Cardiff Metropolitan University

Doctoral Thesis

The effects of the menopause on left ventricular mechanics

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"Life is a journey, not a destination."

Ralph Waldo Emerson

Abstract

Ageing is associated with sex-specific decreases in cardiac function that may be explained by the menopause. However, the effects of the menopause on regional myocardial function and the practical implications of menopause-related differences in cardiac function are unknown. The aim of this thesis was to investigate the effects of the menopause on left ventricular (LV) function and mechanics at rest, and in response to physiological tests and exercise training. In the first study, resting LV function and mechanics were compared between middle-aged pre- and post-menopausal women, as part of an ageing study comprising young adult and middle-aged men and women. Post-menopausal women had lower LV function and mechanics than pre-menopausal women. Middle-aged men had greater peak systolic apical LV mechanics compared with middle-aged women, but there was no evidence that apical mechanics differed between younger men and women. These findings suggest that the menopause lowers LV mechanics, but may only partly explain sex-specific differences in LV apical mechanics with ageing. In the second study, the effects of 12 weeks of high-intensity aerobic interval training on LV function and mechanics were compared between pre- and post-menopausal women. Post-menopausal women had a smaller increase in peak aerobic capacity after exercise training, compared with pre-menopausal women. Physiological testing with lower body negative pressure and submaximal supine cycling revealed higher LV basal mechanics in pre-menopausal women after exercise training compared with post-menopausal women, in contrast to the age-related sex differences observed at the apex in the first study. These findings suggest that the menopause may reduce aerobic adaptability and influence regional LV mechanics. To investigate whether exercise training mitigates the effects of the menopause on LV function and mechanics, the Bayes factor was used to complement statistical inferences from P-values in the third study. Exercise training in post-menopausal women caused an average 5% increase in likelihood of similar LV mechanics relative to untrained pre-menopausal women. Collectively, this thesis provides new evidence for the effects of the menopause on regional LV mechanics and aerobic adaptability. Future work should investigate the potential effects of exercise training intensity and duration on LV mechanics and aerobic adaptability between pre- and post-menopausal women.

Acknowledgements

I would like to thank my PhD supervisors, Eric Stöhr and Barry McDonnell, for the academic training that has culminated in this thesis. A special note to Eric for teaching me to scan and analyse echocardiographic images, which was key to the novel data collected in this thesis. Thank you for your unwavering support through this journey.

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Glossary

Action potential a phenomenon involving temporal changes in membrane potential, typically with a fast depolarisation and slower repolarisation back to the resting state.

Allometric scaling A technique to normalise left ventricular size for differences in body size, by assuming an exponential relationship between the two variables.

Cardiac excitation-contraction coupling The process by which the electrical stimulation of cardiac myocytes leads to muscular contraction.

Cardiac output Total volume of blood ejected from the left ventricle every minute.

Chronotropy Heart rate.

Diastole One of two distinct components of the cardiac cycle; comprising isovolumic relaxation and filling.

Ejection fraction Ratio of stroke volume to end-diastolic volume, expressed as a percentage.

End-systolic volume The residual volume of blood in the ventricle after ejection.

End-diastolic volume The maximum volume of blood in the ventricle after filling.

Heart rate Number of heartbeats per minute.

Inotropy Cardiac contractility.

Isovolumic contraction Contraction of the left ventricle with no change in volume; occurs between mitral valve closure and aortic valve opening.

Isovolumic relaxation Relaxation of the left ventricle with no change in volume; occurs between aortic valve closure and mitral valve opening.

Left ventricular torsion Twist per unit length of the left ventricle (deg/cm).

Left ventricular twist The net difference in rotation between the left ventricular base and the apex.

Left ventricular base Cross-section of the left ventricle at the level of the mitral valve.

Left ventricular apex Cross-section of the tip of the left ventricle; just proximal to end-systolic luminal obliteration in echocardiography.

Left ventricular mechanics The umbrella term for the rotation and deformation of the left ventricle across the cardiac cycle.

Left ventricular deformation Changes in length/thickness along the longitudinal, circumferential and radial coordinates of the left ventricle.

Lusitropy Cardiac relaxation.

Menopause The time in a woman's lifespan when menstruation ceases, marking the end of the woman's reproductive years.

Myocardium Heart muscle.

Oestrogen A primary female sex hormone.

Population ageing A global phenomenon of rising proportions of older persons (aged 60 years or older) in the total population.

Progesterone A primary female sex hormone.

Ratiometric scaling A technique to normalise left ventricular size for differences in body size, by assuming a directly proportional relationship between the two variables.

