0.51	>0.2
Left atrial size (cm)	4.19	4.10	- 4.28	4.21	4.12	- 4.30	>0.2

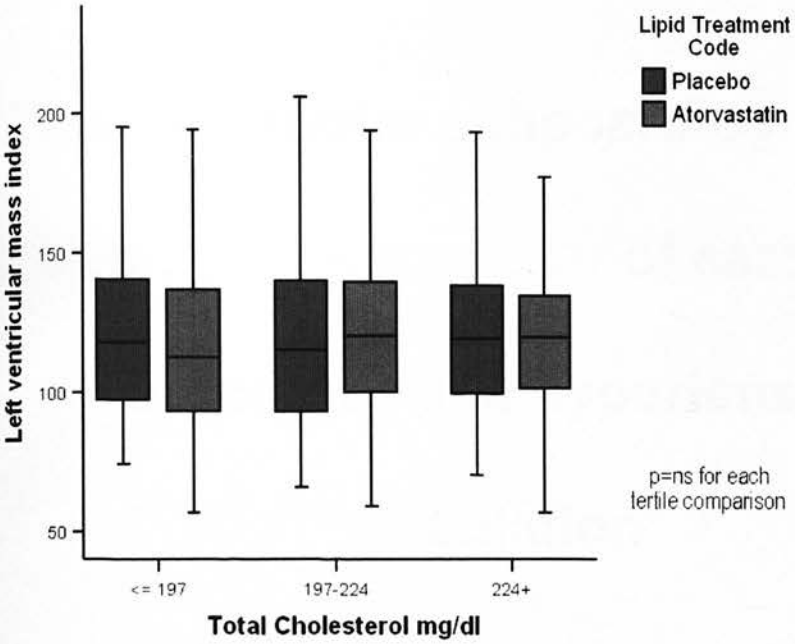
Data are means (95% confidence intervals), p values were calculated using a Student's t-test.

Table 7.3. Conventional and tissue Doppler echocardiographic data.

	Placebo		Atorvastatin		
	Mean	CI	Mean	CI	p value
Transmitral Doppler					
E wave (cm/s)	60.32	58.33 - 62.31	61.17	59.38 - 62.97	>0.2
A wave (cm/s)	70.40	68.01 - 72.80	71.20	69.38 - 73.03	>0.2
E/A ratio	0.90	0.85 - 0.95	0.88	0.85 - 0.90	>0.2
E wave deceleration time (s)	0.19	0.18 - 0.20	0.19	0.18 - 0.20	>0.2
Tissue Doppler (cm/s)					
Mean early diastolic velocity (E') cm/s	8.37	8.09 - 8.65	8.34	8.08 - 8.60	>0.2
Mean early diastolic velocity (A') cm/s	11.40	11.08 - 11.75	11.78	11.48 - 12.09	0.11
Mean early diastolic velocity (S') cm/s	8.87	8.57 - 9.17	8.95	8.69 - 9.21	>0.2
Mean E/E' ratio	7.80	7.47 - 8.13	7.80	7.59 - 8.08	>0.2

Data are means (95% confidence intervals), p values were calculated using Student's t-test.

Figure 7.1. The relationship of LVMI with tertile of cholesterol according to lipid treatment arm.



Chapter 8.

Tissue Doppler echocardiography is a strong predictor of cardiac outcomes in a hypertensive population.

Abstract

Background

Patients with controlled hypertension are at risk of future cardiac events, but predicting first events remains difficult.

We hypothesized that modern echocardiographic measures of left ventricular diastolic function may be more sensitive than traditional echocardiographic methods of risk prediction and set out to test this in a cohort of patients with well controlled hypertension.

Methods

Conventional and tissue Doppler echocardiography was performed on a subset of participants in the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). All subjects had hypertension but no known cardiac disease. Cardiac events were defined as fatal and non-fatal myocardial infarction (including silent myocardial infarction), coronary revascularization procedures, new-onset angina (stable or unstable), fatal and non-fatal heart failure and life-threatening arrhythmias. Analysis was performed by a single, blinded observer.

Results

There were 1006 subjects recruited, of whom 980 patients completed protocols suitable for this sub-study. Within this cohort, there were 56 primary cardiac events during 4.2±0.7 years follow-up. The ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity (E/E') was the strongest predictor of first cardiac events in Cox-proportional hazards models. Following adjustment for covariates, a unit rise in the E/E' ratio was associated with a 17% increment in risk of a cardiac event (HR 1.17, CI 1.05-1.29; p=0.003).

Conclusion

Tissue Doppler E/E', a non-invasive estimate of left atrial filling pressure, independently predicts primary cardiac events in a hypertensive population and out-performed traditional echocardiographic measures in this moderately sized, well-treated hypertensive population. E/E' represents a simple, effective tool for assessing cardiac risk in a hypertensive population.

Background

Reliable methods for predicting primary cardiac events in individuals with well controlled hypertension remains limited (Black 1997). Inclusion of cardiac measures of hypertensive structural and functional change, such as left ventricular hypertrophy(Verdecchia 1998; Levy 1990), raised left atrial size (Benjamin 1995) and transmitral Doppler assessment of diastolic blood flow (Schillaci 2002) have improved risk prediction, but it is unclear which measure best predicts outcome and provides the most appropriate tool for risk stratification.

By combining the early filling velocity on transmitral Doppler (E) with the early relaxation velocity on tissue Doppler (E'), the E/E' ratio of diastolic function has been shown to have good prognostic value in established and advanced cardiac disease (Nagueh 1997; Ommen 2000; Nagueh 1998; Okura 2006, Hillis 2004) but this measure has not been shown to predict primary cardiac events.

