The impact of ageing and exercise training on cardiac structure and function in healthy females

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Figure 1.2 page 6

Abstract

In recent years it has become clear that Western societies face a rapidly increasing ageing population. With ageing comes a significant reduction in functional capacity, cardiovascular function, increased cardiovascular disease risk and thus increasing health care costs. Exercise interventions in the elderly may prove to be a valuable tool in coping with an ageing population. It was, therefore, the purpose of this thesis to investigate; a) the effects of healthy ageing upon cardiac structure and function in adult males and females, b) the effects of a progressive aerobic exercise training programme upon cardiac structure and function in post-menopausal females as well as c) the effects of competitive exercise training on cardiac structure and function in post-menopausal female athletes and controls. The exact nature of left ventricular (LV) remodelling with age is the source of some controversy. Within a cross-sectional design cardiac structure and function was assessed in 124 women and 74 men (18-76 years). Left ventricular mass was maintained across the adult age-span in females ($r= 0.02$, $P>0.05$) but was significantly and negatively associated with age in males ($r= -0.36$, $P<0.05$). The maintenance of LV mass in females despite an age-related decrease in LV volume suggested that remodelling of the LV with age was concentric in nature in females, with a relative wall thickening. In males, however, the large decrease in LV mass along with a smaller decrease in LV volume suggested a form of "eccentric atrophy" of the LV. Other data suggested an increase in male RV volume with age (c. 25%), no depression in LV and RV systolic function with age in either males or females and an expected age-related decrease in LV and RV diastolic filling (E:A ratio). Twenty post-menopausal females completed a progressive 12-month aerobic exercise training programme. Despite a significant and progressive increase in maximal aerobic capacity (pre, 23.7 ± 3.1 $ml.kg^{-1}.min^{-1}$; post, 32.2 ± 4.1 $ml.kg^{-}$

^{1}.min^{-1}) there were few alterations in cardiac structure and function. It seems, therefore, that healthy sedentary females do indeed lose the ability to induce LV hypertrophy (LV mass pre, 155 ± 41 g; post, 136 ± 30 g) with training. There was some evidence of an increase in LV volume with training (and a much smaller trend toward an increase in SV). Other data showed no change with progressive exercise including LV systolic and diastolic function as well as volume data, systolic and diastolic function in the RV. Finally, nine post-menopausal female athletes were compared to an age- and lean body mass-matched control group. In agreement with the intervention study LV mass was not different in the athletes and controls (sedentary, 146 ± 31; active, 143 ± 25 g). To support and extend the training study LV volume (18%) and SV (25%), as well as RV volume (15%), were significantly greater in the athletes than the controls. The athletes also demonstrated an enhanced LV E:A ratio (sedentary 1.12; active, 1.53) although the increase in RV E:A was non significant. Both LV and RV systolic function were not different between groups. In conclusion, there is some evidence that healthy ageing of cardiac structure and function is different in males and females. Further, whilst functional capacity increases with exercise training in post-menopausal women there seems to be a lack of a LV hypertrophic response.

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List of abbreviations

2-D	Two-dimensional
3-D	Three-dimensional
A	Atrial filling peak velocity
ANOVA	Analysis of variance
BM	Body mass
BMI	Body mass index
BV	Blood volume
Ca^{2+}	Calcium ions
CO	Cardiac output
CV	Cardiovascular
DBP	Diastolic blood pressure
E	Early filling peak velocity
E:A	Ratio of E and A
ECG	Electrocardiography
EF	Ejection fraction
FS	Fractional shortening
HR	Heart rate
HRmax	HR maximum
LV	Left ventricular
ICC	Intra-class correlation coefficient
LBM	Lean body mass
LoA	Limits of agreement
LVEDD	Left ventricular end-diastolic dimension
LVEDV	Left ventricular end-diastolic volume
LVESD	Left ventricular end-systolic dimension
LVESV	Left ventricular end-systolic volume
LVPWT	Left ventricular posterior wall thickness
LVL	Left ventricular length

MAP	Mean arterial pressure	
Mit	Mitral	
Mo	Month	
MRI	Magnetic resonance imaging	
NM	Not measured	
O_2	Oxygen	
PFR	Peak filling rate	
RV	Right ventricle	
SBP	Systolic blood pressure	
SD	Standard deviation	
SV	Stroke volume	
TP	Training programme	
TPFR	Time to peak filling rate	
Tri	Tricupsid	
$\dot{V}O_{2max}$	Maximum oxygen consumption	

1. Introduction

There are a number of important public health issues within the UK that are currently high on the agenda of the general population, the media, the National Health Service (NHS), research and educational institutions and the government. Two of these are the rapidly increasing proportion of our population who can be termed "old" or "aged" and the continuous struggle with the prevention, identification, treatment and management of cardiovascular disease, which remains the number one killer in the UK. The two issues are clearly linked as cardiovascular disease rates increase with age as it is primarily an acquired disease that develops over prolonged periods of time. On this basis the investigation of ageing, the cardiovascular system and exercise as a potentially positive intervention is obviously warranted.

1.1 Ageing

Ageing is an important and essential part of human existence that has impact beyond the biological with sociological, economic consequences to name but a few. Age is defined as the period of time that a person, animal or living organism has lived. Ageing or aging refers to the fact or process of growing old. This is known as chronological age. It is an objective means of quantifying how old a person is and is usually, but wholly arbitrarily, measured in years. However, there are wide inter-individual differences in functional status at any given chronological age. In terms of maximal oxygen intake, muscle strength and flexibility, the best-preserved 65-year-old may out-perform a sedentary 25-year-old. Confusion often arises in the interpretation of differences between older and younger individuals because of a failure to acknowledge or control for interactions among age, disease, and lifestyle (Lakatta, 2001). Genetic components of ageing, disease and lifestyle, which remain largely unknown in the literature, complicate the picture further (Lakatta, 2002); therefore it is clear that extreme care must be taken in an attempt to control for some of these factors in future research. Exclusion criteria incorporating physical activity habits, dietary information, family medical history, blood pressure limits all must be controlled for.

The alterations that occur in the body with ageing are comparable to those apparent with deconditioning and disuse. This deterioration of the major biological systems in

the body with age has led to questions regarding the mechanisms responsible for this deterioration. Debate has arisen amongst professionals asking the question of the basic theories of ageing: Is age an adaptation or a progressive disease state? Although it is well known that age is synonymous with disease many people achieve "old age" without evidence of these diseases, therefore age cannot be described as a progressive disease state. Conversely, age contributes to an increased exposure time to other risk factors i.e. diet, physical activity patterns, age-related blood pressure increases. Due to the relationship between these factors, time itself becomes a risk-factor and consequently so does age. Such continuing discussion has also led to the search for possible interventions to delay and perhaps reverse some consequences of the ageing process. Consequently, research into ageing is now viewed as a significant area and of relative high priority for funding bodies.

The percentage of the UK population aged 65 years and over has risen dramatically in the past 10 years. In England, this rise has meant that in 2001 16% of the national population was aged 65 years and over. In the USA estimations are that by the year 2035 nearly one in four individuals will be age 65 years or over (Lakatta, 2003). It is well known that the older body is more susceptible to illness, and that the prevalence of high blood pressure, diabetes, osteoporosis and coronary artery disease increases in such people (Lakatta, 1993). As a result of this the quality of life for older people can decrease dramatically. Everyday tasks become difficult to perform due to restrictive factors such as localised chest, muscle or bone pain, breathlessness and risk of falls (ACSM, 1998).

With a large proportion of people over 65 years taking prescribed medication, it is reported that the NHS spent around 40% of its budget on people over 65 years in 1998/1999 (National Statistics, 1999). Existing projections show that the number of people aged over 65 years is expected to increase to 21% of the population by 2026 (National Statistics, 1999). The consequence of this is a continuation of the rising cost to the NHS of caring for the elderly. This represents a real and increasing burden that requires management, intervention and research.

Possibly the most widely reported ageing effect is the decline in maximal oxygen uptake ($\dot{V}O_{2max}$) and associated physical performance capacity. The rate of decline of

$\dot{V}O_{2max}$ has been reported to approach 10% per decade in sedentary populations and in healthy, active older individuals the age-associated decrement was approximately 3% in men and 2.5% in women per decade (Fleg *et al.*, 1988). The amount of decrease is dependant on many factors such as health status and lifestyle (past and present), however the primary ageing process, although having a genetic component, occurs in the absence of disease and is independent of lifestyle (Lakatta, 1993). What is clear with this specific variable, but also with many measures of cardiovascular structure and function, is that the process of separating healthy ageing from disease and effect of lifestyle is difficult and our current knowledge base of ageing *per se* is somewhat limited.

1.2 Cardiovascular (CV) disease

Cardiovascular disease is the most important single cause of morbidity and mortality in Western society, killing more than 110,000 people in England each year (National Statistics, 1999). Key lifestyle risk factors are smoking, poor diet and lack of physical activity, however, age is an independent risk factor in itself. Worryingly, almost 80% of all cardiovascular deaths occur in people 65 years or over (Lakatta, 2003).

It is clear from a wide range of research, both epidemiologic and intervention, that exercise/physical activity can play an important role in CV disease development/prevention as well as rehabilitation. In a review article, Clausen (1976) concluded that training can improve exercise tolerance in coronary artery disease (CAD) patients by inducing a reduction in HR and SBP that decreases myocardial O_2 demand at a given pulmonary O_2 uptake. In a more recent review Coats (1993) maintained that physical training can lead to $\approx$ 25% increases in exercise duration, $\dot{V}O_{2peak}$ and improvements in patients' perception of their own condition. Central cardiac changes in response to exercise training in the diseased heart have also been reported including an increase in end-diastolic diameter suggesting that with coronary artery disease patients continue to elicit cardiac adaptation to aerobic training (Coats, 1993).

In summary, exercise is an intervention that can possibly be used to delay or prevent physiological deterioration caused by ageing and/or CV disease. Whether these occur

independently or together it is pertinent to investigate the role and impact of exercise upon the heart and cardiovascular system in the ageing human body.

1.3 Cardiac structure and function

The heart is a hollow muscular organ weighing approximately 300*g*. It sits within the thoracic cavity in between the left and right lungs and predominantly behind the sternum. Because of a slight apex-base tilt and left-right rotation at the left sternal border in the 3-5th intercostals space the heart can be viewed with the right ventricle anterior and left ventricle posterior to the chest wall. An apex view of the heart can be obtained in the mid-chest region around the fourth/fifth intercostal space. The heart is divided into four chambers, two atria and two ventricles (see Figure 1.1). The right and left sides are separated by the septum. The tricuspid and mitral valves separate the atria from the ventricles and prevent back flow into the upper chambers (the atria) during ventricular systole and the pulmonary and aortic valves separate the ventricles from the major arteries and guard against reflux from outflow tracts into the ventricles during diastole.

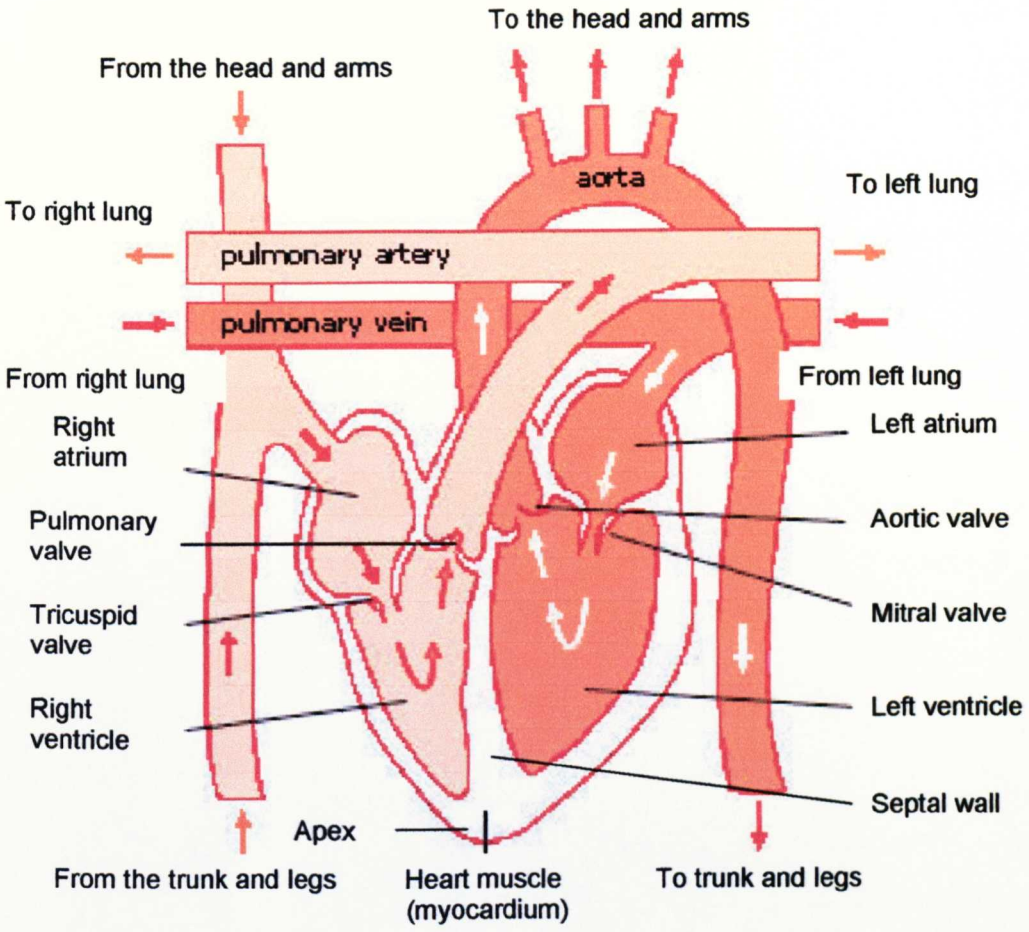


Figure 1.1 The heart and major vessels

In the study of exercise and its impact upon the heart it is common to concentrate on the left ventricle (LV). This is due to the role of the LV in pumping blood to the working muscles and the fact that the LV, due to its orientation, is much easier to image in non-invasive studies utilising tools such as echocardiography.

There are a variety of structures associated with the LV and here description is limited to those that will be central to the empirical data collection in this thesis. Left ventricular mass (LV mass) is a common variable in cardiac assessment as well as an important and independent clinical predictor for cardiovascular disease risk (Savage *et al.*, 1990). The assessment of LV mass can be accomplished in a variety of ways using echocardiography but in all cases estimation includes the measurement of interventricular septal wall and posterior ventricular wall dimensions as well as determination of LV chamber size, normally recorded at end-diastole. Left

ventricular volume (LV volume) is a measurement of the size of the LV chamber *per se*. It does not include any measurement of wall thickness, it is simply assessed as the LV internal area combined with the LV internal length (from mitral valve to apex – endocardial border). Right ventricular volume (RV volume) can also be estimated, however, due to the difference in shape and size compared to the LV, different calculations are necessary. Among a range of possible ways to assess RV volume we chose to determine RV area at the tricuspid valve level and RV length to facilitate the calculation of RV volume.

The assessment of LV volume can be made at the end of both the diastolic and systolic phase of the cardiac cycle and this is the basis for the estimation of the variables stroke volume (SV) and ejection fraction (EF).

(1) Mitral valve closes, (2) Aortic valve opens, (3) Aortic valve closes, (4) Mitral valve reopens

(1) Mitral valve closes

Figure 1.2 The cardiac cycle (Birch *et al.*, 2004)

The function of the heart relies upon the electrical signals that pass between the cardiac muscle cells. The heart is unique in that some specific cells can initiate the process of depolarisation independently of external influences. In this way the heart is described as autorhythmic. It is only after the cardiac muscle cells are depolarised by a 'wave' of electrical activity that the muscle cells can contract and blood is pumped. The intergration of all cardiac cell action potentials can be assessed at the surface of the body via electrocardiography (ECG). The ECG is split into various component parts and periods that are commonly referred to as PQRST (see Figure 1.2). The P component or 'wave' represents the simultaneous depolarisation of the right and left atria. A small delay (P-R interval) represents the electrical signal transmission through the atrio-ventricular node where transmission is slowed to allow full ventricular filling (see Figure 1.2). The QRS complex, or again 'wave', is normally a thin large spike of electrical activity that represents the simultaneous depolarisation of the left and right ventricles. The QRS complex masks the repolarisation of the atrial cells. The S-T segment represents the refractory period of ventricular cells, this is followed by the T-wave which represents ventricular repolarisation. A period of baseline electrical membrane potential precedes the next P-wave. The ECG is an invaluable clinical tool, not only due to the ability to determine heart rate, but its ability to provide direct and indirect evidence of health and disease within the heart.

The cardiac cycle integrates the time sequence of electrical activity, changes in pressure, flow and volume of blood in the left and right side of the heart. When the onset of QRS complex occurs this registers the beginning of systole. Ventricular pressure begins to rise which results in closure of the mitral valve. This volume of the ventricle at this point is called the preload. Preload is essentially dictated by how far heart fibres are able to stretch when the heart is fullest. This can be affected by different factors including age and elastic compliance of the ventricle. The aortic valve remains shut for a short period whilst ventricular pressure rises to meet and then exceed aortic pressure. This period where both mitral and aortic valves are shut is called the iso-volumetric contraction period or pre-ejection period. As ventricular pressure exceeds aortic pressure the aortic valve is opened and blood flow occurs out of the LV. The velocity of blood flowing through the aortic (and pulmonary) valves

can be measured, and reflects ventricular pumping performance. The blood flow (or volume) produced by ventricular contraction is called SV. This is controlled by a range of factors including LV filling or preload, resistance to flow (afterload), and the intrinsic contractile properties of the ventricle (contractility). Ejection fraction and fractional shortening (FS) are both measures of contractility and represent the ratio of SV to LV volume at end-diastole. After ventricular contraction is finished ventricular pressure begins to drop. When this falls below aortic pressure the aortic valve closes to prevent blood flow back into the ventricle. This indicates the end of systole and the beginning of diastole. The ventricular pressure must drop below atrial pressure in order to open the mitral valve. The period where both aortic and mitral valves are closed is called iso-volumetric relaxation. As ventricular pressure drops below atrial pressure the mitral valve opens and the refilling process begins.

Within the filling or diastolic phase there are two primary components. Firstly, early (passive filling; E), occurs as the atrial-ventricular pressure gradient causes the mitral valve to open, and by way of gravity and suction of the relaxing ventricle the blood flows down from the atria into the ventricles. After a period of diastasis (this occurs at rest but effectively disappears at HR over 100-120 beats.min^{-1}) where there is little if any flow and an isoelectric ECG, the second component of filling begins with the P-wave or atrial depolarisation. This results in further diastolic flow and filling of the ventricle. This is known as atrial or active (A) filling and completes the transfer of blood to the ventricle. The ratio of E/A can be used as an indicator of diastolic compliance. At rest, in young, healthy individuals 2/3 of ventricular filling occurs in the early/passive phase whilst only 1/3 of filling is as a consequence of atrial systole. After active filling and the onset of the QRS complex diastole is complete.

In summary, after introducing background concepts and issues relevant to this thesis, ageing as well as cardiovascular function and disease, it is appropriate to state the aims of the empirical studies that follow. The aims of this PhD are as follows;

1) To investigate the impact of healthy, sedentary ageing upon cardiac structure and function in adult males and females.
2) To investigate the impact of a 12-month aerobic training programme upon cardiac structure and function in healthy, sedentary post-menopausal females.

3) To investigate the difference in cardiac structure and function between highly trained compared with healthy, sedentary post-menopausal females.

2. Literature Review

The structure of this literature review is such that it begins by appraising current evidence related to the impact of ageing upon cardiac structure and function that provides insight into the background for study one. The literature review then considers cross-sectional and longitudinal evidence for the impact of exercise upon cardiac structure and function, primarily in ageing populations that provide the rationale for studies two and three in the thesis. The literature review concludes with a summary and specific hypotheses on which the empirical studies are based.

2.1 Effect of age on the CV structure and function

2.1.1 Cardiac Structure

Various methods of assessment have been used in the quest to answer the question of what happens to the structure of the myocardium with advancing age. Autopsy has been considered as an important technique and has been used in a small number of studies. Total heart weight (including atria and epicardial fat) has been reported to increase as a function of age by approximately 0.36 g/year (Olivetti *et al.*, 1991). The amount of epicardial fat (dissected off and weighed separately) correlated significantly with age, total heart weight, and amount of subcutaneous fat (Reiner *et al.*, 1959).

Results from Olivetti *et al.* (1991), however, demonstrated that when the muscle mass of the left ventricle and septal wall (LV mass) alone were analysed (not including connective tissue and epicardial fat), a *reduction* in LV mass of 0.70 g/year was reported. Interestingly, RV mass also decreased by 0.21 g/year – or at one third of the rate of the reduction in the LV. The decrease in LV mass seemed to be the result of the diminished septal wall thickness as LV free wall thickness has been reported to decrease only slightly (Klein *et al.*, 1994) or as with the RV, remain relatively constant (Kitzman and Edwards, 1990). The basis of the diminution seems to be that with ageing the number of myocyte nuclei decreased in both ventricles. This loss was concomitant with an *increase* in myocyte volume where volume per each nucleus increased by 110 and 118 mm^3/yr in LV and RV, respectively. Despite this hypertrophy the decrease in LV mass indicated that hypertrophy does not seem to fully compensate for the myocyte loss (Olivetti *et al.*, 1991).

In living organisms echocardiography (echo) has been reported as an accurate and cost-effective (Feigenbaum, 1994) clinical tool in the assessment of cardiac structure and function and in the clinical diagnosis of LV hypertrophy. When interpreting echo data in ageing studies we must bear in mind that a range of echo techniques; M-mode, 2-D, 3-D, have been employed to evaluate LV structure and function. Initial data collected with M-mode, due to its earlier development, may be limited by technical problems and geometric assumptions. Indeed M-mode echo data assessing the relationship between age and LV mass, although sometimes inconsistent, has led to the general belief that LV mass increases with age (Gardin *et al.*, 1979), caused by an increasing wall thickness (Gerstenblith *et al.*, 1977, Gardin *et al.*, 1979, Pearson *et al.*, 1991, Shub *et al.*, 1994, Slotwiner *et al.*, 1998) whilst LV end diastolic dimension and thus LV volume remains unchanged (Dannenburg *et al.*, 1989; Ganau *et al.*, 1995). However, M-mode echocardiography has been criticised in its ability to accurately depict changes in LV geometry and mass due to the limited views it uses to calculate LV mass (see Chapter 3). For example the M-mode and autopsy data of Deveruex and Reichek (1977) suggested a potential error from M-mode of between 25 and 50g. With LV mass as an important and independent clinical indicator for CV disease progression both in diagnosing LV hypertrophy, systolic and diastolic dysfunction and in the use of echo is predicting CV disease risk it is extremely valuable to investigate the ageing heart utilising 2-D echo to determine a more accurate picture of the LV mass response to increasing age.

More recently the use of magnetic resonance imaging (MRI) has been employed to investigate the effects of ageing on CV structure and function. The introduction of this technique has further illuminated problems with previous M-mode echocardiographic investigations. Sandstede *et al.* (2000) demonstrated that both absolute and indexed LV mass was lower in older males (although not significant) than younger males and in females LV mass did not alter with age. Hees *et al.* (2002) reported that LV volume decreased in males and remained stable in females with advancing age. Kitzman and Edwards (1990) also described altered chamber geometry with ageing. This was due to a shortening of the long-axis (base to apex) dimension compared to the short-axis. This results in a mild decline in internal systolic and diastolic LV dimensions, dilation and rightward shifting of aortic root

and dilation of the left atria. The mechanisms for this change remain unclear (Kitzman and Edwards, 1990).

It is clear from existing literature, likely due to methodological differences, that the impact of increasing age upon cardiac structure still requires some clarification. This maybe illuminated by the use of 2-D echo assessment techniques, due to the geometric advantages that this method holds over previously utilised M-mode methods. It is also pertinent to note that echo is a painless non-invasive technique that is much more common in routine clinical use and cardiological research than MRI despite its potential advantages in accuracy.

2.1.2 LV function (systolic)

Although the exact mechanisms behind heart rate changes with increasing age remain essentially unresolved it is generally established that heart rate at rest, submaximal and maximal level of exercise decreases with age (Lakatta, 2000). At rest in the general population an average heart rate is between 60-80 beats.min^{-1} and with ageing there is a reported decrease of up to 10% between the ages of 20 and 80 years. During maximal exercise this change is more evident with a decrease in maximal exercise heart rate (HR_{max}) of up to 25% (Lakatta, 2000). In humans HR_{max} is approximately 210 beats.min^{-1} at age 10 and decreases to approximately 155 b.min^{-1} by age 65 years possibly caused by a reduction in the number of pacemaker cells in the sinus node (these may decrease by 90%) between the ages of 20-75 years (Kitzman and Edards, 1990). It has also been reported that there is a loss of muscle fibers in the internodal tracts and a partial loss of left bundle fascicles originating in the bundle of His may contribute to a reduction in HR_{max} (Kitzman and Edwards, 1990). Corre *et al.* (1976) concluded that the reduction in HR_{max} in rats during maturation is due to intrinsic changes in the heart cells as opposed to changes in neural influences. Changes in pacemaker permeability and/or resting and threshold depolarisation potentials, increased connective tissue or other factors may account for these observations. In addition, Cavoto *et al.* (1974) reported changes in atrial cell potentials with ageing including a decrease in the rate of rise of the action potential and a more negative resting potential. Other proposed mechanisms include reduced automaticity of the sinus node and structural changes in the paranodal tissue (Patel *et al.*, 1998).

Whatever the case there has been a consistent observation in the scientific literature and age-induced reduction in HR_{max} that likely partially underpins reduced $\dot{V}O_{2max}$.

Elevated blood pressure is considered one of the major risk factors for coronary heart disease (CHD). Large vessel wall thickening and subsequent stiffening in humans results from an increase in intimal thickness and collagen within the vascular media. This is associated with an increased central vascular systolic blood pressure and brachial systolic blood pressure even within a clinically normal range of 110 mmHg at age 20 to c. 140 mmHg at age 80 years (Lakatta, 2000). The biggest increase in blood pressure occurs between the ages of 40-60 years where there is a 50% increase reported in the prevalence of hypertension (defined as BP > 140/90) (Franklin *et al.*, 1997). Schoenberger *et al.* (1986) reported that more than half of US elderly population of 22 million has borderline or definite systemic hypertension with an elevation of both systolic (5-10 mmHg higher from age 40-70 years) and diastolic blood pressure (5-6 mmHg higher). Interestingly after the initial increase reported in diastolic blood pressure a *decline* after age 60 years has been described (Franklin *et al.*, 1997). There have been a number of explanations postulated for this, however, Frankin *et al.*, (1997) suggest that the most likely explanation is an increase in large artery stiffness. In essence the importance of any increase in blood pressure with age is that it will place an increased afterload on the LV that may serve to compromise function as well as a stimulus for pathological increases in LV mass.

Given evidence for an increased afterload (BP) with age and some evidence of a decline in preload with age (LV volume) it is interesting to report on the effects of ageing on pump function, specifically stroke volume (SV), cardiac output (CO), and ejection fraction (EF). In healthy, but exclusively sedentary, ageing there have been conflicting reports regarding the ability of older individuals to effectively maintain SV and CO with ageing. Previous echocardiographic data suggests no change in resting SV with ageing (Slotwiner *et al.*, 1998). Accordingly in a healthy community dwelling population (mildly active) Rodeheffer *et al.* (1984) reported that there was no significant age-related decline in CO at rest or during exercise between the ages of 25-79 years. However, the hemodynamic mechanisms by which CO is augmented during acute exercise may change. In younger subjects there is a greater reliance on increased heart rate and reduction in end-systolic volume (hence increased

contractility) to enhance CO. However, with increasing age there seems to be a greater reliance on the Frank Starling mechanism, which is manifested by an increase in end-diastolic volume and SV (Rodeheffer *et al.*, 1984). Other techniques including MRI have reported declines in SV with age likely as a consequence of a reduction in LV volume (Sandstede *et al.*, 2000; De Bondt *et al.*, 2001).

Contractility *per se* is very difficult to accurately examine in man and intact animal models, due to the effects of differences in loading conditions (Patel *et al.*, 1998). The extent to which myofilaments become Ca^{2+} activated during systole is determined by degree of diastolic stretch (preload – Frank Starling mechanism), afterload – forces that resist myocardial fiber shortening after onset of filament Ca^{2+} activation (vascular blood pressure) and inotropic stimulants – adrenaline, noradrenaline (Lakatta, 2000). Evidence obtained in isolated cardiac muscle tissues has indicated relatively little age-related change in cardiac muscle function contractility (Grossman, 1980). This is despite evidence that the β-adrenergic stimulation of HR, myocardial contractility and arterial vascular tone decreases with advancing age (Lakatta, 1979). Previous studies have shown that in normal healthy volunteers aged 20-95 years there were no age-related changes in resting EF (the most commonly reported surrogate of contractility) via radionuclide angiography (Port *et al.*, 1980) and echocardiography (Pearson *et al.*, 1991). In contrast, however, Salmasi *et al.* (2003) described a *decrease* in EF in males and females with age and De Bondt *et al.* (2001) described an *increase* in EF in females with age utilising echocardiography. This conflict requires further study where perhaps 2-D echocardiography may serve to clarify existing literature.

2.1.3 LV function (diastolic)

There is a marked change in LV inflow velocities in humans with advancing age. Klein *et al.* (1994) showed that in 56 subjects (mean age, 63 years), peak early filling velocity was decreased with a concomitant increase in peak late filling velocity with increasing age which resulted in a decrease in the ratio of E/A (see Figure 2.3 a and b). In a study by Voutilian *et al.* (1991) age alone explained 45-68% of the variation in the peak early and late diastolic velocities, their ratio, atrial filling fraction and peak filling rate in a general population.

Suggested mechanisms underpinning these changes vary. Such patterns of change in the Doppler measurements of diastolic function with age have been suggested to reflect a reduction in the rate of LV relaxation and thus likely represents an alteration of the intrinsic diastolic properties of the heart muscle. There is also a reported increase in interstitial collagen and elastin (Hutchins, 1980), distributed diffusely in the sub-endocardium and myocardium. This increase in connective tissue probably contributes to enhanced myocardial stiffness and decreased compliance (Lakatta and Yin, 1982). This is also consistent with reports of increased duration of the action potential in isolated rat cardiac myocytes with ageing (Capasso *et al.*, 1983).

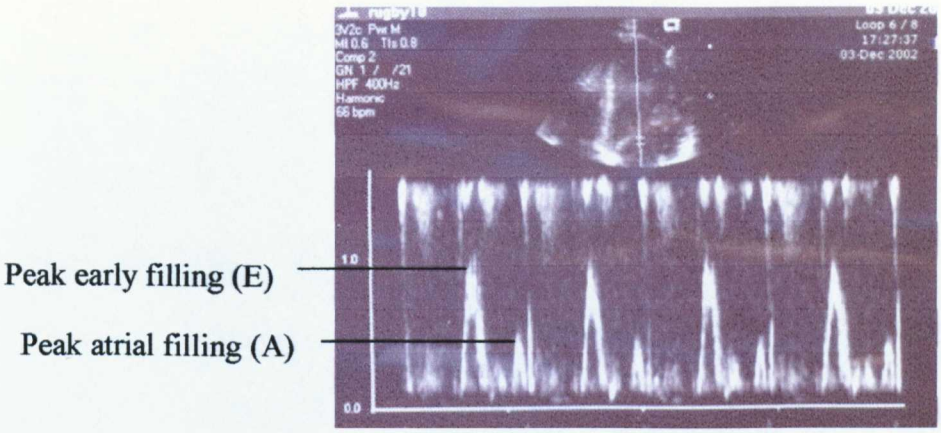


Figure 2.1 (a) Doppler trace of LV filling at approximately age 20

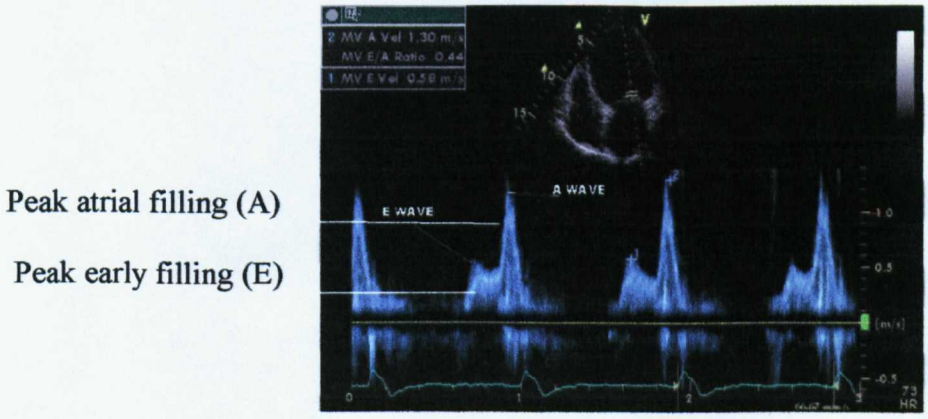


Figure 2.1 (b) Doppler trace of LV filling at approximately age 70

Recently, Hees *et al.* (2004) using tissue Doppler, standard Doppler and invasive techniques, suggested that the reduced E/A with ageing could reflect a reduced early

diastolic atrial pressure as well as changes in LV pressure decay in early diastole. Interestingly, when early diastolic filling was indexed to LA pressure, age was no longer significantly correlated suggesting that age may exert its effects on E wave velocity and isovolumetric relaxation time (IVRT) through a reduction in early LA pressure.

2.1.4 CV structure and function and ageing in females

The male heart becomes more susceptible to CV events from ≈ age 40 years, whereas in females this susceptibility comes much later at ≈ age 60 years, or after the menopause, but can deteriorate more rapidly than males (Mendelson and Hendel, 1995). This observation has prompted many investigations to search for gender differences in the myocardium with advancing age. Olivetti *et al.* (1995) investigated this phenomenon in autopsy patients and reported that between the ages of 20 and 95 years the number, size, shape and proportion of myocytes did not change in females, however, in males between the ages of 17 and 89 years there was a significant loss of myocytes which was accompanied by an increase in myocyte cell volume leading to some preservation of wall thickness. Although the primary culprit for this disparity has been suggested to be the presence/absence of female sex hormones, results reported by Olivetti *et al.* (1995) do not support this, with no tendency towards cell loss after the menopause (ages 55-95 years) in females. Using M-mode echo, Dannenburg *et al.* (1989) reported that there was only a minor change in indexed LV mass with age in both males and females, however, when results were investigated separately a small decrease (c. 10%) in males and a small increase (c. 10%) in females was detected. Such data has been supported by Shub *et al.* (1994) and Gardin *et al.* (1995). Suggested causes for these data have included an increased plasma volume in women (de Simone *et al.*, 1991), blood viscosity differences, atrial natriuretic factor differences, plasma renin activity differences as well as the influence of sex hormones (Shub *et al.*, 1994), however, mechanisms are still largely unknown.

Whether a sex difference in LV systolic function with age exists is unclear. Magnetic resonance imaging studies have suggested sex-differences in LV structure, which could affect function, with LV volume decreasing with age in males but remaining constant in females (Sandstede *et al.*, 2000; Hees *et al.*, 2002). Celentano *et al.* (2003) reported that younger and middle aged females demonstrated significantly

higher EF than males however, Salmasi *et al.* (2003) recently reported EF, measured by 2-D echocardiography, to decrease with age in both males and females ($r = -0.42$), and Slotwiner *et al.* (1998) reported no change in SV across the age range. In both of these studies males and females were not separated in the data analysis. Thus the influence of sex upon indices of LV systolic function across the adult age span requires further investigation.

Large diastolic filling changes are observed with ageing (Oxenham and Sharp, 1999), however the presence of any sex-differences in this change is less clear. Hinderliter *et al.* (1992) investigated young sedentary adults (mean age 30 years) with results showing no differences in diastolic function between sexes. Schirmer *et al.* (2000) reported that females (aged $\approx$ 60 years) exhibited higher E and A peak velocities than age matched males with no overall change in E:A ratio due to a comparable increase in both E and A. Early deceleration time was significantly longer in males suggesting that females demonstrated a more efficient filling. Therefore although age differences in diastolic filling are an accepted phenomenon, sex-related differences are less clear, particularly sex-related differences with ageing. This requires further investigation.

2.1.5 The RV and ageing

Right ventricular changes with ageing have been reported in an autopsy study (Olivetti *et al*, 1995) and an MRI study (Sandstede *et al.*, 2000). Both Olivetti *et al.* (1995) and Sandstede *et al.* (2000) reported that RV volume decreased significantly with age in both males and females. Data from echo studies of the RV in ageing populations is limited primarily because the RV is notoriously difficult to image. Due to the tilted nature of the myocardium the RV is often out of direct view. In addition to this the shape of the RV means that simple geometric assumptions, as with the LV, are inappropriate in any calculations. The complexity of the RV means that an ellipsoid must be combined with a crescentic shape, which defies simple geometrical description (Levine *et al.*, 1984). There have been a number of attempts to accurately depict RV shape and volume using echocardiography, however, the resulting complex formulae (Levine *et al.*, 1984; Feneley *et al.*, 1990) left the data exposed to error, which, due to potentially limited picture quality rendered this technique relatively obsolete. More recently there have been attempts to simplify the RV volume

determination by echocardiography (Denslow *et al.*, 1998; Aebischer *et al.*, 1998). Aebischer and colleagues (1998) demonstrated that a new crescentic area-length model yielded the best correlation in comparison to MRI derived volume. Due to the use of echocardiography in clinical settings and the important role the RV plays in LV mechanics as well as reflecting pulmonary circulatory health, it is important to investigate the impact of healthy human ageing upon right ventricular (RV) structure and function. Thus it is of interest to evaluate age-related changes in RV structure and function using appropriately validated 2-D echocardiographic models.

2.1.6 The association between changes in the CV system and functional capacity with age

Along with age-related changes in cardiac structure and function it is also widely reported that physical work capacity (PWC) and maximal O_2 uptake also declines with advancing age in both males and females (deVries, 1970; Kasch *et al.*, 1973; Pollock *et al.*, 1975). Whether there exists any strong causal link between changes in cardiac structure and function with age-related alterations in $\dot{V}O_{2max}$ has rarely been assessed. This issue has been addressed in younger populations with mixed results. George *et al.* (2005) reported no link between changes in $\dot{V}O_{2max}$ with alterations in cardiac structure and function in children undergoing a 12 week aerobic exercise training programme. Other work (Obert *et al.*, 2001), also in children, provided somewhat contradictory evidence suggesting delta values for LV mass, LV volume and SV following exercise were closely linked to changes in maximal aerobic capacity. In adults most data has been cross-sectional linking high and low fitness with bigger and smaller hearts, respectively, but again the evidence is far from clear (Hutchinson *et al.*, 1991: Osborne *et al.*, 1992). In older adults again the literature is sparse and somewhat ambiguous. A number of studies have not clearly evaluated a link and in the one study in older women to demonstrate cardiac adaptations to training (Morrison *et al.*, 1986) these changes were un-related to the observed increase in $\dot{V}O_{2max}$. This area requires further work to clarify these relationships.