Sarcoplasmic reticulum An extensive branching tubular network that regulates intracellular calcium concentrations, which is key to contraction and relaxation.

Speckle In ultrasound imaging, the observed texture in tissues due to scattering of ultrasound energy.

Speckle tracking echocardiography A technique to analyse the motion of cardiac tissue, based on the scattering of ultrasound energy to produce "speckles"; the technique used to measure left ventricular mechanics in this thesis.

Stroke volume Volume of blood ejected from the ventricle during one cardiac cycle; the difference between end-diastolic volume and end-systolic volume.

Sub-epicardium Outer layer of the myocardium.

Sub-endocardium Inner layer of the myocardium.

Systole One of two distinct components of the cardiac cycle; comprising isovolumic contraction and ejection.

Ultrasound The portion of the sound spectrum characterised by a frequency of greater than 20,000 Hz, which is considerably higher than the audible range of human hearing.

Dedicated to my mum.

Chapter 1

Introduction

1.1 Background

The menopause is a natural part of female ageing and marks the end of a woman's reproductive years (Harlow et al., 2012). Women experience a permanent cessation of menstruation due to an underlying loss of ovarian follicular activity (Burger, 2006). The menopause usually occurs between 46–54 years of age (Ley et al., 2017), with some geographical variation between a median age of menopause of 49 years in Latin America and 54 years in Europe (Palacios et al., 2010).

Classic life-history theory predicts that an extended lifespan after the menopause should not occur because there should be no selection for survival after the cessation of reproduction (Johnstone and Cant, 2019). However, women do live a significant proportion of their lives after reproductive senescence. Among mammals, only four species of toothed whales show a similar pattern of a prolonged post-reproductive life — killer whales, short-finned pilot whales, narwhals and beluga whales (Figure 1.1). Based on kinship dynamics, theories such as the grandmother hypothesis and the reproductive conflict hypothesis have been proposed to explain this prolonged post-reproductive life (Johnstone and Cant, 2019). The grandmother hypothesis posits that the prolonged post-reproductive life enables women to assist their offspring to reproduce successfully (Hawkes et al., 1998), while the reproductive conflict hypothesis proposes that the

costs of co-breeding with kin are greater for older than younger females (Croft et al., 2017). Within the broader theme of ageing, these hypotheses to explain the menopause and a prolonged post-menopausal lifespan may be classified as programmed theories of ageing — a class of theories positing that humans have generally evolved mechanisms that purposely limit their lifespans for an evolutionary benefit (Goldsmith, 2014). In contrast, non-programmed or error theories of ageing posit that ageing results from natural deteriorative processes. The different theories of ageing are not mutually exclusive, and it is likely that a combination of theories may be necessary to adequately describe the complex and multi-factorial process of ageing.