We set out to test whether this measure is able to predict first events in a sub-study of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) and to compare performance against traditional echocardiographic measures of risk.

Methods

A full description of ASCOT can be found elsewhere (Sever 2001). In brief, ASCOT was a clinical trial of antihypertensive therapy (amlodipine ± perindopril vs. atenolol ± bendroflumethiazide) in 19,257 men and women aged 40 - 79 years with hypertension. Detailed cardiovascular phenotypic data were purposefully collected on a subset of 1006 participants recruited from two centers (579 subjects from St Mary's Hospital, London and 427 subjects from the Adapt Centre, Beaumont Hospital, Dublin) as part of the Hypertension Associated Cardiovascular Disease (HACVD) sub-study (Stanton 2001).

A detailed description of the blood pressure protocols for this sub-study, including quality control measures used for acquisition of data, can be found in detail elsewhere (Stanton 2001). Briefly, brachial BP was measured as the mean of three readings made in a seated position using an Omron HEM 705-CP semiautomatic oscillometric recorder. Participants rested for 5 minutes before testing. The measures quoted in this manuscript represent those taken at the time of echocardiography.

Height and weight were measured in light clothing by a trained observer. Body mass index (BMI) was calculated as weight (kg) / height (m^2). Plasma glucose and serum total cholesterol were measured using standard enzymatic methods on a Roche / Hitachi 921 (Roche Diagnostics, Basel, Switzerland) automated analyzer. The values quoted for lipids and glucose in this manuscript represent those taken at the time of echocardiography. eGFR was calculated using the MDRD formula (Stanton 2001)

Inclusion and exclusion criteria

The criteria for inclusion were hypertension (either untreated hypertension: systolic BP ≥160mmHg and/or diastolic BP ≥100mmHg at both screening and randomization; or treated hypertension: systolic BP ≥140mmHg and/or diastolic BP ≥90mmHg at randomization) with any three of the following cardiovascular risk factors: type 2 diabetes, peripheral vascular disease, previous TIA/stroke, male sex, age > 55, microalbuminuria/proteinuria, current smoker, plasma total cholesterol/HDL ratio > 6, family history of ischaemic heart disease (IHD) in a first degree relative. The final inclusion criterion was 'previously identified echo or ECG LVH'. This was required to have been identified within normal clinical practice prior to any assessment for the study and was not influenced by any subsequent investigation performed as part of ASCOT.

Exclusion criteria included those with a diagnosis of, or symptoms consistent with, ischaemic heart disease or heart failure. Patients demonstrated through the study to have severe valvular heart disease were excluded from analysis.

Echocardiography

Following a one year period of blood pressure control according to the ASCOT protocol, 980 patients completed the appropriate echocardiography protocols for this study. Those with poor echocardiographic windows or incomplete data sets were excluded and adequate on-axis images and tissue Doppler tracings from all three left ventricular

territories were available in 828 subjects (84%). Of these, 12 suffered first cardiac events between randomization and the time of echocardiography, and so were also excluded from analysis, leaving a total of 816 subjects.

Scanning was performed by one experienced echocardiographer at the Dublin site, and two experienced echocardiographers at the St Mary's site using ATL HDI 5000 ultrasound machines. Each patient underwent standard two-dimensional echocardiography in the left lateral position using a standard multi-frequency probe, with detailed LV measurements made from M-Mode in the parasternal long-axis according to the American Society of Echocardiography guidelines (Lang 2005). If the technical quality of the M-mode was sub-optimal, measurements were required to be made from the two-dimensional images.

LV mass was calculated according to the formula:

$$\text{LV mass (g)} = 0.8\,[1.04\,(\text{IVSd}+\text{LVIDd}+\text{PWTd})^3 - (\text{LVIDd})^3] + 0.6$$

IVSd = Intraventricular Septal Thickness in diastole (cm), LVIDd = LV Diameter in diastole (cm),

PWTd = Posterior Wall Thickness in diastole

This was then divided by body surface area to give LV mass index (LVMI) (Devereux 1986).

Relative wall thickness was calculated according to the formula:

Relative wall thickness = 2*PWTd/LVIDd

Pulsed Spectral Doppler echocardiography was performed using a 5mm sample volume placed at the tips of the mitral leaflets parallel to inflow during diastole at end-expiration, with a sweep speed of 100mm/s. Tissue Doppler measurements were sampled at the level of the mitral annulus over the septal, lateral and inferior walls with filters adjusted to obtain the lowest wall filter settings and the minimal optimal gain; Nyquist limits of 15-20 cm/sec and a frame rate of 200Hz were used. Each spectral trace was downloaded for offline analysis using the HDILab software program by a single researcher (AS), who was masked to all patient outcome data until the final cleaned dataset was presented for outcome analysis.

Values for each component of the TDE waveform (S', E' and A') were measured and averaged over 3 consecutive cardiac cycles. A composite mean for each of these parameters was then formed by taking the average of the values from the septal, lateral and inferior wall. The ratio of the transmitral Doppler E wave velocity and the composite mean of E' was then used to calculate the E/E' ratio. This therefore means that the mean tissue Doppler variable E' incorporates data from nine cardiac cycles, minimizing the effect of beat to beat variation or respiratory artefact.

Reproducibility of data

Between centre and between echocardiographer variability of echocardiographic measurements was assessed before commencement of the study and subsequently at regular intervals during the study. Using the Bland-Altman method (Bland 1986), the standard deviation of difference of individual variables (e.g. E/E') was found to be less than 7.5% of the mean value, demonstrating that our reproducibility measurements were in keeping with other studies (Palmieri 2003). We also confirmed within a separate multivariate model that the centre of origin of the subject had no effect on outcome, further eliminating the possibility of bias from differences in acquisition of data between echocardiographers.