2.1.7 Scaling: appropriate scaling technique and body size variable

Batterham *et al.* (1999) stated that body dimensions influence a vast number of physiological variables or anatomic structures (e.g. heart size, LV mass) whereby an increase in body dimension parameter (e.g. body surface area) is associated generally

with an increase in the structural or functional variable. This of course has significant potential impact upon the reporting of data from the LV and RV. Furthermore we know that both age and sex can exert independent effects on body size and body composition (Shub *et al.*, 1994). Thus in any studies comparing cardiac data between men and women and/or between the young and the old then some account should be taken of differences in body size and body composition for a meaningful data analysis and interpretation to be completed.

Studies of LV structure have long acknowledged that the size of a subject could be related to echocardiographic measurements of heart size (Henry *et al.*, 1978; Gardin *et al.*, 1979). However, it is only recently that original research studies have systematically investigated the best, or most appropriate, way to account for differences in body size (i.e. scaling) so as to facilitate data interpretation (e.g. de Simone *et al.*, 1992; Batterham *et al.*, 1999; George *et al.*, 2001).

When attempting to scale cardiac data for differences in body size and composition the key outcome is that once scaled the data is then size-independent. This is easy to confirm via correlational analysis but has been ignored by many research studies investigating the LV. The way to achieve size-independence is an area of some debate but must address the most appropriate method and adopt the best scaling variable. These issues have been dealt with in a relatively recent review article (Batterham *et al.*, 1999), however such guidance has often been overlooked in examples of cardiac studies in ageing (e.g. Dannenburg *et al.*, 1989; Carroll *et al.*, 1992; Shub *et al.*, 1994; Gardin *et al.*, 1995; Schirmer *et al.*, 2000; Hayward *et al.*, 2001; Hees *et al.*, 2002; Celentano *et al.*, 2003; Haykowsky *et al.*, 2005). Current available evidence would suggest, both theoretically and empirically, that allometric scaling of cardiac structures for individual differences in lean body mass (LBM) is the most elegant way to scale (e.g. Daniels *et al.*, 1995; George *et al.*, 2001).

Within this thesis we will attempt to address a number of issues related to the scaling of cardiac data in ageing research. Firstly, we will derive sample-specific allometric power function ratios, as such specificity is an important part of the theory behind the

use of allometric scaling (Schmidt-Nielsen, 1994). Secondly, the generation of sample-specific allometric relationships will be specifically evaluated in its efficacy in producing size-independent data, compared to simple ratio scaling. Thirdly, we will extend the current scaling database that has concentrated on LV mass (e.g. de Simone *et al.*, 1992) to assess the relationships between LV volume, LV length, SV and RV volume with a range of body size indices. In performing these analyses it is hoped to provide a more appropriate and informed interpretation of cardiac structural and functional changes with advancing age.

2.1.8 Summary

Although the effects of ageing on the human body and CV system have been investigated, particularly in recent years, it becomes clear when assessing existing literature that there are still many questions that remain to be answered. With disparate reports on the effects of ageing *per se,* as well as potential sex-differences with ageing, the exact impact of healthy ageing upon cardiac structure and function in community-dwelling males and females remains somewhat unclear.

Further to this the echocardiographic techniques utilised in the assessment of the CV system to date may undermine the value of any data generated and thus existing assumptions of the effect of ageing on the LV and RV need to be redefined. Therefore a cross-sectional investigation of cardiac structure and function utilising 2-D echocardiography, in males and females over the entire adult age range is required to enhance our understanding of the complex phenomenon of ageing.

2.2 Exercise and CV system

There are many mechanisms that contribute to the decline in the ability of older people to utilize oxygen. As humans age from sexual maturity to senescence the structure and function of the cardiovascular and musculoskeletal systems decline. This leads to a diminishing ability of the elderly to undertake simple tasks. In turn this inability to adequately complete essential tasks create a cyclic effect whereby disuse will result only in a continued decline in the ability to perform.

Several investigators have found that habitual exercise can at least partially reverse the decline in PWC (deVries, 1970; Kasch *et al.*, 1973; Pollock *et al.*, 1975). Endurance exercise training can also improve exercise capacity and quality of life, and has been shown to improve LV function in patients with CAD (Ehsani *et al.*, 1986) and induce a modest but significant regression of LV hypertrophy and remodeling in older adults with hypertension (Ehsani, 2001).

Research investigating the consequences of aerobic conditioning on cardiac structure and function has been performed using both cross-sectional (athlete vs. control) and longitudinal training studies. There are advantages and disadvantages to both of these methods with cross-sectional investigations generally allowing larger sample sizes to be tested in shorter periods of time. It is also attractive due to the fact that athletes represent the maximal exercise exposure and thus the potential for maximal cardiac adaptation. In this way cardiac structural and functional indices in athletes are often reported as representing "the upper normal limits of human physiological adaptation" (George *et al.*, 1991). Drawbacks in cross-sectional studies relate to the increased between subject variability demonstrated in independent groups research and the lack of causality that can be deduced from such descriptive designs. Longitudinal training studies solve these two problems with an assessment of a specific intervention in a within group research design. Problems associated with longitudinal research are primarily derived from logistical constraints, notably, they are time consuming and often limited in sample size. Whether cross-sectional or longitudinal in nature previous cardiac research has tended to focus on males. This is likely for two reasons; firstly, due to a larger number of active males in competitive sports available for recruitment; secondly, the reproductive/hormone changes in females dictate that it is perhaps difficult to control for these changes in studies with eumenorrheic women.

2.2.1 Cardiac structural and functional data in younger males and females

2.2.1.1 Mixed group data

One of the major problems in understanding the impact of sex upon cardiac adaptations to exercise training has been the number of studies that have combined

men and women in a single study group. This does not allow an easy comparison of male and female responses.

In a cross-sectional comparison of active males and females compared to sedentary controls (mean age 31 years) Douglas *et al.* (1986) reported a greater wall thickness and LV mass in the trained group. Contractility (FS) was similar in both groups and diastolic filling indices revealed that although early filling velocity was similar in both groups, E/A ratio was significantly higher suggestive of a smaller atrial contribution in the triathletes. In a longitudinal study DeMaria *et al.* (1978) investigated an 11-week aerobic training programme on 24 normal subjects (mean age 26 years). M-mode echo detected training effects included an increased LV mass, due to both an increase in wall thickness and an increase in LV end-diastolic dimension, a decrease in LV end-systolic dimension and a resultant increase in EF and SV. Similarly, Stein *et al.* (1980) reported increases in end-diastolic dimension at rest with significant increases in SV and dimensional shortening (defined as stroke shortening/end-diastolic dimension) in males and females (mean age 20 years) after 14 weeks of training and also Cox *et al.* (1986) concluded that younger males and females elicited increases in absolute SV and EF in addition to LV mass and LV volume increases after 7 weeks of aerobic training.

2.2.1.2 Male data

In younger male subjects there have been a number of studies conducted, both cross-sectional and longitudinal. Cross-sectional studies report larger LV volumes and LV mass in younger endurance trained athletes (Morganroth *et al.*, 1975; Milkinen *et al.*, 1988) compared to sedentary controls. In addition, Finkelhor *et al.* (1986) reported a significantly greater LV mass (absolute and indexed to BSA) in endurance-trained males caused by a larger wall thickness and LV cavity dimension. The trained group also demonstrated a greater indexed SV, with no significant difference in contractility (EF). For diastolic filling subjects demonstrated a slightly higher E wave and slightly lower A wave (both non significant) leading to a more efficient filling ratio (E/A) in the endurance trained group. The authors postulated that the more efficient diastolic filling was probably caused by a longer filling time and slower heart rate in the trained subjects (Finkelhor *et al.*, 1986).

Longitudinal studies in younger males have yielded similar findings. Shapiro and Smith (1983) investigated the effects of 6-12 weeks endurance training on LV structure and function in younger males using echo. Maximum O_2 consumption was increased at 6 weeks but not further increased at 12 weeks. This increase at 6 weeks was accompanied by increases in septal and posterior wall thickness, and therefore LV mass, with no concomitant increase in LV cavity dimension. No changes in FS or diastolic function were observed. Ehsani *et al.* (1978) reported increases in LV dimensions and mass after 9 weeks aerobic training. Articles reviewing training adaptations have attributed the increased maximal aerobic capacity observed in young and middle aged males after training to augmented cardiac output and an increased arteriovenous O_2 difference during exercise (Clausen, 1976; Holloszy, 1976). The consensus of data detailing training-related adaptations in cardiac structure and function in younger males has been further supported by review articles (George *et al.*, 1991; Urhausen and Kindermann, 1998).

2.2.1.3 Female data

In an early M-mode echo study absolute and indexed LV end-systolic and diastolic diameter, LV volume and LV mass were significantly higher in younger endurance trained females than age-matched controls (Zeldis *et al.*, 1978). Wall thickness was similar in both groups. In more recent studies LV end-diastolic dimension, LV mass and volume as well as septal and posterior wall thickness were all reported to be greater in endurance trained female athletes (George *et al.*, 1999). In addition SV has been reported to be higher in endurance-trained females than in moderately active females (Ferguson *et al.*, 2001). Wernstedt *et al.* (2002) reported that endurance-trained females had significantly higher LV volumes and mass than both strength-trained and control subjects in a study utilising both echo and MRI. In a recent meta-analysis, Whyte *et al.* (2004) studied a total of 890 female athletes and concluded that training (endurance and strength) elicits a physiologic stimulus resulting in LV enlargement and enhanced SV that reflects those observed in the trained male heart. There is, somewhat surprisingly, a limited database of training interventions in young women.

Taken together these studies clearly demonstrate that in younger males and females training, both habitual and competitive, can induce changes in resting LV structure

and function that are associated with a more efficient and healthy CV system and likely underpin the enhanced physical work capacity.

2.2.2 Cardiac structural and functional data in older males and females

Up until 30 years ago it was believed that older individuals did not adapt to exercise training in the same way as younger subjects, with some research suggesting that as ageing occurs, there was a diminished ability to adapt to endurance training (Niinimaa and Shepard, 1978). Subsequent studies that have adapted a more rigorous training protocol, e.g. working subjects at 60-80% VO_{2max}, 4 sessions per week for 11 months (Ehsani *et al.*, 1991) have found there to be similar increases in maximal oxygen uptake and cardiac output with training in both younger and older age groups. These findings advocate similar cardiovascular adaptations to training, irrespective of age.

2.2.2.1 Male data

A number of cross-sectional comparative studies have consistently reported the presence of central cardiac adaptations to endurance training in older males. Douglas and O'Toole (1992) compared trained and sedentary older males and reported that although in the older athletes both absolute and indexed LV end-diastolic diameter was larger in the athletes, this did not result in a larger LV mass due to smaller wall dimensions. Diastolic filling was also similar between athletes and sedentary groups. Jungblut and colleagues (2000) revealed that trained older men demonstrated a higher LV mass purely from larger LV dimensions with no difference in posterior or septal wall thickness compared with sedentary controls. However, contractile function and Doppler derived diastolic filling indices were not different in the trained individuals. Similarly, when comparing younger trained and untrained males with older trained and untrained males, Baldi *et al.* (2003) reported no difference in diastolic filling indices with the exception of shorter isovolumetric relaxation time in older trained males than older sedentary males. Ogawa *et al.* (1992) reported significantly higher SV in endurance trained older subjects that were comparable to the larger SV observed in younger endurance trained males.

A number of longitudinal training studies in older men have been published and a cross-section of these has been presented in Table 2.1.

Table 2.1 Training studies in older men investigating cardiac structure and function.

Year; Author	Mean Age (years)	Length of TP	VO_{2max}	Structure	Systolic function	Diastolic function
1991; Ehsani *et al.*	64	12 mo	↑	LVEDV ↑ LVEDD↑ LVPWT↑	SV ↔ EF ↔	NM
1993; Levy *et al.*	68	6 mo	↑	LVMI ↑ LVEDV ↑	HR ↓	PEFR ↑
1994; Stratton *et al.*	68	6 mo	↑	LVEDVi ↑	HR ↓ SV ↑ EF ↔	NM
1996; Spina *et al.*	66	9 mo	↑	NM	EF ↔	TPFR ↔ PFR ↔

NM; not measured, **LVEDV(i)**; left ventricular end-diastolic volume (indexed), **LVEDD**; left ventricular end-diastolic dimension, **LVPWT**; left ventricular posterior wall thickness, **LVMI**; left ventricular mass indexed, **HR**; heart rate, **SV**; stroke volume, **EF**; ejection fraction, **RRi**; ECG R-R interval, **PEFR**; peak early diastolic filling rate, **TPFR**; time to peak filling rate, **PFR**; peak filling rate, **TP**; training programme.

It is clear from both cross-sectional and longitudinal investigations that older men have the ability to adapt LV structure, decrease HR and BP, and increase SV in response to exercise training. These improvements with training are comparable to that of younger males and females and indicate that with advancing age the male heart does not lose the ability to adapt to physiological stress.

2.2.2.2. Female data

Although Douglas and O'Toole (1992) suggested that both older male and female athletes could adapt centrally to endurance exercise, males and females were not separated in analysis so this must be interpreted with caution. In a cross-sectional comparison Ogawa *et al.* (1992) reported that at rest there was no significant difference in SV in older females regardless of training status, in contrast to the higher SV found in older endurance-trained males. Conversely, recently Hagmar *et al.* (2005) compared 20 postmenopausal former elite female athletes to sedentary controls and reported greater exercise capacity in addition to a larger LV diameter, LV volume

and SV. This database is significantly limited and the inconclusive cross-sectional results here are not entirely resolved by data from longitudinal investigation. Suffice it to say that there is evidently a need for cross-sectional studies in postmenopausal female athletes to add to our current and limited knowledge of the upper limit of cardiac adaptation to exercise in such groups.

Longitudinal aerobic training programmes have produced similar results with respect to an improvement in aerobic capacity in males and females. In subjects aged 60 years and over both males and females exhibited enhanced $\dot{V}O_{2peak}$ of 10-15% after 4 months training increasing again up to 6% with additional training (Blumenthal *et al.*, 1991). Accordingly, in females aged 70 years and over, 12 weeks of aerobic training resulted in a 12% improvement in $\dot{V}O_{2max}$ (Warren *et al.*, 1993). Although we know that age *per se* does not impede the extent to which older females improve their maximal oxygen uptake with training, the weight of longitudinal training data suggests that older females adapt differently to exercise than males (Spina, 1999). It would seem that older females may lack the ability to adapt centrally to aerobic training indicating that they must rely solely on peripheral adaptations to enhance aerobic capacity (Spina, 1999). This phenomenon has engaged the attention of a number of investigators (see Table 2.2). However, a range of subject ages, training programme durations and investigative techniques have been employed making conclusions difficult to draw. Specifically, Morrison *et al.* (1986), Park *et al.* (2003), and Haykowsky *et al.* (2005) all utilised M-mode echo in their investigations with only Park *et al.* (2003) reporting some 2-D echo data.

It is interesting to note that Morrison *et al.* (1986) reported a small but significant increase in end-diastolic diameter with a concomitant increase in EF (see Table 2.2). The increase in EF is surprising due to the general lack of any reported increase in resting contractile indices in cross-sectional and longitudinal studies in both younger men and women as well as older males. Further it is pertinent to note that echo changes could not be correlated to improvements in aerobic capacity.

Table 2.2 Training studies in older women investigating cardiac structure and function.

Year; Author	Mean Age (years)	Length of TP	VO_{2max}	Structure	Systolic function	Diastolic function
1986; Morrison *et al.*	51	8 mo	↑	LVEDD ↑	SBP ↓ EF ↑	NM
1993; Spina *et al.*	63	9 mo	↑	EDV ↔ ESV ↔	SBP ↔ HR ↓ SV ↔ EF ↔	NM
1996; Spina *et al.*	64	9 mo	↑	NM	EF ↔	PFR ↔ TPFR ↔
2003; Park *et al.*	63	9 mo	↑	LVEDD ↔ LVESD ↔ LVEDV ↔ LVESV ↔	SBP ↓ SV ↔ EF ↔ FS ↔	NM
2005; Haykowsky *et al.*	68	3 mo	↑	LVEDD ↔ LVESD ↔ LVM ↔	NM	E ↔ A ↔ E:A ↔

NM; not measured, **LVEDV**; left ventricular end-diastolic volume, **LVESV**; left ventricular end-systolic volume, **LVEDD**; left ventricular end-diastolic dimension, **LVESD**; left ventricular end-systolic dimension, **LVPWT**; left ventricular posterior wall thickness, **LVM**; left ventricular mass, **HR**; heart rate, **SV**; stroke volume, **EF**; ejection fraction, **FS**; fractional shortening, **BV**; blood volume, **TPFR**; time to peak filling rate, **PFR**; peak filling rate, **E**; peak early filling velocity, **A**; peak atrial filling velocity, **E:A**; E/A ratio, **TP**; training programme.

In subsequent studies, results seem fairly consistent with a lack of alterations in both LV structural and functional indices after training in older females. All studies, with the exception of Park *et al.* (2003) utilised M-mode echocardiography in their studies. The disadvantages of using this technique have already been noted within this review. Although Park *et al.* (2003) employed 2-D echocardiography in addition to M-mode measurements; these were limited to LV end-diastolic volume and not used in the calculation of LV mass, SV and EF. Moreover, although in combination these studies have investigated LV structure, systolic function and diastolic filling no single study has explored all of these parameters together. In addition LV mass was only reported by Haykowsky *et al.* (2005), despite LV mass being a key indicator of CV disease risk (Savage *et al.*, 1990) and its widespread use in younger male and female

investigations (Morganroth *et al.*, 1975; George *et al.*, 1999; Jungblut *et al.*, 2000; Wernstedt *et al.*, 2002). Furthermore, sample sizes were generally small in the longer training studies. Spina *et al.* (1993) were only able to collect echocardiographic images from six of the total sixteen women participating in the training programme and Park *et al.* (2003) had only a total of 8 women complete the study. Finally the maximum duration of any study was 9 months which may limit physiological adaptation in subjects who must, by their very nature, start at a low exercise intensity and volume and progress carefully and slowly.

With a lack of cross-sectional investigations and incongruent findings in longitudinal data it is difficult to summarise adaptations in post-menopausal women with training. Notwithstanding the inconsistency in that females can indeed increase LV structure and function with training (Morrison *et al.*, 1986) and those contradictory studies reporting no such adaptations to training, there have been no studies which have investigated LV structure, systolic and diastolic performance in a 12 month training programme utilising 2-D echocardiography.

2.3 Summary

There is a distinct lack of cross-sectional evaluation of post-menopausal endurance trained females compared with sedentary controls. The upper normal limit of cardiac adaptation in older women is not clear and prolonged training in athletes is the obvious way to investigate this issue. This is particularly relevant when attempting to investigate the effects of an exercise training intervention on previously sedentary older females where intensity of exercise and duration of a training programme may raise the question 'was the intensity great enough? or, ' was the training programme long enough?'.

Further there is a need to add to the body of training literature in older women and employ a comprehensive assessment of LV structure and function with a prolonged and progressive training programme. Importantly this should include evaluation of cardiac indices derived from 2-D echo and employ rigorous health screening. There is also no literature examining the effects of training on RV structure or diastolic filling in older females although RV differences have been shown to mirror LV adaptations in younger athletes (Henriksen *et al.*, 1999).

2.4 Research hypotheses

On the basis of the above literature review and areas highlighted for research attention, we can state the following null hypotheses that will be tested in our empirical studies.

1) There is a significant positive relationship between age and a decline in LV and RV structure.
2) There is a significant positive relationship between age and a decline in LV and RV function.
3) There is a difference in the relationship between age and cardiac structure and between sexes.
4) There is a difference in the relationship between age and cardiac function between sexes.
5) There are no training related changes in LV and RV structure after a 12-month intervention in post-menopausal females.
6) There are no training related changes in LV and RV function after a 12-month intervention in post-menopausal females.
7) There are significant differences in LV and RV structure between sedentary and highly trained post-menopausal females.
8) There are significant differences in LV and RV function between sedentary and highly trained post-menopausal females.

3 General methods

3.1 Ethics and subject recruitment

Liverpool John Moores Ethic's Committee granted ethical approval for all the studies presented in this thesis. Written informed consent was obtained from all subjects in each investigation subsequent to a verbal and written explanation of all procedures and testing equipment and the subjects' meeting inclusion criteria.

To recruit and assess subject suitability a number of steps were taken. Media coverage, both by newspaper and radio was utilised in the recruitment of the main body of volunteers for the cross-sectional and longitudinal study. This consisted of various newspaper articles and two radio interviews prior to recruitment of the cross-sectional study (November 2002– June 2003) and similarly newspaper articles and a radio interview for the longitudinal study (September 2003). In addition to this, advertisements and information on the project were placed in the local Liverpool Women's Hospital and a local Women's Health Information Facility. Word of mouth also facilitated in the attraction of a large number of male and female volunteers. Various open evenings were held for both the cross-sectional and longitudinal studies involving presentations and demonstrations of equipment employed. These were extremely successful and ensured a large number of participants. Recruitment for the trained versus untrained female cross-sectional study was more difficult due to the lack of trained 60 year old females in the Liverpool vicinity. Although the ultrasound equipment is portable, the DEXA scan for body composition, and exercise equipment used to determine $\dot{V}O_{2max}$ is fixed which meant that all subjects for this study had to visit the lab. As a result of this the sample size for this study is relatively low. Subjects were recruited by way of contacting all of the athletics and sports clubs in the Liverpool and Greater Manchester areas. While response was limited, an adequate number of females volunteered to participate, and training status was determined via similar means for the male/female cross-sectional and longitudinal training study.

After first contact, normally via the phone, the assessment of subject's eligibility was initiated. A lifestyle questionnaire was administered that included questions

regarding any current or previous medical diagnosis/condition, a history of any cardiovascular symptoms, medication or supplementation, lifestyle habits (dietary, alcohol or tobacco) and habitual exercise level taking into account frequency, duration and intensity of exercise. Only healthy, non-smokers with a low – moderate alcohol intake, low to moderate habitual exercise levels (for the cross-sectional and longitudinal only) and no known cardiovascular disease and/or medication were recruited to an initial laboratory visit. Participants were considered to be healthy but not active if their total physical activity levels per week (assessed by questionnaire) was less than 3x30 minutes of moderate level activity (i.e. brisk walking/swimming) and were considered to be sedentary if their total physical activity was less than or equal to 2 sessions of mild activity per week.

At the first laboratory visit a physical examination was undertaken which included measurements of height (HT), body mass (BM), blood pressure (BP), 12-lead ECG, resting echocardiogram and maximal treadmill exercise test. From these data inclusion criteria consisted of; body mass index (BMI) of <30, blood pressure <160/90 mmHg (determined following 10 minutes of seated rest by Dynamap [GE systems, Tampa, FL] automated blood pressure monitor over two measures), a normal resting ECG (Schiller, Switzerland), an EF of >50% (Rodeheffer *et al.*, 1984) to rule out subjects with LV systolic dysfunction and successful completion of an exercise treadmill stress test conducted according to a revised Bruce ramp protocol, with no evidence of exercise induced ischemia/angina. The exercise test was performed in the presence of a cardiologist on all subjects over 50 years.

3.2 Data collection

Having met inclusion criteria subjects were recruited to a number of data collection sessions based on the study they were involved in. All participants were instructed to refrain from food/alcohol/caffeine intake for 3 hours prior to examination. Subjects were also asked to avoid exercise for 24 hours prior to the session. Subjects went through a standard rotation of data collection procedures.

3.2.1 Body size and composition assessments

Standard procedures were employed in the measurement of HT (cm), BM (kg) and body surface area (BSA, cm^2; Dubois and Dubois, 1916). Whole body and regional body composition were measured via dual energy x-ray absorptiometry (DEXA, Hologic Inc, Horizon Park, Levensesteenweg, Belgium; see Figure 3.2.1).

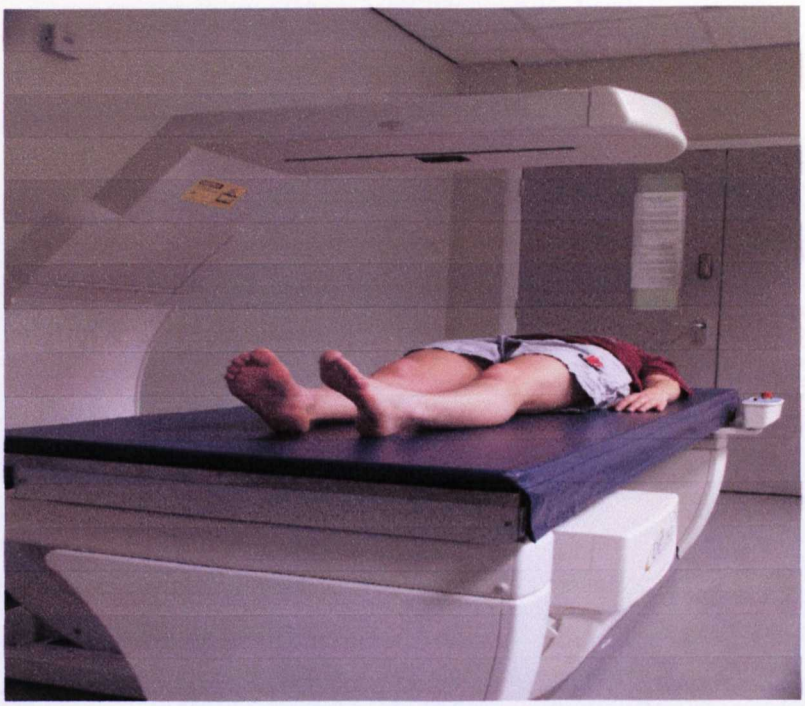


Figure 3.2.1 Dual energy x-ray absorptiometry

The equipment and software (Delphi A S/N 70719) facilitated the assessment of the mass of fat free tissue, fat tissue and bone (Hologic QDR software for Windows 11.2). Limbs were isolated from the trunk and head by using regional computer-generated default lines with manual adjustment. The DEXA unit consists of a bed on which the subject lies supine, while a collimated dual energy x-ray fan beam originating from a source under the bed passes through the subject (Figure 3.2.1). The detectors above the subject measured the beam's attenuation. The dual energy of the beam allowed quantification of fat-free tissue and fat tissue in boneless regions.

Body size components were defined as the following for this thesis:

- Body mass (BM) = fat mass + fat free mass + bone mass.
- Lean body mass (LBM) = the non-adipose tissue of body mass (i.e. skeletal muscle, brain, heart, liver, kidneys and gastrointestinal tract). Accurate assessment of LBM was important primarily to facilitate scaling of cardiac structural data (see section 3.4).

Calibration of the DEXA was performed daily using a spine phantom that tested the system's calibration and its precision of imaging. A step phantom was also used consisting of a variety of known densities. Studies have shown that DEXA provides highly reproducible and accurate measures for body composition variables including LBM when compared to underwater weighing (Hansen *et al.*, 1993; Kohrt, 1998). When scanned all subjects wore light clothing having previously been advised to remove any metal objects (jewellery, underwired bras, zippers etc).

3.2.2 Maximal oxygen uptake

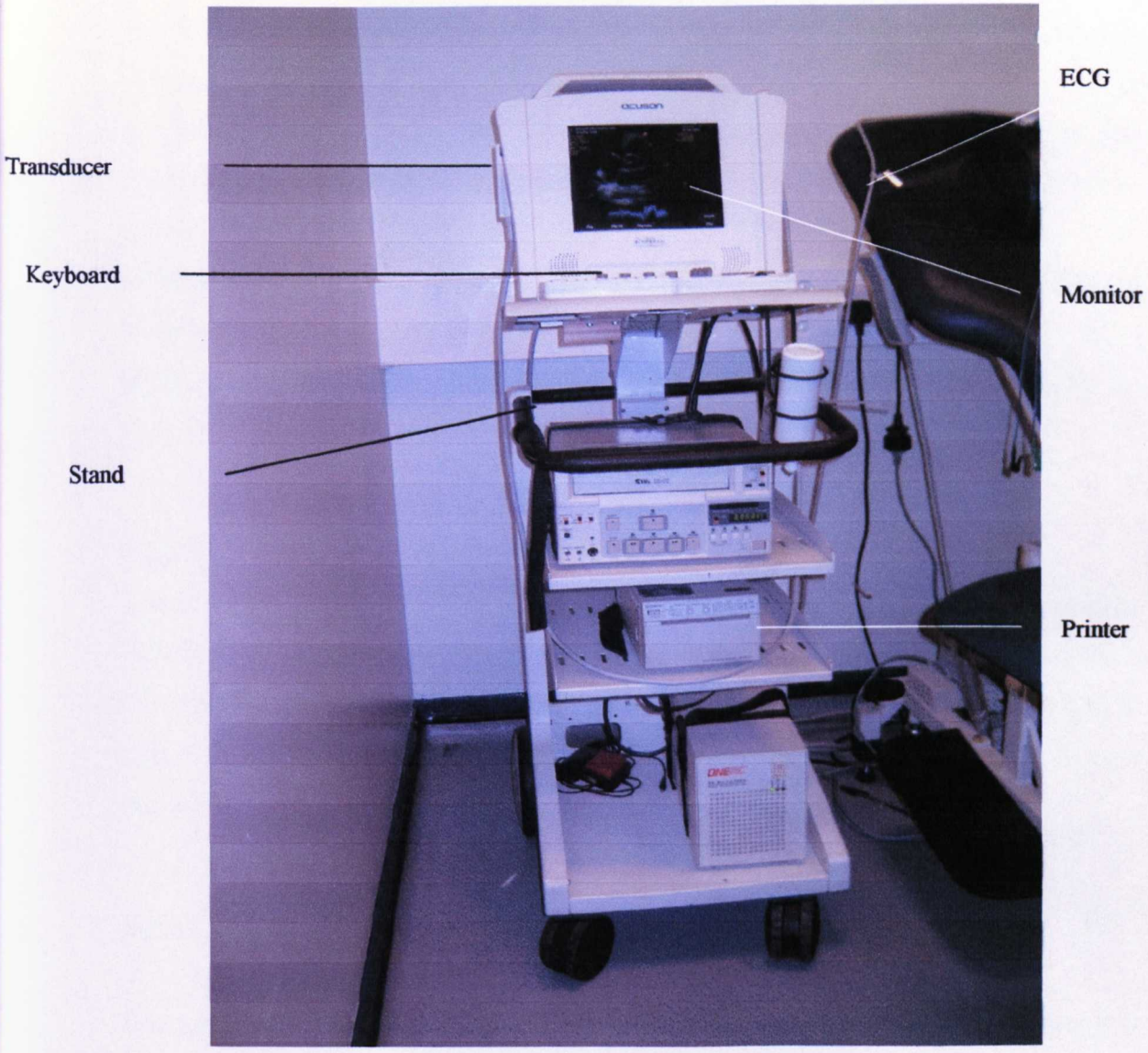
The exercise protocol that assisted in the assessment of $\dot{V}O_{2max}$ was a modified ramped Bruce protocol performed on a treadmill (Cosmos: Nussdorf-Traunstein, Germany). Breath-by-breath analysis of oxygen consumpion ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), tidal ventilation and respiratory rate were all measured using the Medgraphics CPX-D system (Medgraphics Corporation, St. Paul, Minnesota, USA). Criteria for reaching $\dot{V}O_{2max}$ were 1) failure of heart rate to increase further as intensity increases, 2) a plateau in oxygen uptake with increased workload and 3) a respiratory exchange ratio of over 1.15 (ACSM, 2000).

3.2.3 Echocardiography

Subsequent to a supine rest period of no less than 15 minutes and measurement of resting BP, subjects underwent echocardiographic assessment with simultaneous ECG recording to assess resting heart rate and timing of the cardiac cycle. Echocardiography is a non-invasive investigative technique that utilises ultrasound waves to provide images of the myocardium that can differentiate between specific structures (i.e. myocardial walls, chambers and valve leaflets). By analysing these images (discussed in more detail later in this subsection) information about structure

and function of the cardiac muscle can be determined. The ultrasound system (Acuson Cypress Echocardiography System, Acuson Corporation, CA) utilised in these investigations is displayed in Figure 3.2.2 and consists of a monitor, a keyboard and various transducer and ECG attachments.

Figure 3.2.2 Overview of the Cypress Echocardiography System



3.2.3.1 Assessment and measurement conventions

Accurate imaging of the myocardium requires practice and skill on the part of the sonographer. Variations in body size and composition, tissue density, anatomical orientation of the heart within the thoracic cavity form the rationale for the adoption

of standardized assessment and measurement procedures between serial testing sessions (i.e. pre-post measures), between separate subjects (cross-sectional studies) and between sonographers. There have been a variety of different guidelines and criteria published regarding M-mode, 2-D and Doppler echocardiographic assessments and measurements. Standard procedures for any M-mode echocardiographic assessments presented in this thesis were adopted from those set by the Penn convention (Devereux and Reichek, 1977; Devereux *et al.*, 1986). For 2-D assessment and measurements, the Schiller *et al.* (1989) consensus guidelines from the American Society of Echocardiography (ASE) were adopted as they are the only well recognised guidelines utilised in 2-D echocardiographic research. These recommendations advocate the routine reporting of LV EF, diastolic volume, and mass from more geometrically sound models than the Penn Convention (Devereux and Reichek, 1977). Recommendations from the Canadian Consensus Group were adopted in this thesis (Rakowski *et al.*, 1996) for the assessment and measurement of Doppler echocardiography. Whilst primarily for use in patients with filling abnormalities, it is suggested that they are pertinent in the normal ageing populations also.

3.2.3.2 Assessment and measurement procedures

Imaging of the LV was achieved by placing the 2.5 MHz phased-array transducer on the chest wall at both the parasternal and apical windows with subjects examined in left lateral decubitus position. Each individual scan required minor adjustments in gain, compress and power settings to optimise image quality. In line with manufacturer's guidelines tissue harmonics were active for all scans.

Two-dimensional echocardiography

Although M-mode has been more commonly used in the assessment of EF, SV, LV volume and LV mass in previous research, in this thesis 2-D imaging and measurements were adopted. The rationale for this choice originated from criticism that cubed M-mode volume calculations are insufficient due to the limited mathematical representation of LV shape (Hees *et al.*, 2002). 2-D sector scanning of the LV in 2 and 4-chamber views (Figure 3.2.3. and 3.2.4, respectively) from the

apical position, as well as the parasternal short-axis position provided a much more accurate depiction of LV shape in systole and diastole as well as facilitating the assessment of the right ventricle (RV).

Therefore LV end-diastolic volume (LV volume) was calculated via Simpson's Rule biplane method (Schiller *et al.*, 1989). Left ventricular mass was estimated using a modified Simpson's rule method (Dickuth *et al.*, 1983) that has recently been favourably compared to MRI-determined LV mass in healthy trained and untrained individuals (Scharhag *et al.*, 2003). To determine LV mass via this method short axis views of epicardial and endocardial LV area in end diastole at the level of the mitral valve and papillary muscle were digitised (Figure 3.2.5 and 3.2.6.) and used in conjunction with longest LV length measured from either 2 or 4 chamber views (Dickhuth *et al.*, 1983).