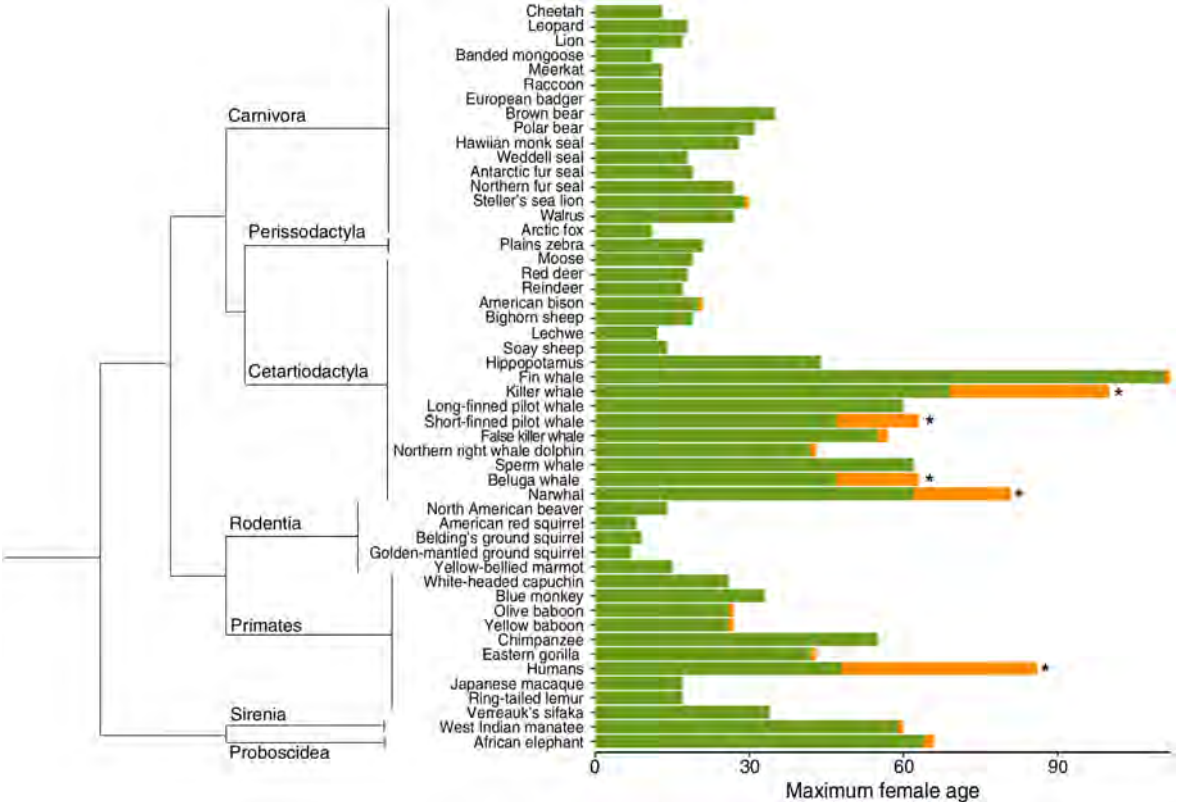


FIGURE 1.1: Reproductive (green) and post-reproductive (orange) years in females from 51 species of mammals. For five species — humans, killer whales, short-finned pilot whales narwhals and beluga whales — the proportion of post-reproductive years is significantly different from zero; extracted from Johnstone and Cant (2019).

Ageing may occur via one of three trajectories: (i) disease and disability, (ii) usual ageing, characterised by an absence of overt pathology but some decline in function, or (iii) successful ageing, characterised by little or no physiological loss and no pathology (Weinert and Timiras, 2003). This thesis addresses the trajectory of usual ageing, by

studying healthy but untrained women who had a natural menopause. The experimental studies will focus on menopause-related effects on the heart, and in particular, on the rotation and deformation of the left ventricle ("LV mechanics") that underlie the function of the heart. In addition, an exercise training intervention that was conducted as part of this thesis will have crossover implications for the other two trajectories of ageing — in disease prevention and improved ageing in middle-aged women.

1.2 Research gaps and novelty of this thesis

The menopause has been associated with a concentric remodelling of the LV (Hinderliter et al., 2002; Pines et al., 1993), lower systolic and diastolic function (Düzenli et al., 2007; Hayward et al., 2000; Kangro et al., 1995; Schillaci et al., 1998), and lower longitudinal strain (Keskin Kurt et al., 2014; cf. Literature review Section 2.4.3). However, the effects of the menopause in the wider context of human ageing in men and women are unclear. With ageing, men have been suggested to experience a greater decline in left ventricular (LV) mass (Dannenberg et al., 1989; Hees et al., 2002; Natori et al., 2006), and women a greater decline in diastolic function (Daimon et al., 2011; Grandi et al., 1992; Okura et al., 2009; cf. Literature review Section 2.3.3). Sex hormones are a likely culprit for differences between men and women (Khosla et al., 1998; cf. Literature review Section 2.4.1–2.4.2), and it is likely that the sharp fall in circulating levels of oestrogen and progesterone (the primary female sex hormones) after the menopause (Harlow et al., 2012; Soules et al., 2001) contributes to sex differences in middle-aged adults. However, the effects of the menopause are rarely considered in large-scale ageing studies. The first research study in this thesis will tackle this gap — by comparing LV structure, function and mechanics in pre- and post-menopausal women, within a cross-sectional study of ageing in young adult and middle-aged men and women.

The effects of the menopause on the heart have been largely garnered from observational studies of women under resting conditions. An understanding of the impact of the menopause on the functional capacity of the heart is therefore lacking. This would provide insight into a woman's ability to complete the activities of daily living, and

would likely reveal information on cardiac function that cannot be deduced from resting data alone (e.g. Tan et al., 2010). To tackle this dearth in the scientific literature, the second research study in this thesis will assess LV function and mechanics during two physiological tests — lower body negative pressure (cf. Literature review Section 2.5.1) and submaximal supine cycling (cf. Literature review Section 2.5.2). In addition to these acute physiological tests, the second and third studies in this thesis will investigate the effects of the menopause on changes in LV function and mechanics after a short-term (12-week) exercise training intervention. Exercise training studies are resource-intensive and conducted even less frequently than acute functional tests, but provide valuable insight into physiological adaptability by accounting for baseline differences.