Endpoints

The cardiac end-points used for this sub-study were pre-specified in the ASCOT protocol as non-fatal myocardial infarction (including silent myocardial infarction), fatal myocardial infarction, coronary revascularisation procedures, new-onset angina (stable or unstable), fatal and non-fatal heart failure and life-threatening arrhythmias. All endpoint events were confirmed by the ASCOT endpoints committee by procedures detailed elsewhere (Sever 2001). Where a patient suffered more than one cardiac event during the course of the study, only the first cardiac event for that patient was incorporated into the analysis.

Statistical Methods

Statistical analysis was performed using SPSS v14.0 for Windows (SPSS Inc., 2005, Chicago, IL). Two groups were established for the purposes of analysis – those who were event-free, and those who had suffered a first cardiac event during the course of the study. For those subjects who suffered multiple events, only the first event was used for analysis.

All continuous variables were tested for normality and log transformed where skewed distributions were demonstrated. These are presented as mean ± standard deviation. Demographic and echocardiographic factors were analysed between groups using ANOVA for continuous variables and chi-square for categorical variables.

Multivariate analysis

Multivariate analysis was performed using tiered Cox proportional hazards models incorporating factors with predictive significance on univariate analysis and hypothesised important factors.

- Model 1 adjusted for age and gender.
- Model 2 adjusted for age, gender, diabetes and systolic blood pressure.
- Model 3 corrected for the traditional Framingham risk factors of age, diabetes, total cholesterol, HDL, smoking, gender and systolic blood pressure.

We then compared the ability of E/E' to predict risk against other traditional measures quoted in the literature (LVMI, RWT, left atrial size, ejection fraction, transmitral E/A ratio, transmitral E wave velocity, mean tissue Doppler E' wave velocity, mean tissue Doppler S' wave velocity). This was done by substituting E/E' for each value in turn in the models listed above.

During analysis, we also incorporated several measures of blood pressure into the modelling process to ensure adequate representation of the distribution of hypertension within our cohort. These included baseline systolic and diastolic blood pressure, systolic and diastolic blood pressure taken at the time of echocardiography and the average blood pressure measured over time (between study enrolment and time of echocardiography ((Baseline systolic BP + Echocardiography systolic BP)/2)). Using each of these measures within the modelling process in place of systolic blood pressure did not change our findings and therefore data is not shown.

Results

Demographic data for the population are shown in Table 8.1. Only diastolic blood pressure differed between those who were event-free and those who suffered a cardiac event at follow up ($p=0.047$); however, after adjustment for age and sex, this difference was no longer significant. Mean follow-up period was 4.2 ± 0.7 years. During this time there were 101 cardiac events in total, of which 56 were first cardiac events (table 8.2).

We then compared the ability of E/E' to predict risk against other traditional measures quoted in the literature (LVMI, RWT, left atrial size, ejection fraction, transmitral E/A ratio, transmitral E wave velocity, mean tissue Doppler E' wave velocity, mean tissue Doppler S' wave velocity). This was done by substituting E/E' for each value in turn in the models listed above.

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The cardiac event rates reported in this sub-study. Echocardiographic parameters are presented according to whether patients had an event, or did not (Table 8.3).

On univariate analysis of echocardiographic parameters, tissue Doppler E/E' was the only parameter to predict first cardiac events.

Multivariate analysis

After adjustment for age and gender, E/E' remained the only significant predictor of first cardiac events (HR 1.17, CI 1.05-1.29; p=0.003. Table 8.4). Additional adjustment for diabetes and systolic blood pressure had little effect (HR 1.17, CI 1.05-1.30; p=0.003). Model 3 adjusted for traditional Framingham risk factors (age, gender, diabetes, systolic BP, smoker, total cholesterol and HDL) and E/E' remained a highly significant predictor of events (HR 1.17, CI 1.05-1.29; p=0.003). When E/E' was adjusted for the 10-year coronary heart disease Framingham risk score itself, it remained a powerful predictor of events (HR 1.14; p=0.006). The Framingham score did not significantly predict outcomes in this model.

Each of these models were then re-run, sequentially replacing E/E' with: LVMI, RWT, LA size, ejection fraction, transmitral E wave velocity, transmitral E/A ratio, tissue Doppler E' wave velocity and tissue Doppler S' wave velocity. None of these measures significantly predicted primary cardiac events, with or without adjustment for covariates. We then forced E/E', LVMI and LA size into the same model with age, gender, diabetes

and systolic BP, in order to demonstrate the additional value of E/E' over existing echocardiographic variables. The only significant echocardiographic predictor of risk within this model remained E/E' (p=0.02). Finally, incorporating centre of origin of the subject into model 3 did not significantly alter our data (data not shown).

Quartile analysis

E/E' values were next divided into four equal groups for the purposes of Kaplan-Meier survival analysis (E/E'<6.43, 6.44-7.52, 7.53-9.18 and >9.18. Using the log rank test, increasing risk was demonstrated with each rise in quartile (p=0.03).

The highest quartile (E/E'>9.19) had more than twice the risk of events (figure 8.1.) as the lowest (E/E'<6.43), with a hazard ratio of 2.42 (CI 1.15-5.10, log-rank =0.048).

Discussion

We demonstrate that in a cohort of patients with well controlled hypertension and three cardiac risk factors, the E/E' ratio of transmitral flow to mitral annular velocity is a strong, independent predictor of cardiac outcomes. For each unit rise in the E/E' ratio, there was a 15% increase in first cardiac events, which rose to 17% when adjusted for Framingham risk factors. Those with an E/E' in the uppermost quartile of this population (> 9.18) had a hazard ratio of 2.4 times that of patients in the lowest quartile (<6.43).