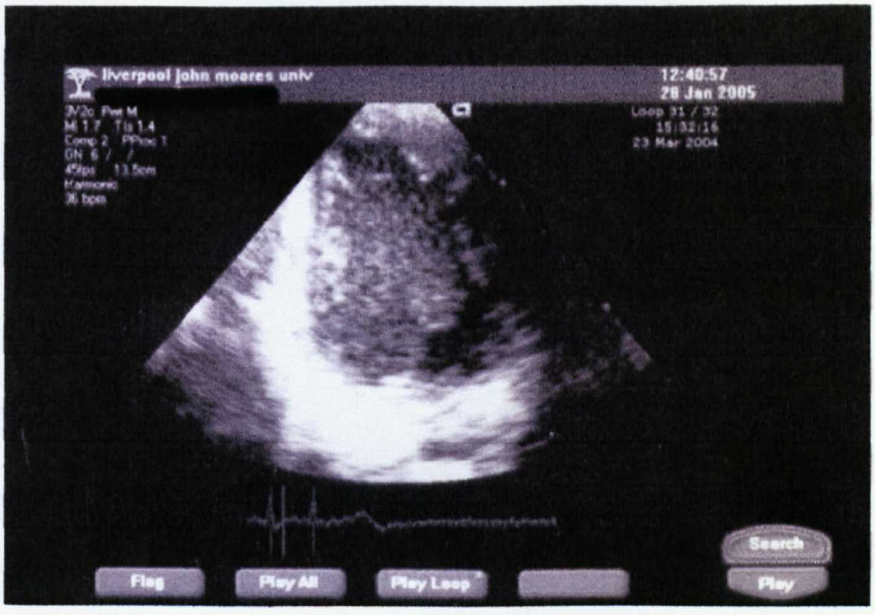


Figure 3.2.3 LV 2-chamber image from apical view

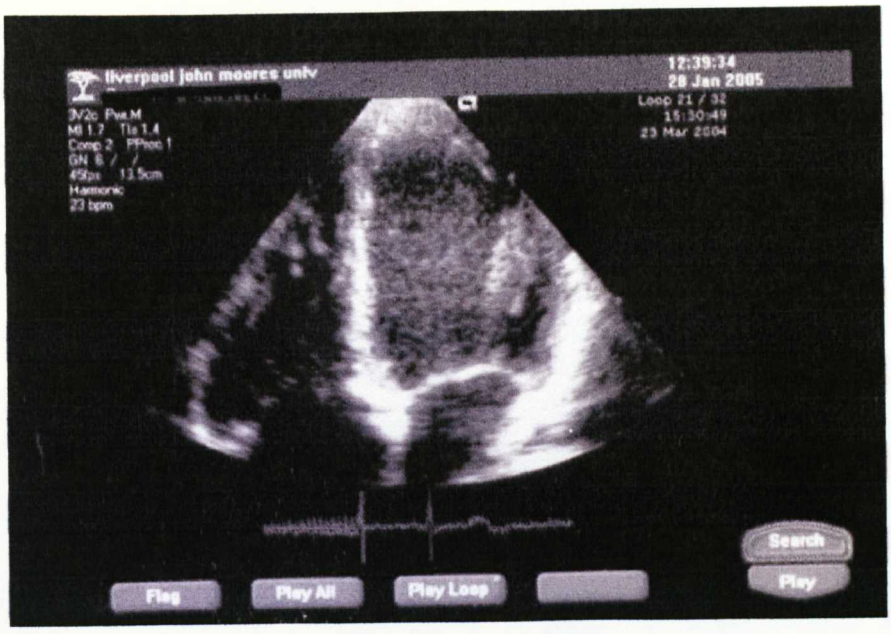


Figure 3.2.4 LV 4-chamber image from apical view

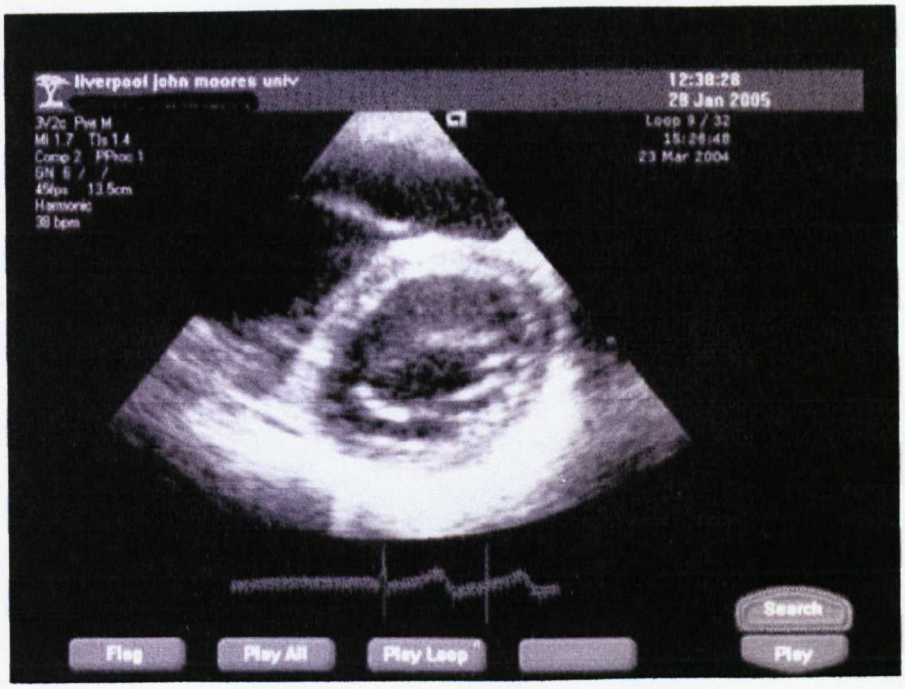


Figure 3.2.5 LV short axis view at mitral level

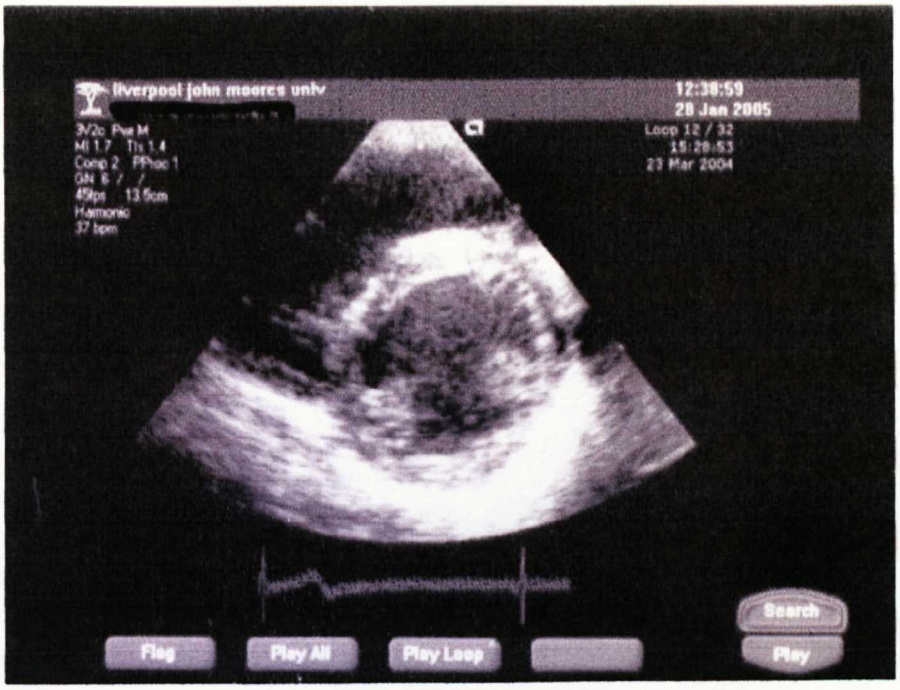


Figure 3.2.6 LV short axis view at papillary level

Calculated equations LV mass, LV volume, EF, SV and RV volume are detailed in the following pages.

LV volume (biplane)

$$V = \frac{\pi}{4} \sum_{i=1}^{20} a_{di} \; b_{di} \bullet \frac{L}{20}$$

a_{di} is the ith disk diameter of LV apical 2 chamber (cm)
b_{di} is the ith disk diameter of the LV apical 4 chamber (cm)
L is the chamber length from either 2ch or 4ch, whichever is longest (cm)
20 is the number of disk segments into which the ventricle is divided

Ejection fraction and SV were calculated using the following formulae derived from biplane measurements of LVEDV and LVESV.

Ejection Fraction

$$EF = \left(\frac{Vd - Vs}{Vd}\right) \bullet 100$$

V is LV volume (ml)
s is systole
d is diastole

Stroke Volume

$$SV = Vd - Vs$$

V is LV volume (ml)
s is systole
d is diastole

LV mass

LVM = (LVEDVepi - LVEDVendo) * 1.05

Where,

LVEDV = (Am*L/3) + [(Am + Ap/2) * L/3] + (1/3 Ap * L/3)

LVEDV is calculated for both epicardial and endocardial areas.
Where Am is area at mitral valve level and Ap is area at papillary level

There have been a number of methods investigated for the assessment of RV volumes from echocardiography (Levine *et al.*, 1984; Tomita *et al.*, 1992; Denslow and Wiles, 1998). Right ventricular end diastolic volume (RV volume) is difficult to assess due to it's resemblance to both an ellipsoid and crescent shape. In addition, obtaining the appropriate views required for these volume calculations in an ageing population can be laborious and inaccurate. However, due to limited data relating to RV structure and function in ageing populations it was deemed appropriate and informative to assess this within the studies contained in this thesis. It is on this basis that the simplistic formula proposed by Aebischer *et al.* (1998) was chosen to represent RV

volume in this thesis. This model, referred to as the crescentic area-length method, requires measurement of the crescent area from a short axis view (Figure 3.2.7) in conjunction with endocardial length of the free wall obtained by measuring the distance from the tricuspid annulus to the RV apex on an apical 4-chamber view (Figure 3.2.8). In the Aebischer *et al.* (1998) study, five methods of RV volume determination were compared to MRI. The crescentic area-length method along with a similar, but more complex, crescentic method and an ellipsoid method revealed the highest intra-class correlations with MRI data. The crescentic area-length method was shown to slightly underestimate RV volume, however, other methods such as tapering and pyramidical underestimated to a much larger extent.

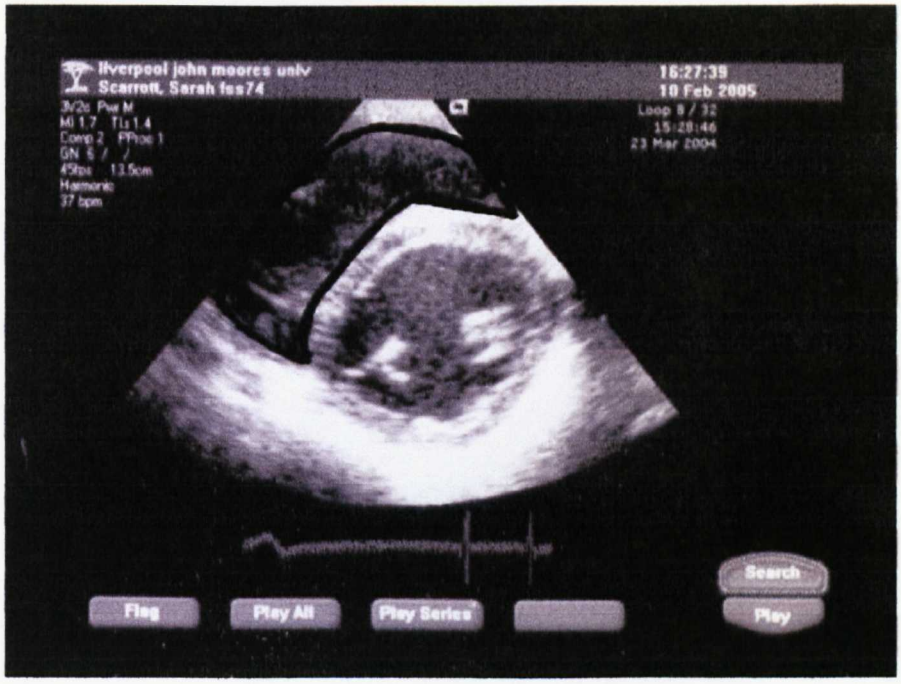


Figure 3.2.7 RV short axis view

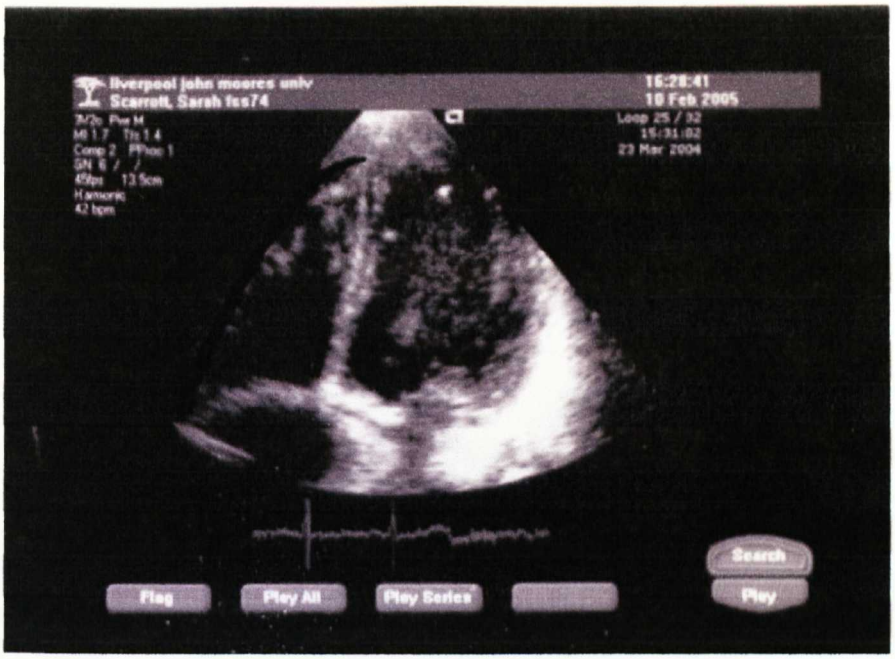


Figure 3.2.8 RV 4-chamber view

RV Volume

RVV = RVA * RVL

RVA – RV Area derived from SA view
RVL – RV Length derived from 4-chamber view

Although 2-D assumptions for LV and RV volumes are considered superior to that of M-mode, the reduced resolution and edge detection cause measurements to be extremely dependant on experience and skills of the sonographer. Thus, extreme care was taken in all assessments to produce the clearest possible image with the use of depth and gain control and with the cursor line or sector scan passing perpendicularly to the major structures of the left ventricle. Achieving an optimum apical view was generally made easier with the palpation of the beat from the apex just below the inferior border of the nipple.

Doppler echocardiography

Doppler echocardiography facilitated estimation of LV and RV diastolic filling parameters and ventricular outflow peak velocities. A 2-D sector scan of a 4-chamber view from the apex position allowed placement of the sample volume at the level of the mitral and tricuspid valves parallel to inflow (Figure 3.2.9). This allowed maximal flow determination by using both visual (clear spectral envelopes) and audio cues (a crisp, clear sound). The peak-flow velocities ($cm.s^{-1}$) of LV and RV inflow in early passive (E) and late atrial contraction filling (A) were measured from baseline to the maximal flow velocity. From this the ratio (E:A) of filling velocities was calculated.

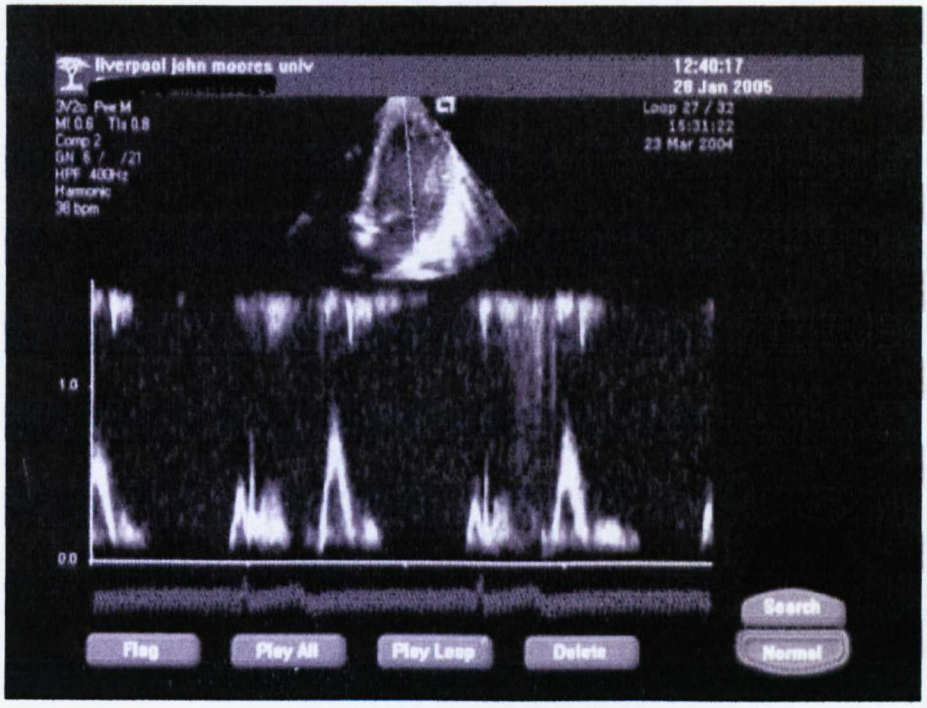


Figure 3.2.9 LV Doppler trace of early (E) and atrial (A) filling phases

Peak aortic flow velocity was then measured by the re-alignment of the sample volume to a position parallel to aortic outflow in a 5-chamber view again taking care to gain the optimal flow. In addition to LV outflow, pulmonary peak flow velocity was also measured in order to report both LV and RV performance. The pulmonary

valve was imaged while in short axis view at the level of the aortic root (Figure 3.2.10). These variables give an indication of LV and RV outflow capabilities in addition to the LV and RV inflow capacity variables (see Figure 3.2.11 for exemplar aortic Doppler trace).

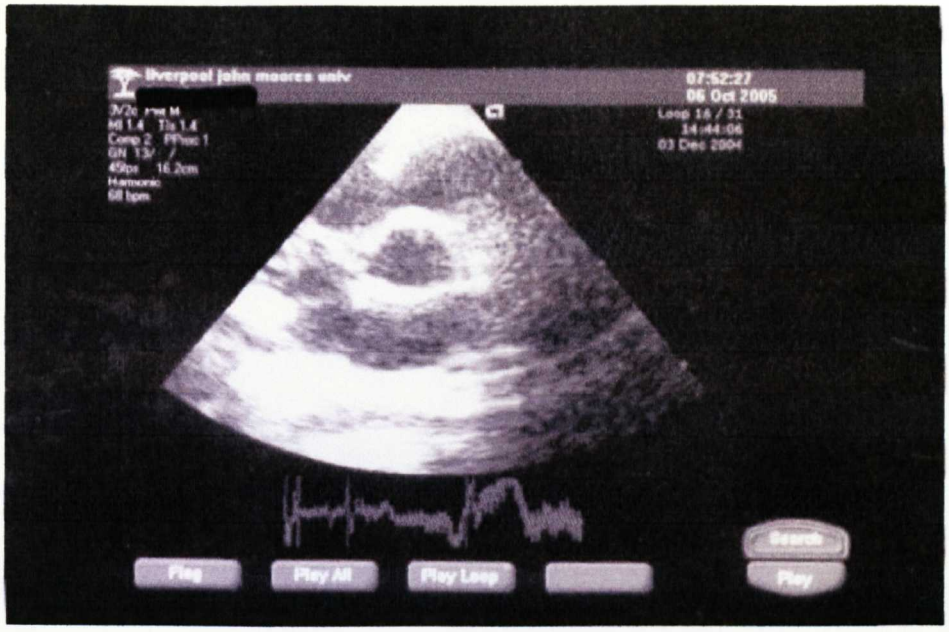


Figure 3.2.10 Short axis view at the level of aortic root (for determination of peak pulmonary flow velocity)

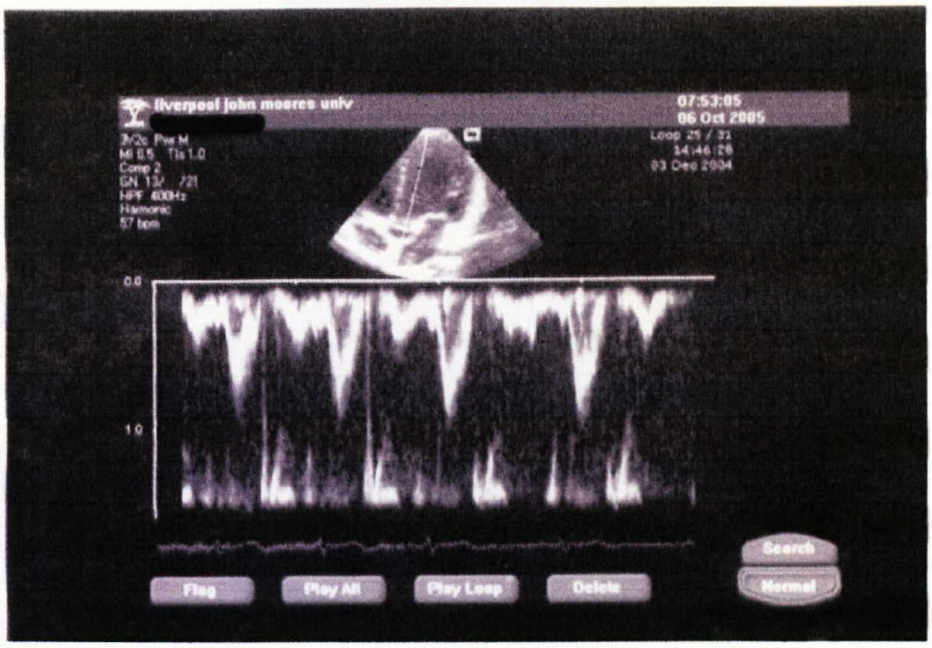


Figure 3.2.11 Aortic flow velocity Doppler trace

3.2.3.3 Technical issues

All images were obtained at end expiration as the respiratory cycle can interfere with imaging (Rubal and Moody, 1990). All data presented used a single experienced sonographer for both imaging and assessment, who at the time of echocardiographic analysis was unaware of sex or age in the cross-sectional investigation and unaware of subject identification in both the longitudinal training study and trained versus untrained female cross-sectional study. This was achieved by the use of a coding system that did not include information related to age, sex or group.

Each measurement was derived from three consecutive cardiac cycles and the mean reported. Traces, which could not be analysed with confidence due to unclear images, were not included.

3.2.3.4 Error in assessment and measurement

Due to the nature of echocardiography it is pertinent to discuss potential sources of error and detail remedial actions adopted and discuss any empirical data obtained. Potential sources of error during scanning which may reduce confidence in data are:

1) Transducer selection – High frequency transducers produce better resolution but lack penetration. For most studies of cardiac anatomy the structures relevant to the investigation lie in a field 2-16 cm from the transducer. For this reason a 2.5 MHz frequency transducer was chosen to allow good resolution to such depths. The resolution of the system for differentiating between specific cardiac structures using this frequency is often quoted at about 1 mm (Feigenbaum, 1994) but with improving software and image processing this has been decreased. This frequency transducer can also measure higher maximum blood flow velocities in Doppler mode than transducers with a higher frequency. Due to the healthy condition of subjects, this was deemed a suitable transducer frequency.

2) Display size – The display was adjusted for each individual subject, particularly due to the sex and body size differences so that the particular element of interest filled a large portion of the screen.

3) Patient variability – Patients with technical difficulties resulting in unclear images were removed from data analysis (n=27 female; n=20 male). Due to the nature of the ageing process it was accepted *a priori* that generally younger patients would have clearer scans than that of older subjects. For this reason great care was taken when scanning to obtain the best image possible using the gain, depth and frequency control settings.

4) Operator variability – Skill, care and experience of the sonographer were foremost when preparing for these investigations. A 6-month training period was completed before testing included in this thesis had commenced.

5) Speed of sound – The image processing algorithms of diagnostic ultrasound assumes that the speed of sound in body tissue is 1540 meters per second. However, different types of body tissue have slightly different speeds of sound transmission (Hewlett Packard, 1987). In soft tissue there is approximately a 2% error, which may be as high as 5% particularly if fatty tissue is present in the area the image is being measured. For this reason there was a BMI limit of <30 (n=6 female were excluded due to this criteria).

6) Doppler alignment - As mentioned earlier the sample volume must be as parallel to flow as possible. Misalignment errors are typically approximately ± 5%.

7) Formulae – As already reported some variables (LV mass, LV volume, SV, EF) are calculated from other measured data and use specific mathematical formulae. These

formulae are based upon assumptions that can introduce error into the calculation. For this reason, and to increase data confidence, formulas have been validated against autopsy data or other 'gold standard' imaging techniques such as radionuclide angiography or MRI (Hees *et al.*, 2002). As a result of the potential for error inflation in derived calculations extreme care must be taken when making the initial direct measurements.

8) Reliability in the assessment and measurement of presented variables is essential. There have been a great number of studies that have reported both intra and inter-reliability across M-mode, 2-D and Doppler parameters. Variations in intra-observer reliability were smaller in all studies (Stefadouros and Canedo, 1977; Lapido *et al.*, 1980; Wong *et al.*, 1981; Pollick *et al.*, 1983) when adequate, experienced sonographers were employed for assessment and quantitation. Based on this information it was decided to use only one sonographer for both scanning and analysis. It was also decided to determine, empirically, intra-observer reliability for this single sonographer (see 2.3) to aid data interpretation in subsequent chapters.

3.3 The reliability of echocardiographic measurements in a group of healthy subjects of disparate age

3.3.1 Introduction

Due to the nature of echocardiography, it was felt necessary to quantify intra-observer reliability of variables intrinsic to this thesis in order to be confident in data presented and to aid in data interpretation in the empirical studies contained in this thesis. The determination of intra-observer reliability was deemed particularly necessary due to the age of the population scanned (the majority being >50 years). Image quality can be affected by age (Feigenbaum, 1994) and so in an ageing population any reliability study should be representative of that population. Interestingly, existing literature rarely provides details of age of participants in reliability studies, those detailing age report mean ages of 32 years (Kuecherer *et al.*, 1991) and 35 years (Crawford *et al.*, 1980). In addition, females are seldom included in reliability studies. Therefore previous reliability data may have been somewhat artificially improved due to the use of echogenic, younger, leaner individuals. Sample size is also an issue with a number

of studies reporting no more than 15 subjects (Pye *et al.*, 1991; Kuecherer *et al.*, 1991).

In addition, although M-mode techniques have been widely scrutinised with regards to reliability and repeatability, 2-D techniques have received less attention, particularly in an ageing population. Specifically it was important to determine reliability data for LV volume, LV mass as well as EF and SV derived through these techniques.

One of the unique aspects of this thesis was the investigation of RV volume across a healthy adult age range in males and females. Again, there are limited reliability data for this specific variable. Further, Doppler echocardiography has been a relatively recent addition to the portfolio of scanning modes. This provides potentially more direct assessment of blood flow and therefore LV and RV function than looking at structural changes via M-mode and 2-D. Reliability data are limited and where presented (George, 1998) suggest that certain aspects of Doppler flow measurements may be open to greater degrees of assessment variability. The determination of test-retest variability for Doppler derived indices of LV and RV inflow and outflow will be informative for this thesis.

It was therefore the aim of this study to empirically establish reliability data for the single sonographer used in this thesis. This is pertinent to the interpretation of echocardiographically derived variables reported in chapters 3-5. Furthermore, data were collected that assessed reliability of two measurements of the same scan as well as two separate scans.

3.3.2. Methods

Subjects

A total of 30 subjects (age range 22-62 years) were scanned on two separate occasions. Scans were performed at approximately the same time of day to avoid any circadian variation, although circadian variation has not been seen to significantly

affect the outcome of echocardiographic scans (Pollick *et al.*, 1983, Gates, 1995, George, 1998). Analysis was performed twice on the primary scan (within-scan), taking care to evaluate the same cycle, and then again on the secondary scan compared to an initial scan (between-scan).

Variables assessed

Directly measured variables included in this study are as follows;

- 2-D (short axis) - LV area (endocardial), RV area
- 2-D (apical) - LV area, LV length, RV endocardial length
- Doppler – LV and RV E, A

Calculated variables included in this study are as follows;

- 2-D - LV mass, LV volume, EF, SV, RV volume
- Doppler - LV E:A, RV E:A

Data Analysis

Statistical analysis for reliability was determined on the basis of providing data that were comparable to previous research and also taking account of current debate with relation to appropriate reliability analysis procedures (Atkinson and Nevill, 2000). To this end we chose three data analysis techniques, all of which provide some estimate of systematic error and random error in test-retest data. Initially, repeated measures ANOVA's were performed to compare the within-scan and between-scan. Significant F ratios would indicate systematic error. Subsequent to the ANOVA, we calculated intra-class correlations (ICC) to quantify random error. Statistically significant and relatively high ICC would represent lower levels of random error. The second analysis was the use of limits of agreement (LoA, Bland and Altman, 1986). The mean of the differences between the within-scan and also the between-scan provided an indication of the degree of systematic error. The upper and lower LoA (± 1.96 * SD of the differences between the within-scan and the between-scan) provided information with respect to random error and also if either the upper or lower LoA did not straddle zero this provided further information on systematic error. The intrinsic

benefit of LoA is that it provides information about random and systematic error in the units of the measured variable and is therefore intuitively easier to interpret. However, it is pertinent to note at this stage that LoA is a statistic which describes the "worst case scenario" error for individuals (the largest error one would expect to find for any individual sampled from the population of interest). The third analysis tool was that of coefficient of variation (CV): the standard deviation as a percentage of the mean. The use of this method would allow direct comparison with previous echocardiographic reliability data, as this is a common method of reporting reliability within cardiology literature. Statistical analyses were completed on SPSS (version 12.0) and excel and critical alpha was set at 0.05.

3.3.3 Results and Discussion

Directly measured 2-D variables are reported in Table 3.3.1. There were no significant ANOVA data and all ICCs were statistically significant ranging from r= 0.90 to 0.99.

This would suggest low levels of random and systematic error in these measurements. The lowest ICC (r=0.90) was reported for LVL. This may be due to relative difficulty in defining the endocardial border of the left ventricle at the apex.

Limits of agreement for all 2-D variables were within c. 10% of the mean score for the within-scan and within c. 20% for between-scan. This likely reflects the decreased spatial resolution in 2-D scans compared to M-mode scans. Coefficients of variation for each of these values are all within 5% for both the within-scan and between-scan. Previous 2-D reproducibility data have reported similar, if not less favourable, results for CV. This is also the case for 2-D calculated variables (see Table 3.3.2) where CV for LV mass was 13% (Kuecherer *et al.*, 1991) compared to 7% in this study.

Table 3.3.1 Two-dimensional echocardiography: reliability data for directly measured variables

Variable	Test	Mean SD	Comparison	ANOVA		ICC		LoA +	LoA -	CV (%)
LVSA (cm^2)	1	22.11	1v2	F =	2.175	r =	0.997	0.994	0.706	1.5
		3.80		P =	0.158	P =	0			
	2	21.96	1v3	F =	1.432	r =	0.985	2.097	1.591	3.2
		3.78		P =	0.247	P =	0			
	3	21.85								
		3.89								
LVA (cm^2)	1	30.15	1v2	F =	0.043	r =	0.992	1.866	1.796	2.3
		5.18		P =	0.837	P =	0			
	2	30.11	1v3	F =	0.597	r =	0.976	3.466	3.011	4.7
		5.05		P =	0.446	P =	0			
	3	29.92								
		5.39								
LVL (cm)	1	7.91	1v2	F =	1.058	r =	0.956	0.745	0.617	2.8
		0.82		P =	0.312	P =	0			
	2	7.84	1v3	F =	0.668	r =	0.901	1.018	0.876	3.7
		0.84		P =	0.420	P =	0			
	3	7.84								
		0.74								
RVSA (cm^2)	1	18.80	1v2	F =	1.443	r =	0.994	1.355	1.034	2.3
		3.86		P =	0.244	P =	0			
	2	18.64	1v3	F =	0.318	r =	0.973	2.41	2.619	4.6
		3.81		P =	0.714	P =	0			
	3	18.91								
		3.85								
RVA (cm^2)	1	8.94	1v2	F =	0.221	r =	0.981	0.579	0.631	3.0
		1.08		P =	0.642	P =	0			
	2	8.96	1v3	F =	0.054	r =	0.959	0.881	0.845	4.2
		1.18		P =	0.818	P =	0			
	3	8.92								
		1.11								
RVL (cm)	1	3.92	1v2	F =	3.360	r =	0.976	0.344	0.482	3.0
		0.65		P =	0.077	P =	0			
	2	3.99	1v3	F =	0.002	r =	0.925	0.64	0.679	4.2
		0.70		P =	0.966	P =	0			
	3	3.92								
		0.64								

L(R)VSA – Left (right) ventricular short-axis area, **L(R)VA** – Left (right) ventricular 4-chamber area, **L(R)VL** – Left (right) ventricular 4-chamber length

Variables calculated from direct 2-D measurements are contained in Table 3.3.2. Of all of the derived variables LV mass has been the most commonly reported variable in cardiological and exercise science literature. As such there are comparative reliability data available in the literature (e.g. George, 1998) although direct comparisons to previous literature are somewhat problematic given the different methods of estimating LV mass used in other research and disparate samples studied. The LV mass ANOVAs were not significant and the ICC's were high ($r = 0.98$-0.99, $P<0.05$) suggesting no systematic and/or random error. This is supported by the LoA data. All statistical outcomes were superior for the within-scan as compared to the between-scan.

Less comparative reliability data are available for LV volume. Somewhat interestingly there was a significant ANOVA F ratio for the within-scan comparison, suggestive of systematic error between the two scans. However, this systematic difference equated to 2 ml which is c. 2% of the mean LV volume values and likely statistical error and therefore biologically meaningless. It is also pertinent to note that there was no evidence of a meaningful systematic error from LoA data. Again, error was marginally greater for the between-scan with LoA representing c. 10% of the mean value and the CV was less than previously reported (Kuecherer *et al.*, 1991).

The functional variables of EF and SV both produced significant ANOVAs, again, suggesting some degree of systematic bias. This was not surprising given the fact that these variables are both derived from LV volume data. As with LV volume further analysis demonstrated that the systematic bias represented c. 2 ml and 1% for SV and EF, respectively. Again, as with LV volume, this difference is biologically meaningless and LoA do not support evidence for any systematic measurement bias. The ICC values were significant for all EF and SV correlations ($r = 0.84 - 0.98$). LoA represented c. 7% of mean EF values and c. 14ml of mean SV values.

Table 3.3.2 Two-dimensional echocardiography: reliability data for directly measured variables

Variable	Test	Mean SD	Comparison	ANOVA		ICC		LoA +	LoA -	CV (%)
LVM (g)	1	169.18	1v2	F =	0.817	r =	0.992	17.37	21.392	3.8
		52.96		P =	0.378	P =	0			
	2	171.19	1v3	F =	2.760	r =	0.977	26.486	38.955	7.1
		53.46		P =	0.114	P =	0			
	3	175.42								
		55.82								
LVV (ml)	1	101.13	1v2	F =	5.351	r =	0.992	10.898	7.098	2.8
		25.88		P =	0.028	P =	0			
	2	99.23	1v3	F =	3.533	r =	0.983	15.614	11.041	6.2
		25.67		P =	0.071	P =	0			
	3	98.85								
		26.23								
RVV (ml)	1	161.84	1v2	F =	0.165	r =	0.994	15.091	13.713	4.1
		46.09		P =	0.690	P =	0			
	2	161.15	1v3	F =	0.002	r =	0.981	24.791	25.041	5.3
		46.73		P =	0.967	P =	0			
	3	161.97								
		44.41								
SV (ml)	1	70.88	1v2	F =	5.144	r =	0.981	11.508	7.561	4.5
		17.31		P =	0.031	P =	0			
	2	68.90	1v3	F =	5.118	r =	0.97	14.527	9.554	8.6
		17.79		P =	0.031	P =	0			
	3	68.39								
		17.74								
EF (%)	1	70	1v2	F =	4.569	r =	0.889	5.716	3.851	2.9
		3		P =	0.041	P =	0			
	2	69	1v3	F =	3.346	r =	0.837	6.988	4.988	3.6
		4		P =	0.078	P =	0			
	3	69								
		4								

LVM – Left ventricular mass, **LVV** – Left ventricular volume at end diastole, **EF** – Ejection Fraction, **SV** – Stroke Volume

These are slightly higher than previous data in younger subject cohorts (George, 1998), however, greater image clarity in younger subjects is likely to explain for this discrepancy. Coefficient of variation data were comparable to those previously reported by Kuecherer *et al.* (1991) indicating that reliability was within acceptable limits.

Table 3.3.3 Doppler Echocardiography: Reliability data for LV parameters

Variable	Test	Mean SD	Comparison	ANOVA		ICC		LoA +	LoA -	CV (%)
Mit E ($m.sec^{-1}$)	1	0.67	1v2	F =	0.191	r =	0.989	0.064	0.056	2.4
		0.14		P =	0.666	P =	0			
	2	0.67	1v3	F =	0.293	r =	0.921	0.149	0.135	8.2
		0.14		P =	0.592	P =	0			
	3	0.67								
		0.11								
Mit A ($m.sec^{-1}$)	1	0.44	1v2	F =	0.125	r =	0.986	0.041	0.043	3.8
		0.08		P =	0.726	P =	0			
	2	0.44	1v3	F =	2.239	r =	0.665	0.139	0.183	9.0
		0.09		P =	0.145	P =	0.002			
	3	0.46								
		0.07								
Mit E:A	1	1.58	1v2	F =	0.302	r =	0.979	0.476	0.429	4.9
		0.41		P =	0.588	P =	0			
	2	1.61	1v3	F =	2.990	r =	0.865	0.638	0.464	11.7
		0.42		P =	0.094	P =	0			
	3	1.49								
		0.38								

Mit E – Mitral valve early filling velocity, **Mit A** – Mitral valve atrial filling velocity, **Mit E:A** – Mitral valve ratio of early to atrial filling velocities

Variables directly measured and calculated from Doppler assessments of the LV and RV are presented in Tables 3.3.3 and 3.3.4 respectively. All ANOVAs were non-significant with higher ICCs (r = 0.67 - 0.99) in LV flow data suggestive of low systematic and random error. These data are supported by LoA data which tended to represent c.10% of mean Doppler data for within-scan. Error was greater in the between-scan approaching 20% of mean scores on most occasions. Again whilst there is limited LoA data for Doppler flow parameters the current data set derived from a broad age range are generally compatible with data collected in a younger cohort (George *et al.*, 1998). This LV Doppler data also compares favourably with

data reported by Kuecherer *et al.* (1991) where CV for E, A and E:A values were between 16-20%.

In the RV ANOVAs were significant, suggesting systematic error, for peak tricuspid A flow velocity and E:A ratio for the between-scan. Differences of 5 $cm.s^{-1}$ (A flow velocity) and 0.14 (E:A ratio) are not completely trivial and should therefore be used to guide careful examination of these variables in future studies within this thesis. Interestingly LoA did not support meaningful systematic bias with upper and lower limits spread either side of zero. The RV ICCs were all significant ($r = 0.93 - 0.98$) and LoA are generally in line with LV Doppler data. We could not find any previous reliability data for RV Doppler data for comparative purposes.

The LoA, especially for E:A ratios, are generally wider in Doppler variables compared to 2-D data. It is not entirely clear why this might be the case but there are a substantial amount of technical details that must be considered in the scanning and assessment of Doppler flow data (Rakowski *et al.*, 1996). For example, small errors in placing sample volume parallel to flow may produce substantial error. It is also apparent that error propagation is not linear in nature with errors in flow measurement getting exponentially larger with quite small angle deviations. Pye *et al.* (1991) also reported these difficulties, particularly in RV views and recommended a shift in transducer position to lengthen the RV inflow region allowing easier alignment with flow.

Table 3.3.4 Doppler Echocardiography: Reliability data for RV parameters

Variable	Test	Mean SD	Comparison	ANOVA		ICC		LoA +	LoA -	CV (%)
Tri E ($m.sec^{-1}$)	1	0.78	1v2	F =	0.013	r =	0.984	0.065	0.064	3.2
		0.12		P =	0.911	P =	0			
	2	0.78	1v3	F =	1.015	r =	0.94	0.115	0.138	7.1
		0.13		P =	0.322	P =	0			
	3	0.80								
		0.14								
Tri A ($m.sec^{-1}$)	1	0.50	1v2	F =	0.098	r =	0.995	0.034	0.036	2.2
		0.12		P =	0.756	P =	0			
	2	0.50	1v3	F =	18.341	r =	0.931	0.084	0.192	8.2
		0.12		P =	0	P =	0			
	3	0.56								
		0.14								
Tri E:A	1	1.65	1v2	F =	0.231	r =	0.993	0.175	0.160	4.0
		0.51		P =	0.635	P =	0			
	2	1.64	1v3	F =	12.465	r =	0.952	0.558	0.286	8.3
		0.49		P =	0.011	P =	0			
	3	1.51								
		0.47								

Tri E – Tricuspid valve early filling velocity, **Tri A** – Tricuspid valve atrial filling velocity, **Tri E:A** – Tricuspid valve early to atrial filling velocity ratio

An appropriate comparison of any pair of repeated measurements is "the Bland-Altman plot" proposed by Bland and Altman (1986), which explicitly shows differences between the two measures (on the Y axis) over their range (on the X axis). These are common methods of visually reporting error but in this study the use of Bland-Altman Plots over the tables would have resulted in 32 separate plots or Figures. To save space we have included two exemplar plots from the variable LV volume that is intrinsic to future studies in this thesis. Figure 2.3.1 shows an exemplar plot of differences between the mean of scan 1 and scan 2 (within-scan) for LV volume. Similarly, Figure 3.3.2 reveals the same for scans 1 and 3 (between-scan). There is no clear evidence of heteroscedasticity of error in either plot and it is noticeable that the LoA in the within-scan are broader than in the between-scan.