Traditional echocardiographic measures of cardiac target organ damage such as LVMI and left atrial size, only trended towards predicting cardiac events in this population. This likely reflects the size of this cohort, rather than the validity of these proven methods. The power of tissue Doppler measures to predict events is all the more impressive in this context.

E/E' and risk prediction

The current study is the first to prospectively demonstrate the ability of the E/E' ratio to predict primary cardiac events in a hypertensive population without established cardiac disease. Our findings therefore significantly extend previous observations showing that the E/E' ratio predicts outcomes in individuals with established, symptomatic heart disease. These data also, for the first time, emphasize that values previously thought to be within the normal range can be associated with an increased risk of cardiac events.

Elevated E/E' has been shown to be a strong predictor of death following myocardial infarction (Hillis 2004) and to be superior in this regard to other clinical or echocardiographic features. More recently, it was also demonstrated to predict cardiac events in subjects following coronary angioplasty (Naqvi 2006) and survival in those with established cardiac arrhythmias Okura 2006), but has not been looked at prospectively in the field of primary prevention until now.

Comparison with other echocardiographic identifiers of risk.

Left ventricular mass index is known to predict events in high-risk hypertensive populations (Verdecchia 1998; Schillaci 2000), but was not able to predict events in this population, though mean values in each group did trend towards prediction. Similarly, left atrial size did not predict outcomes nor did other methods for the indirect assessment of diastolic function such as transmitral E/A ratio or each component of the E/E' ratio when considered individually.

The explanation for why recognized risk markers proved less effective may lie in the size of the cohort and in the overall likelihood of this cohort developing cardiovascular disease. This was a group of hypertensive patients who were at only moderate risk, selected on the basis of an estimated 5-year cardiovascular risk of 5%, which makes the power of any outcome prediction measure all the more impressive. Mean LVMI (125.3 g/m2) and LA size (4.29cm) were only marginally above the normal in those patients with events, suggesting that in well-treated populations such as these, additional methods are likely be required to further stratify cardiovascular risk.

Why would E/E' predict cardiac outcomes?

All of these patients were asymptomatic and most of the values for E/E' were within what is thought to be the 'normal range'. Why, therefore, would this measure predict future myocardial infarctions or new onset angina?

One possible explanation is that we are detecting occult coronary artery disease. When coronary disease causes regional hibernation of the myocardium, the E' velocity drops. This velocity has been shown to rise again after percutaneous coronary intervention (Diller 2009). Those who went on to have coronary events may, therefore, have had a higher E/E' ratio due to regional changes in myocardium, caused by sub-clinical coronary disease.

An alternative hypothesis is that the cumulative burden of hypertension per patient is proportionate to the degree of diastolic 'dysfunction'. This measure may therefore be acting as a surrogate for the overall effect hypertension has had on the myocardium to date, which in turn may predict outcomes.

Study Limitations

The ASCOT study took hypertensive subjects with three cardiovascular risk factors and then aggressively treated them to a strict blood pressure target. Such aggressive treatment is not uniform across the worldwide hypertensive population, and therefore we cannot say with certainty that E/E' would predict risk in a less well treated hypertensive population, though it seems likely.

Conclusion

Tissue Doppler E/E' independently predicts primary cardiac events in a hypertensive population and out-performed traditional echocardiographic measures in this moderately

sized, well-treated hypertensive population. E/E' represents a simple, effective tool for assessing cardiac risk in a hypertensive population and provides additional information over and above that of clinical risk factor assessment.

Tables

Table 8.1. Patient Characteristics

	Event free n=760		Cardiac event n=56		p value
Continuous variables	**Mean**	**S.D.**	**Mean**	**S.D.**	
Age (years)	62.2	± 7.9	64.1	± 7.1	0.079
Systolic Blood pressure (mmHg)	143.5	± 16.1	142.6	± 14.6	0.685
Diastolic Blood pressure (mmHg)	81.9	± 9.0	79.4	± 8.4	0.047
Heart Rate (bpm)	64.4	± 13.0	64.1	± 11.7	0.863
BMI (kg/m^2)	28.9	± 4.6	28.1	± 3.7	0.220
Total Cholesterol (mmol/L)	5.2	± 1.1	5.1	± 1.0	0.783
HDL (mmol/L)	1.3	± 0.4	1.3	± 0.3	0.610
Creatinine (mmol/L)	99.2	± 17.2	103.0	± 15.6	0.116
Calculated GFR (mL/min/1.73m2)	69.5	± 12.4	66.7	± 13.6	0.102
Glucose (mmol/L)	5.9	± 2.0	6.1	± 1.9	0.449
Categorical variables	**%**		**%**		
Males n (%)	79.1		87.5		0.086
Diabetes Mellitus	20.4		25.0		0.253
Cerebrovascular disease	8.7		8.9		0.549
Smoker	24.5		33.9		0.081
Amlodipine based treatment	49.9		51.8		0.445

Table 8.2. Cardiac Events

Endpoint	n	%
Fatal coronary heart disease	11	18.3
Non-fatal and fatal MI (including silent)	23	38.3
New onset angina	24	40.0
Coronary revascularization procedures	35	58.3
Fatal and non-fatal heart failure	3	5.0
Life-threatening arrythmia	5	8.3
Total events	101	