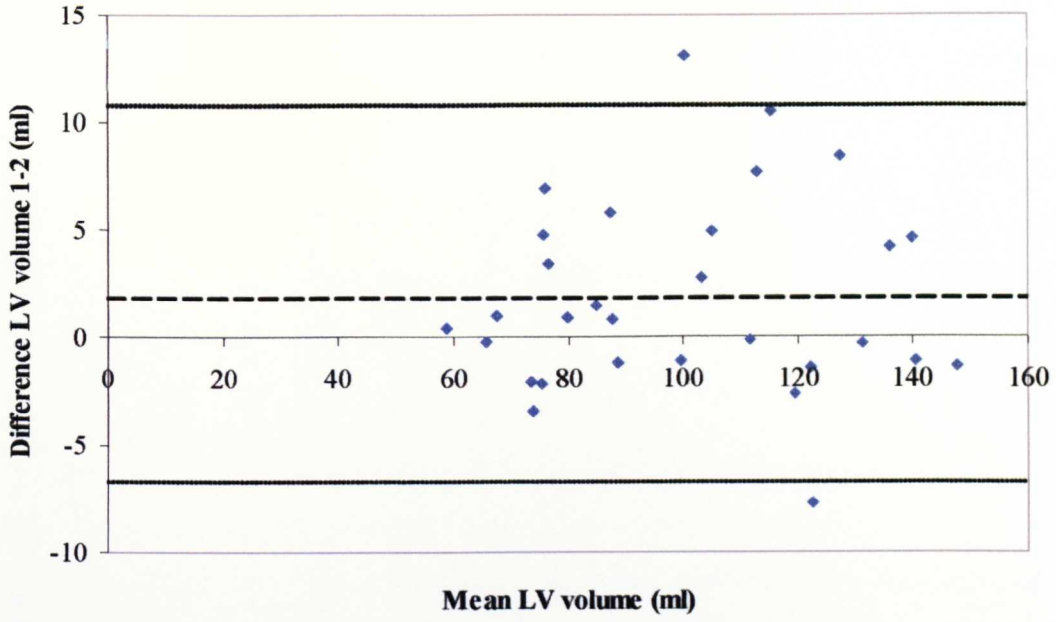


Figure 3.3.1 An exemplar Bland Altman plot for LV volume (scan 1-2).

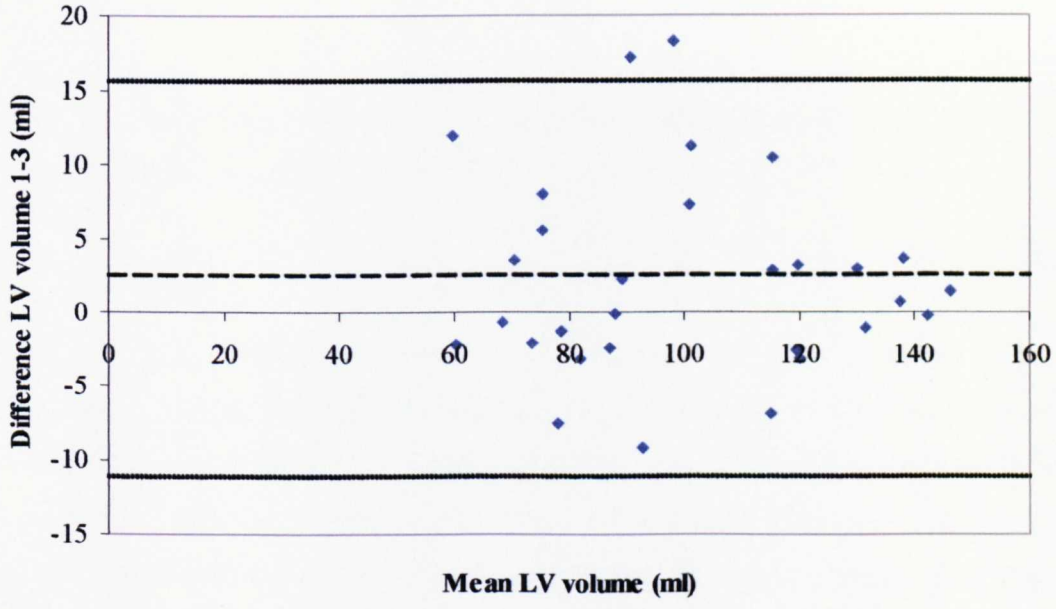


Figure 3.3.2 An exemplar Bland Altman plot for LV volume (scan 1-3).

(Note: The dashed lines are the mean difference and the upper and lower complete lines represent the upper and lower LoA.)

3.3.4 Conclusions

Echocardiography is susceptible to variability (both systematic and random error) due to a number of factors (i.e. subject, equipment and observer). This variability is increased when using an "arduous" subject sample such as an ageing population. The sample characteristics (age range 22-62 years) likely accounts for the slightly greater scan-scan systematic and random error reported here compared to previous literature derived from younger subject samples.

It is important to be aware of the limitations of the methods employed in ageing and echocardiographic studies. Indeed, empirical reliability data are central to correctly interpreting results of future studies. Although different echocardiographically derived variables produce a range of reliability findings all are acceptable in the biological study of human ageing and render echocardiography an appropriate method for use within this thesis.

3.4 The investigation of appropriate body size scaling of cardiac structural and function data in an ageing population

3.4.1 Introduction

Batterham *et al.*, (1999) stated that body dimensions influence a vast number of physiological variables or anatomic structures (e.g. LV mass) whereby an increase in body dimension parameter (e.g. BM) is generally associated with an increase in the structural or functional variable. On this basis the scaling of cardiac data for body size and/or composition will facilitate more meaningful comparisons between groups or individuals. For example, it is well documented that men and women have, on average, significantly different body sizes and body composition (Wilmore and Costill, 1996). Likewise there are notable changes in body size/mass with the ageing process (Sezginsoy *et al.*, 2004). Indeed age and sex have specifically been reported to influence echocardiographic measurements (Shub *et al.*, 1994). As a result of this information it is clear that any comparison of cardiac structure and function in males and females across a broad age range cannot be interpreted with any clarity unless data are scaled to remove the effects of differences in body size and composition.

Only in this way will the "true" effects of ageing and sex upon cardiac structure and function likely be unveiled.

Although it has long been acknowledged that the size of a subject is related to echocardiographic measurements (Henry *et al.*, 1978; Gardin *et al.*, 1979; Roge *et al.*, 1978) there has been a degree of controversy and lack of clarity with regards to scaling of data. In order to scale cardiac structures and functional variables appropriately two specific issues must be resolved. Firstly, which method of scaling is the most appropriate to use for these data, and secondly, which scaling variable to use.

There are a number of scaling methods used in the clinical and scientific cardiac literatures. The most common, and simplest, scaling method is the per-ratio standard approach where a cardiac variable (y) is simply divided by the body size or composition variable (x; y/x), exemplars of which can be found in current exercise science and cardiological literature (e.g. Haykowsky *et al.*, 2005). This method assumes a linear, proportional relationship between the dependent variable (y; e.g. LV mass) and the body size variable (x; e.g. BM) with the line of best fit passing through the origin. Whilst simplistic and commonly used this technique has been criticized, initially as long ago as the 1940s (Tanner *et al.*, 1949), primarily because there is ample empirical and theoretical evidence that many body size-cardiac variable relationships do not display such a simple linear relationship. Even regression standards models ($y = a + bx + e$, where e is the error term) which have improved data fit and resulted in less residual error (Batterham *et al.*, 1999) still assumes a linear relationship when biological literature suggests a curvilinear relationship between body size and cardiac parameters (Schmidt-Nielson, 1984).

More recently in exercise science and cardiological literature allometric scaling methods have been evaluated and applied (Daniels *et al.*, 1995; Batterham *et al.*, 1997; Batterham and George, 1998). This approach adopts an allometric power function model ($y = ax^b \cdot e$), which reflects a curvilinear relationship with the least-squares regression line constrained to pass through the origin. This type of scaling

can result in an improved fit to the data, with less residual error in comparison with other models (Batterham *et al.*, 1999) as well as being theoretically more plausible than relationships with positive or negative y-intercepts. Whilst there are some available data that have investigated the relationship between cardiac structures and a body size variable across a broad age range (De Simone *et al.*, 1991; De Simone *et al.*, 1992; Daniels *et al.*, 1995) this has almost exclusively used only LV mass as the cardiac dimension of interest, likely because of its importance as a clinical risk factor (Savage *et al.*, 1990). Thus it was important for this thesis to determine the appropriate relationships and scaling model to apply to a broader range of structural and functional variables (i.e. LV volume, RV volume, SV).

The appropriate choice of body size variable to be used in any allometric scaling model is also contentious (Batterham *et al.*, 1999). The most common body size variable reported in previous literature is BSA as this combines measurements of HT and BM in a simplistic mathematical calculation. This is useful in clinical practice because of the speed and ease of calculation (Batterham *et al.*, 1999). Concerns with regard to the use of BSA include the accuracy of the measurement, which is based on norms, as well as the fact that is does not adequately differentiate changes in fat and LBM that might alter BSA (Batterham *et al.*, 1999). Based on criticisms of BSA data clinical literature have adopted HT as a simple and convenient scaling variable (De Simone *et al.*, 1991; 1992). Other work has questioned the use of height especially in adult only studies with a restricted range of data (Batterham *et al.*, 1997; George *et al.*, 2001). The most appropriate body size variable to use must meet theoretical as well as empirical requirements. In this regard LBM holds some potential as LBM is mostly comprised of active muscle tissue which provides the haemodynamic "sink" for increased flow as well as the site of increased resistance for changes in afterload. Such an important haemodynamic role for LBM has led to its investigation in scaling studies and empirical analysis has supported its role as the most appropriate scaling variable (compared to HT, BM and BSA) with reduced residual error (George *et al.*, 2001). A criticism, however, of the use of LBM has been the accuracy with which it has or can be assessed (Batterham *et al.*, 1999). The use of DEXA in this regard is an important and unique part of this study as it has been described as a potential gold standard in body composition assessment (Myerson *et al.*, 2002).

Thus the purpose of this study was to evaluate the "true" nature of the relationships between cardiac structural and functional variables with a range of body size parameters in a broad age cohort of healthy adult males and females. The adoption of the most appropriate scaling method and scaling variable should lead to the reporting of size independent data.

3.4.2 Methods

Subjects

198 males and females (males, 48 ± 16 years; females, 49 ± 13 years) were assessed for this scaling investigation. These subjects also formed the subjects for Chapter 3. Therefore the results of this investigation will directly influence scaling technique utilised within this thesis.

Variables assessed

Cardiac variables measured included LV mass, LV volume, SV and RV measures included RV volume. Scaling variables utilised included HT, BM, BSA, and LBM. These are most commonly used within age and training related literature.

Data Analysis

The initial stage in any analysis of scaling method and variable identification is to verify that significant relationships exist between the independent and dependent variables such that scaling, of some sort, is therefore warranted. This was accomplished very simply through a (linear) Pearson Product-moment Correlation test that would likely identify significant relationships even if the "true" nature of association were curvilinear (see Table 3.4.1).

Table 3.4.1 Cardiac variable and body size variable correlation coefficients (*p<0.05, **p<0.01)

	HT (cm)	BM (kg)	BSA (m^2)	LBM (kg)
LV mass (g)	0.32**	0.52**	0.51**	0.54**
LV volume (ml)	0.72**	0.54**	0.60**	0.75**
RV volume (ml)	0.60**	0.57**	0.61**	0.70**
SV (ml)	0.68**	0.50**	0.56**	0.70**

LV-left ventricular, **RV**-right ventricular, **SV**-stroke volume

It is apparent from Table 3.4.1 that some form of scaling is appropriate to facilitate the interpretation of cardiac data from male and female subjects across a broad, adult age range. To investigate which method and variable would provide the best combination for scaling of cardiac structural and functional data we adopted two different approaches and used all body size variables for comparison.

The initial method of per-ratio scaling simply involved the division of individual LV mass, LV volume, RV volume and SV data for individual data for HT, BM, BSA and LBM. To determine the utility of the methods and variables the scaled data (e.g. LVM/HT) were then plotted against the body size variable (in this case HT) to see if the method had removed the impact of the body size variable. Specifically, any residual significant correlation (Pearson Product-moment) would identify that the impact of body size had either not been removed fully or had been overcompensated for. Data for both males and females were pooled together.

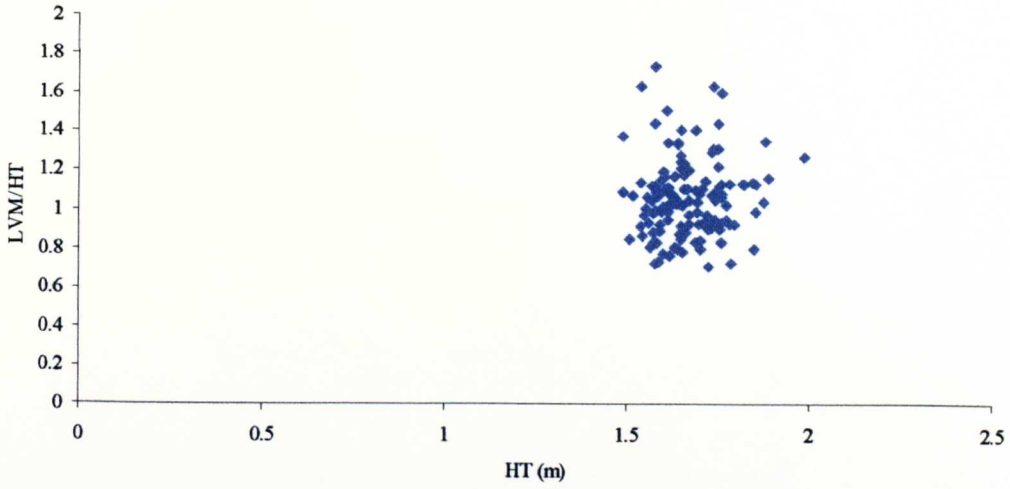
The second method involved the determination of the allometric exponent (***b***) taking the natural log of all data. This allowed a linear regression approach to be adopted to determine the slope of the regression equation, which equated to the ***b*** exponent. It was possible to then construct a power-function ratio (y/x^b; Nevill *et al.*, 1992) in an attempt to derive a body-size independent cardiac variable. The allometric exponents were derived from a multivariate scaling approach that produced the best compromise

b exponent for a combined male and female population (Batterham *et al.*, 1997) to allow comparison with the per-ratio approach. Within the multivariate model all interaction terms for gender and body size variables were not statistically significant. Thus, the interaction term could be removed and the analysis was completed including both male and female subjects. This was then used in the determination of individual power function ratios. As with per-ratio scaling, the scaled variable was then plotted against the body size variable to check to see if the influence of the body size variable had been removed or not (non-significant correlation coefficient). To investigate which body size variable would be the most appropriate to use we assessed the relative width of ***b*** exponent confidence intervals and the multiple ***r*** from the log-linear regression analysis. This was combined with theoretical discussion to make a final selection.

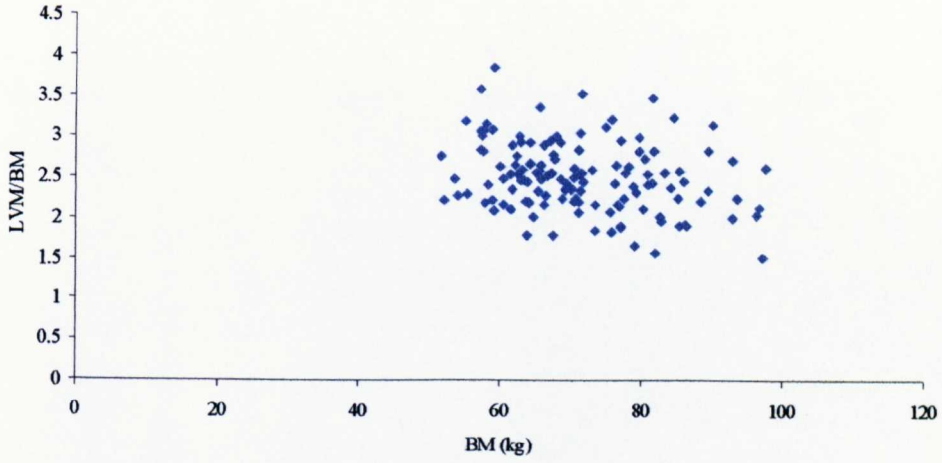
3.4.3 Results and discussion

When per ratio scaling was performed and then correlated with body size variables significant associations remained in a number of relationships. These data are presented in the exemplar Figure 3.4.1a, b, c, d for LV mass plotted against all body size variables and in Table 3.4.2 which summarises the relationships for all other variable combinations. It is clear that most relationships are still statistically significant and thus the scaling model has not removed all of the influence of the body size variables. These are comparable findings to previous research in different cohorts and with slightly different variables (Batterham and George, 1998; George *et al.*, 1998; George *et al.*, 2001). The remaining influence is not surprising given that both theoretical (Gutgesell and Rembold, 1990) and empirical (George *et al.*, 2001) reports support curvi-linear relationships.

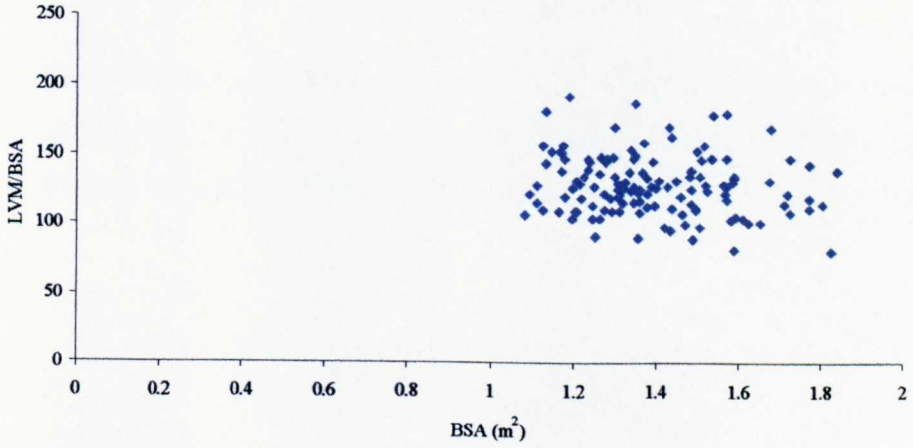
a) Scaled LVM and HT (r=0.05)



b) Scaled LVM and BM (r= -0.0253)



c) Scaled LVM and BSA (r= -0.129)



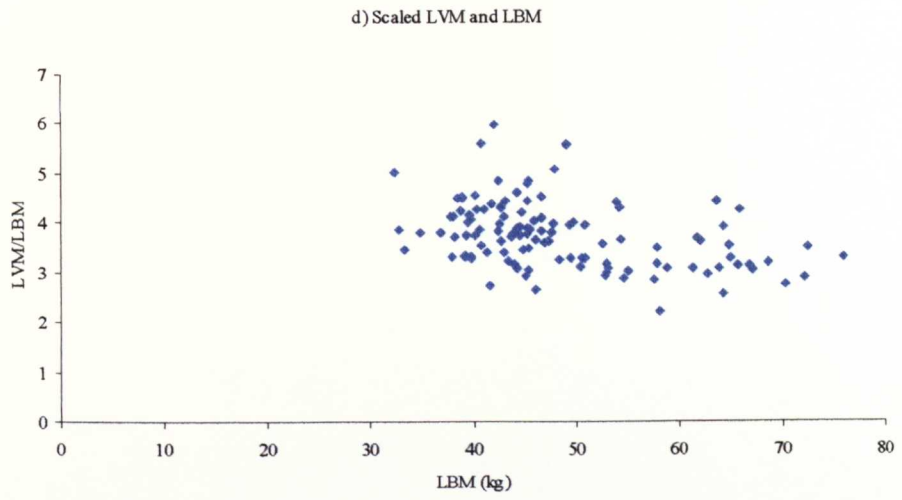


Figure 3.4.1 Per-ratio linear correlation plots for a) LV mass/HT and HT, b) LV mass/BM and BM, c) LV mass/BSA and BSA, and d) LV mass/LBM and LBM.

Table 3.4.2 Per-ratio scaled data correlated to body size variables (* p<0.05, ** p<0.01)

R=	HT	BM	BSA	LBM
LV volume (ml)	0.62**	0.07	0.25**	0.21**
RV volume (ml)	0.46**	0.11	0.25**	0.11
SV (ml)	0.57**	0.03	0.20**	0.13

When the allometric model was evaluated the first step was to generate log-linear regression data to derive *b* exponents and investigate the relative "goodness of fit" for the differing body size variables. These data are presented in Table 3.4.3.

Table 3.4.3. Log-linear data for all body size and cardiac parameters.

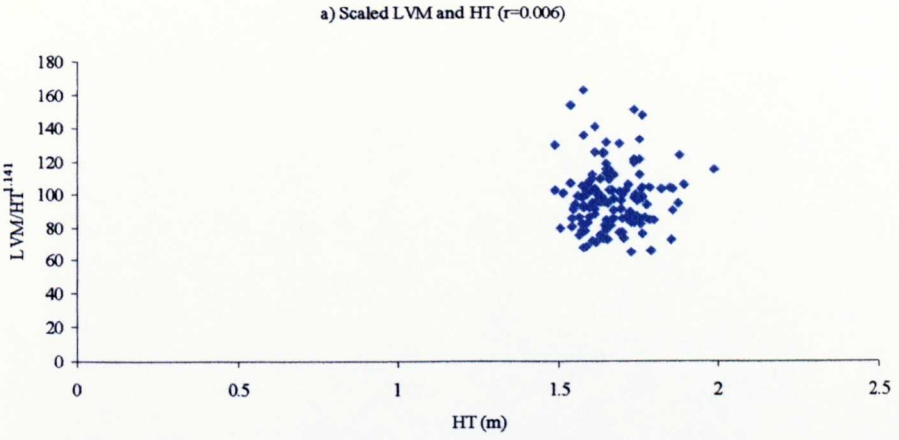
	HT				BM			
	R^2	*b*	CI (u)	CI (l)	R^2	*b*	CI (u)	CI (l)
LV mass (g)	0.096	1.141	1.752	0.530	0.269	0.694	0.494	
LV volume (ml)	0.479	3.982	4.645	3.319	0.281	1.039	0.774	1.304
RV volume (ml)	0.301	3.282	4.291	2.273	0.310	1.136	0.797	1.474
SV (ml)	0.410	3.292	4.631	3.153	0.225	0.981	1.271	0.692

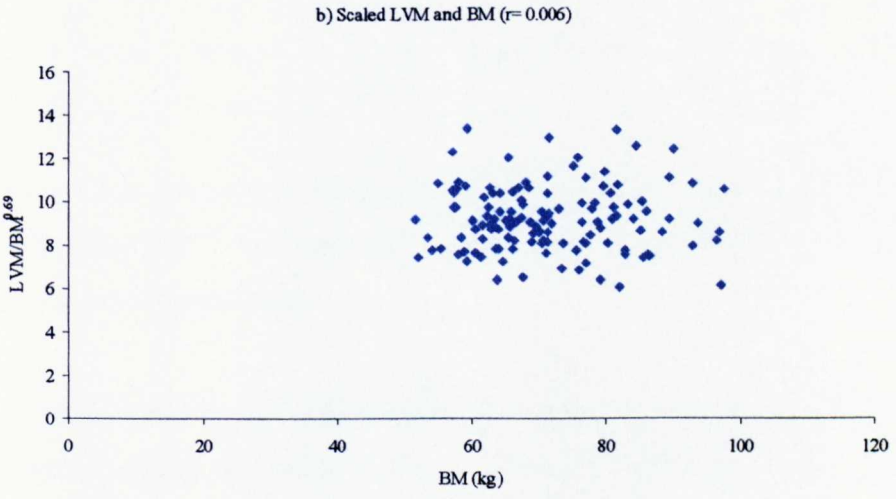
	BSA				LBM			
	R^2	*b*	CI (u)	CI (l)	R^2	*b*	CI (u)	CI (l)
LV mass (g)	0.258	0.814	0.573	1.054	0.272	0.559	0.389	0.730
LV volume (ml)	0.343	1.387	1.081	1.693	0.528	1.179	0.988	1.371
RV volume (ml)	0.338	1.432	1.032	1.831	0.424	1.089	0.814	1.364
SV (ml)	0.279	1.321	1.658	0.983	0.435	1.109	1.325	0.893

CI - confidence intervals, **u** - upper, **l** - lower

A range of *b* exponents was reported for the log-linear regression calculations. This is not surprising given the range of different body size variables selected and their place within the concept of the theory of "geometric similarity". Simply this suggests that independent and dependent variables in any scaling procedure must possess the same dimensional quantity to be scaled to a *b* exponent of 1.0 (linear). This might be the case with LV mass and LBM (both 3-dimensional variables) but is not the case with LV mass and BSA (a 2-dimensional variable). The latter variable pairing would, bound by the theory of geometric similarity have a curvilinear relationship (with a *b* exponent of 1.5). Data for R^2 and relative breadth of CI would suggest that the best variable to scale cardiac data with is LBM. This supports the theoretical standpoint that has been supported by this and other research as well as editorial statement (Roman, 1998).

The next step having generated the **b** exponents for each pairing of cardiac and body size variables is to correlate the power function ratio and the body size variable to determine if the scaling process has removed the impact of body size. These data are presented in exemplar figures for LV mass (Figure 3.4.2) as well as tabulated for all other variables (Table 3.4.4).





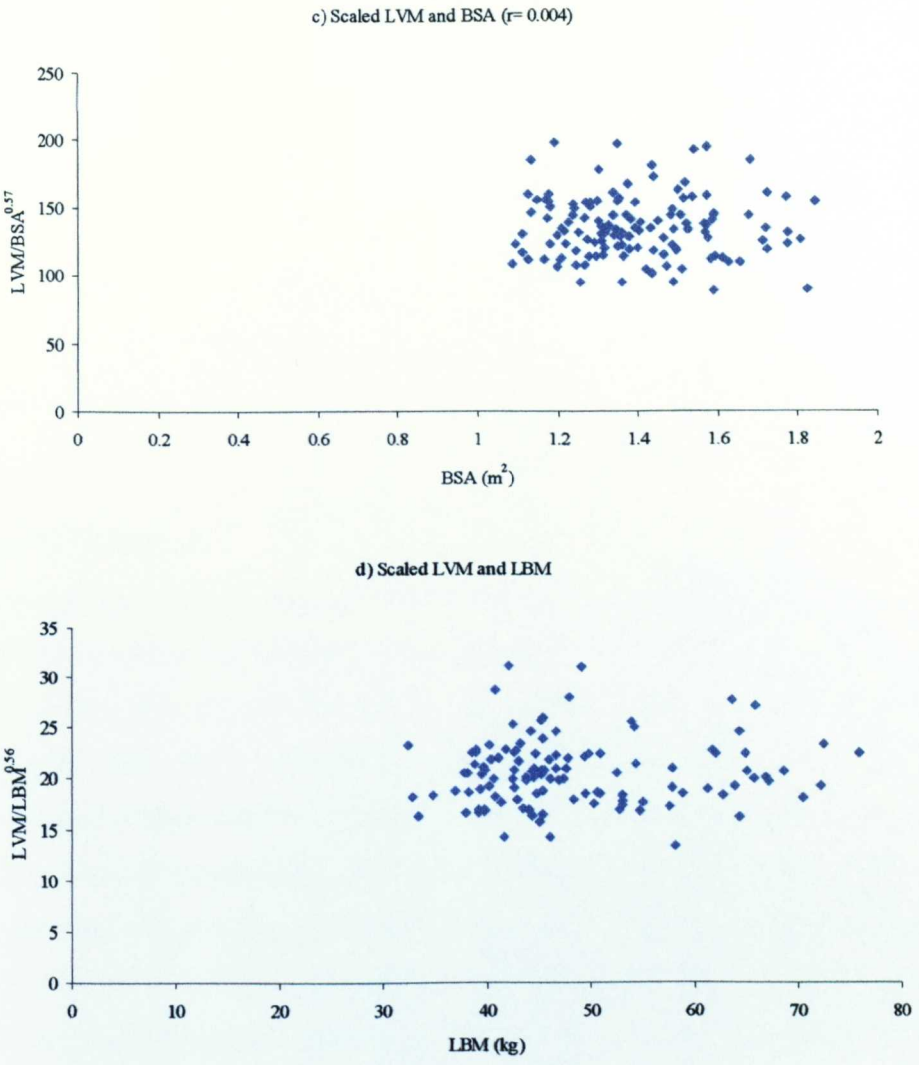


Figure 3.4.2 Allometric linear correlation plots for a) LV mass/$HT^{1.141}$ and HT, b) LV mass/$BM^{0.69}$ and BM, c) LV mass/$BSA^{0.57}$ and BSA, and d) LV mass/$LBM^{0.56}$ and LBM.

It is clear that body size effects have been removed in nearly all cases with the use of allometric scaling with no relationship being observed between any structural variable and body size variable (Figure 3.4.2 and Table 3.4.4). This supports the use of allometric scaling over per ratio scaling and is consistent with previous research (Batterham *et al.*, 1997; George *et al.*, 1998; George *et al.*, 2001).

Table 3.4.4 Correlation coefficients for cardiac data scaled as power function ratios plotted against the body size variable (* p<0.05).

	HT (m)	BM (kg)	BSA (m2)	LBM (kg)
LV volume (ml)	0.01	0.046	0.045	0.042
RV volume (ml)	0.03	0.033	0.031	0.039
SV (ml)	0.15*	-0.03	0.15*	0.04

3.4.4 Conclusions

Although per-ratio is the most commonly used form of scaling due to the ease and simplicity of the calculations, it is obvious from this study that it does not eradicate the effects of body size for any given cardiac variable. Although somewhat more complex and time consuming, data here demonstrate that allometric scaling successfully removes the effects of body size upon cardiac parameters and permits a more accurate interpretation of cardiac data in populations or cohorts who differ with respect to body size data (such as men and women and across the adult age spectrum). The allometric scaling exponents derived for LBM and its association with LV mass, LV volume, SV and RV volume will be utilised in the next chapter to normalise data.

4 An echocardiographic study of age-related changes in cardiac structure and function in males and females

4.1 Introduction

Age-related changes in the structure and function of the myocardium have been increasingly targeted in scientific investigations over the past decade due to the escalation in the percentage of population aged over 60 years and the fact that cardiovascular disease is prevalent in approximately 60% of this expanding population group (National Statistics, 1998). It is for this reason that an accurate picture of myocardial ageing must be determined.

Left ventricular structural data in both males and females have demonstrated both increases (Gardin *et al.*, 1979) and little or no change (Dannenburg *et al.*, 1989; Ganau *et al.*, 1995) in echocardiographically determined LV mass with age. Although wall thicknesses; both posterior and septal, have consistently demonstrated an increase with age (Gerstenblith *et al.*, 1977, Gardin *et al.*, 1979, Pearson *et al.*, 1991, Shub *et al.*, 1994, Slotwiner *et al.*, 1998), the extent of this increase along with conflicting LV internal dimension data have led to uncertainty regarding changes in LV structure with ageing. The same problem exists with echocardiographic studies of LV functional changes with ageing. Previous echocardiographic data suggest no change in resting SV (Slotwiner *et al.*, 1998) and both no change (Pearson *et al.*, 1991) and a decrease (Salmasi *et al.*, 2003) in EF in males or females with age.

Part of the reason for such equivocal data may reside in the common use of the M-mode echocardiographic technique in past literature (Gerstenblith *et al.*, 1977, Gardin *et al.*, 1979; 1995, Henry *et al*, 1980, Levy *et al.*, 1987, Dannenburg *et al.*, 1989, Pearson *et al.*, 1991, Shub *et al.*, 1994, Slotwiner *et al.*, 1998). M-mode echocardiography portrays a single slice ("ice-pick") of ventricular tissue for measurement purposes and assumes uniform wall thickness and simple geometry of the ventricle when calculating LV mass, LV volume as well as estimating EF and SV. These problems are now widely recognised (Feigenbaum, 1994) and have led to the reinvestigation of ageing related changes in the heart via other more accurate imaging techniques. The "gold-standard" imaging technique is thought to be MRI (Myerson *et al.*, 1999) and this was employed by Hees *et al.* (2002) to investigate age-related

changes in LV morphology. Hees *et al.* (2002) reported no change in LV mass with age in females as opposed to a significant decrease in LV mass with age in males. This was explained in females by a reduction in the internal LV long axis length (c. 9% over 7 decades) associated with an increase in mean wall thickness that contributed to the maintenance of LV mass. Conversely, in men a decrease in long axis length (c. 11%) was associated with no increase in wall thickness resulting in a decrease in LV mass. Hees *et al.* (2002) therefore concluded that M-mode echocardiography and MRI produced diverse findings for progression of changes in LV structure with age. Other MRI studies have addressed age-related changes in LV function with De Bondt *et al.* (2001) reporting that females have better-preserved EF than age-matched males. Magnetic resonance imaging, however, is not the standard frontline clinical tool for assessing cardiac structure and function. This role still lies with echocardiography and currently 2-dimensional (2-D) echocardiographic techniques represent the most accurate ways to assess LV mass, LV volume, EF and SV (Schiller *et al.*, 1989) via echocardiography. Despite this there are very few 2-D echocardiographic studies examining the age-related changes in ventricular morphology and function in males and females.

Further factors have complicated the interpretation of previous age-related studies of cardiac structure and function, irrespective of imaging technique used. One such factor is the health and activity status of the subjects. It is well known that activity status and disease can affect CV structure and function (George *et al.*, 1991). Although many studies have screened subjects to exclude those with overt cardiovascular disease the same rigour has not been applied to controlling activity status. Activity status is rarely reported, in spite of comments in a number of ageing review articles (Lakatta, 1993; 2001) that promote the need for a more stringent standardisation of inclusion/exclusion criteria, or more detailed descriptions of population samples in the literature.

In addition, the impact of healthy human ageing upon right ventricular (RV) structure and function has rarely been investigated despite the important role it plays in LV mechanics as well as reflecting the health of pulmonary circulation. Right ventricular changes with ageing have been reported in an autopsy study (Olivetti *et al.*, 1995) and an MRI study (Sandstede *et al.*, 2000). Sandstede *et al.* (2000) reported that RV

volume decreased significantly with age in both males and females. Despite these data it is of interest to evaluate age-related changes in RV structure and function using appropriately validated 2-D echocardiographic models.

Previous LV research has consistently reported, via Doppler echocardiography that with increasing age there is a shift in diastolic filling from a reliance on the early stage of LV filling in young subjects, to an almost equal reliance on the latter (atrial) phase in older individuals. This causes a decrease in overall ratio of early to late (E:A) filling (Miyatake *et al.*, 1984; Pasierski *et al.*, 1991; Sagie *et al.*, 1993; Schirmer *et al.*, 2000), however, RV values have rarely been reported despite the importance of RV function.

Cardiac structural variables such as LV mass are strongly associated with body size (Gardin *et al.*, 1987, Savage *et al.*, 1990; Chapter 2.4). Consequently the normalisation or scaling of cardiac structural variables by body size indices (e.g. body surface area) has become relatively standard in the reporting of cardiac data (Batterham *et al.*, 1999). There is, however, still a degree of controversy surrounding the theory and practice of scaling cardiac variables. Tanner (1949) and later Gutgesell and Rembold (1990), amongst others, both theoretically and empirically criticised the ratio-scaling of cardiac variables. Despite many warnings these practices are still widespread in the scientific literature, even in recent literature (e.g. Pearson *et al.*, 1991; Shub *et al.*, 1994; Lorenz *et al.*, 1999; Sandstede *et al.*, 2000; De Bondt *et al.*, 2001). Allometric scaling has been suggested as a more appropriate method for normalising cardiac dimensions for body size (De Simone *et al.*, 1992; Batterham *et al*, 1999; Chapter 2.4). Moreover, lean-body mass (LBM) has been reported to be the most theoretically and empirically appropriate body size index for scaling physiological (Kohrt *et al.*, 1998) and cardiological variables (Roman, 1998). This was further endorsed by a comparison of the per-ratio and allometric scaling methods on the data presented in this study (see Chapter 2.4). These results illustrated that per ratio scaling was less adequate in removing the influence of body size in comparison to that of the allometric scaling model and thus this was adopted.

Consequently, the purpose of this cross-sectional study was to investigate three issues. Firstly, the study assessed the impact of healthy ageing on LV structure and function in sedentary but healthy males and females using 2-D echocardiography with

stringent inclusion and exclusion criteria for cardiovascular disease and habitual activity status. Secondly, this study sought to generate new data related to the effects of healthy ageing upon RV structure and function. Finally, the evaluation of ageing-related changes in LV and RV structure and function was further illuminated through the use of empirically supported scaling procedures to remove the impact of body size and composition.

4.2 Methods

4.2.1 Subjects

Out of over 350 applications received, due to strict inclusion criteria and structured screening (see General Methods), only 124 females and 74 males (age range 20-77 years) were recruited. Written informed consent was obtained from each subject subsequent to a full written and verbal explanation of procedures.

4.2.2 Variables Assessed

For the purpose of this investigation all subjects visited the laboratory on a single occasion. Height (HT, m) and body mass (BM, kg) were assessed by standard techniques. This was followed by a DEXA scan to determine lean body mass [LBM] (see General Methods for specific procedures).

Subjects were then seated quietly for 5–10 minutes in preparation for resting blood pressure evaluation. Blood pressure evaluation was then followed by echocardiographic interrogation. Subjects were asked to lie in lateral decubitus position in order to enhance imaging and all images were recorded at end-exhalation, Two-dimensional echocardiographic views from parasternal and apical acoustic windows were utilised in the assessment of cardiac structure. Variables estimated included LV mass, LV Volume, LV endocardial length, and RV volume. Two-dimensionally generated LV systolic functional measurements included SV and EF. Doppler echocardiography facilitated measurement of diastolic filling (E, A and E:A) in both the LV and RV. Similarly both LV and RV peak outflow velocities were assessed via pulsed-wave Doppler (see General Methods for specific procedures).

4.2.3 Data Analysis

All structural echocardiographic data (LV mass, LV volume, RV volume) were expressed in absolute units and then scaled for individual differences in LBM as determined empirically in Chapter 2.4. All variables were compared between males and females using student T-tests after assessing the normality of distribution via the Kolmogrov-Smirnov test. All variables were then correlated to age, in discrete sex cohorts, using Pearson's Product Moment correlation and equations for lines of best fit were generated through linear regression. Statistical significance was reached at $P<0.05$. Data were analysed using SPSS version 10.