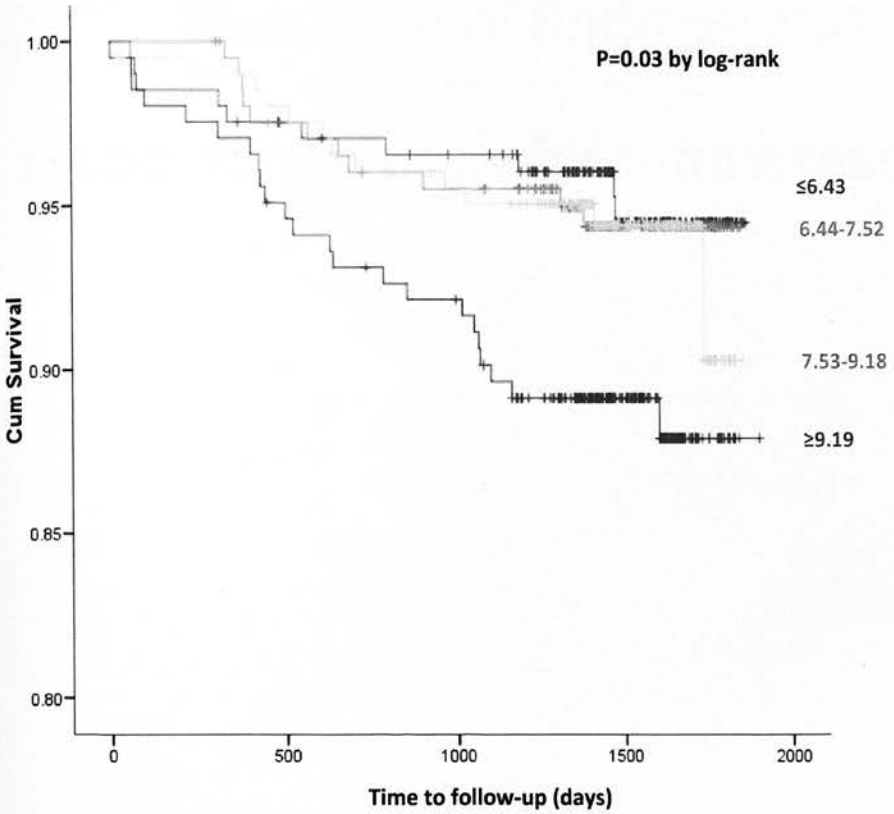
Table 8.3. Echocardiographic results

	Event free n=760	Cardiac event n=56	p value
Echocardiographic data			
Mean E/E' ratio	7.87 ± 2.15	8.77 ± 2.94	0.003
LVMI (g/m^2)	120.4 ± 30.9	125.3 ± 34.4	0.282
RWT	0.51 ± 0.10	0.53 ± 0.12	0.097
Left atrial size (cm)	4.19 ± 0.62	4.29 ± 0.62	0.273
Ejection Fraction (%)	69.4 ± 11.8	69.2 ± 10.8	0.899
Mean tissue Doppler E' velocity (cm/s)	8.36 ± 1.96	8.02 ± 2.41	0.215
Mean tissue Doppler A' velocity (cm/s)	11.57 ± 2.34	11.35 ± 2.25	0.501
Mean tissue Doppler S' velocity (cm/s)	8.86 ± 2.10	8.77 ± 2.18	0.766
Transmitral E wave velocity (cm/s)	61.49 ± 14.98	64.00 ± 15.67	0.228
Transmitral E/A wave ratio	0.88 ± 0.24	0.85 ± 0.23	0.320

Table 8.4. Multivariate analysis: Hazard ratios for E/E'

Model	Adjusted for	Hazard ratio	CI	p value
Unadjusted		1.15	1.05 - 1.26	0.003
Model 1.	Age, Gender	1.17	1.05 - 1.29	0.003
Model 2.	Age, Gender, Diabetes, Systolic BP	1.17	1.05 - 1.30	0.003
Model 3.	Age, Gender, Diabetes, Systolic BP, Heart Rate,	1.18	1.06 - 1.32	0.003
Model 4.	Age, Gender, Systolic BP, Total Chol, HDL, Diabetes, Smoker	1.17	1.05 - 1.29	0.003

Figure 8.1. Kaplan-Meier curves showing freedom from cardiac events according to quartile of Mean Tissue Doppler E/E'



Chapter 9

Summary of findings and recommendations for future research

Summary of findings

This thesis used tissue Doppler echocardiography to characterise, in detail, left ventricular function in 1006 subjects and produced several novel findings.

Firstly, transmitral Doppler had limited ability to identify impaired diastolic function in a cohort at high risk of diastolic dysfunction. Further, the Valsalva maneouvre was found to be unreliable as a discriminator in subjects with an apparently normal transmitral Doppler flow profile.

Secondly, using tissue Doppler early mitral annular velocities (E'), subjects of African-Caribbean ethnicity were demonstrated to have more impaired diastolic function than populations of White European origin.

Thirdly, a correlation was demonstrated between fasting plasma glucose levels and left ventricular diastolic function, supporting the concept of a 'diabetic cardiomyopathy'.

Fourthly, the anti-hypertensive combination regimen atenolol+/- bendroflumethiazide was demonstrated to be associated with relatively adverse measures of diastolic function when compared to a regimen of amlodipine+/- perindopril.

Finally, a large cohort of hypertensive subjects underwent echocardiography and was followed for 4.2 years. The strongest predictor of future cardiac events proved to be the

subject's diastolic function, as measured using the ratio of the transmitral pulsed Doppler early filling velocity (E) to the tissue Doppler early mitral annular velocity (E').

Three of the chapters in this thesis formed the basis for publications in major cardiac journals, as described at the outset of this thesis. One of the aims of this body of research was to provide further support for the inclusion of Tissue Doppler echocardiography in standard clinical echocardiographic protocols. This is now the case, with most echo labs including TDE measures in their routine studies as a result of the large body of work now existing in the literature, of which this thesis forms one small component. The non-invasive assessment of left atrial filling pressure using the E/E' ratio is now routinely quoted in the average echocardiographic report and the data reported in this thesis add further focused support for that strategy.

Areas for future research

There are several ongoing areas of research which are designed to expand on the work in this thesis.

Firstly, patients within the HACVD sub-study of ASCOT underwent a second echocardiographic scan 3 years into their treatment protocol. This means our group has paired echocardiographic data obtained at 1 year (the data that form the basis of this thesis) and at 3 years. Analysis of the effects of anti-hypertensive treatment over this two year intervening period is ongoing. Our research group hopes to be able to report on

degree of change in indices of diastolic function over time, thus allowing further understanding of the impact of the two drug treatment strategies within ASCOT on the myocardium.

The impact of statins on LV mass and diastolic function will also be re-examined, with analysis of changes observed between the two echocardiographic scans over time. It may be that an impact of statins on left ventricular structure will be demonstrated through paired scans and after a longer period of treatment with statin therapy.

Secondly, consideration will be given to whether E/E' represents a potential target for treatment, just as systolic and diastolic blood pressure currently represent targets for treatment. Examining this would represent a substantial undertaking and so analysis of outcomes in those patients who exhibit the most and the least change in this parameter, between one year and three year scans, will provide important supporting evidence for this novel concept.

Thirdly, technology has moved on considerably since the outset of the ASCOT study. Strain rate imaging and speckle-tracking echocardiography now provide detailed information on regional long-axis myocardial function. Rather than summating an entire wall from base to apex, these technologies allow examination of localised regions of myocardium and modern software has reduced the amount of time it takes to perform analyses. The use of such technologies in future studies of the hypertensive heart would

better delineate the interaction of coronary blood supply with long axis function, by allowing selective interrogation of myocardial relaxation properties within single coronary artery territories. Protocols have also changed over time, with the importance of the measurement of left atrial volume now established.

Furthermore, the impact of fibrosis on the hypertensive ventricle remains incompletely understood. It is unclear what component of the fibrotic process, if any, can be reversed with drug treatment. This fibrotic process is thought to be a key component of the inexorable progression from normal myocardial function to severe impairment of function and therefore demonstrating its extent, clinical impact and subsequently the effect of various hypertensive treatments is essential to understanding how to slow, or even reverse this process.

The wide availability of cardiac MRI means we now have a new technology to improve our understanding in this area. MRI provides detailed information on the degree of fibrosis within myocardium, though the use of gadolinium to examine late enhancement within muscle. Demonstrating a difference between the two ASCOT drug treatment cohorts in the degree of late gadolinium enhancement on MRI (and hence fibrosis) might help us better understand why some subjects who received the amlodipine/perindopril combination fared better, in terms of clinical outcomes, than some of those who received the atenolol/BFZ-K combination.

There are sound physiological reasons why this might be the case, as aldosterone is thought to be an important factor in the establishment of myocardial fibrosis. It is therefore plausible that ACE inhibition might limit the degree of fibrosis in myocardium and that this might have detected in a cohort of the size studied in ASCOT using sensitive measures such as cardiac MRI. Certainly, a reduction in the degree of myocardial fibrosis might be expected to lead to reduced rates of heart failure.

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Appendix 1a. Sample of Doppler measurement reproducibility analysis. Similar measures were performed for all Doppler and Tissue Doppler variables.

Lateral wall E wave	Op 1	Op 2	Average	Difference	Difference2	SD	CV
Patient 1	14.2	15.1	14.7	-0.9	0.8	0.6	4.3
Patient 2	14.0	13.8	13.9	0.2	0.0	0.1	1.0
Patient 3	8.3	8.1	8.2	0.2	0.0	0.1	1.7
Patient 4	12.4	13.1	12.8	-0.7	0.5	0.5	3.9
Patient 5	16.2	16.6	16.4	-0.4	0.2	0.3	1.7
Patient 6	9.9	10.3	10.1	-0.4	0.2	0.3	2.8
Patient 7	12.8	12.9	12.9	-0.1	0.0	0.1	0.6
Patient 8	16.4	16.9	16.7	-0.5	0.3	0.4	2.1
Patient 9	13.1	11.8	12.5	1.3	1.7	0.9	7.4
Patient 10	15.5	15.9	15.7	-0.4	0.2	0.3	1.8
Patient 11	14.0	13.1	13.6	0.9	0.8	0.6	4.7
Patient 12	14.8	15.7	15.3	-0.9	0.8	0.6	4.2
Patient 13	7.7	7.3	7.5	0.4	0.2	0.3	3.8
Patient 14	8.1	8.6	8.4	-0.5	0.3	0.4	4.2
Patient 15	17.2	16.7	17.0	0.5	0.3	0.4	2.1
Patient 16	10.2	10.6	10.4	-0.4	0.2	0.3	2.7
Patient 17	15.4	15.4	15.4	0.0	0.0	0.0	0.0
Patient 18	12.2	12.4	12.3	-0.2	0.0	0.1	1.1
Patient 19	13.3	13.7	13.5	-0.4	0.2	0.3	2.1
Patient 20	16.4	17.0	16.7	-0.6	0.4	0.4	2.5
Average	12.8	12.9		-0.1	Average CV	2.9	
SD	2.9	3.0		0.6	SD CV	1.7	
					Sum diff	8.5	
					zw	0.4	
					Repeatability	1.1	

Appendix 1b. Sample of LV measurement reproducibility analysis. Similar measures were performed for all M-Mode acquired variables.