4.3 Results

Anthropometric and resting cardiovascular functional data for males and females are presented in Table 4.1. The mean ages of the two cohorts were not significantly different. Males demonstrated significantly higher values for all anthropometric variables than females with males having on average c.17 kg more LBM than females. Such anthropometric differences further supported the necessity for appropriate scaling of cardiac data. Both systolic and diastolic blood pressure were significantly greater in males than females ($P<0.05$) whereas resting HR was not different between the sexes.

Correlation analysis of anthropometric data revealed that both males and females demonstrated a significant decrease in height, and a significant increase in SBP with age. Although not significant both males and females demonstrated a small increase in body mass with age, however, LBM decreased with age in males (significantly) and females (non-significantly). These patterns of change in LBM again support the importance of scaling of cardiac data in lifespan studies. Females demonstrated a small but significant increase in HR with increasing age with no such association in males.

Table 4.1 Anthropometric and resting cardiovascular data for male and female cohorts. (Data are mean ± SD and Pearson's correlation coefficient, r, with age, *P<0.05, ** P<0.01)

	Males (n= 74)	R	Females (n= 124)	R
Age (years)	48 ± 16		49 ± 13	
Height (m)	1.85 ± 0.07**	-0.25**	1.62 ± 0.06	-0.30**
Body Mass (kg)	80.3 ± 10.1**	0.09	67.5 ± 9.8	0.16*
Lean body mass (kg)	59.5 ± 6.9**	-0.19**	42.9 ± 4.9	-0.05
Systolic Blood Pressure (mmHg)	140 ± 18*	0.39**	124 ± 17	0.47**
Diastolic Blood Pressure (mmHg)	77 ± 8*	0.11	73 ± 9	0.15*
Heart rate ($beats.min^{-1}$)	62 ± 11	0.06	63 ± 9	0.18*

Left ventricular data

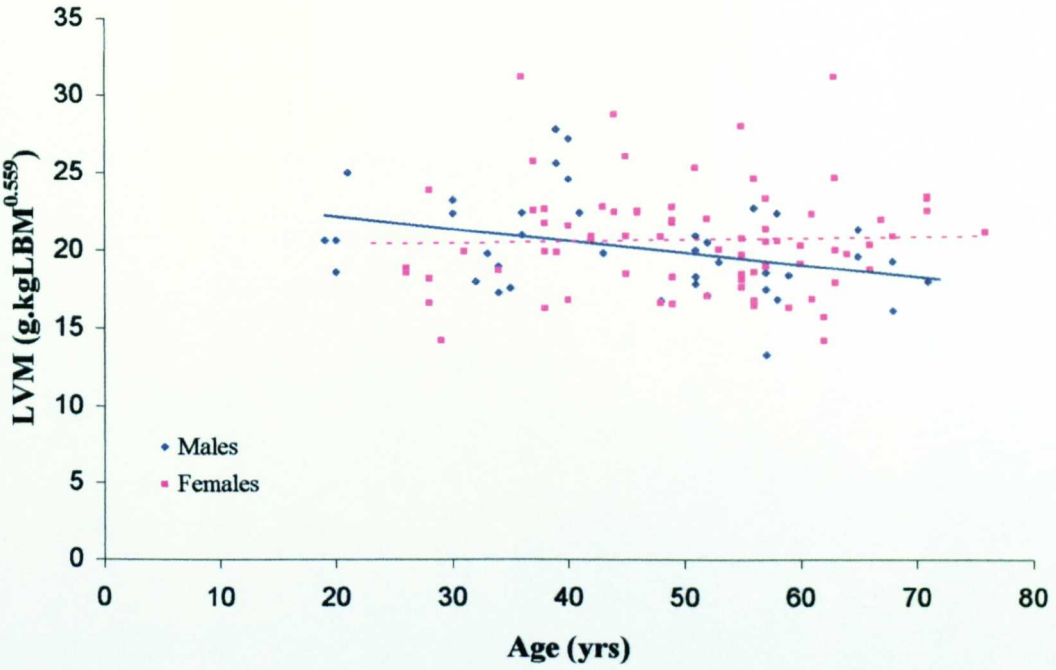
Left ventricular structural and functional data are presented in Table 4.2. Scaled and absolute data are presented for comparison. Absolute LV mass data were significantly larger in males than females ($P<0.05$), however, when scaled for LBM this difference was removed. This pattern was repeated in data for LV volume, LV endocardial length and SV. Ejection fraction and aortic velocity were not scaled.

In the following set of figures the data for LV structural (scaled only) and functional (scaled SV) variables are displayed plotted against age with best fit lines (and regression equations) applied to male and female data separately. A decline in absolute LV mass was significantly associated with age in males but not in females (see Table 4.1 and Figure 4.1). This decline with age was still present in males after scaling for LBM, and the normalisation process had no affect upon the relationship between age and LV mass in the females (see Figure 4.1).

Table 4.2 Left ventricular structural and functional data (Data are mean±SD and Pearson's correlation coefficient, r, with age, * P<0.05, ** P<0.01)

	Males	R	Females	r
LV Mass (g)	195.7 ± 38.8*	-0.24**	169.0 ± 30.9	-0.03
LV Mass ($g.kg.LBM^{0.559}$)	20.2 ± 3.1	-0.36**	20.7 ± 3.4	0.02
LV Volume (ml)	93.8 ± 23.8*	-0.19**	59.5 ± 12.1	-0.38**
LV Volume ($ml.kg.LBM^{1.179}$)	0.76 ± 0.16	-0.13	0.71 ± 0.14	-0.26**
LV endocardial length (cm)	8.65 ± 0.66*	-0.23**	7.22 ± 0.52	-0.14
LV endocardial length ($cm.kg.LBM^{0.390}$)	1.76 ± 0.13	-0.22**	1.67 ± 0.14	-0.11
Stroke volume (ml)	60.5 ± 16.7*	-0.16*	39.2 ± 8.9	-0.31**
Stroke Volume ($ml.kg.LBM^{1.109}$)	0.66 ± 0.15	-0.13	0.61 ± 0.14	-0.22**
Ejection fraction (%)	64 ± 6	0.07	67 ± 7	0.03
Peak aortic flow velocity ($m.s^{-1}$)	1.01 ± 0.18	-0.03	0.97 ± 0.15	-0.30**

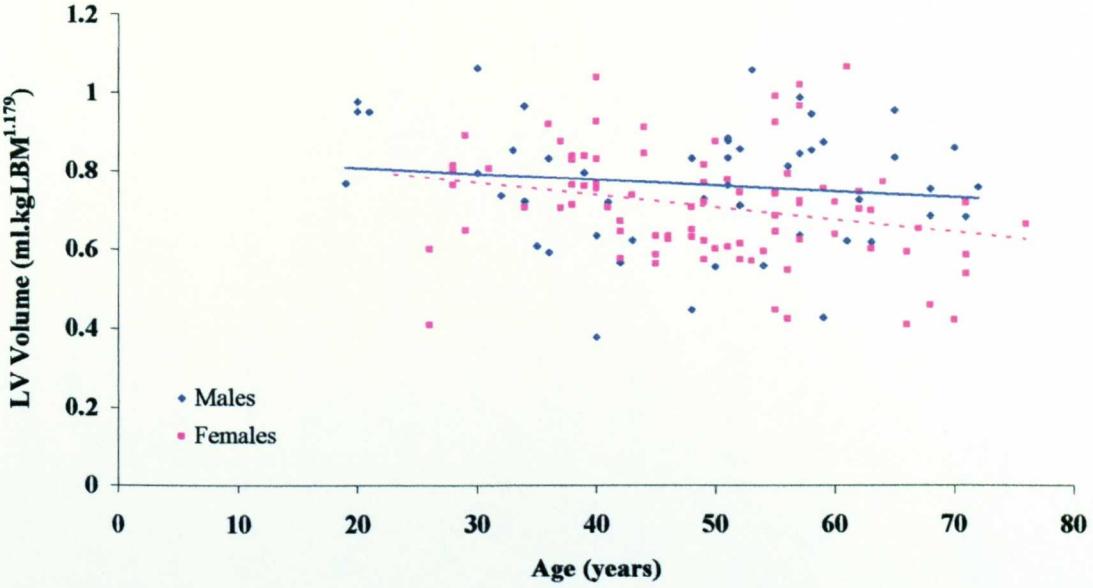
LV-left ventricular



Male: $LVM/LBM^{0.559} = -0.80age + 23.8$; Females: $LVM/LBM^{0.559} = 0.005age + 20.4$

Figure 4.1. Association between scaled LV mass and age in males and females.

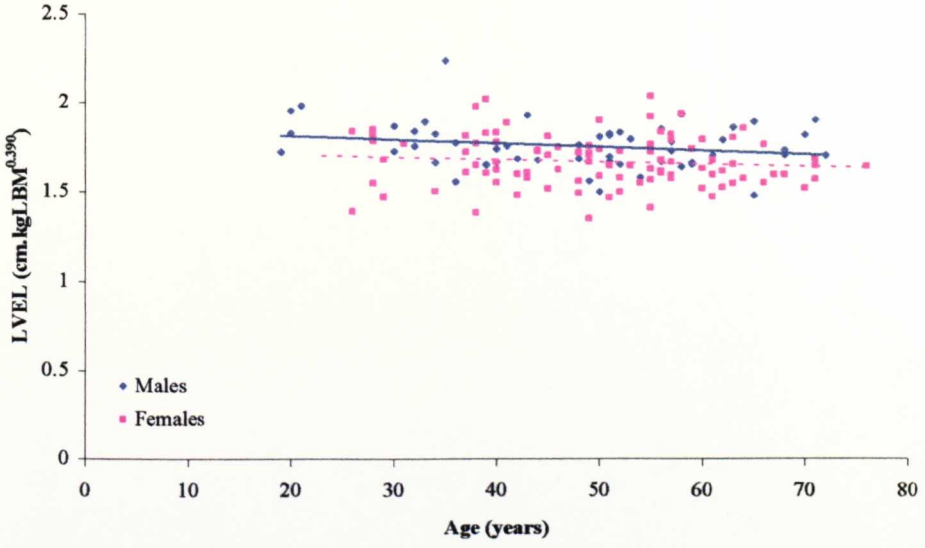
Increasing age was associated with a significant decrease in LV volume in both males and females with a slightly greater decline observed in females (see Table 4.2). After scaling, this relationship was no longer significant in males, however, there remained a significant association between age and declining LV volume in females (see Table 4.2 and Figure 4.2).



Male: $LVV/LBM^{1.179} = -0.002age + 0.839$; Females: $LVV/LBM^{1.179} = -0.003age + 0.857$

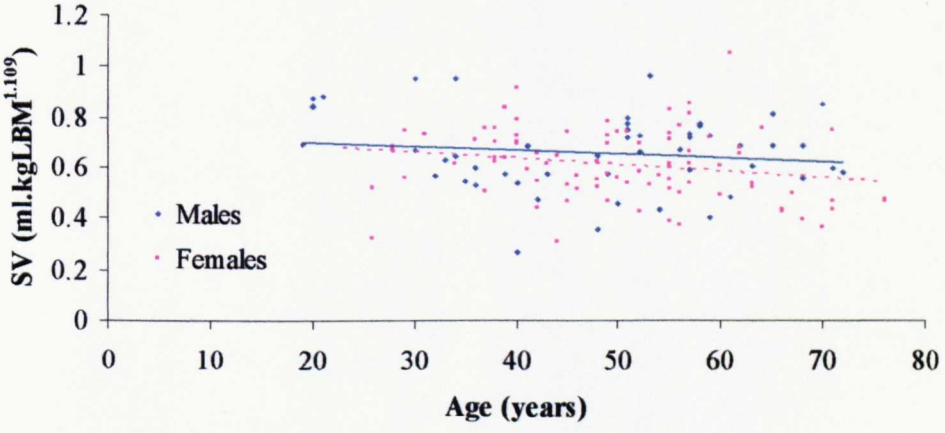
Figure 4.2 Association between scaled LV volume and age in males and females.

The relationship between age and LV endocardial length is presented in Figure 4.3. Although only statistically significant in males there also appears to be a trend towards a decline in females. A small age-related decline in scaled SV, like LV volume, was significant in both males and females (see Figure 4.4). There was no apparent association between age and changes in EF in either males or females (see Figure 4.5).



Male: $LVEL/LBM^{0.390} = -0.002age + 1.856$; Females: $LVEL/LBM^{0.390} = -0.001age + 1.727$

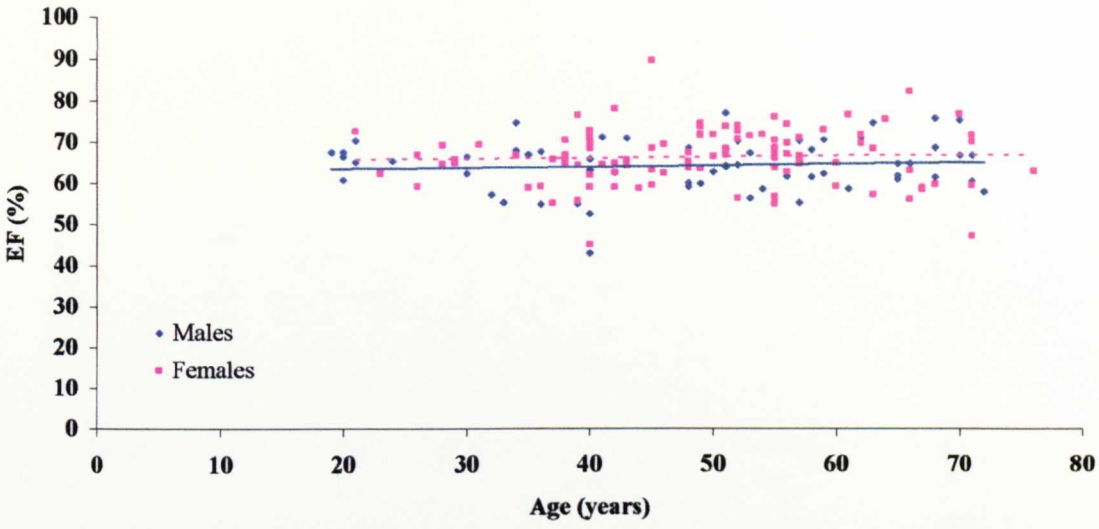
Figure 4.3 Association of scaled LV endocardial length with age in males and females.



Male: $SV/LBM^{0.109} = -0.001age + 0.729$; Females: $SV/LBM^{0.109} = -0.002age + 0.729$

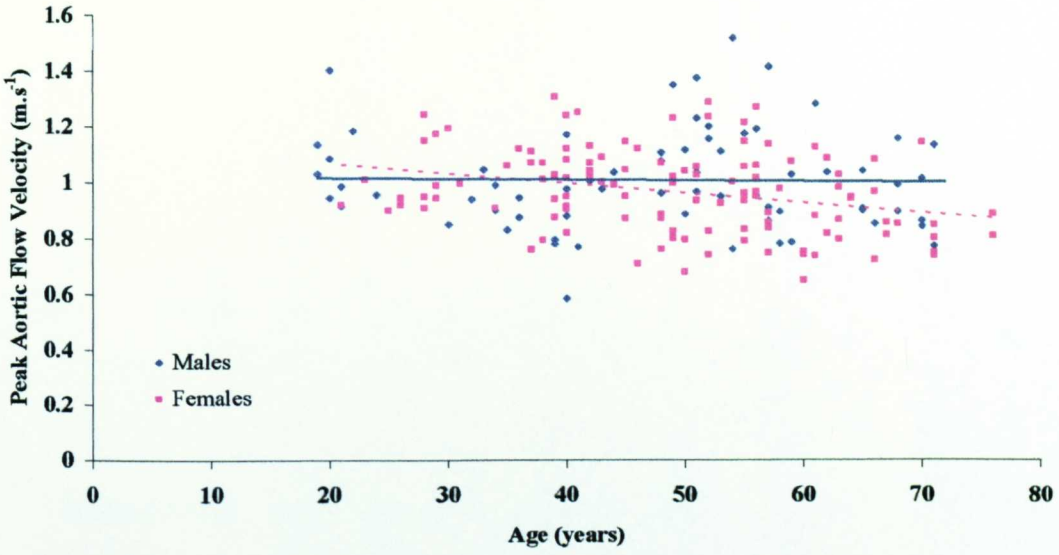
Figure 4.4. Association of scaled SV with age in males and females.

Although there was no difference between males and females for LV outflow velocity (see Table 4.2) similar to EF, it is apparent that there was a significant association between declining peak aortic flow velocity and age in females with no such association observed in males (see Figure 4.6).



Male: EF = 0.026age + 63.1; Females: EF = 0.018age + 65.7

Figure 4.5. Association of EF with age in males and females



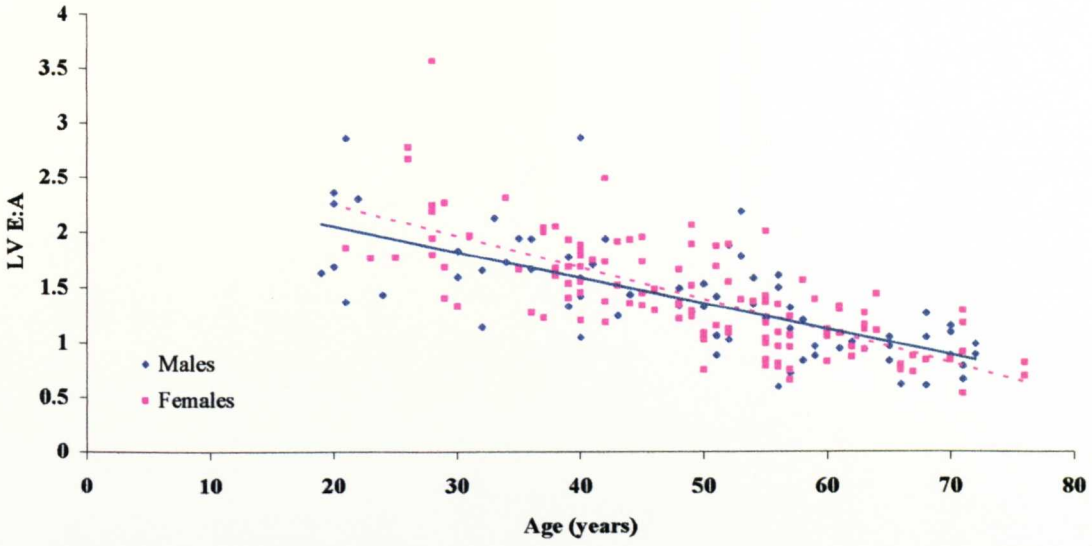
Male: PAFV = 0.00age + 1.032; Females: PAFV = -0.03age + 1.136

Figure 4.6. Association of LV aortic flow velocity and age in males and females.

Diastolic LV data are summarised in Table 4.3. Data for LV inflow are comparable between males and females ($P>0.05$) as well as producing similar correlations with increasing age (for E:A ratio see Figure 4.7).

Table 4.3. Doppler derived diastolic flow data for the LV (data are mean±SD and Pearson's correlation coefficient, r, with age, *P<0.05, **P<0.01)

	Males	r	Females	r
Peak Early filling velocity ($m.s^{-1}$)	0.75 ± 0.16	-0.41**	0.83 ± 0.18	-0.49**
Peak atrial filling velocity ($m.s^{-1}$)	0.59 ± 0.17	0.63**	0.63 ± 0.16	0.54**
Early:Atrial ratio	1.38 ± 0.50	-0.71**	1.42 ± 0.50	-0.73**



Male: LVE:A = -0.023age + 2.52; Females: LVE:A = -0.029age + 2.81

Figure 4.7. Association between LV E:A ratio and age in males and females

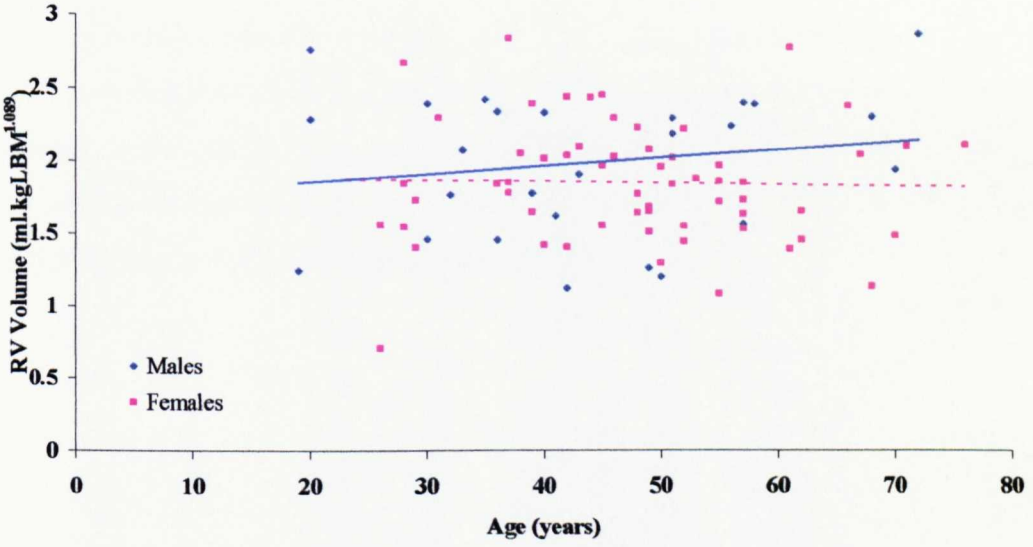
Right ventricular data

Right ventricular volume, peak pulmonary artery blood flow velocity and diastolic filling data are presented in Table 4.4. Males and females demonstrated similar mean values for all RV variables with the exception of absolute RV volume, which was significantly greater in males ($P<0.05$). As with LV data, scaling RV volume for LBM eradicated any sex difference.

The association between age and absolute RV volume was not significant in males and females (see Table 4.4). After scaling the association between age and RV volume was such that RV volume slightly increased with age in males but not in females (see Figure 4.8).

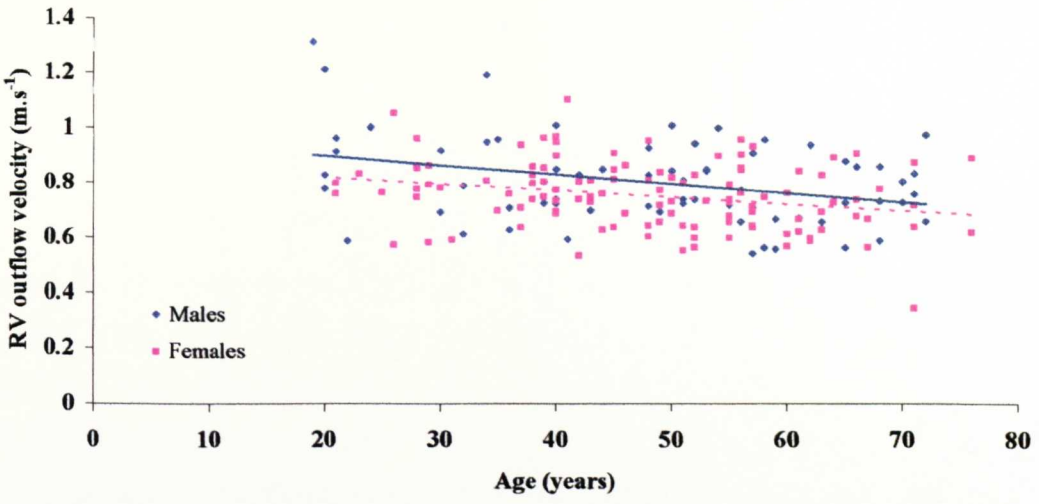
Table 4.4. Right ventricular volume and Doppler data (data are mean±SD and Pearson's correlation coefficient, r, with age,*P<0.05, **P<0.01)

	Males	r	Females	R
RV Volume (ml)	167.3±49.5*	0.03	108.9±25.4	-0.13
RV Volume ($ml.kgLBM^{1.089}$)	1.98±0.49	0.17*	1.85±0.41	-0.03
Peak Early filling velocity ($m.s^{-1}$)	0.65±0.14	-0.69**	0.66±0.12	-0.59**
Peak Atrial filling velocity ($m.s^{-1}$)	0.46±0.10	0.10	0.49±0.11	0.15*
Early:Atrial filling ratio	1.50±0.49	-0.53**	1.43±0.40	-0.53**
Peak pulmonary artery velocity ($m.s^{-1}$)	0.80±0.16	-0.33**	0.75±0.12	-0.26**



Male: $RVV/LBM^{1.089} = 0.005age + 1.742$; Females: $RVV/LBM^{1.089} = -0.001age + 1.895$

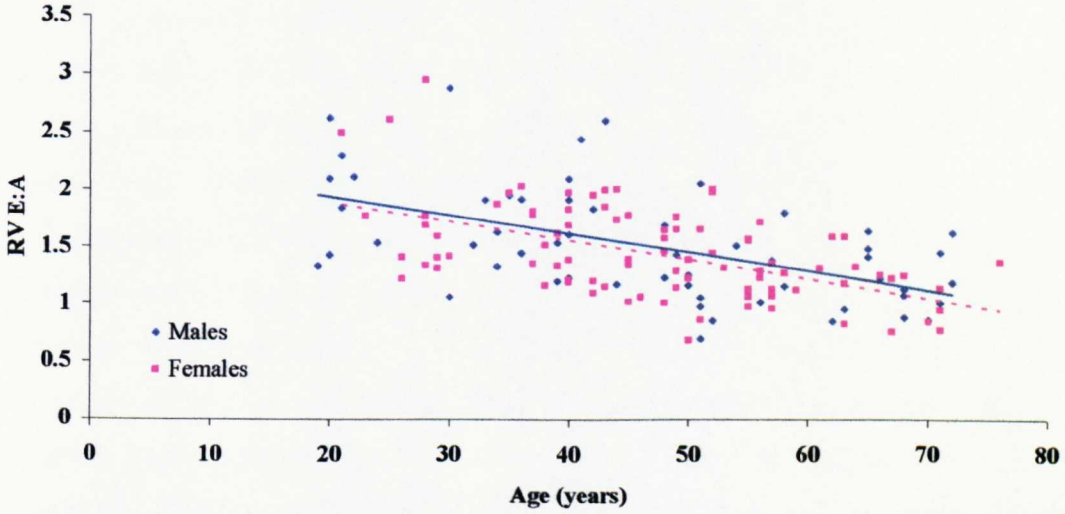
Figure 4.8 Association between scaled RV volume and age in males and females.



Male: PPFV = -0.003age + 2.027; Females: PPFV = -0.009age + 2.289

Figure 4.9 Association between RV outflow velocity and age in males and females.

Right ventricular outflow (see Figure 4.9) significantly declined with age in both males and females. Mean RV diastolic filling data, as with LV, were comparable between males and females. Likewise, the impact of ageing upon RV Doppler data was similar to that observed in the LV; with a significant decline in RV E:A with increasing age in both males and females (see Figure 4.10).



Male: RVE:A = -0.018age + 2.34; Females: RVE:A = -0.017age + 2.23

Figure 4.10 Association between RV E:A ratio and age in males and females.

4.4 Discussion

Left ventricular changes with ageing

Data for the impact of ageing on LV mass from the current study report a c.10% decline in men between the ages of 20 and 75 years with no change in women. This is generally in agreement with previously reported MRI and autopsy data (Olivetti *et al.*, 1991; 1995; Hees *et al.*, 2002). The data presented are at odds with previous M-mode echocardiography studies of ageing (Henry *et al.*, 1978; Gardin *et al.*, 1979; Dannenburg *et al.*, 1989; Pearson *et al.*, 1991; Shub *et al.*, 1994; Slotwiner *et al.*, 1998) and this can likely be explained by the limitations of the M-mode technique compared to 2-D echocardiography. The geometric assumptions of 2-D are substantial improvements over the "cubed" formulae for LV mass estimation used in M-mode studies (Devereux and Reichek, 1977). Whilst still not assessing the LV mass in three-dimensions (3-D) as occurs in MRI and newer 3-D echocardiography, 2-D echocardiography has validated geometric models that progress towards MRI data from original M-mode approaches. As 2-D echocardiography is still the most common clinical scanning mode it is important that such agreement exists between 2-D derived data and MRI-derived data in the ageing and cardiovascular literature.

Although it is difficult to provide an explanation for the sex-based differences in LV mass change with age, previous autopsy studies may shed some light. Olivetti *et al.* (1991, 1995) suggested a decrease in myocyte number that is associated with cell necrosis as a potential explanation for the decline in LV mass with age in men. It was also suggested that although a compensatory hypertrophic mechanism of existing myocytes seems to exist in males, it does not fully compensate for cell loss and therefore explains decreased heart weight in males with age. In females, however, the total number of myocytes stayed essentially the same from ages 20-95 years. Possible mechanisms suggested for this have included female sex hormones (Shub *et al.*, 1994), however, with no tendency for cell loss in females from ages 55-95 years this explanation seems unlikely. Other mechanisms are difficult to identify, however, the investigation of intrinsic myocardial properties in animal models may shed some light on this important issue. At a cellular level a number of factors may be important in underpinning the gender differences in structural responses of the LV to ageing. There is a general decrease in transcription of genes controlling protein synthesis, the

β-adrenergic system and the interaction of these factors with some, as yet unclarified or undetermined, gender difference may be responsible for the age-related data observed. What is apparent is that cellular factors may become more important in the clear explanation of phenotypic differences in the responses to ageing and other physiological processes (e.g. exercise) as our knowledge of genes (genomics) and important regulatory cellular proteins (proteomics) becomes clearer due to recent technical advances.

Interestingly, data for LV volume in the current study demonstrated a decrease in males (c.10%), whereas the decline in females was much bigger (c.25%). Such data are rarely reported in echocardiographic studies whose primary outcome variable has normally been LV mass. Combined with age-related changes in LV mass, already discussed, these data would suggest that remodelling of the LV with progressive ageing is different in males and females. With maintenance of LV mass and a reduction in LV volume mean LV wall thickness must be increasing with age in women, but not in men. This finding agrees with previously reported autopsy data (Olivetti *et al.*, 1995), and is a testament to the ability of 2-D echocardiography to accurately measure LV mass and volume changes observed with age (Hees *et al.*, 2002). Hees *et al.* (2002) reported a decrease in LV mass in males predominantly caused by a decrease in LV endocardial length. Although a decrease in LV endocardial length was apparent but to a smaller extent in females, it was accompanied by an increase in wall thickness. In males, the decline in LV mass and LV volume with age again suggests that hypertrophy did not compensate for age-related myocyte loss.

The clinical relevance of this data is impossible to specifically determine but some postulation is possible. Firstly, when interpreting age-related changes in LV mass it must be clear that appropriate imaging and data handling (scaling) have been utilised otherwise the conclusions reached may be flawed. Secondly, the decrease in LV mass in males, compared to the maintenance in LV mass in females, may reflect a process of age-related degeneration in cardiac mass that potentially leaves the male heart at more risk if a pathological process (e.g. CAD) is superimposed upon the ageing.

Absolute stroke volume was both lower in females compared to males and a decline in SV was significantly associated with age irrespective of sex. The sex-based difference is likely a primary consequence of a smaller heart size due to differences in body size and this is supported by the scaled SV data which eradicates the difference. The negative correlation of SV to age, in both absolute and scaled is likely explained by the combination of a decrease in LV volume with age and no change in EF. Thus both males and females report lower SV as they get older because of decreasing LV filling and thus preload (see E:A data). The current data are at odds with previous echocardiographic studies that reported no change in SV with age (Merino *et al.*, 1988; Pearson *et al.*, 1991: Slotwiner *et al.*, 1998). However, their data are estimated from one-dimensional M-mode traces and thus further substantiates the purpose of this investigation and it's relevance in questioning the use of M-mode echocardiography when investigating the ageing consequences for the structure and function of the LV.

Despite the recent interest in systolic performance indices of the LV within MRI studies (Lorenz *et al.*, 1999; Sandstede *et al.*, 2000) the inclusion of these variables, particularly EF, in routine clinical diagnostics justifies their assessment within this investigation. This rationale is also supported by the remaining question mark over sex and age-related differences in EF (Merino *et al.*, 1988; Gardin *et al.*, 1995; Slotwiner *et al.*, 1998; Celentano *et al.*, 2003). The current data suggest that there is no affect of sex upon EF and this supports MRI (Lorenz *et al.*, 1999) and echocardiographic (Merino *et al.*, 1988) literature. The impact of ageing upon EF in males and females was negligible which is at odds with a study by Gardin *et al.* (1995) that reported a decrease in EF in both males and females with ageing. Ruan and Nagueh (2005) utilised the recently validated acceleration rate during isovolumetric contraction, which is much less affected by loading conditions and reported that all indices of systolic function had no significant correlation with age, therefore also supporting our findings.

As well as differences in M-mode (Gardin *et al.*, 1995) and 2D assessment of EF, it may also be that the process of pre-assessment health and physical activity screening may explain some of these discrepancies. Health screening measures in previous studies may have not been particularly stringent for the developing CHF/CAD in

older populations which could lead to a reduction in EF. We can be relatively confident that the current study employed sedentary males and females with limited underlying cardiac pathology due to the strict inclusion criteria and screening procedures employed. The lack of sex difference or change in EF in this study with increasing age suggests that regardless of a decline in myocyte number in males, both males and females are able to maintain LV contractility.

The impact of age upon peak LV outflow velocity has rarely been studied with research groups concentrating on variables such as EF within the heart or parameters such as the loss of aortic elasticity with increasing age, using techniques such as pulse wave velocity (Fried *et al.*, 1991; Gates *et al.*, 2002). In this study peak aortic outflow velocity demonstrated no mean sex differences and the changes with healthy ageing were quite small with a tendency for a decline to be observed in the female cohort. This difference is difficult to interpret but may be biologically meaningless especially in light of the maintenance of EF across the age range in both sexes however; further investigation of this variable in healthy ageing is warranted. Data for age-related changes in EF and aortic flow velocity are likely to be similar because they will both be similarly affected by age-related changes in preload or afterload.

No sex differences in E, A and E:A were reported in our data and this confirms previous studies. In a sample size of over 3000 Schirmer *et al.* (2000) found no sex-related differences in early and late filling velocities. In a recent review article, Oxenham and Sharpe (2003) described age-related changes in transmitral filling dynamics. The gradual, progressive decline in the early/passive filling phase was characterised by a 50% decrease in the E wave velocity, with a concomitant 40% increase in atrial/late velocity wave between the ages of 30 and 70 years. The current study supports this statement and agrees with a range of original research studies (Kitzman *et al.*, 1991; Wandt *et al.*, 1998; Gardin *et al.*, 1998; Schirmer *et al.*, 2000). The basis for age-related changes in LV filling dynamics remains to be fully elucidated, however multiple regression analysis by Pasierski *et al.* (1991) reported that in normal subjects transmitral flow indexes were related to age, increases in relative wall thickness and increases in systolic blood pressure but not related to heart rate. Burslew (2004) suggested that a significant factor in the change in diastolic function in the elderly could be the maladaptive remodelling of interstitium resulting

in an increase in interstitial collagen content. As a result of this increase in myocardial collagen diastolic suction is compromised and LV diastolic pressure increases. Another explanation of the underlying mechanisms of this could be the result of a slowing rate of LV pressure decline characterised by a prolongation of isovolumetric relaxation time with a concomitant reduction in early diastolic suction (Thomas and Weyman, 1991), however, Hees *et al.* (2004) postulated that changes in passive or active LV filling properties could be related to a reduced early diastolic LA pressure rather than a slower LV pressure decline.

Right ventricular changes with ageing

Data for RV volume have often been difficult to compile and interpret. This has been primarily due to difficulty in obtaining optimal image clarity when attempting to image the RV. As a consequence echocardiographic studies have tended to concentrate on the LV with fewer RV studies. The current study suggests that absolute RV volume is significantly bigger in males than females. This is likely a consequence of the bigger body size of males and this is substantiated by the lack of a sex difference in the scaled RV volume data. Correlation data suggest that both absolute and scaled RV volume do not change with age in women and in men only the scaled data show a small but significant increase with progression of age. There are no comparative echocardiographical studies but Sandstede *et al.* (2000) reported a significant decline in MRI determined RV volume with age, regardless of sex. Again differences in subject population may account for minor disparities. The fact that RV volume does not decline with age in women whereas LV volume does is somewhat surprising. An explanation for this is beyond the scope of the current study but is worthy of further research. One possible clinical explanation for the lack of decline in RV volume could be the presence of some degree of pulmonary disease (asymptomatic), the prevalence of which is known to increase with age (Sin and Man, 2005). Finally, on the basis that we cannot discount the influence of inflated measurement error in echocardiographic imaging of the RV as a partial explanation for our results future research should adopt MRI as the gold standard imaging tool.

Both males and females demonstrated a similar but significant decline in pulmonary outflow velocity with age, which possibly substantiates the possible existence of some

pre-clinical pulmonary disease. This may be related to the altered RV tricuspid flow but also warrants further investigation, possibly using tissue Doppler techniques.

Data for RV diastolic filling and pulmonary artery outflow velocities were not different between males and females. The current study suggests, similar to the LV, that RV E:A declines in a similar fashion with age in both males and females. However, RV diastolic filling remains widely unreported in studies of healthy ageing humans despite RV diastolic dysfunction being reported in ischemic heart disease. Again of comparative interest, but using a different scanning mode, recent tissue Doppler data (Kukulski *et al.*, 2000) has suggested that there may be different responses in the RV and LV to increasing age. This would suggest the necessity for further studies assessing the impact of ageing upon RV diastolic function in males and females.

4.5 Limitations

The limitations to this study are similar to many previous ageing studies. It was difficult to recruit significantly large numbers of subjects, especially males in this cohort, particularly when inclusion and exclusion criteria were strict and rigorously applied. However, this study has provided a useful template for similar and on-going assessment of the response of the heart to healthy ageing.

4.6 Conclusions

This study demonstrated that when investigating the influence of ageing and sex upon cardiac structure and function the use of 2-D echocardiographic methods are informative and can provide similar data to autopsy and MRI. This supports the utility of 2-D echocardiography in on-going age-related research. Ageing is associated with a change in cardiac structure with advancing age in both males and females however this remodelling occurs differently in the sexes and this requires further investigation. Whilst there are age-related changes in preload that lead to a reduction in SV, contractility remains essentially unchanged in both males and females with age. Diastolic filling is affected by the ageing process in a similar manner in both sexes.

Echocardiographically determined RV structure and function are prone to some technical limitations in a proportion of subjects due to anthropometric and anatomic variability, even with 2-D echocardiographic analysis. Although reliability for this study places confidence in the data limitations should be taken into account. However, changes in the RV function seem to mirror age and sex related changes in the LV although further studies should seek to confirm or refute these findings.

Finally, scaling cardiac data has a substantial affect on data interpretation. Certainly scaling removed most of the sex-related differences in mean cohort data. It is important to always utilise the most appropriate scaling method and variable to arrive at confident conclusions as to the impact of ageing and sex upon cardiac structure and function.