IVSd	Op 1	Op 2	Average	Difference	Difference^2	SD	CV
Patient 1	1.1	1.1	1.1	0.0	0.0	0.0	0.0
Patient 2	1.5	1.3	1.4	0.2	0.0	0.1	10.1
Patient 3	0.9	0.9	0.9	0.0	0.0	0.0	0.0
Patient 4	1.3	1.5	1.4	-0.2	0.0	0.1	10.1
Patient 5	1.3	1.4	1.4	-0.1	0.0	0.1	5.2
Patient 6	1.0	1.0	1.0	0.0	0.0	0.0	0.0
Patient 7	1.2	1.1	1.2	0.1	0.0	0.1	6.1
Patient 8	1.2	1.1	1.2	0.1	0.0	0.1	6.1
Patient 9	1.1	1.1	1.1	0.0	0.0	0.0	0.0
Patient 10	1.0	1.0	1.0	0.0	0.0	0.0	0.0
Patient 11	1.7	1.7	1.7	0.0	0.0	0.0	0.0
Patient 12	1.1	1.0	1.1	0.1	0.0	0.1	6.7
Patient 13	1.2	1.1	1.2	0.1	0.0	0.1	6.1
Patient 14	1.2	1.4	1.3	-0.2	0.0	0.1	10.9
Patient 15	1.3	1.2	1.3	0.1	0.0	0.1	5.7
Patient 16	1.2	1.2	1.2	0.0	0.0	0.0	0.0
Patient 17	0.9	0.9	0.9	0.0	0.0	0.0	0.0
Patient 18	1.3	1.2	1.3	0.1	0.0	0.1	5.7
Patient 19	1.1	1.2	1.2	-0.1	0.0	0.1	6.1
Patient 20	1.4	1.3	1.4	0.1	0.0	0.1	5.2
Patient 21	1.3	1.1	1.2	0.2	0.0	0.1	11.8
Patient 22	1.3	1.0	1.2	0.3	0.1	0.2	18.4
Patient 23	1.0	1.2	1.1	-0.2	0.0	0.1	12.9
Patient 24	1.2	1.0	1.1	0.2	0.0	0.1	12.9
Patient 25	0.8	1.0	0.9	-0.2	0.0	0.1	15.7
Patient 26	1.4	1.2	1.3	0.2	0.0	0.1	10.9
Average	1.2	1.2		0.0	Average CV	6.4	
SD	0.2	0.2		0.1	SD CV	5.5	
					Sum diff	0.5	
					zw	0.1	
					Repeatability	0.3	

Appendix 1c. Biomarker definitions for myocardial infarction

	Decision limit	Timing	Number of occasions	Evidence of rise and fall required?
Creatine kinase (CK) / B fraction of CK / aspartate aminotransferase (AST) / Lactate dehydrogenase (LDH)	At least twice the upper limit of normal for that institution	Within 72 hours or 3 calendar days of onset of symptoms, admission to hospital, or any recurrence of symptoms.	1	No
Troponin T or I	>99th percentile	<24 hours from index clinical event	1	No; but troponins may be elevated for 7 days
CK-MB	>99th percentile	not stated	2	Yes
CK-MB	at least twice upper limit of normal for that institution	"first hours"	1	No

Appendix 1d. Edinburgh claudication questionnaire.

1. Do you get pain or discomfort in your leg(s) when you walk?

   Yes θ

   No θ

   I am unable to walk θ

   If you answered "YES" to question 1 – please answer the following questions. Otherwise you need not continue.

2. Does this pain ever begin when you are standing still or sitting?

   Yes θ No θ

3. Do you get it if you walk uphill or hurry?

   Yes θ No θ

4. Do you get it when you walk at an ordinary pace on the level?

   Yes θ No θ

5. What happens to it if you stand still?

   Usually continues more than 10 minutes θ

   Usually disappears in 10 minutes or less θ

6. Where do you get this pain or discomfort?
   Mark the place(s) with "X" on the diagram below.

Scoring the Edinburgh claudication questionnaire

A classification of definite claudication requires all the following responses:

Question 1 – Yes

Question 2 – No

Question 3 – Yes

Question 5 – Usually disappears in 10 minutes or less

Question 6 – Pain clearly indicated within the calf muscle, regardless of whether pain is also marked in other sites

A classification of atypical claudication requires all the following responses:

Question 1-5 – as above

Question 6 – Pain indicated in the thigh or buttock, in the absence of any calf pain

Grading degree of claudication

Question 4 - Yes = Grade 2

No = Grade 1

Sites excluding claudication

A diagnosis of intermittent claudication should be excluded if pain is indicated in the hamstrings, feet, shins, joints, or appears to radiate, in the absence of any pain in the calf.

Appendix 1e. Patient information sheet for ASCOT enrolment.

Your blood pressure is higher than normal. This is associated with an increased risk of developing cardiovascular side effects such as heart attacks, heart failure and stroke. The risk is further increased if you also have additional risk factors such as smoking and high cholesterol levels. Treatment of high blood pressure has been shown to reduce the risk of these complications. Treatment measures include improving dietary habits, increasing exercise and stopping smoking. However, many patients also require drug treatment for controlling high blood pressure and cholesterol.

The Aim of the Study

Pressure Lowering Therapy

You are invited to participate in a study, in which we want to compare the long term effects of blood pressure reduction by using two combinations of antihypertensive drugs. The study is taking place in the UK, Ireland, Denmark Finland, Norway and Sweden, and will comprise 18000 patients in total. If you agree to participate in the trial, and we hope you will, you will receive one of two well known drugs used to treat high blood pressure - either atenolol, a beta-blocker or amlodipine, a calcium channel blocker.

The decision whether to treat you with atenolol or amlodopine will be made "at random" which means that there is a fifty-fifty chance that you will receive either drug. If the first drug you receive is not enough to lower your blood pressure to an acceptable level, you will get additional treatment. A thiazide diuretic (bendroflumethiazide with potassium supplementation) will be added to those on atenolol, and an ACE inhibitor (perindopril), will be added to those initially supplied with amlodipine. If these treatments are not enough, you will then receive an alpha blocker, (doxazosin),

All the drugs used in the study are well-documented effective blood pressure lowering drugs, and are usually well tolerated by patients. However, each of these drugs may occasionally cause side effects e.g. atenolol may produce cold hands and feet and tiredness; amlodipine may produce swollen ankles; bendroflumethiazide and potassium may cause gout; perindopril may cause coughing and doxazosin may produce dizziness when standing.

All the study drugs will be provided free of charge throughout the study.

Cholesterol Lowering Therapy

If your level of blood cholesterol is below a certain level, you will be invited to participate in a parallel part of the study. In this part of the study we will compare the effects of atorvastatin, a drug which lowers blood cholesterol, with the effect of a placebo (a harmless, inactive substance). If you take the placebo, you will still receive all the advice and medical check-ups included in the study. In order to judge this part of the study fairly, it will be double-blinded (i.e. neither you nor your doctor will know if you are receiving atorvastatin or the placebo, which, like the blood pressure lowering drugs, are assigned randomly). The drugs in this part of the study will also be supplied free of charge.

Advantages and Disadvantages of Joining the ASCOT Study

Advantages

1. Patients will be followed and treated more systematically than is usual for their disease and complications
2. Patients will achieve more stringent BP control than is usually achieved on average in routine clinical procedure
3. Patients will have more complete investigation and evaluation of their cardiovascular health than is usual on average in routine clinical practice.
4. The study medication is provided free of charge

Disadvantages

1. Treatment choice and administration schedule is theoretically more rigid than in routine practice but total flexibility is permitted within the trial for efficacy or safety reasons
2. Patients with relatively modest elevations in cholesterol or even 'normal' blood cholesterol levels may receive low-dose atorvastatin. Although this group of drugs is extremely well tolerated (as well as placebo) and other data suggest benefits may accrue from their use among such patients, the size of the benefits relative to any possible adverse effects has not yet been fully established

Your contribution to this research will help us to select those treatments which are most effective at reducing the level of chronic illness and death caused by cardiovascular illness and will help to improve treatment for patients in the future.

Study Procedure

About one month before the study starts you will be invited to come for a screening visit when a few investigations, including blood and urine tests and an ECG (electrocardiogram) will be carried out. If you are then suitable for inclusion in the study you will be asked to attend for a second visit when you will be supplied treatment with either atenolol or amlodipine. You will also receive information regarding your diet and lifestyle, which will help you to reduce your risk of cardiovascular disease.

During the course of the study you will be asked to visit your study doctor and/or nurse about twice each year. At each visit they will measure blood pressure and carry out a general check-up. At some of the visits blood and urine tests will be taken. If the original drugs are not enough to lower blood pressure to an acceptable level, there are possibilities to either increase the dose or to add another drug as described above. All of this will be discussed with you in detail if you decide to join the study. At any time during the trial you can consult the study doctor or nurse if you have any questions about your part in the study (see below).

The study will continue for approximately five years. If you change your address during the study period it may still be possible to remain in the study.

The results of the study will be analysed and reported according to the requirements of official authorities. Your results can only be identified through a special patient number and your birth date, but not by your name, address or any other means of identification. Only your study nurse and study doctor can identify you from the patient number. If you choose to participate in the study your signed consent form will be kept in an archive. Any information that you give will be held in the strictest confidence and used solely for research purposes. Authorised study personnel may occasionally be required to examine your medical files in order to verify that the study in which you are participating is conducted accurately and properly. The study has been approved by the appropriate Ethics Committee.

All significant new findings developed during the course of the research will be made available to you.

Your participation in the study is entirely voluntary and you can withdraw your consent to participate at any time, without giving a reason. In that situation, your own doctor will give you the best alternative treatment, but your study doctor or nurse will still want to keep contact with you until the study is finished. This would not affect your future medical care or your relationship with any of the study staff involved in providing it.
The telephone numbers of the doctor and nurse responsible for the study are listed below. If you have any questions relating to this research project do not hesitate to contact them.

Doctor_____

Telephone_____

Nurse_____

Telephone_____

Appendix 1f: Patient consent form, agreement to participate in the ascot research project.

I, [name of subject] _____

Of [address] _____

_____

Agree to take part in the ASCOT research project

I confirm that I have received verbal and written information about participation in the clinical trial called ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial). I have been given time to consider the information and the opportunity to ask questions about this trial. The nature and demands of the research have been explained to me. I fully understand and accept them.

I understand that my medical records relevant to this trial may be examined by authorised ASCOT staff, who have my permission to do so.

I understand that my consent is entirely voluntary, and that I may withdraw from the research project if I find that I am unable to continue for any reason and this will not affect my medical care.

Signed: _____

[print name] _____

Witness _____

[print name] _____

Date_____

Investigator's Statement:

I have explained the study outline, nature, demands and foreseeable risks of the above research project to the subject. I have given the subject time to consider the information. I have received the patient's informed consent to participate.

I will save a copy of this consent form for archive according to the present rules.

To be filled in by responsible doctor/nurse

Signature_____

Date _____

Patient consent form checklist

The participant should complete the whole of this checklist him/herself (please delete as necessary)

.....

- Have you read the patient information sheet?
  YES/NO
- Have you had an opportunity to ask questions and discuss this study?
  YES/NO
- Have you received satisfactory answers to all of your questions?
  YES/NO
- Have you received enough information about the study?
  YES/NO
- Who have you spoken to?
- Dr/Mrs/Ms/Mr_____
- Do you understand that your decision to consent is entirely voluntary and that you are free to withdraw from the study at any time, without having to give a reason and without affecting your future medical care?
  YES/NO
- Do you agree to take part in this study?
  YES/NO

Signed_____Date:_____

Name in block
letters_____