5 The impact of a progressive intensity, 12-month endurance training programme upon left and right ventricular structure and function in post-menopausal women.

5.1 Introduction

As the initial study in this thesis has verified normal healthy ageing is associated with changes in cardiac structure and function. Due to rising numbers of people aged 65 years and over, and the increasing prevalence of cardiovascular disease in this population, it seems pertinent to investigate ways of delaying or even reversing this ageing process that may serve to increase functional capacity, cardiovascular function and quality of life. In recent years, exercise physiologists, gerontologists and other health professionals have studied aerobic exercise as a possible intervention to offset the age-related decline in cardiovascular function (Green and Crouse, 1993).

Data from both cross-sectional and longitudinal studies in males suggest that endurance exercise can improve cardiovascular structure and function as well as work capacity in older men (Douglas *et al.*, 1986; Douglas and Toole, 1992; Makrides *et al.*, 1990; Levy *et al.*, 1993; Stratton *et al.*, 1994; Spina *et al.*, 1996). These studies have demonstrated a range of cardiac structural adaptations to training in older men including increases in left ventricular wall thickness, LV mass and proportional increases in end-diastolic diameter and LV wall thickness, suggesting eccentric remodelling of the LV (Makrides *et al.*, 1990; Douglas *et al.*, 1986; Douglas and O'Toole, 1992). In addition endurance training has induced improvements in systolic and diastolic function (Levy *et al.*, 1993; Spina *et al.*, 1996).

Despite ample evidence of the positive cardiovascular benefits of endurance training in older men there is limited and contradictory data available in women (Spina, 1999). One of the first studies to investigate endurance-training effects on the cardiovascular system of postmenopausal women was that of Morrison *et al.* (1986). This M-mode echocardiographic study demonstrated significant increases in LV volume, EF and $\dot{V}O_{2max}$ after an 8-month aerobic training programme. More recently, however, a number of research studies have reported no central cardiovascular adaptations to training in post-menopausal women (Spina *et al.*, 1993; 1996; Park *et al.*, 2003; Haykowsky *et al.*, 2005). In training studies of 12-weeks (Haykowsky *et al.*, 2005),

8-months (Spina *et al.*, 1993) and 9-months (Spina *et al.*, 1996; Park *et al.*, 2003) significant increases in aerobic capacity were not accompanied by any changes in resting LV structure and function. Spina (1999) concluded that any increase in maximal oxygen uptake with training in older women was only associated with peripheral adaptations evidenced by widening of arterio-venous oxygen content difference.

The discrepancies evident in existing data may be a consequence of study differences in echocardiographic mode of scanning, training programme duration, exercise intensity and progression employed as well as potential sample differences. Despite known limitations, M-mode echocardiography has been employed to assess LV structure and function in some ageing studies (Morrison *et al.*, 1986; Haykowsky *et al.*, 2005). Spina *et al.* (1993, 1996) utilised radionuclide angiography which provides accurate LV volume and functional data but cannot detail LV structural responses to training. Whilst Park *et al.* (2003) performed 2-dimensional echocardiographic scans to assess LV structure they provided no Doppler interrogation of LV inflow or outflow blood velocity profiles. Therefore, in the current study we chose to combine 2-D and Doppler echocardiography to provide the most complete picture of LV structural and functional responses to aerobic training and also to determine if any LV adaptation was related to changes in functional capacity ($\dot{V}O_{2max}$).

Data investigating the RV response to endurance training are limited. In younger male and female subjects Henriksen *et al.* (1999) reported similar LV and RV structural adaptations to training. In older subjects only cross-sectional data from male athletes and controls exist (Owen *et al.*, 2004) that reported no differences in both LV and RV diastolic function. The response of the RV to endurance training has never been examined in post-menopausal females.

Therefore the purposes of this study was to investigate whether post-menopausal females can improve aerobic capacity and alter resting LV and RV structure or function as a result of a progressive 12-month aerobic exercise training programme using 2-D echocardiography. Secondarily the research investigated the potential link

between changes in functional capacity and alterations in resting LV structure and function.

5.2 Methods

5.2.1 Subjects

Fifty three healthy, sedentary post-menopausal women (59 ± 3 years) volunteered for this study. All subjects provided written informed consent. The criteria for inclusion in this study included 1) aged between 55-65 years, 2) postmenopausal ≥1 year, 3) sedentary, 4) no apparent underlying cardiovascular disease or medication usage (see General Methods). In order to accommodate likely drop-outs 36 subjects were recruited for exercise training and 17 were recruited as age-matched non-exercising controls for comparison. Subjects were randomly allocated to either control or exercise groups. There was a larger number allocated to the training group due to a likelihood of a higher drop out rate in this group.

5.2.2 Design

In the training group (mean age 60 ± 3 years) all measurements were taken at baseline and repeated after 6 weeks, 12 weeks, 24 weeks, 36 weeks and 48 weeks of exercise. In the control group (mean age 61 ± 4 years) measurements were taken at baseline and after 24 and 48 weeks.

5.2.3 Variables assessed

Height and BM data were recorded by standard procedures. As detailed in the general method LBM was estimated from whole body DEXA scans. Due to technical difficulties, however, it was not possible to obtain LBM measurements in the training group at the 24 week mark.

After a 10 minute period of seated rest, blood pressure was taken via automated sphygmomanometer followed by echocardiographic interrogation with simultaneous ECG. Two-dimensionally measured structural variables included LV mass, LV volume, LV length and RV volume. Systolic functional variables assessed were ejection fraction (EF) and stroke volume (SV). Diastolic filling was measured via

Doppler echocardiography and included early and atrial peak filling velocity (E:A ratio) in both the LV and RV as well as pulmonary and aortic peak outflow velocity (see General Methods).

Following 30 min of supine rest, during which resting HR was assessed, subjects underwent a graded exercise test to exhaustion. At peak exercise the highest HR achieved was recorded as maximal HR for exercise intensity calculations (as detailed in General methods).

5.2.4 Exercise Programme

Training programmes are difficult to administer, particularly in older sedentary populations, due to the necessity to familiarise participants and start at low exercise intensities/volumes. Furthermore, there are no specific published guidelines for exercise mode, intensity, duration (volume), progression as well as the length of the training programme. Recent studies in older sedentary populations have thus used diverse exercise programmes but seem to be consistent in implementing a simple, and transferable exercise programme consisting of walking and/or cycling initially at a low-moderate intensity (30% VO_2 reserve) between 3-5 times per week (Asikainen *et al.*, 2002; Swain and Franklin, 2002; Park *et al.*, 2003; Haykowsky *et al.*, 2005). These principles were adopted in the current study and the use of a progressive 12-month training regime added, uniquely, to the current literature base.

The mode of exercise employed included both walking/running on a treadmill, and cycling on a cycle ergometer for each 30 min session (20 min walk/run 10 min cycle). Subjects were advised on stretching pre and post-exercise. Exercise intensity was initially set at 30% heart rate reserve (HRR). This was a low intensity in order to familiarise participants with equipment and avoid injury and minimise dropout. Subjects were taught the use of a standard polar heart rate monitor, to ensure they exercised to the correct predetermined HR. At 12 weeks intensity was increased to 45% HRR, at 24 weeks intensity was increased to 60% HRR, and at 36 weeks to 75% HRR. Each progression occurred subsequent to a maximal graded exercise test and a new assessment of resting and maximal HR. Initially 3 sessions per week was considered sufficient to introduce the subjects to the exercise protocol and again familiarise the participants with the training protocol, and minimise dropout. After 6-

weeks, exercise frequency was increased to 5 times per week at the same intensity, the additional sessions were either at the gym or walking, running and cycling outside. All sessions were recorded in an exercise diary including details of any problems experienced as well as comments about HR achieved. At the 12-week mark subjects were allowed the use of a total of 11 gyms around the city of Liverpool with a key-card system to perform their exercise sessions. They reported at the University gym at least once per week and carried out subsequent sessions in their own time at the gyms or at home. The choice of this training model, we felt, increased the chance of adoption into the subject's regular lifestyle and therefore would result in the continuation of exercise after conclusion of the study.

Control subjects completed the same testing protocols as exercise participants at baseline, 24 and 48 weeks and were asked to continue with normal activity and dietary habits. The reduced testing requirement was deemed vital to maintain subject involvement in a group receiving no exercise training. It was not deemed scientifically or physiologically vital to test every 12 weeks as age-related changes would be minor over this time span.

5.2.5 Data analysis

Statistical analysis was performed using the SPSS Statistical Software version 12.0 (SPSS, USA). Data were expressed descriptively as mean ± SD. A one-way analysis of variance was used to assess differences across time in the training group at all testing times with Bonferroni post-hoc analysis completed if significant F-ratios were reported. Due to the different number of repeat assessment times between controls and the training group a comparison of groups was employed, via two-way ANOVA for data obtained baseline, 24-weeks and 48-weeks. Due to the nature of this statistical analysis any graphical representation of results will show only baseline, 24 and 48 weeks data. Any markedly different data at 6, 12 or 36 weeks in the training group would be described in the text and/or tables. Pearson product-moment correlations were used to assess the degree of association between changes in $\dot{V}O_{2max}$ and LV volume or SV within the training group.

5.3 Results

Of the 36 women recruited for the training study 16 did not complete the entire 12 months (although 28 women completed up to 6 months). Reasons for this included family and work commitments, lack of time, problems with travel to various gyms, two ladies due to pains whilst exercising (one back, one knee) and finally one lady left due to a close family death. Surprisingly there was a higher drop out in the control group than expected. This was due to back problems from two ladies rendering them unable to exercise, one lady was injured from a mugging, one lady moved away from the area and the remaining three ladies chose not to return due to lack of time and motivation. Therefore final number for the training and control group were 20 and 9, respectively. Despite notable individual variability there were no significant differences in baseline data between groups for BM, LBM blood pressures or resting heart rate ($P>0.05$). There were no significant changes in BM, LBM, blood pressure or HR across assessments in the control group ($P>0.05$, see Table 5.1). Despite a trend for a reduction in BM, BP and HR there were no significant changes in these variables in the exercise group ($P>0.05$).

Table 5.1 Basic anthropometric and cardiovascular data for the training and control groups

Variable	T/C	Baseline	6 wk	12 wk	24 wk	36 wk	48 wk
BM (kg)	**T**	69.4 ± 10.9	68.6 ± 8.3	67.3 ± 8.9	66.9 ± 8.7	67.4 ± 9.1	67.3 ± 7.8
	C	66.1 ± 10.9			66.6 ± 8.7		66.8 ± 7.7
LBM (kg)	**T**	42.6 ± 4.3	40.9 ± 8.6	41.7 ± 3.9		40.9 ± 2.6	42.2 ± 3.4
	C	40.6 ± 4.3			40.1 ± 4.1		40.7 ± 3.4
SBP (mmHg)	**T**	127 ± 14	122 ± 17	118 ± 13	117 ± 13	124 ± 11	123 ± 13
	C	117 ± 19			117 ± 18		116 ± 11
DBP (mmHg)	**T**	72 ± 8	69 ± 8	70 ± 8	67 ± 8	70 ± 8	72 ± 8
	C	67 ± 12			66 ± 9		63 ± 6
HR (beats.min^{-1})	**T**	66 ± 8	63 ± 9	63 ± 7	60 ± 7	63 ± 10	61 ± 10
	C	66 ± 12			68 ± 7		69 ± 8

T - training group, **C** - control group, **wk** - weeks, **BM** - body mass , **LBM** - lean body mass, **SBP** - systolic blood pressure, **DBP** - diastolic blood pressure, **HR** - heart rate.

All subjects attained at least 2 criteria for reaching $\dot{V}O_{2max}$ and thus data were considered as max and not peak. $\dot{V}O_{2max}$ was not significantly different between groups at baseline ($P>0.05$, see Figure 5.1). A significant time by group interaction ($P<0.05$) was found reflective of a progressive and significant increase in $\dot{V}O_{2max}$ in the exercise group that represented an increase of c. 9 $ml.kg^{-1}.min^{-1}$ (30%) over the 12 months. In the control group $\dot{V}O_{2max}$ increased ($P>0.05$) from 24-28 $ml.kg^{-1}.min^{-1}$ over the first 6 months and then did not change at 12 months whereas $\dot{V}O_{2max}$ in the exercise group was significantly greater than in the control group ($P<0.05$).

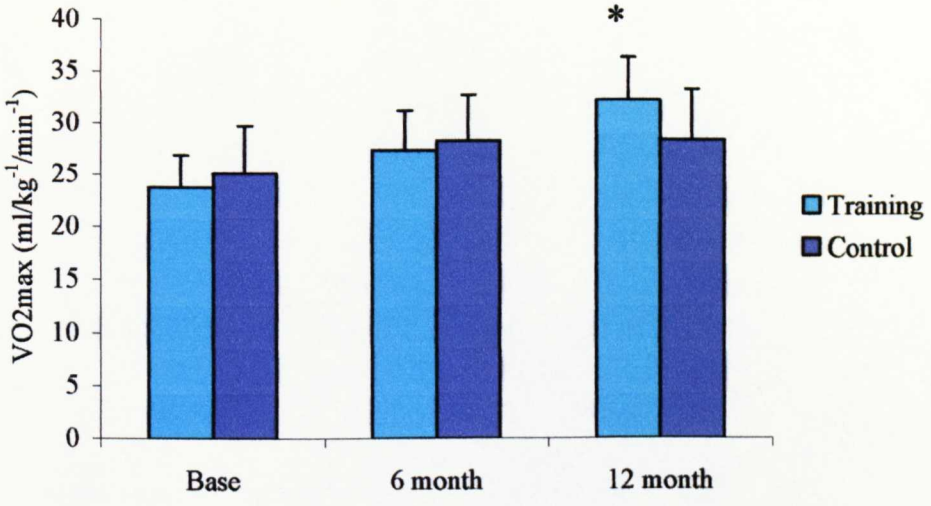


Figure 5.1 $\dot{V}O_{2max}$ changes in the training and control groups (*P<0.05)

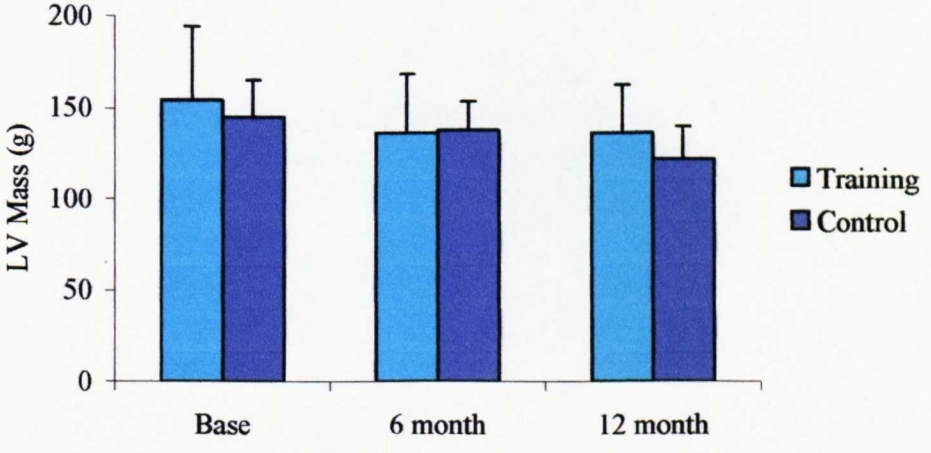


Figure 5.2 LV mass over the 12-month period in the training and control groups.

There were no significant group differences in LV mass at baseline (P>0.05) between exercise and control groups (see Figure 5.2). Despite individual variability and small changes in group mean data, LV mass did not significantly alter in either group over 12 months (P>0.05).

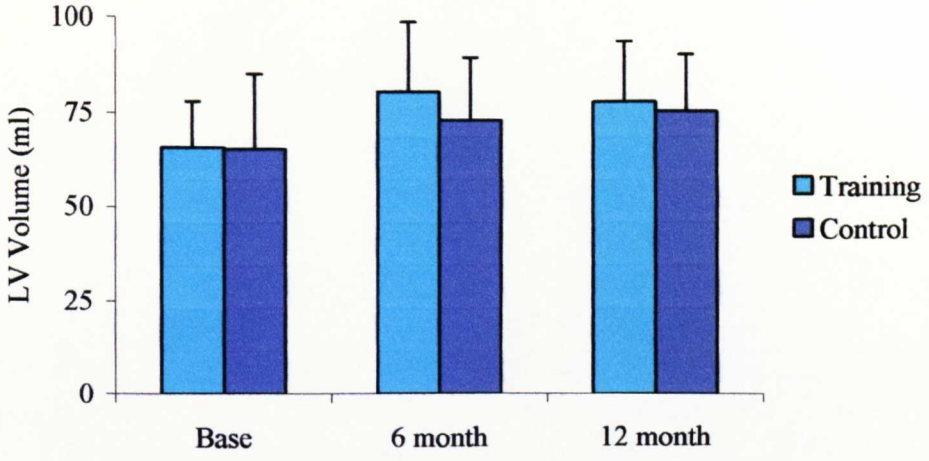


Figure 5.3 LV Volume over the 12-month period in the training and control groups.

LV volume was not different between control and training group at baseline (P>0.05, see Figure 5.3). Despite a non-significant (P=0.11) time by group interaction there was evidence of an increase in LV volume from baseline to 24 weeks. This was supported by a significant main effect of time (P<0.05) as well as when analysis of variance was run on the training group only. The increase of c.19% at 24 weeks was not further advanced at 48 weeks. Interestingly, LV volume also increased in the control group to a lesser extent (13%), contributing to the main effect for time. This change was just outside limits of reliability for this variable.

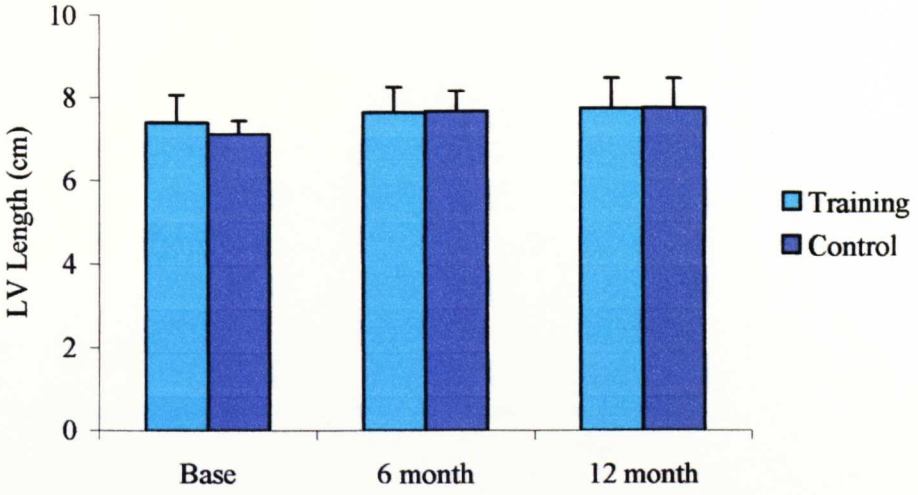


Figure 5.4 LV length over the 12-month period in the training and control groups

LV length was not significantly different between groups at baseline (P>0.05, see Figure 5.4). Neither group demonstrated any significant change in LV length over the training period (P>0.05).

Data for LV systolic and diastolic functional parameters in both the trained and control group over the 12-month period are presented in Table 5.2. There were no significant differences in SV between the control and exercise groups at baseline (P>0.05). Despite an increase in SV of 8 ml (within limits of reliability) from baseline to 48 weeks there was no significant change with training (P>0.05). A smaller change of 3 ml was observed in the control group (P>0.05). There was no significant difference in EF between groups at baseline (P>0.05). Although EF did not significantly alter in the training group over the 12 month period (P>0.05) there was a significant main effect for group (P<0.05), probably due to the decrease in EF in the control group to 65% at 48 weeks from a baseline of 68%. This change is within the limits of the repeatability for this variable and is therefore likely to be statistically and biologically meaningless.

There was no change in E or A filling velocity or E:A ratio over time in either the control or training group. However, E and E:A ratio were significantly, and

consistently lower in the control group (12% and 16%, respectively, $P<0.05$). There was no change in aortic flow velocity over time in either the control or training group ($P>0.05$). However, mean data were significantly, and consistently lower in the control group (6%, $P<0.05$). These values are within reliability limits for this technique in an ageing population.

Table 5.2 Left ventricular functional data over the 12-month period in the training and control groups (no significant effects for time or group were recorded).

Variable	T/C	Baseline	6 wk	12 wk	24 wk	36 wk	48 wk
SV (ml)	T	44.7 ± 8.2	48.2 ± 6.7	53.8 ± 10.8	55.0 ± 15.1	57.4 ± 9.5	52.7 ± 11.3
	C	43.7 ± 18.1			46.2 ± 10.0		46.1 ± 9.6
EF (ml)	T	69 ± 5	68 ± 5	71 ± 4	70 ± 4	71 ± 3	71 ± 3
	C	68 ± 4			68 ± 5		65 ± 3
E ($msec^{-1}$)	T	0.81 ± 0.18	0.79 ± 0.19	0.76 ± 0.20	0.77 ± 0.17	0.75 ± 0.20	0.82 ± 0.18
	C	0.71 ± 0.17			0.61 ± 0.13		0.60 ± 0.11
A ($msec^{-1}$)	T	0.68 ± 0.14	0.67 ± 0.10	0.65 ± 0.12	0.68 ± 0.14	0.65 ± 0.16	0.69 ± 0.14
	C	0.69 ± 0.13			0.67 ± 0.13		0.69 ± 0.11
E:A	T	1.22 ± 0.30	1.20 ± 0.28	1.18 ± 0.28	1.17 ± 0.33	1.20 ± 0.34	1.24 ± 0.42
	C	1.03 ± 0.24			0.93 ± 0.21		0.90 ± 0.17
Ao vel ($m.sec^{-1}$)	T	0.94 ± 0.15	0.95 ± 0.14	0.95 ± 0.15	0.98 ± 0.17	0.96 ± 0.13	0.97 ± 0.12
	C	0.89 ± 0.15			0.83 ± 0.16		0.81 ± 0.13

SV - stroke volume , **EF** - ejection fraction , **E** - early filling velocity, **A** - atrial filling velocity, **Ao vel** - peak aortic blood flow velocity.

In order to investigate the potential link between central cardiac adaptations and training induced increases in $\dot{V}O_{2max}$ it was decided to plot the relationships between LV volume and SV with $\dot{V}O_{2max}$ as these were the only two variables to demonstrate any trend for increase over the 48 weeks.

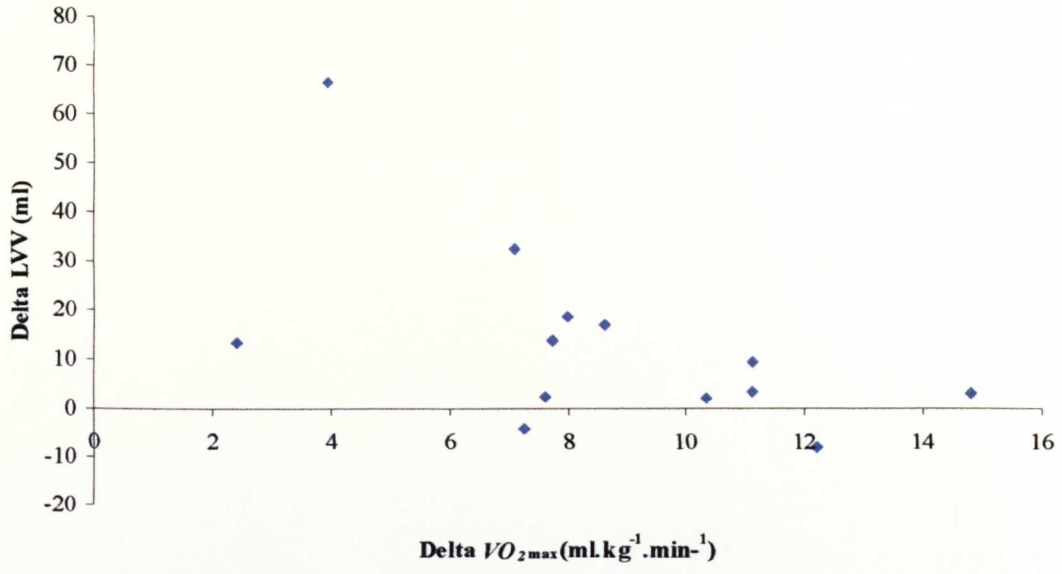


Figure 5.5 The relationship between changes in LV volume and changes in VO_{2max} in the training group

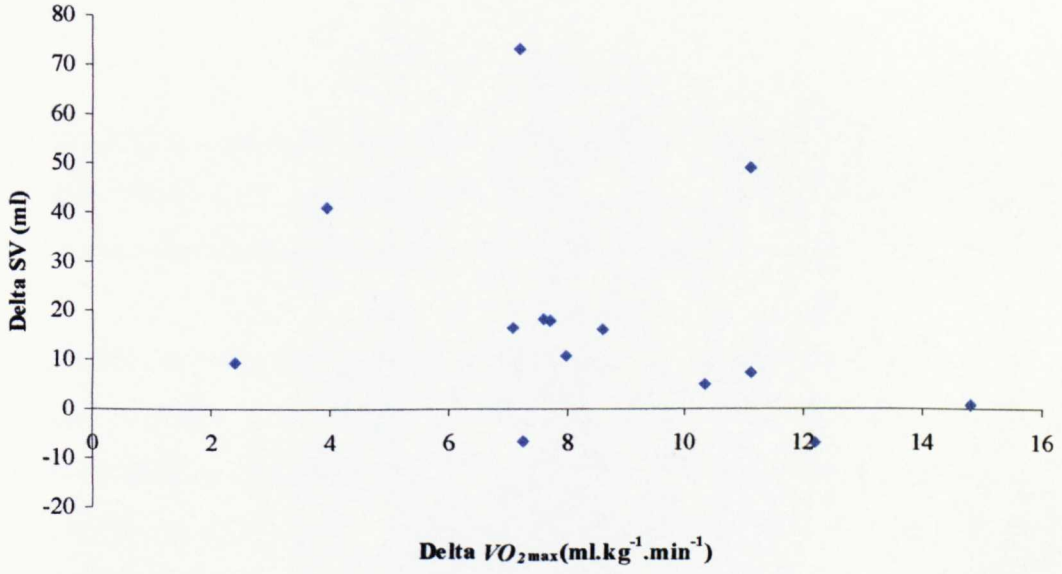


Figure 5.6 The relationship between changes in SV and changes in VO_{2max} in the training group

There was no significant correlation between the change in LV volume and the increase in aerobic capacity ($r = -0.15$, $r^2 = 0.02$, $P>0.05$ – see Figure 5.5). There was, however, a moderate correlation between changes in SV and delta VO_{2max} ($r = -0.61$,

$r^2 = 0.37$, $P<0.05$, see Figure 5.6). However, the fact that the relationship is negative suggests that as SV increased $\dot{V}O_{2max}$ was decreasing.

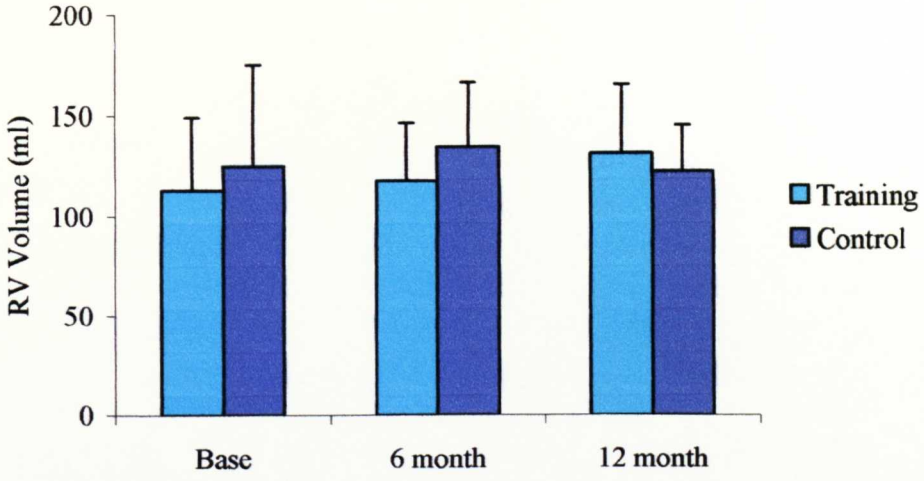


Figure 5.7 RV volume over the 12-month period in the training and control groups.

Data for RV volume were not significantly different between groups at baseline ($P>0.05$). Whilst some test-to-test variability is noted, RV volume did not alter over the 12-month period ($P>0.05$) in either the training or control groups (see Figure 5.7).

Data for RV diastolic filling and pulmonary artery flow velocity in both groups are presented in Table 5.3. Right ventricular E, A and E:A were not significantly different at baseline between the training and control groups ($P>0.05$). In the training group RV E, A and E:A did not change over time. In the control group A was moderately increased at 6 months (0.07 $m \cdot sec^{-1}$) which contributed to a significant time by group interaction ($P<0.05$) with a decrease in E:A (0.16) at 6 months in the control group. The changes in A and E:A in the control group, although, statistically significant, were within the limits of reliability for these variables and are likely to be statistically and biologically meaningless.

Peak pulmonary artery blood flow velocity was not significantly different at baseline between the training and control groups ($P>0.05$). Data for pulmonary artery blood flow velocity did not alter over 12 months in either group ($P>0.05$). A main effect for

group was significant and reflected a consistently lower (0.06 - 0.10 $m \cdot sec^{-1}$) peak pulmonary artery blood flow velocity in the control group.

Table 5.3 RV functional changes over the 12-month period in the training and control groups (no significant effects for time or group were recorded).

Variable	T/C	Base	6 wk	12 wk	24 wk	36 wk	48 wk
E ($msec^{-1}$)	T	0.60 ± 0.10	0.62 ± 0.11	0.61 ± 0.10	0.59 ± 0.10	0.55 ± 0.08	0.60 ± 0.08
	C	0.59 ± 0.09			0.56 ± 0.10		0.54 ± 0.07
A ($msec^{-1}$)	T	0.48 ± 0.09	0.51 ± 0.11	0.50 ± 0.11	0.46 ± 0.10	0.47 ± 0.11	0.50 ± 0.10
	C	0.49 ± 0.05			0.56 ± 0.14		0.46 ± 0.13
E:A	T	1.28 ± 0.21	1.27 ± 0.36	1.29 ± 0.34	1.33 ± 0.31	1.23 ± 0.30	1.27 ± 0.45
	C	1.20 ± 0.21			1.04 ± 0.24		1.25 ± 0.38
Pulm vel ($m.sec^{-1}$)	T	0.74 ± 0.10	0.73 ± 0.13	0.71 ± 0.12	0.76 ± 0.11	0.76 ± 0.12	0.74 ± 0.11
	C	0.68 ± 0.09			0.69 ± 0.04		0.64 ± 0.08

Pulm vel- peak pulmonary artery blood flow velocity.

5.4 Discussion

The key findings from this study were that with the imposition of a prolonged and progressive endurance exercise programme there was only limited evidence of any alteration to cardiac structure and function despite a significant and sizeable increase in maximal oxygen uptake of c. 30%. Interestingly, at the onset of the training programme $\dot{V}O_{2max}$ data for both the control and training groups were in the 20th percentile for maximal oxygen uptake in females of that age reported by the Aerobic Centre Longitudinal Study (ACSM, 2006). The 30% increase observed in the training group at 12 months corresponded to 70th percentile.

Left ventricular structural variables including LV mass and LV length did not change in the training group. Even though there was a significant increase in LV volume over time two important considerations must be made. Firstly, there was a moderate increase in LV volume of the control group (this was just outside reliability limits for this variable – see General Methods) that suggests the training-related increase may be quite modest (although this was outside of the limits of reliability – see General

Methods). Secondly the increase in LV volume came as LV length and LV mass did not change. The simple interpretation of this is that with no change in LV length the increase in LV volume must be due to an increase in the cross-sectional area of the ventricle with a degree of generalised LV wall thinning. Thus clearly the muscle mass of the myocardium is not hypertrophying in response to the exercise training, as has been suggested in older males who undergo training (Levy *et al.*, 1993; Jungblut *et al.*, 2000).

Alongside the LV structural data there were no significant changes in LV function with training, although there was a small trend for an increase in SV that was likely associated with the observed change in LV volume. Taken together the data confirm earlier studies that a significant and progressive period of endurance exercise training has no impact upon the cardiac function (Spina *et al.*, 1993; Spina *et al.*, 1996; Haykowsky *et al.*, 2005) and cardiac morphology (Park *et al.*, 2003; Haykowsky *et al.*, 2005). It is pertinent to note that even the 3 month period of 5 training sessions at 75% HRR (weeks 36-48) did not provoke any or additional central cardiac adaptation. In all other studies, as with the current data, post-menopausal female subjects improved their $\dot{V}O_{2max}$ with training with no apparent alteration to the central circulation. We could therefore theorise, as have others previously (Spina, 1999), that the alteration in functional capacity is probably largely due to alterations in the peripheral circulation and skeletal muscles.

It is pertinent to note that whilst current data agree with most previous literature it does partially contradict the earliest study of training in post-menopausal females (Morrison *et al.*, 1986). Morrison *et al.* (1986) demonstrated increases in both end-diastolic volume, and ejection fraction after 8 months of training. The reason for the disparity between the current study and Morrison's data is difficult to specifically explain, however, a key issue may be Morrison's use of "ice-pick" views of the LV by which LV volume, LV mass, SV and EF are estimated in M-mode echocardiography. The development of more geometrically-sound measurement processes via 2-D echocardiography as well as the use of Doppler lends greater credence to the current data. In addition to technical differences, subject differences may be a reason for the disparity as the subjects in the current study were approximately 10 years older than those of Morrison *et al.* (1986). Small

dissimilarities between exercise programmes are unlikely to be responsible for different results, however in the previous study subjects exercised for 10 minutes longer but only 3 days per week at a higher intensity over the 8-month period. The initial low intensity adopted before progression to higher intensities in this study may have inhibited a training effect; however, a lack of significant increases in all parameters with the exception of $\dot{V}O_{2max}$ at 48 weeks renders this unlikely.

Despite these overall statements it is important to speculate as to why LV volume demonstrated some evidence of a training-related increase. Despite not being fundamentally a true structural component (it is a cavity not a part of myocardium) LV volume is still important as a potential determinant of LV function. The increase in LV volume peaked at 6/9 months with little or no change at 12 months. The lack of change at the highest exercise intensity/volume was quite surprising given the trend to that point, and is difficult to explain. It is possible that the small changes in LV volume and consequently SV (although non-significant) may be due to an increase in blood volume, and thus a "stretch" of the LV with a preload increase, rather than eccentric hypertrophy. It is well documented in younger subjects that plasma volume expands after training (Convertino, 1991). A recent study, however, into effects of 10 days aerobic training on blood volume and LV performance in young and postmenopausal females revealed that plasma volume (PV) and blood volume (BV) only increased in the younger females, regardless of hormone supplementation taken in the older women (Katyal *et al.*, 2003). This was despite the fact that all groups increased their aerobic capacity. Additionally, Stachenfeld *et al.* (1998) reported no change in blood volume following 6-months of training in postmenopausal females. This is interesting because at 6-months in the current study LV volume was significantly higher than baseline. This disparity may be explained by the difference between studies. Although Stachenfeld *et al.* (1998) utilised a longer training period for both training modalities used (treadmill, 24wk, n=4; trampoline, 16wk, n=3) there were only modest increases in $\dot{V}O_{2peak}$ which may indicate that training intensity was not sufficient to induce BV expansion. The smaller trend in SV data in the current study would likely be due to the changes in LV volume (a preload effect) as there were no training related changes in EF (contractility) or BP (afterload).

Although Morrison *et al.* (1986) reported an increase in LV dimensions and $\dot{V}O_{2max}$ these two changes did not correlate significantly. Also Katyal and colleagues (2003) reported only a weak association between an enhanced BV and the increase in maximal aerobic capacity with training. They concluded, as others have previously, that improvements in aerobic fitness in postmenopausal women are most likely due to peripheral adaptations, rather than central. In the current study correlation analysis of LV volume and SV changes alongside training-induced increases in $\dot{V}O_{2max}$ supported the findings of Morrison *et al.* (1986), Spina *et al.* (1993) and Katyal *et al.* (2003) where cardiac functional changes are not seemingly related to changes in maximal aerobic capacity. Again this suggests that training related alterations in $\dot{V}O_{2max}$ are determined by peripheral adaptation or alteration in cardiac performance curing exercise that cannot be observed at rest.

Whilst the LV is a relatively common topic of research interest with respect to ageing and exercise, the same cannot be said of the RV. Less attention has been given to possible RV changes with exercise training despite the important role of the RV in the maintenance and control of pulmonary circulation and thus supplying the body and initially the LV with oxygenated blood. In the present study no significant alterations were observed in RV volume, RV filling dynamics or peak pulmonary artery blood flow velocity. The lack of change in the functional data mirrors the lack of change in diastolic and systolic blood flow parameters in the LV. The lack of change in RV volume with training is interesting especially given some evidence for an increase in LV volume. It is possible that the lack of change in RV volume reflects the difficulty and limitations in assessing the RV via echocardiography or the fact that the exercise stimulus applied simply did not initiate a dilatation of the RV. The latter point is unlikely to fully explain the data if the reason for a LV volume increase was due to an enhanced BV. This increase in BV would have affected the RV as well as the LV. Indeed in most cases where the LV and RV have been studied in younger athletes or training groups a more global pattern of cardiac adaptations is present with concomitant changes in both the RV and LV (Henriksen *et al.*, 1999). One final issue that cannot be ruled out is some sub-clinical level of pulmonary disease that was undetected by screening procedures. Whatever the explanation for these complex outcomes, it is suggested that further research should continue to investigate the

responses of the RV to training in older populations, possibly using newer techniques with better RV structural and functional measurement resolution.

So, why are postmenopausal women incapable of demonstrating central cardiac adaptation to prolonged and progressive endurance training? There are, seemingly no clear, unambiguous and empirically supported answers to this fundamental question. Many have speculated including Spina (1999) who postulated, rather vaguely, that the lack of significant changes in cardiac structure and function in post-menopausal females with training was due to the complex hormonal changes associated with the menopause. There is some evidence that the menopause leads to alterations in LV structure and haemodynamics (Schillaci *et al.*, 1998; Hinderliter *et al.*, 2002). Specifically, Schillaci *et al.* (1998) reported an increased wall thickness and a reduced fractional shortening in early menopause and similarly Hinderliter *et al.* (2002) described a significantly larger LV wall thickness and a trend toward a decreased chamber size in post-menopausal (>9 months amenorrheic) compared to pre-menopausal women of a similar age and LV mass. Despite similar blood pressures, peripheral resistance was higher in the post-menopausal group. Whilst this suggests some haemodynamic and consequent structural changes post menopause, it is not clear how such changes might influence training adaptation. Other interesting and potentially relevant data have been produced from estrogen-replacement studies in post-menopausal women. Pines *et al.* (1991) reported increased plasma volume and SV with hormone replacement therapy (HRT). As a follow on Pines *et al.* (1992) also reported that the long-term use of HRT can influence cardiac function with a significant improvement in measures of aortic flow after 10 weeks of HRT. Again, whilst suggesting that haemodynamics are altered post-menopause it is still unclear how or why such alterations may limit central cardiac adaptation to training, as increases in peripheral resistance and a decrease in baseline SV have not prevented central changes in response to training in hypertensives (Ehsani, 2001). Whilst this evidence *may* implicate the menopause and hormonal alterations in the ability of the heart to respond to training in older women other data contest this. Kirwan *et al.* (2003) investigated the effects of a 6-month aerobic training programme on a group of postmenopausal females with heart disease and, like most previous research, reported improved aerobic capacity due to changes in peripheral function. What was interesting was that these results were independent of whether the women were on

HRT or not. On the basis of this inconclusive and complex issue further research is required to substantiate any role for the lack of (or presence of) estrogens in the central cardiac adaptation to exercise training in older women. Whilst speculative it could be postulated, at a cellular level, that there are other gender differences that could account for the lack of hypertrophic response to exercise in post-menopausal females. Firstly, the role of adrenergic receptors are important in determining the size of male, but not female, hearts in their response to a physiological stimuli, such as training. The explanation for this could be that there is a different amount of adrenergic signalling or sensitivity to that signalling in males and females. This may mean that either males operate at a different range of sensitivity to physiological stimuli compared to females or that a higher effective threshold in adrenergic signalling may be required in females in order to obtain a structural response to ageing. This speculation has not been verified in animal models or with *in vitro* studies but may be a useful line of enquiry. Secondly, insulin-like growth factor (IGF-1), a regulatory protein complex, is observed to decrease with age. The decline is similar in males and females however females have a reduced sensitivity to circulating IGF-1 and thus the different adaptation to exercise training in older females may reflect a reduced circulating IGF-1 coupled with a down-regulation in sensitivity. Again this requires verification in future research.

Other data from the present study are interesting and worthy of comment. There were no significant changes in anthropometric variables although there was a c.2-3 kg loss of BM at 6 months with maintenance of LBM. The maintenance of LBM supports the concept that scaling of absolute cardiac data for changes in body size would have been pointless. Any changes in BM and LBM were not large and suggest either that the training programme was not sufficient to induce substantial alterations in whole body morphology or composition or that energy intake increased in response to training. This latter point was not assessed but is worthy of further research. The lack of significant change in SBP and HR may be somewhat misleading as they both demonstrated trends for a decrease up to 36 weeks that was not progressed at 48 weeks. Although all training diaries demonstrated strict adherence to the training programme it is possible that because the final period of the training programme ran over the Christmas period for some subjects this may have disrupted the programme to some degree.

The implications of this research are such that it would seem that although women do indeed lack the ability to alter cardiac structure or function in response to a 12-month aerobic training protocol, they are capable of increasing functional capacity. Their peripheral vascular function and skeletal muscles are indeed still trainable and this is probably important for healthy sedentary women as well as those with CV disease. Manson and colleagues (2003) reported that walking could reduce cardiovascular disease risk by 12-40% over 3 years in post-menopausal women regardless of age, ethnic origin or BMI. This is similar to more vigorous exercise but much more achievable in this older cohort. Thus exercise prescription in healthy older women will still lead to significant and important physiological alterations, as well as a likely improvement in psychological profile that was not assessed in this study.

Whilst we have proposed some future research to clarify a number of issues raised by the data in this study it would seem that the adoption of a lengthy and progressive intensity aerobic exercise training programme does not alter cardiac structure and LV and RV function at rest in healthy but sedentary post-menopausal women. This does not entirely negate the question as to whether prolonged and competitive level training are required to observe differences in cardiac structure and function. This cannot be achieved, realistically, with an initially sedentary group and suggests that a study of well-trained post-menopausal female "masters" athletes is the only way to evaluate the full potential, or upper limits, of cardiac adaptation in older women. This forms the basis for the third and final study in this thesis.

5.5 Limitations

The number of control subjects was over half of the number of training subjects due to a number of "drop-outs" in the control group which was unexpected. This is not ideal for the comparison between groups; however, both groups remained matched for age and body mass. The end of the training programme coincided with the festive period which the subjects commented was a particularly difficult time to complete five exercise sessions. Finally, the last stage of the exercise programme was at a particularly high level which often required a jog or run in some of the ladies. This was difficult for many of them, for psychological reasons as well as physiological strain that running caused them.

Many researchers suggest that data analysis in clinical trials should adopt an "intention to treat" analysis that would include all those who drop out and do not adhere as this provides a more realistic estimate of the impact of the intervention. Within the current study we attempted all possible (within ethical remit) methods of persuading subjects to attend for follow-up tests but due to the nature of the exercise protocols this became very difficult. Therefore, an appropriate intention to treat analysis could not be realistically completed. To counter some of the issues related by the drop-out and intention to treat discussion it was possible to revisit the 6 month data as at this point the drop out in the exercise and control group was substantially smaller than at 12-months. The outcomes and data trends at 6 months were not noticeably different from those of the smaller cohort who adhered to the 12-month programme.

5.6 Conclusions

A 12-month period of progressive aerobic exercise training did not result in any increase/improvement in LV mass, LV length, EF, LV diastolic/systolic blood flow velocities, RV volume and RV diastolic/systolic blood flow velocities. Whilst some evidence of an increase in LV volume may be present in the training group this likely represents a functional alteration of the LV and not any sign of LV hypertrophy. Despite a limited LV response to this training programme there was a 30% increase in maximal oxygen uptake that is likely due to adaptations to the peripheral circulation and/or skeletal muscle metabolism.

6 Cardiac Structure and Function in Post-menopausal Female Master Athletes

6.1. Introduction

The impact of exercise training on cardiac structure and function can be assessed in two ways. Firstly, via longitudinal training studies where a specific (and normally quantifiable) exercise stimulus is imposed for a chosen period of time. Such intervention studies, as carried out in Chapter 5, are supported as a direct way to assess cause and effect between the training and any cardiac adaptation. An alternate research design often adopted in the literature is a cross-sectional comparison of two groups with disparate training histories. This normally involves an "athlete-sedentary control" comparison (George *et al.*, 1991). Cross-sectional studies have been criticised (Urhausen and Kindermann, 1998) for a number of reasons including selection bias and an inability to separate environmental effects (training) from genetic effects (i.e. they became athletes because they had big hearts and not vice versa). There is, however, one potential benefit of the cross-sectional research that cannot be matched by longitudinal design. Specifically if well-trained athletes are selected and tested it provides some estimation of an upper-limit of cardiac structural and functional adaptation due to many years and high volumes of training (Whyte *et al.*, 2004). This provides an upper-normal limit for cardiac structure and function that may be of use when differentiating the athletic heart from pathologies (such as hypertrophic cardiomyopathy) that may predispose to sudden cardiac death (Maron, 1986).

A cross-sectional study of cardiac structure and function in postmenopausal female athletes, compared to sedentary controls, will add to our understanding of cardiac adaptations to exercise in such populations. This is partially because the evidence from longitudinal studies (Spina *et al.*, 1992: Park *et al.*, 2003; Haykowsky *et al.*, 2005; Chapter 5) suggests that older, postmenopausal women lose the ability to adapt centrally to exercise training programmes whilst still improving exercise capacity or $\dot{V}O_{2max}$. This is the opposite conclusion compared to that derived from cross-sectional athlete-control or longitudinal training studies of older men who retain the ability to alter central cardiac structure and function, alongside peripheral vascular and metabolic adaptations (Spina, 1999). Specifically LV mass, LV dimensions (Jungblut *et al.*, 2000) and SV (Ogawa *et al.*, 1992) have all been reported to be significantly

higher in trained vs. untrained older males. Further, there is clear evidence that younger trained pre-menopausal women demonstrate larger LV end-diastolic dimension, LV mass, LV volume, LV wall thickness and SV in cross-sectional studies (George *et al.*, 1999; Wernstedt *et al.*, 2002; Ferguson *et al.*, 2001). Recent meta-analyses have confirmed these individual studies (Pluim *et al.*, 1999; Whyte *et al.*, 2004).

One explanation for the lack of central cardiac adaptation to training in older women could be because of the relatively low exercise intensities, frequencies and volumes employed. This is exacerbated by the low initial training status at the beginning of the programme and thus exercise volumes are low with slower progression. Training programme length may also be a factor that contributes to a lack of change in myocardial structure and function. Most training programmes in post-menopausal women are between 12 weeks and 9 months. In this thesis, a 12-month training programme was employed, however, this is still relatively short-term compared to a number of years of intense and structured training that would be apparent in "master" female athletes.

To our knowledge there have been limited cross-sectional investigations of the heart of post-menopausal female athletes in comparison with age-matched controls. Ogawa *et al.* (1992) measured aerobic fitness, SV and a-vO_2 difference in 110 subjects and reported no differences in SV (derived from cardiac output measured via the acetylene [C_2H_2] re-breathe technique) regardless of training status in older females. In contrast, a recent echocardiographic study by Hagmar *et al.* (2005) demonstrated that trained post-menopausal females exhibited greater exercise capacities, larger LV diameters, LV volumes and SV compared to sedentary controls. These two studies constitute the limited available database in this controversial topic.

In an attempt to resolve previous contradictory data, to add to the small available database and specifically to provide unique data for LV diastolic filling performance and RV structure or function, we chose to undertake a cross-sectional study of post-menopausal female athletes and sedentary controls. Therefore the purpose of this study was to investigate the effects of long-term endurance training on LV and RV structure and function in older, postmenopausal females compared with sedentary

controls. This will serve to demonstrate upper limits of cardiac structural and functional data in older females and will also provide a descriptive point of reference for additional interpretation of the changes in LV volume reported as a consequence of the 12-month training programme adopted in the longitudinal study in Chapter 4.

6.2 Methods

6.2.1 Subjects

Athletic and running clubs in the Merseyside, Manchester, Salford areas were contacted in the search for well-trained post-menopausal females athletes free from known cardiovascular disease. Partially due to the limited number of well-trained postmenopausal female athletes in general, only 15 women responded to these advertisements. Of these 15 women, only 9 met the inclusion criteria and were suitable for the study (aged 53 ± 4 years). Specifically the inclusion criteria included; an absence of known cardiovascular disease, postmenopausal status (without menses for a minimum of 12 months, which excluded 5 of the original volunteers who were peri-menopausal on self-report) as well as a documented history (a minimum of 5 years) of high level, structured and/or competitive exercise training (intense exercise was defined as a "breathless" activity which was carried out continuously for a minimum period of 45 minutes e.g. running, cycling). The 9 subjects who entered the study self-reported current levels of moderate to high-intensity training including running, cycling or swimming at least 5 times per week. All subjects had been training for at least 5 years with the majority over 10 years and up to 20 years. Exact intensities could not be determined but the training supported competitive performance in a broad range of activities including 10km, half and full marathon races as well as squash and mountain climbing. It was evident upon interview that the vast majority of training for all well-trained athletes was aerobic in nature.

The control group was selected from subjects tested as part of the cross-sectional study in Chapter 4. These individuals self reported no or low levels of exercise and were therefore considered sedentary (for definition see Chapter 3). Specifically, subjects were selected to provide a postmenopausal and age-matched control group (aged 56 ± 4 years). All subjects were postmenopausal for at least 2 years. Mean ±

SD data for age and LBM are contained in Table 6.1 and were not significantly different between groups.

6.2.2 Variables Assessed

All subjects had a thorough explanation, both oral and written, of equipment and protocols and then gave written informed consent to participate in this study. Each participant attended a single testing session where standard anthropometric measures of BM and stature were taken prior a DEXA assessment to determine LBM, fat mass and percent body fat. After a further 5-10 minutes of seated rest blood pressures was recorded using a standard dynamap automated BP system. This was followed by echocardiographic interrogation. Two-dimensional imaging of parasternal short axis and long axis apical 2 and 4-chamber views facilitated the assessment of LV mass, LV volume, SV, EF and RV volume. Doppler echocardiography assisted in the assessment of diastolic filling velocities across the mitral and tricuspid valves as well as systolic ejection velocities across the pulmonary and aortic valves.

6.2.3 Data analysis

Statistical analysis was completed using SPSS version 12.0 for windows statistical package. Data was tested for normal distribution, using a Kolmogrov-Smirnov test. All except data for SV were normally distributed and this was taken into account for subsequent analysis. Independent t-tests determined significant differences between groups (except for SV where a non-parametric Mann Whitney U test was performed). Alpha level was set at 0.05.

6.3 Results

Anthropometric and other cardiovascular data for both groups are presented in Table 6.1. Resting HR and $\dot{V}O_{2max}$ were significantly different between groups with the active group demonstrating a lower, bradycardic HR and higher $\dot{V}O_{2max}$ ($P<0.05$). Although BM was over 8 kg (>10%) higher in the untrained group this was not statistically significant due to the small sample and quite large variability of body sizes in both groups. Lean body mass was comparable, which negated the necessity to scale the data for between group comparisons (see General Methods). Between groups fat mass and percent body fat were significantly lower ($P<0.05$) in the trained females which was to be expected and explains the lower BM in the trained group.

There were no significant differences in BP. Systolic blood pressure was similar (trained; 124 ± 12 mmHg, untrained; 126 ± 13 mmHg, P>0.05), however, although not significant, the trained group demonstrated a lower DBP (*c.* 12%) compared to the untrained females (trained; 64 ± 11 mmHg, untrained; 72 ± 9 mmHg, P>0.05).

Table 6.1 Anthropometric and cardiovascular data for well-trained post-menopausal female athletes and matched sedentary controls (* P<0.05).

Group	Sedentary	Active
HT (m)	1.59 ± 0.06	1.63 ± 0.07
BM (kg)	69.9 ± 10.0	61.2 ± 7.4
LBM (kg)	43.9 ± 4.6	43.1 ± 4.0
Fat mass (kg)	24.2 ± 4.7	17.5 ± 5.7 *
Body fat (%)	36.1 ± 3.6	26.0 ± 4.6 *
$\dot{V}O_{2max}$ (ml/kg/min^{-1})	23.9 ± 3.0	40.7 ± 8.0 *
SBP (mmHg)	126 ± 13	123 ± 12
DBP (mmHg)	72 ± 9	64 ± 11
HR (beats.min^{-1})	67 ± 7	51 ± 14 *

Left ventricular data

There were no significant differences in LV mass between the trained and untrained group (P>0.05, see Figure 6.1). The small difference (trained 142.6 ± 25.0 g; untrained 146.7 ± 31.1 g) was well within the measurement error for this variable. Of interest mean LV mass of the trained group was very similar to the mean LV mass (136.2 ± 25.9 g) of the subjects who had trained for 12 months in Chapter 5. The trained group, however, demonstrated a significantly larger LV volume compared with the untrained group (P<0.05, see Figure 6.2). The difference of 15 ml (c. 18%) was outside of the measurement error for this variable. Again, of interest mean LV volume of the trained group (82.3 ± 12.6 ml) was higher than the mean LV volume (77.6 ± 15.8 ml) of the subjects who had trained for 12 months in Chapter 5.

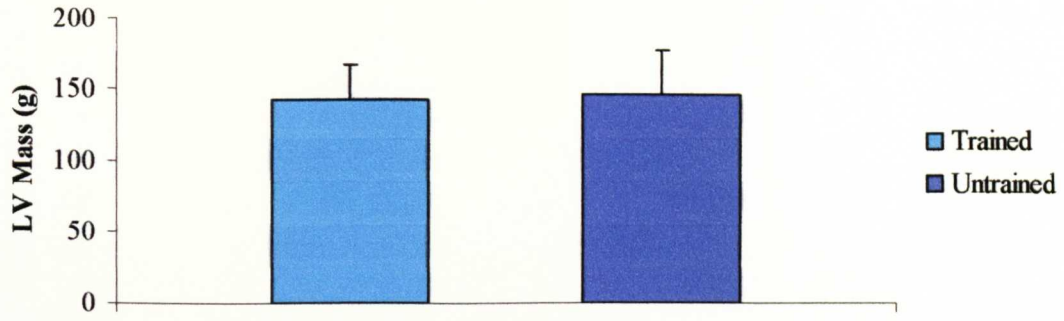


Figure 6.1 Absolute LV mass in trained compared with untrained females (P>0.05).

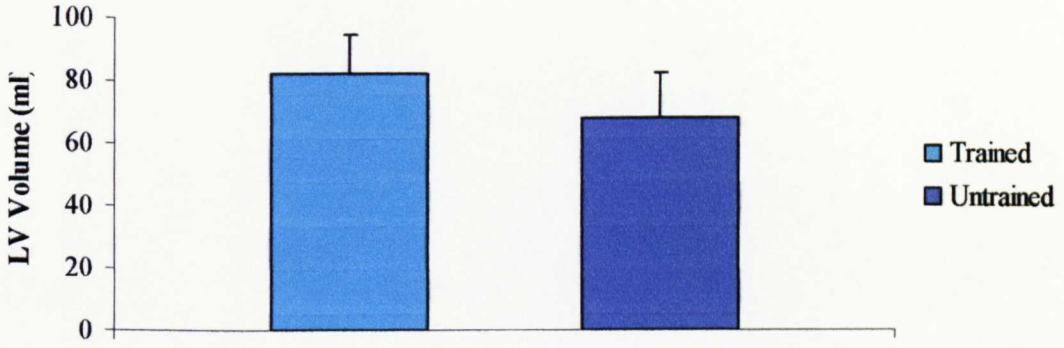


Figure 6.2 Absolute LV volume in trained compared with untrained females (P<0.05).

As a consequence of the increase in LV volume in the trained group it is perhaps, not surprising that SV was greater in the trained than the untrained group (P<0.05, see Figure 6.3). The difference of 15 ml (c. 25%) was outside of the measurement error for this variable. Of interest mean SV of the trained group (60.2 ± 9.5 ml) was slightly higher than the mean SV of the subjects who had trained for 12 months (52.7 ± 11.3 ml) in Chapter 5. The increase in SV was not mirrored by changes in EF. Ejection fraction was comparable between groups (P>0.05, see Figure 6.4). The small difference of 4% between groups was well within the measurement error for this variable. The mean EF of the trained group (72 ± 2%) was very similar to the mean EF (71 ± 3%) of the subjects who had trained for 12 months in Chapter 5.

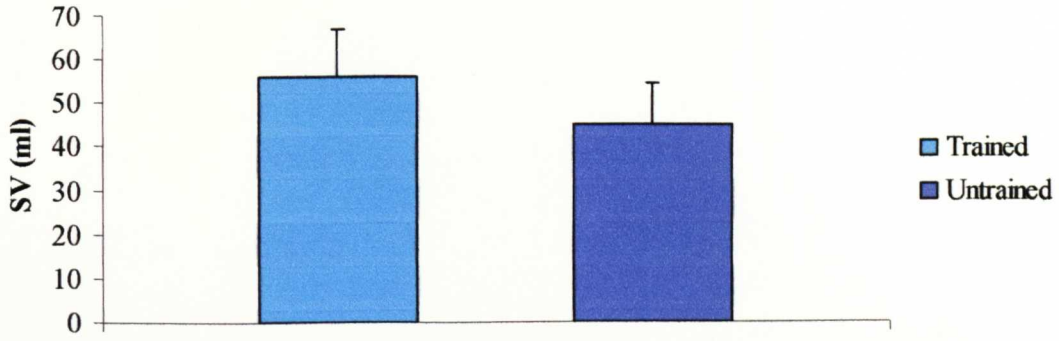


Figure 6.3 Absolute SV in trained compared with untrained females (P<0.05).

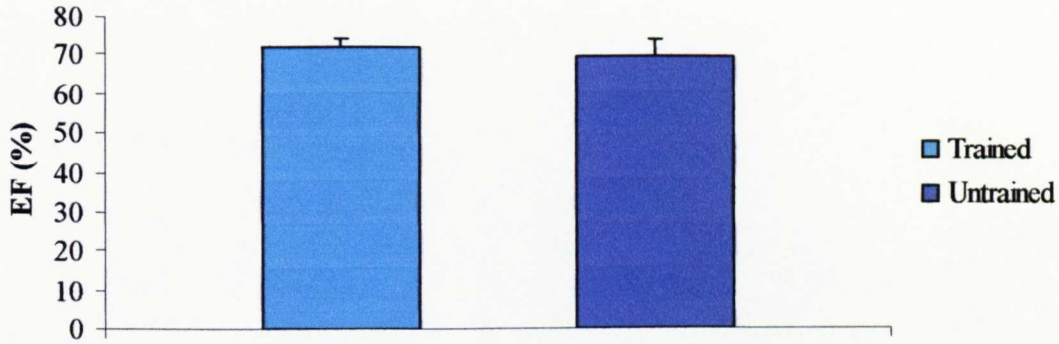


Figure 6.4 Ejection fraction in trained compared with untrained females (P>0.05).

Early mitral filling velocity was similar between groups (trained; 0.79 ± 0.13 $m.sec^{-1}$, untrained; 0.73 ± 0.17 $m.sec^{-1}$). There was, however, a significantly smaller atrial contribution in the trained females (trained; 0.52 ± 0.06 $m.sec^{-1}$, untrained; 0.67 ± 0.16 $m.sec^{-1}$). This led to a significantly greater E:A ratio in the trained compared to the untrained group (P<0.05, see Figure 6.5). The difference of 0.41 between groups was outside of the measurement error for this variable. The mean E:A of the trained group (1.53 ± 0.32 $m.sec^{-1}$) was substantially bigger than the mean E:A (1.24 ± 0.42 $m.sec^{-1}$) of the subjects who had trained for 12 months in Chapter 4.

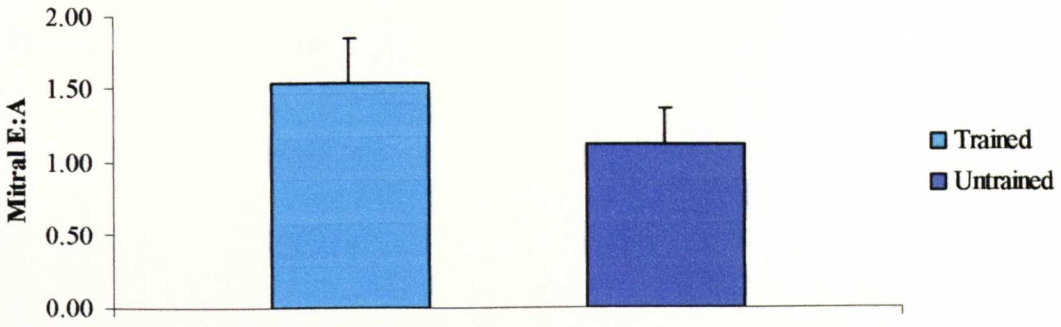


Figure 6.5 LV E:A ratio in trained compared with untrained females (P<0.05).

Peak aortic flow velocity was comparable (within 10%) in both the trained and untrained groups (trained; 1.05 ± 0.18 m.sec^{-1}, untrained; 0.97 ± 0.15 m.sec^{-1} – see Figure 6.6). This is within measurement variability for this variable. These values were also similar to those reported in Chapter 5 in females after 12 months aerobic training (0.97 ± 0.12 m.sec^{-1}).

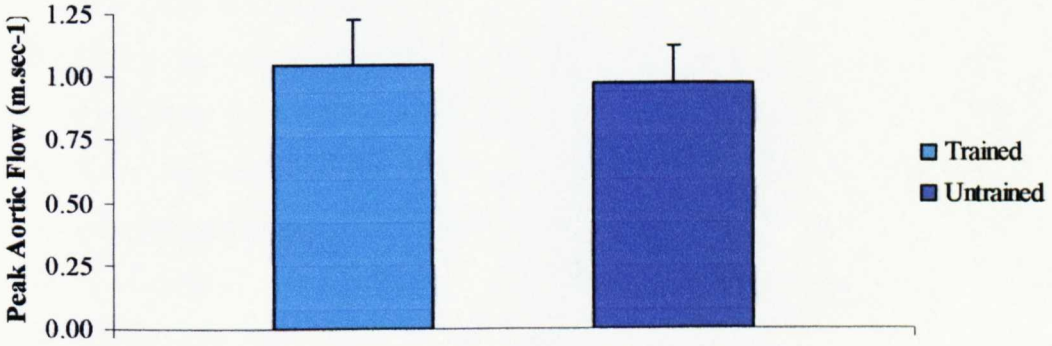


Figure 6.6 Aortic flow velocity in trained compared with untrained females (P>0.05).

Right ventricular data

Right ventricular volume was significantly higher in the trained vs. untrained individuals (P<0.05, see Figure 6.5). This between group difference (14%) was a little lower than the between group difference in LV volume (25%). The difference in RV volume of c. 20 ml between groups was just within the measurement error (LoA 15%) for this variable. Of interest mean RV volume of the trained group (146.7 ±

34.7 ml) was larger than the mean RV volume (131.4 ± 34.5 ml) of the subjects who had trained for 12 months in Chapter 4.

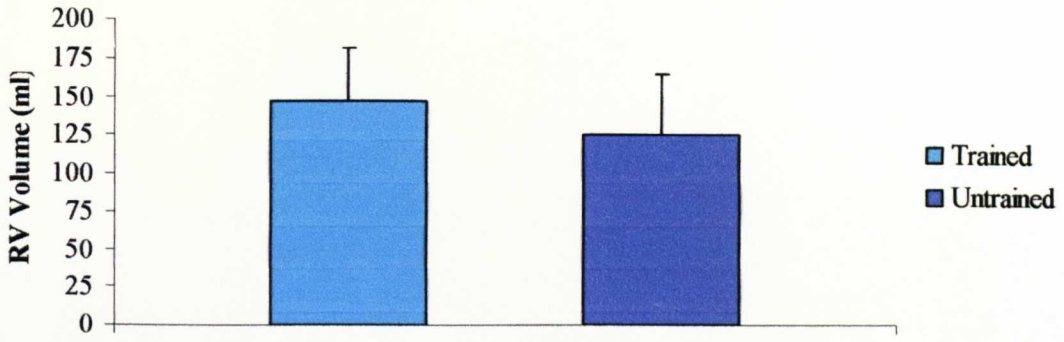


Figure 6.7 Right ventricular volume in trained compared with untrained females (P<0.05).

Early filling of the RV was not significantly different between groups (trained; 0.54 ± 0.13 $m.sec^{-1}$, untrained 0.58 ± 0.12 $m.sec^{-1}$). This was also the case for A (trained 0.40 ± 0.10 $m.sec^{-1}$, untrained 0.47 ± 0.11 $m.sec^{-1}$). Therefore tricuspid RV E:A ratio was not significantly different between the trained athletes and the untrained controls (P>0.05, see Figure 6.8). The small difference of 0.12 (9%) between groups was within the measurement error for this variable. The mean RV E:A of the trained group (1.39 ± 0.45 $m.sec^{-1}$) was slightly higher than the mean RV E:A (1.27 ± 0.45 $m.sec^{-1}$) of the subjects who had trained for 12 months in Chapter 4.

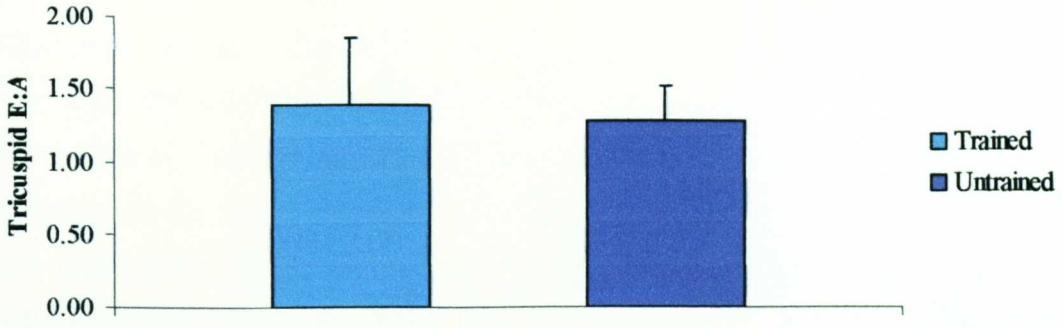


Figure 6.8 Tricuspid E:A ratio in trained compared with untrained females (P>0.05).

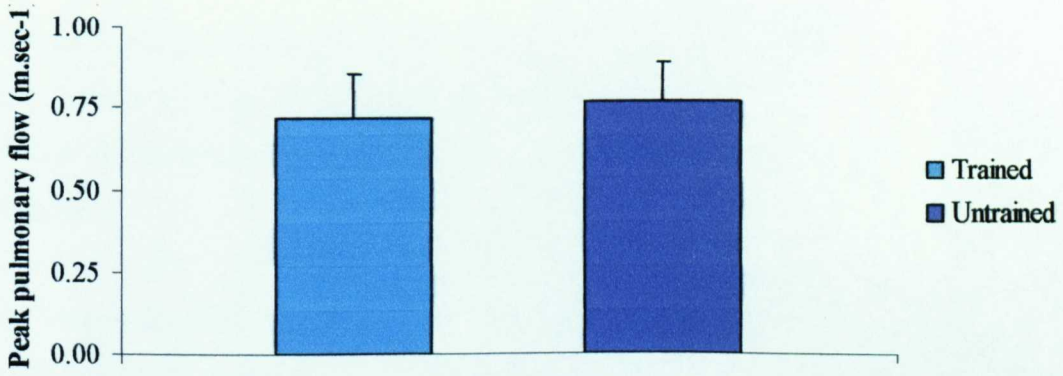


Figure 6.9 Peak pulmonary flow velocity in trained compared with untrained females (P>0.05).

Peak pulmonary flow velocity was not significantly different between groups (trained; 0.72 ± 0.13 m.sec^{-1}, untrained; 0.77 ± 0.13 m.sec^{-1}). These values were also similar to those reported for post 12 months training in Chapter 4 (0.74 ± 0.11 m.sec^{-1}).

6.4 Discussion

The purpose of this study was to investigate cardiovascular structural and functional parameters in post-menopausal females after long-term endurance training compared

to those of sedentary females. The trained group had lower fat mass, percent body fat and resting heart rate as well as a significantly higher $\dot{V}O_{2max}$. This provides strong support for the trained status of these athletes compared to the sedentary, untrained control group. Interestingly $\dot{V}O_{2max}$ data for the trained and control group when compared to the ACLS data (ACSM, 2006) reveal that the control group values correspond to the 10th percentile for females of 60 years, where as the trained group correspond with the 90th percentile. Similar differences in body composition, resting HR and $\dot{V}O_{2max}$ have been reported between younger female athletes and controls as well as young and older male athletes and controls (George *et al.*, 1991; Jungblut *et al.*, 2000) and is also similar to the recent cross-sectional data from Hagmar *et al.* (2005) in post-menopausal women.

The key findings from the study are discussed below but it is pertinent at this stage to note the relative lack of previous cross-sectional studies of post-menopausal female athletes for comparative purposes and thus longitudinal literature is also cited for evaluation.

Left ventricular data

In this study LV mass was not different between groups regardless of training status. This substantiates data from Hagmar *et al.* (2005) where LV mass was not significantly different between formerly elite postmenopausal females and sedentary controls. The current data also support the lack of structural adaptation or hypertrophy in response to training witnessed in longitudinal training studies (Chapter 4; Haykowsky *et al.*, 2005). This suggests an inability to hypertrophy myocardial muscle even in post-menopausal women with a prolonged training history of relatively high intensity and volume. Mechanisms for this are difficult to determine, however, Hagmar *et al.* (2005) proposed that this could be due to a change from competitive to recreational training with age. This was considered relatively unlikely in the current study due to the high volumes of training even at recreational or semi-competitive levels. Other suggestions include the concept that absolute cardiac output and systolic pressure response during exercise is lower in females than males and that there may have been insufficient hemodynamic stimulus to maintain or increase wall thickness. Likewise the relative lack of testosterone could be implicated as animal studies have shown that testosterone plays an important role in generating

LV hypertrophic response to exercise. However, these issues, either individually or in combination, seem unlikely to be able to explain all of this discrepancy due to the fact that young female athletes demonstrate similar cardiac adaptations to male athletes that are significantly different from controls (George *et al.*, 1999; Whyte *et al.*, 2004).

Data for LV volume were significantly greater in the trained group and supports limited cross-sectional data (Hagmar *et al.*, 2005), our previous training study (Chapter 4) and the longitudinal data from Morrison *et al.* (1986). However, there is some contradiction with other training study data (Spina *et al.*, 1999; Park *et al.*, 2003; Haykowsky *et al.*, 2005). The cause of this is difficult to elucidate, although limited subject numbers and the relatively short-term nature of these studies may be the reason for the inconsistent findings. The mechanism(s) that could explain the higher LV volume, and thus greater preload, in the trained females might well include blood volume (BV) expansion. Blood volume has been reported to be augmented post training (Convertino, 1991). Katyal *et al.* (2003) reported that in younger females BV was significantly larger following a short-term training stimulus; however, in postmenopausal females BV did not change. In addition Stachenfeld *et al.* (1998) also reported no increase in BV in postmenopausal females although this was only following a 3-month training protocol. As BV was not directly measured and preload inferred from changes in LV volume in our study it is clear that this requires further investigation however, the short-term duration of some previous training studies may imply that a longer training duration is needed to provoke BV expansion in postmenopausal females.

The larger LV volume, as with our training study, did not lead to a larger LV mass. The lack of a difference in LV mass in spite of a larger LV volume suggests a global thinning or relative atrophy of the ventricular wall. Mechanisms for this could be related to physiological and biochemical alterations occurring with the menopause particularly as trained younger females and younger and older males have all been reported to demonstrate a larger LV mass (Douglas *et al.*, 1986; Finkelhor *et al.*, 1986; George *et al.*, 1991; Levy *et al.*, 1993; Whyte *et al.*, 2004) in both cross-sectional and longitudinal investigations. Although Spina (1999) suggested the menopause might be implicated they provided no further depth or discussion as to which specific aspects might be relevant in this context. Further the role of hormonal

alterations at the menopause have been questioned as some studies have indicated that hormone replacement therapy (HRT) does not positively influence LV structural parameters (Green *et al.*, 2000; Katyal *et al.*, 2005). This suggestion of wall-thinning (here and Chapter 4) is seemingly unique and worthy of further research.

The larger SV in the trained group compares favourably with Hagmar *et al.* (2005) but does contradict with Ogawa *et al.* (1992). An explanation of this could be the difference in investigative techniques employed. Ogawa *et al.* (1992) utilised the acetylene (C_2H_2) rebreathe technique, whereas, echocardiography was employed by both Hagmar *et al.* (2005) and the current study. The fact that re-breathe techniques tend to demonstrate greater levels of variability and thus measurement error at rest compared to during exercise may be relevant in explaining this difference (Warburton *et al.*, 1999). The larger SV in the trained subjects in this study is greater than that observed after 12-months of aerobic training in previously sedentary women and likely reflects the more prolonged exercise exposure. Clearly the difference in SV is a response to the greater end-diastolic LV volume that is possibly the consequence of an enhanced BV as previously discussed. Interestingly, there was no difference in EF between groups and thus enhanced contractility cannot be a mechanism for explaining the increased SV. With the exception of Morrison *et al.* (1988) there have been no studies to report an increase in resting EF after training. This also extends to resting data in older males where EF remains unchanged 6-12 months of aerobic exercise training (Ehsani *et al.*, 1991; Stratton *et al.*, 1994; Spina *et al.*, 1996; Jungblut *et al.*, 2000). Therefore it is possible that if resting contractility is healthy before training then no adaptation is necessary. Data for EF were also supported by the lack of difference between peak aortic outflow velocities in both groups. Although not widely used or reported in previous literature the peak velocity of flow likely represents the peak contractile force of the LV when afterload is not altered, which was the case in this study.

Left ventricular function (diastolic)

In this study trained females demonstrated a significantly higher E:A ratio than controls. This was primarily due to a significantly smaller atrial filling contribution to diastolic inflow. The effects of training on diastolic function from previous cross-

sectional and longitudinal research are unclear. Douglas *et al.* (1986) reported that in younger trained males and females the E:A ratio was significantly higher than untrained individuals. Similarly, in younger males (Finkelhor *et al.*, 1986) the E:A ratio was also significantly higher in trained than untrained males. In older males only Bouvier *et al.* (2001) reported a larger E:A ratio in trained compared to untrained males. In other cross-sectional studies similar diastolic filling performance has been documented (Douglas and O'Toole, 1992; Jungblut *et al.*, 2000; Baldi *et al.*, 2003) in trained and untrained subjects. In longitudinal studies of both older males and females no resting diastolic changes have been reported with aerobic training (Chapter 5; Ehsani *et al.*, 1991; Stratton *et al.*, 1994; Spina *et al.*, 1996; Haykowsky *et al.*, 2005) with the exception of Levy *et al.* (1993) who reported an increased time to peak early filling rate after 6 months training in older males. It may be that previous longitudinal investigations have not reported an increased diastolic filling performance with training because subjects have been previously sedentary (Spina *et al.*, 1996; Haykowsky *et al.*, 2005) and therefore the intensity and volume of exercise may not have been high enough for adaptations to occur. The inconsistent cross-sectional data in males reinforces the need for further research in this area to refute or confirm enhanced diastolic filling capacity with training in older subjects.

These data would suggest LV filling is relatively more passive in the trained subjects and therefore requires less energy expenditure. This acts to reverse some of the natural ageing changes in E:A data (Oxenham and Sharpe, 2003) and thus improve diastolic function. The explanation for this more efficient LV filling in athletes is difficult to explain. It may be that the ventricle has become more compliant. Training may offset some of the biochemical consequences of ageing that increase stiffness and reduce compliance (Lakatta and Yin, 1982) including an increase in interstitial collagen and elastin distributed diffusely in the subendocardium and myocardium (Hutchins, 1980). Another possible mechanism may reflect a training induced improvement in calcium re-uptake by the sarcoplasmic reticulum that has been reported to decrease with healthy ageing (Capasso *et al.*, 1983). This is difficult to measure directly. Further research in this area could utilise tissue Doppler to gain a more detailed segmental and global insight into diastolic filling properties with training. Alternatively animal models could be useful in determining intrinsic changes in cellular biochemistry with training.

Right ventricular structure and function

Right ventricular findings mirror those of the LV in this study. There was a significantly larger RV volume in athletes than controls (14%), as well as a larger E:A ratio (9%). It is difficult to fully discuss these findings due to a paucity of comparative RV data from cross-sectional or longitudinal studies. In a cross-sectional investigation of younger male and female athletes RV volume also mirrored LV volume differences between trained and untrained groups (Henriksen *et al.*, 1999). These data seem to have been replicated here with the addition of differences in RV diastolic filling to mirror the LV. This suggests that prolonged exercise training promotes global adaptation of the myocardium rather than specifically stressing and altering the LV alone. This is logical as exercise places a significant workload on both the RV and LV and thus can act as a stimulus for adaptation in both ventricles (Gurtner *et al.*, 1975).

It is pertinent to note, however, that any findings for RV data, specifically those for RV volume, must be interpreted with some caution given the difficulty associated with imaging the RV with echocardiography. It is clear that this requires further investigation possibly with more consistent imaging techniques such as MRI.

Lastly, although RVEF was impossible to accurately calculate because of the mechanically different form of RV contraction we did collect peak flow velocity data across the pulmonary artery valve. This was not significantly different between groups and suggests, as with the LV, the intrinsic systolic contractile performance was not altered by the prolonged training history. Whilst we have some estimate of LV afterload from brachial artery blood pressure this is not the case with the low-pressure pulmonary circuit, which would require invasive catheterisation to determine blood pressures. Right ventricular performance is a possible target for future research to establish what effect training has on systolic performance.

6.5 Limitations

Perhaps the biggest limitation to this study is the low sample size. Every effort was made to get the largest sample size possible, however, due to the heavy duty DEXA equipment it was not possible to test away from the labs, therefore we were limited to

the North-west. Although statistically significant data for some variables were presented a larger sample size may have resulted in other statistically significant data (i.e. RV E:A differences may have reached statistical significance).

6.6 Conclusions

This study clearly demonstrates that it is possible for postmenopausal females to adapt centrally to exercise training, however, although this adaptation involves a larger LV volume it does not appear to include myocyte hypertrophy and a larger LV mass. This in itself may suggest a relative atrophy of myocardial muscle mass with training in these subjects that requires further investigation. Stroke volume was larger and diastolic filling was more efficient in the trained group, however, again the mechanism(s) behind this require further investigation. It seems clear, however, that older females do not lose all of the ability to adapt centrally to aerobic training that may be present in their youth.

7. General Discussion

The structure of this general discussion is formatted in an attempt to prevent simple repetition of discussion issues from individual data chapters and rather attempt to dissect general issues of importance that have developed across the thesis. Accordingly, after a very brief initial recap of key outcomes in the three data chapters the general discussion will tackle overarching issues of scientific interest, issues of technical importance, key limitations of the current studies, directions for future research and a final set of conclusion statements that address the hypotheses stated at the end of the literature review.

7.1 Key Outcomes

It was the purpose of this thesis to investigate; a) the effects of the healthy ageing upon cardiac structure and function in adult males and females, b) the effects of a progressive aerobic exercise training programme upon cardiac structure and function in post-menopausal females as well as c) the effects of competitive exercise training cardiac structure and function in post-menopausal female athletes and controls.

The exact nature of LV remodelling with age is the source of some controversy. Within in the cross-sectional study LV mass was maintained across the adult age-span in females but was significantly and negatively associated with age in males. The maintenance in LV mass in females despite an age-related decrease in LV volume suggested that remodelling of the LV with age was concentric in nature in females, with a relative wall thickening. In males, however, the large decrease in LV mass along with a smaller decrease in LV volume suggested a form of "eccentric atrophy" of the LV. This aspect of this study alone is unique in nature. Other data suggested an increase in male RV volume with no change in the females, no depression in LV and RV systolic function with age in either males or females, an expected age-related decrease in LV and RV diastolic filling (E:A ratio).

The second study focused purely on post-menopausal females as they undertook a progressive 12-month aerobic exercise training programme. Despite a significant and progressive increase in maximal aerobic capacity there were few alterations in cardiac structure and function. Interestingly, it became clear that as well as ageing having no affect on LV mass in females, exercise training also failed to elicit any change in LV

mass. It seems, therefore, that healthy sedentary females do indeed lose the ability to induce LV hypertrophy with training. There was some evidence of an increase in LV volume with training (and a much smaller trend toward an increase in SV). Other data showed no change with progressive exercise including LV systolic and diastolic function as well as volume data, systolic and diastolic function in the RV.

The final study investigated the cardiac structural and functional response to prolonged, competitive training in post-menopausal female athletes and controls. In agreement with the intervention study LV mass was not different in the athletes and controls. To support and extend the training study LV volume and SV, as well as RV volume, were significantly greater in the athletes than the controls. The athletes also demonstrated an enhanced LV E:A ratio although the increase in RV E:A was non significant. Both LV and RV systolic function were not different between groups.

7.2 Overarching issues

Lack of affect of ageing and exercise training on LV mass

It is generally an accepted phenomenon in clinical practice that with increasing age there is a hypertrophy of the LV (Gardin *et al.*, 1979; Dannenburg *et al.*, 1989; Shub *et al.*, 1994; Slotwiner *et al.*, 1998). This hypertrophy has been attributed to a number of different reasons including an increase in size of cardiomyocytes accompanied by an increase in collagen, increased vascular loading (specifically an increase in blood pressure and afterload), and a decrease in the efficacy of β-adrenergic modulation of both the heart and vasculature with ageing (Lakatta, 2002). It seems, however, that this belief may be erroneous for both autopsy (Olivetti *et al.*, 1991) and recent MRI data (Hees *et al.*, 2002) along with data from this study clearly suggest that LV mass *decreases* with age in males, and remains constant in females. This was despite an age-related increase in systolic blood pressure. The maintenance in LV mass in women has been attributed to the relative lack of myocyte loss with ageing in women compared to that observed in men (Olivetti *et al.*, 1995). The reasons for such sex-based differences are unclear but may have an important role in underpinning the differences in CVD prevalence in men and women, at least until the age of menopause. At a cellular level the general decrease in transcription of genes

controlling protein synthesis, the β-adrenergic system and the interaction of these factors with some, as yet unclarified or undetermined, gender difference may be responsible for the age-related data observed, however, the role of this requires further investigation. The impact of menopause was not addressed in the initial cross-sectional study but there was no clear alteration in age-cardiac variable relationships in the women as they got progressively older.

In postmenopausal females (age 55-65 years) a 12-month progressive aerobic training programme induced no change in LV mass, which was somewhat expected considering previous data (Park *et al.*, 2003; Haykowsky *et al.*, 2005). Further the athlete-control cross-sectional study also reported no increase in LV mass in the trained group. The combination of these two studies does not support the idea that the lack of response to training in previously sedentary post-menopausal women is because of the (initially) low intensity and limited duration exercise stimulus. It is, however, clear from previous studies in younger females and younger and older males that similar training studies or cross-sectional athlete-control comparisons do report alterations in LV mass. The reason for the lack of change in post-menopausal women who train is not abundantly clear. The impact of acute exercise upon LV and RV haemodynamics is not different in males and females, despite some early suggestions (Higginbotham *et al.*, 1984). Further it seems that acute changes in preload, afterload and contractility may not change substantially with age. Therefore a haemodynamic explanation for the lack of change in LV mass in females in not likely. Other male-female differences and ageing–related issues, such as the menopause have been implicated in the explanation of this LV mass response to training in women (Spina, 1999) without a clear and empirically supported mechanism. This is likely a topic of future research. The lack of change in LV mass with age or with exercise training simply suggests that mechanisms and pathways of hypertrophy in older post-menopausal women are simply shut down. This is not as a consequence of cell death (Olivetti *et al.*, 1995), but likely some other and as yet unknown physiological process(es). At a cellular level, other mechanisms for this could be the role of a different amount of adrenergic signalling or sensitivity to that signalling in males and females and the reduced sensitivity to circulating IGF-1 in females leading to different adaptation to exercise training in older females could be responsible for the results

observed in this study but this is speculative and requires further research in animal models.

Left ventricular volume and SV changes

A further and complicating issue in the assessment of LV mass with age and training is the change in internal dimensions or volume of the LV. These data are interesting because they suggest that there is a concentric remodelling of the LV with ageing that is in fact somewhat reversible with training, particularly prolonged intense training in post-menopausal females athletes. Left ventricular volume decreased in both males and females in the ageing study; however, the decrease observed in males was much less that in females (10% and 25%, respectively). Associated with the decline in LV volume was a drop in SV with age in both males and females with the greatest age-related change in women. Whether this decline represents an alteration in preload (blood volume changes), afterload (increased SBP in both sexes), contractility (although EF and peak aortic blood flow velocity did not change) or some intrinsic component of diastolic filling, possibly linked to the age-related decline in E:A is not possible to fully deduce.

What is interesting though is that the drop in LV volume with ageing is not totally irreversible. A progressive increase in LV volume, up to 9 months, was observed in the training study and the post-menopausal female athletes had a greater LV volume and SV than age-matched controls. Interestingly, explanations for the cessation of increases through to 12 months could be due to a number of reasons including a failure of subjects to reach specified heart rate in non-supervised sessions, a failure of subjects to maintain exercise sessions up to 5 days per week (this time period corresponded with Christmas), or finally, a physiological plateau in ability to adapt to exercise training post 9 months (this is unlikely due to the conclusions of the final study). Whether training related changes in LV volume and SV are associated with an increased blood volume are not clear as this was not assessed in the current study, although it remains a likely candidate for future studies, given the lack of other potential explanations. In the athlete-control study the increase in LV volume was also associated with a positive change in LV filling patterns. This is somewhat supportive of a role for an enhanced blood volume and thus central blood volume driving LV filling and preload. However, the training intervention did not see

concomitant changes in LV volume and E:A data. This may represent the smaller and more controversial changes in LV volume with the training programme compared to the athlete-control comparison. Other explanations may include some element of improved intrinsic relaxation of the LV with training, that would be independent of blood volume but this is speculative without a broader evaluation of diastolic filling parameters from non-invasive or invasive measurements.

LV systolic function

Other systolic functional indices more closely linked to contractility, EF and peak aortic blood flow velocity, were unchanged with ageing or exercise. This consistent finding (e.g. Haykowsky *et al.*, 2005) is not really surprising if one considers that the most likely cause of a depression in contractile function of the LV with increasing age is going to be the increasing prevalence of CV disease. Thus the findings of the first study, primarily, are a validation of the rigorous screening process for CV disease. Similar screening was undertaken for subjects in the exercise based studies and one might consider that if no decrement in EF had occurred prior to training then no adaptation would probably take place irrespective of the intensity and/or duration. These findings would support most previous literature in younger and older subjects (Shapiro and Smith, 1983; Finkelhor *et al.*, 1986; Ehsani *et al.*, 1991; Spina *et al.*, 1993, 1996; Stratton *et al.*, 1994; Park *et al.*, 2003). The fact that EF and peak aortic blood flow data were similar in all studies would suggest that both represent similar functional parameters, likely intrinsic contractile force. Whilst it is well known that EF is dependent on preload, afterload and HR (Dawson *et al.*, 2003), it is likely that the small changes in all of these factors with ageing and exercise may have cancelled each other out to some extent. Alternatively in the ageing study both healthy males and females were able to maintain EF despite a reduction in preload and only small changes in HR and BP (afterload) suggesting that LV systolic function may actually improve somewhat with ageing (De Bondt *et al.*, 2001). Conversely, the lack of change in EF despite a potential increase in preload with short-term and long-term training is somewhat puzzling and beyond the ability of our data to explain.

LV Diastolic function

The impact of ageing on LV diastolic filling was similar to previous literature with a large decline in E, accompanied by a compensatory increase in A leading to a

decreased E:A ratio (Oxenham and Sharpe, 2003). This is seen as a significant and primary response of the myocardium to ageing and data have suggested a range of explanations including an impairment of LV pressure decline as well as altered left atrial pressure increase in diastole (Hees *et al.*, 2004). Such a decline without changes in LV systolic performance may seem somewhat surprising but LV diastolic functional deterioration is often a precursor to systolic functional changes seen with CV disease.

As a primary response of the LV to ageing it was of interest to determine whether this could be reversed or slowed with exercise training in older women. No evidence to support this was forthcoming from the 12-month training programme. Uniquely, however, the post-menopausal female athletes demonstrated a higher LV E:A ratio, suggesting an attenuation of the age-related decrease in LV filling reported in the first study within this thesis. This is particularly interesting because diastolic filling improvement at rest has not previously been reported with training in older females (e.g. Haykowsky *et al.*, 2005) and has not been previously reported in a cross-sectional study of this nature. This finding is also rare in older males with the majority of literature reporting no difference in LV E:A ratio in trained and untrained older males (Douglas and O'Toole, 1992; Jungblut *et al.*, 2000; Baldi *et* al., 2003). Only Bouvier *et al.* (2001) has reported improvements in resting LV diastolic filling in older males. The implication for this change with more prolonged training in older women may be important both functionally as well as providing some protection against the onset of any depression in cardiac function as a representation of CV disease. The explanation or mechanisms underpinning this change may be partially preload related but could reflect any number of components of the LV pressure gradient including adaptations in the LV as well as left atria. Further work should address the reasons for this seemingly important outcome.

The right ventricle

Data for the RV was limited to RV volume, peak pulmonary blood flow and RV E:A primarily because of the difficulties in scanning and constructing feasible models of RV function due to its geometric shape. In our data RV volume does not seem to mirror changes in the LV with age which is in contrast to autopsy data (Olivetti *et al.*, 1991). A small increase in male RV volume and maintenance of RV volume in

females was reported which is difficult to explain. However, because the RV volume is a pump for a low pressure system of such restricted size, compared to the systemic circulation there must be the potential for differing responses to healthy ageing in the RV and LV.

After a 12 month training programme there was no change in RV volume in postmenopausal females, however, the increase observed in highly trained compared to sedentary post-menopausal females indicates the trainability of the RV somewhat mirrors that of the LV. This tends to agree with the limited data that has compared RV and LV structure and function in younger athletes and controls (Henriksen *et al.*, 1999). Global adaptation to training is important in ageing women again as it may represent an important way of preventing or slowing CV disease progression in both the RV and LV. These data, in post-menopausal females are unique and require further investigation perhaps in MRI studies. Interestingly RV E:A changes with training in post-menopausal female athletes also mirrored LV E:A changes and suggested a limited reversal or slowing of the age-related decline in RV diastolic function, the aetiology of which requires further research.

7.3 Implications of the data

A range of both practical and theoretical implications can be drawn from individual studies or the combination of data chapters. Some have practical application to future research in cardiovascular ageing and others have broader implications for public health messages for ageing women.

Two-dimensional echocardiography

This study was founded upon the principle of using 2-D echocardiography as apposed to the more common or "standard" M-mode techniques on both theoretical and practical grounds. Theoretically 2-D offers better models of LV and RV geometry and thus function, 2-D thus moves closer to other more complex imaging techniques like radionuclide angiography and MRI and finally 2-D has generally surpassed M-mode in clinical utility (Feigenbaum, 1994). The use of 2-D echocardiography in this thesis has provided novel echocardiographic insights into the impact of ageing upon LV structure and function as well as the capability of the ageing female myocardium to respond to the imposition of exercise. Perhaps the biggest clinical implication of

this thesis with regards to the utility of 2-D echocardiography is the confirmation of autopsy and MRI studies, and thus disagreement with past M-mode studies, LV mass actually decreases with age in males and remains constant in females. As 2-D echocardiography is the most commonly utilised clinical imaging tool tools in both the assessment of CV disease risk and the diagnosis of cardiac events this is an important finding. In future ageing and exercise studies that cannot utilise MRI, for cost or logistical constraints, has an ideal tool to use in 2-D echocardiography. There remains no reason for using M-mode echocardiography in such studies (e.g. Haykowsky *et al.*, 2005).

Scaling of cardiac structural and functional data

This thesis has provided further empirical support for the use of allometric scaling when normalising measures of cardiac structure and function. Specifically the available database (Batterham *et al.*, 1999; George *et al.*, 2001) has been extended by the scaling of RV volume data as well as LV length and SV. In most instances allometric scaling, of general geometric consistency, was far more successful in removing the influence of body size than per-ratio scaling which is surprisingly still common in this field (e.g. Haykowsky *et al.*, 2005) despite now a decade and half of strong empirical and theoretical support for allometric scaling approaches and LBM as the primary scaling variable (Batterham *et al.*, 1999). It is clear that per-ratio does not eliminate bias associated with body size in a male and female population; therefore it is perhaps remiss of scientific investigators to continue to publish comparative sample data in this manner.

The fact that allometric scaling was not adopted in the exercise studies simply reflects the lack of change in LBM with the 12-month training programme and the a priori decision to match controls and athletes for LBM prior to the cross-sectional study. This allowed the analysis of absolute data, which can be more meaningful from the point of view of quick clinical interpretation (few know what an $LVM/BSA^{1.5}$ of 76.2 actually means). Suffice it to say we would support the appropriate adoption of scaling procedures in any future study of the impact of ageing, sex and exercise upon cardiac structure and function as long as empirical (study specific) evidence supports its utility.

Whilst we investigated the most appropriate approach to scaling variables such as RV volume future studies may also include other cardiac structural and functional variables. To date limited data have scaled other structural and functional parameters associated with the RV (RV stroke volume) or other parameters such as left and right atrial dimensions.

Female training in the elderly

An important implication of this thesis is that prolonged and relatively intense exercise is well tolerated by healthy post-menopausal women and it leads to substantial increases in functional capacity, specifically assessed as $\dot{V}O_{2max}$ some adaptation of cardiac function is seen with more prolonged exercise training and this may be important in the preservation of function and delay of onset of CV disease and a deterioration in cardiac function. The trainability of the vascular system and functional capacity in post-menopausal women is similar to similarly aged men as well as younger males and females evident from the final study of this thesis. It is important, however, that the lack of ability to hypertrophy the LV in response to training should be seen as a potentially minor and less important consequence of female ageing that should receive continued research attention, but should not be highlighted above other important adaptations still present.

It is clear that although females have a smaller risk of developing CV disease through to menopause, this risk is drastically increased after the menopause. It is important to note that one of the ways to reduce the increased risk of CV disease post-menopause is the adoption of or maintenance of a healthy diet, and a regular exercise regime. It is also an important point of this thesis that in healthy women it is never too late to begin this regime.

7.4 Limitations

Exercise vs. rest

It is pertinent at this point to note that this study is based on resting measures alone and perhaps sub-maximal and maximal in-exercise data may have produced different conclusions with respect to the impact of ageing on cardiac function and the impact of exercise training on cardiac function in older women. Spina (1999) concluded that

older females lose the ability to centrally adapt to physical training and that this loss of ability does not occur in males, however, it would seem that this difference is only apparent when data are collected during exercise. Indeed Wilmore *et al.* (2001) reported training related increases in both SV and arterial venous oxygen difference at sub-maximal exercise intensities that were similar in younger and older males and females after a 5-month aerobic training intervention. The authors recognised that their older females were in most cases younger than those studied by Spina and colleagues (1996) and some were taking estrogen replacement therapy, which is reported to enhance cardiovascular performance (Pines *et al.*, 1991). However such data still suggest that exercise-related cardiac adaptation to training may be more revealing than resting parameters. Whilst this may seem logical we must recognise the limitations of cardiac evaluation during exercise, particularly with respect to echocardiography and other imaging modalities. Needless to say this area requires further empirical studies with some adaptation to echocardiographical scans particularly in a subject sample which is notoriously difficult to image.

A topic that requires some discussion is that of the menopause which was not included as a part of the initial study of cardiovascular changes with ageing. The study included pre-, peri- and post-menopausal subjects. Clearly the menopause is a significant event for the female CV system as Mendelson and Hendel (1996) concluded that after menopause women become much more susceptible to suffering a cardiac event and are less likely to recover from such an event than their age-matched male counterparts. Some researchers have suggested that the menopause can alter cardiac structure and function. Hayward *et al.* (2001) postulated that sex hormones could be influential in modulating LV mass. Recently Hinderliter *et al.* (2002) reported that the menopause was associated with concentric modelling of the LV. Although the effects of menopause *per se* were not directly measured in our cross-sectional study, it would seem that the results of the cross-sectional study provide some support of this suggestion although without a clearly defined age cut-off from the development of a concentric remodelling of the LV.

The impact of the menopause in study 2 and 3 related to training was controlled by the selection of only post-menopausal subjects. The importance of the menopause here is related to the explanation of the studies outcomes. Specifically, Spina (1999)

attributed the lack of change in cardiac structure and function after aerobic training to the menopause. It was suggested in this review article that oestrogen deficiency can affect vascular stiffness, which can lead to an increase in aortic impedance which can raise afterload and prevent an increase in stroke volume. This is questionable, however, because in our training study there was a trend towards an increase in SV and in our final study trained females demonstrated a significantly higher SV. Although it is entirely feasible that the menopause has an influence on cardiac structure and function, the extent to which this may alter the ability to induce central training effects has been brought into question by not only the exercise/training studies presented in this thesis but a number of previous investigations (Morrison *et al.*, 1986; Olivetti *et al.* 1995; Wilmore *et al.*, 2001; Hagmar *et al.*, 2005).

It was originally the plan of this thesis to include the use of HRT within both the second and third studies. This, however, became impossible as there were insufficient numbers of post-menopausal women taking HRT. An explanation for this may be the controversial nature of HRT. Specifically, recent investigations have suggested that, in contrast to some previous literature (Williams *et al.*, 1980), HRT in fact increases the risk of a cardiac event in those women with more than one risk factor present (Heart and Estrogen-Progestin Replacement Study [HERS], 1998). A great number of women previously taking HRT have now stopped creating a considerable problem for human research into HRT, it's affects on the cardiovascular system and it's response to training. Whilst again the lack of endogenous female sex hormone production and/or the use of HRT have been implicated in the lack of a central response to training in women there is in fact little direct evidence to support this assertion. Green *et al.* (2002) investigated the effects of ageing, HRT and training on CV function in 395 females. There were no differences in $\dot{V}O_{2max}$, HR, SV, a-vO_{2diff}, DBP between groups taking HRT and not taking HRT. The only differences reported were SBP and subsequently mean arterial pressure (MAP), and total peripheral resistance (TPR) suggesting that HRT may not influence central cardiac function. Furthermore after training there were significant changes in $\dot{V}O_{2max}$, exercise HR and SV in both HRT and non-HRT groups, with no change in exercise a-vO_{2diff} or TPR, again indicating that training effects were consistent between groups regardless of HRT group (doses not reported). Subsequently both Kirwan *et al.* (2003) and Katyal

et al. (2003) have also reported that cardiovascular training responses were consistent despite HRT status.

7.5 Future Research

It is clear that there are a number of issues pertaining to results presented in this thesis that require further investigation. In this respect it is important that this work is confirmed using 2-D echo techniques or MRI with the possible addition of BV measurement in order to confirm or refute possible mechanisms for training related responses.

As previously stated the occurrence of the menopause as well as the use of HRT and their effects on cardiac parameters with ageing and the CV response to training are contentious issues. These areas are in need of further investigation although subject recruitment may remain a potential problem. Although it was an area that we would have liked to investigate within this thesis, there was not a sufficient number of females taking HRT applying to take part in either the training study or the trained female cross-sectional study in order to compare groups. It would also be of value to determine specifically what happens to cardiac structure and function as healthy, sedentary women pass from pre-, through peri-, to post-menopausal status. Further, it may be pertinent to investigate the cardiac trainability of similarly aged pre-, peri-, and post-menopausal women. Additionally, this thesis has focused on aerobic endurance exercise interventions and the value of other types of exercise training (e.g. resistance training) for maintaining or improving CV function in elderly females (and males) has received limited attention to date.

In addition to investigating the effects of training on healthy women it is of great interest to investigate the effects of exercise on women with cardiac disease. Can post-menopausal women with CV disease improve cardiovascular parameters after exercise training?

7.6 Conclusions

On the basis of the data presented in chapters 3-5 the following conclusions are made relative to the research hypotheses tested for each study.

1) There is a significant relationship between age and aspects of LV and RV structure.

2) There is a significant relationship between age and aspects LV and RV function.

3) There is a significant difference in the relationship between age and cardiac structure, but not function, between sexes.

4) With the exception of a moderate increase in LV volume, there are no training related changes in LV and RV structure after a 12-month training intervention in post-menopausal females.

5) There are no significant training related changes in LV and RV function after a 12-month training intervention in post-menopausal females.

6) There are significant differences in LV and RV structure between sedentary and highly trained post-menopausal females.

7) There are significant differences in LV and RV function between sedentary and highly trained post-menopausal females.

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9. Appendices

9.1 Subject health questionnaire

Personal Information

1. Name: _____

2. DOB: Age:

3. Height: Weight:

4. What is your ethnic group (please tick box)

Caucasian	Hispanic	Black	Asian	Chinese	Other
☐	☐	☐	☐	☐	☐

Section One

Personal Medical History Assessment	(Circle answer)	
5. Has your doctor ever said that you have had a heart condition?	Yes	No
6. Have you ever been instructed to perform physical activity only when recommended by a doctor?	Yes	No
7. Have you ever had a real, or suspected, heart attack?	Yes	No
8. If so when did it occur _____		
9. Have you ever experienced rapid heart beating or palpitations?	Yes	No
10. Have you ever had angina or a sharp heavy pain in your chest as the result of physical activity?	Yes	No
11. Do you lose your balance because of dizziness?	Yes	No
12. Do you ever lose consciousness?	Yes	No
13. Have you ever had a resting or exercise ECG taken?	Yes	No
14. Was the ECG normal?	Yes	No

15. Have you ever been severely breathless as a result of low/moderate level of exercise? Yes No

16. Do you suffer from high or low blood pressure? Yes No

17. If so which one? Low High

18. Are you currently taking prescribed medication to control your blood pressure? Yes No

19. Have you ever been told your blood cholesterol is too high? Yes No

20. Are you currently taking prescribed medication to control your cholesterol ? Yes No

21. Do you suffer from any kidney problems now or in the past? Yes No

If yes please specify _____

22. Do you suffer from diabetes? Yes No

23. If yes, how is it controlled **(please tick)**

a) Dietary means ☐ b) Insulin injection ☐

c) Oral medication ☐ c) Uncontrolled ☐

24. Do you suffer from asthma, or any respiratory disorders? Yes No

25. Do you have any musculo-skeletal problems that could be made worse by a change in physical activity? Yes No

26. Do you know of any other reason why you should not undertake physical activity? Yes No

If yes why _____

Section 2.

Hormonal Status.

27. Have you ever menstruated (had a period) before? Yes No

If YES, how old were you when you first menstruated? _____

28. Are you using hormone contraceptives
(the pill, progesterone injection, patch)? Yes No

If NO, go to question 31.
If YES, please state what type you use, it's hormonal contents and dosage, if known

_____
_____
_____

29. How long have you been using hormone contraceptives?

- □ Less than 1 year.
- □ 1-2 years.
- □ 3-5 years.
- □ More than 5 years. Please state how long _____

30. Why do you use them?

- □ Contraception alone.
- □ Contraception and regulation of the menstrual cycle.
- □ Contraception and regulation of menstrual cycle symptoms (depression, pain, back ache etc.)
- □ Other reasons, please describe _____

31. If you do not currently use hormone contraceptives,
have you ever used them in the past? Yes No

If YES, please continue (Q.32).
If NO, go to question 35.

32. How long were you using them?

- □ Less than 1 year.
- □ 1-2 years.
- □ 3-5 years.
- □ More than 5 years. Please state how long _____

33. How long ago did you stop using hormone contraceptives?

- □ Less than 1 year ago.
- □ 1-2 years ago.
- □ 3-5 years ago.
- □ More than 5 years ago. Please state how long _____

34. Why did you stop using them?

- □ Irregular bleeding.
- □ Side effects (cycle irregularities, weight gain, mood disturbances).
- □ Planning a pregnancy.
- □ Other reasons. Please state _____

35. Have you reached the menopause?

- □ Yes, surgical hysterectomy.
- □ Yes, naturally, confirmed by my doctor.
- □ Yes, naturally, unconfirmed by my doctor.
- □ Unsure.
- □ No.

If YES, please continue (Q.36).
If NO, go to section 3.

36. At what age did you reach the menopause? _____

37. Are you taking any Hormone Replacement Therapy (HRT)? Yes No

If NO, go to question 41.
If YES, please continue (Q.38).

38. Please list what HRT name, hormonal contents and dosage _____
_____
_____

39. Why do you use HRT?

- □ Treatment of short term symptoms (hot flushes, night sweats, vaginal dryness)
- □ Prevention or treatment of osteoporosis.
- □ Prevention of heart disease.

□ Other. Please describe. _____

40. How long have you been taking HRT?

□ Less than 1 year
□ 1-2 years
□ 3-5 years

□ Longer than 5 years , please state how long _____

41. If you are not taking HRT now, have you ever used it in the past? Yes No

If YES, please continue (Q.16).
If NO, go to section 3.

42. When did you stop taking HRT?

□ Less than 1 year ago
□ 1-2 years ago
□ 3-5 years ago

□ Longer than 5 years ago, please state how long _____

43. How long did you take HRT for?

□ Less than 1 year
□ 1-2 years
□ 3-5 years
□ Longer than 5 years, please state how long _____

44. Why did you stop taking HRT?

□ Side effects (headache, acne, bloating, breast discomfort)
□ Worried about health risks (cancer, hypertension, blood clots, etc.)
□ No longer needed.
□ Other. Please describe. _____

Section Three

Physical Activity Assessment

45. Considering a typical **7-day period** (week), how **many times** do you do the following kinds of exercise for during your free time (write on each line the appropriate number).

	Times Per Week	**Duration (to the nearest 5mins)**
a) Strenuous Exercise (Heart beats rapidly)		
(e.g running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous longer distance cycling)	_____	_____
b) Moderate Exercise (Not Exhausting)		
(e.g. fast walking, baseball, tennis, easy cycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____	_____
c) Mild Exercise (Minimal Effort)		
(e.g. yoga, archery, fishing from river bed, bowling, horseshoes, golf, easy walk)	_____	_____

46. Considering a typical **7-day period** (week), during your leisure time, how often to do you engage in regular activity long enough to work up a sweat with your heart beating rapidly?

OFTEN	SOMETIMES	NEVER/RARELY
☐	☐	☐

Section four

Diet Assessment
(please circle)

48. Are you a vegetarian Yes No

49. During a typical day what do you eat/drink

_____

_____

_____

50. Do you take any food supplements Yes No

If yes, please specify _____

Participant signature: _____

Thank you for completing this questionnaire

9.2 Subject Information Sheet

Thank you on behalf of the Cardiovascular Ageing Research Group for expressing an interest in this study. If you need to contact anyone for any reason then please call on 07899 798641 or e-mail, Claire Stephenson (spscstep@livjm.ac.uk).

Title of Study: Cardiovascular structure and function: effects of age and training.

There are an ever-increasing number of elderly individuals in today's society in response to an increased average life span. Ageing is a natural process, characterised by a deterioration in the functional capacity of various organs and systems within the body. There is also an age-related decline in physical activity levels, which further enhances this process. Ageing results in a loss of muscle cells (myocytes) in the heart, which leads to a decrease in heart size. Furthermore, this deterioration of the heart seems to be more pronounced in males than females, illustrating gender differences in the ageing process. It is therefore important to determine how the performance of the heart is affected by age, and also how exercise interacts with this process and possibly reversing some of the effects of ageing. As many studies have already been conducted in males, a female study is our priority at present.

Experimental Procedures

1. At this stage you should have an initial screening form (questionnaire). This is to establish if you are healthy and not suffering from any cardiovascular or respiratory disease. Please fill this in and send it back to us to the address below.

2. You will then be invited into the laboratories for your assessment. A full explanation of all the equipment you encounter will be given and if you have any questions then please feel free to ask. It is important to us that you are completely comfortable with all of the procedures before participating.

3. Measurements of your height, weight and body composition (using skinfold calipers) will be taken. Resting blood pressure will also be measured (using the

usual procedure of an inflatable cuff on your upper arm) and we made need to take a sample of blood (4 ml, about a teaspoon full). This will be taken from your arm. If this is a problem, we fully understand and it is not a compulsory part of the testing procedures.

Echocardiogram (ultrasound scan of the heart) and Forearm Blood Flow

This will be a single session lasting approximately 1 hour after the initial measurements (detailed above) will have been taken. There will be an explanation and familiarisation with the equipment and then testing will commence.

Echocardiograph

1. A resting elecrocardiogram (ECG) will be performed. This is inherent to the ultrasound scan.
2. For the scan you will be lying on a bed in a quiet, semi-dark room. You will then be asked to relax as much as possible. ECG electrodes will be placed on your skin (shoulders and bottom of rib cage) and attached to wires linked to the echocardiogram. You will then have an ultrasound transducer (with gel) placed on your chest at various points (suprasternal, apical and parasternal) to allow imaging and recording of cardiac structure and function.

Control and regulation of forearm blood flow

1. Forearm blood flow

- Forearm blood flow will be measured using a mercury-in-strain gauge placed around the forearm, this records changes in forearm volume.
- An inflatable cuff will be placed around the wrist and a second around the upper arm, these will be used to temporarily occlude blood flow.
- Resting blood flow - Inflating of the wrist cuff for 1 minute, then the cuff on the upper arm will be inflated for 10 seconds and deflated for 5 seconds, will cycle will be repeated 8 times.

- Maximal blood flow – Inflation of the upper arm cuff for 5 minutes, after 4 minutes have elapsed the wrist cuff will be inflated. Once the upper arm cuff has been inflated for 5 minutes it will be inflated and deflated as occurred in the previous protocol.

2. Skin blood flow

- Skin blood flow of the forearm will also be assessed, this will be measured with the use of laser Doppler probes. These measure the movement of red blood cells through the vessels of the skin.
- Resting skin blood flow - this will be assessed by the placement of two probes on the forearm, and will record data for approximately 10 minutes.
- Maximal skin blood flow - this will be recorded by heating the probes to 45°C, and recording the data for 20 minutes.

DEXA scanning

The DEXA is a whole body scanning machine. It provides us with information regarding your bone structure such as bone mineral density and content. The duration of the test lasts approximately 20 minutes during which time involves you lying down on a flat surface whilst the scan passes over you. Each scan will last approximately 3 minutes.

Depending on numbers of participants we may or may not add in a test which involves the participant performing an exercise test on 2 separate occasions. The details are below and we will keep you posted if these tests are to be carried out or not.

Exercise (one of two types)

First Session

- At the end of the first session and whilst resting, you will be asked to breath in a mixture of gases containing a slightly higher than normal concentration of carbon dioxide (this is in no way harmful) through the mouthpiece for approximately 4 deep breaths. This will give us an estimate of the amount of blood your heart is pumping around the body each minute. (This may be conducted in your second session depending on time restraints).

- You will then be asked to perform some exercise on a treadmill (walking machine). You will be given an initial familiarisation session if needed and then asked to step onto the treadmill and start to walk at a slow comfortable speed. Thereafter, with appropriate advanced notice, the speed and gradient of the treadmill will increase every 1 minute. The exercise will continue until to you reach the point where you can no longer continue with the exercise. Throughout this exercise period you will wear a nose clip and a mouthpiece to enable us measure the amount of oxygen your body is consuming. Also, ECG and blood pressure measurements will be taken.

Second Session

- Following from the results of the first session you will perform similar exercise by returning to the treadmill and quickly progressing to the same level of maximal exercise that was achieved in the 1st test in the first session. This will take approximately 3 mins. The speed and gradient of the treadmill will then be decreased to allow you to recover. This will be repeated for a 2nd time again after approximately 5 minutes, and perhaps a 3rd time. During these exercise periods your blood pressure will be measured. Also you will breathe another mixture of gases (slightly less concentration of carbon dioxide than at rest) for approximately 6 deep breaths.

As a volunteer subject you have the right to withdraw at any time without prejudice. However your involvement to complete these

9.3 Subject Consent Form

Liverpool John Moores University

Form of consent to participate as a subject in a research project

Name:..... **Date of Birth**:...../...../.....

Researchers: Mr Paul Chantler **Supervisor**: Prof. David Goldspink

Mr Richard Clements

Miss Lisa Sharp

Miss Claire Stephenson Dr. Keith George

Mr Gary Hodges Prof. N. Tim Cable

Project Title: Ageing and cardiac power output

Ageing and cardiac structure and function

Ageing and the control of peripheral blood flow

Purpose of the Study: The purpose of this study is to examine the effects of ageing on cardiac power output, the structural and functional parameters of the heart and the control and regulation of peripheral blood flow.

For these studies the subject will be required to attend the Research Institute for Sports and Exercise Sciences, at Liverpool John Moores University, Henry Cotton Campus, Webster Street.

Determination of Cardiac Power Output

A full introduction to the treadmill, gas analysis system, ECG and blood pressure measurements will be carried out, in addition an initial health screen in the form of a questionnaire will also be performed. On the first occasion you will be requested to remain seated for 5-10 min while resting blood pressure and heart rate are taken. Immediately after, you will exercise on a treadmill until your maximum oxygen uptake ($\dot{V}O_{2\ max,}$) has been determined. On the second session, you will perform another bout of exercise at 100% $\dot{V}O_{2\ max,}$ during which cardiac output and blood

pressure will be measured. N.B. Prior to testing it is essential that no food, alcohol or caffeine are consumed for at least 3 hours.

Structural and Functional Determination

This will be one single session lasting approximately 45 minutes. There will be an initial explanation and familiarisation with the equipment and then testing will commence.

If so desired, it is possible for another person to be present at the time of testing.
In this study the procedures used to analyse the structure and function of the heart will involve electrocardiography and echocardiography. Electrocardiography measures the electrical conductance of the heart through 3 leads placed just below each shoulder and above the left hip. Echocardiography involves taking an ultrasound picture of the heart taken through the use of a transducer placed immediately to the left of the chest bone, level with the heart and then just below it.

Assessment of the control of peripheral blood flow

This will be a single session lasting approximately 35 minutes. There will be an initial explanation and familiarisation with the equipment and then testing will commence.

This study assesses the control of peripheral blood flow (whole arm blood flow) and skin blood flow, of the forearms. Forearm blood flow will be assessed using a plethysmograph, which is used to determine changes in forearm volume, which will be measured with a mercury-in-strain gauge placed around the forearm. Skin blood flow will be measured using laser Doppler flowmetry, this measures blood flow in the vessels of the skin by using a beam of light to record the movement of red blood cells.

YOU HAVE THE RIGHT TO WITHDRAW FROM THE RESEARCH AT ANY TIME.

All Information Given to the Researcher Will Be Treated As Confidential

I, ...agree to take part in the above named project/procedure, the details of which have been fully explained to me and described in writing, along with the demands, risks and benefits of the study. I am aware of the risks involved and understand that I can withdraw from this study at any time without penalty.

Signed (Subject) ..... Date .....

I, ...certify that the details of this project/procedure have been fully explained and described in writing to the subject named above and have been understood by him/her.

Signed (Researcher) ..... Date .....

I, ...certify that the details of this project/procedure have been fully explained and described in writing to the subject named above and have been understood by him/her.

Signed (Witness)..... Date .....