The underlying mechanics of the LV have been implicated in the healthy functioning of the heart both at rest and during physiological tests, by enabling filling of the LV from the left atrium and the ejection of blood from the LV into the aorta (Doucende et al., 2010; Esch et al., 2010; Takeuchi et al., 2007; Yoneyama et al., 2012; cf. Literature review Section 2.5). Of particular relevance to this thesis, previous work suggests that regional differences in calcium handling and sympathetic drive may manifest as differences in basal and apical mechanics of the LV between pre- and post-menopausal women. This hypothesis is founded upon (i) a localised effect of oestrogen on increasing the L-type calcium current in cardiomyocytes isolated from the basal epicardium (Yang et al., 2012), and (ii) a menopause-related surge in cardiac sympathetic nerve activity (Sakata et al., 2009) stimulating more nerve endings at the base than at the apex (Kawano et al., 2003; Pianca et al., 2019). By tracking the speckle pattern of the myocardium in basal and apical short-axis ultrasound images across the cardiac cycle (speckle tracking echocardiography; Helle-Valle et al., 2005; cf. Methods Section 3.3), LV mechanics will be investigated in all of the three research studies in this thesis. A literature search indicates that the effects of the menopause on basal and apical mechanics of the LV are unknown. Therefore, the data on LV mechanics that will be acquired for this thesis may begin to build the missing link between *in vitro* mechanistic studies on the effects of oestrogen and sympathetic drive on cardiomyocytes (typically conducted with animal models) — and *in vivo* effects in women.

1.3 Thesis overview

The aim of this thesis is to investigate the effects of the menopause on the heart, with a focus on the underlying mechanics of the LV. In the following chapter, a review of the literature pertaining to the work in this thesis will first be presented (Chapter 2). This will include an overview of cardiac physiology and LV mechanics, a review of sex differences with cardiac ageing, a summary of the impact of female sex hormones on the heart, and an overview of the typical cardiovascular responses to lower body negative pressure, incremental exercise and exercise training. The possible effects of the menopause on LV structure, function and mechanics will be discussed across the chapter based on the existing literature.

In the chapter after the literature review, the experimental methods used in this thesis will be described in detail (Chapter 3). Data for this thesis were collected from August 2011 to May 2014 in the School of Sport at Cardiff Metropolitan University. The experimental results of this thesis will then be presented in three consecutive chapters, in which the experimental methods used will be reiterated, albeit succinctly.

In the first experimental study, the effects of the menopause on LV structure, function and mechanics will be investigated within a cross-sectional study of ageing in young adult and middle-aged men and women (Chapter 4). In the second experimental study, the effects of the menopause on LV function and mechanics after exercise training will be investigated, both at rest and during the physiological tests of lower body negative pressure and submaximal supine cycling (Chapter 5). The third and final experimental study will investigate whether exercise training mitigates the effects of the menopause on LV function and mechanics in post-menopausal women (Chapter 6). A general discussion in the last chapter of this thesis (Chapter 7) will combine all of the experimental work under the unifying research question of this thesis — *the effects of the menopause on LV mechanics*.

Chapter 2

Literature review

Part of this chapter has been published: Nio AQX, Stöhr EJ, Shave R (2015). The female human heart at rest and during exercise: A review. *Eur J Sport Sci*, 15:286–295.

2.1 Introduction

Ageing affects the hearts of men and women differently: men experience a greater decline in left ventricular (LV) mass (Dannenberg et al., 1989; Hees et al., 2002; Natori et al., 2006), and women a greater decline in diastolic function (Daimon et al., 2011; Grandi et al., 1992; Okura et al., 2009). In fact, cardiovascular disease typically occurs at a later age in women compared with men (Maas et al., 2011). One potential player in the age-related changes in women is the menopause, which has been associated with a concentric remodelling of the LV (Düzenli et al., 2007; Hinderliter et al., 2002; Pines et al., 1993; Schillaci et al., 1998) and lower systolic and diastolic function (Düzenli et al., 2007; Hayward et al., 2000; Kangro et al., 1995; Schillaci et al., 1998). Contradictions, limitations and gaps in the existing literature, however, highlight a need for further work to elucidate the effects of ageing and the menopause on the female heart. In addition, most of our knowledge is built upon measures of the heart under resting conditions — an experimental model that lacks input on the functional capacity of the heart (Goldspink, 2005). Further work is therefore especially important to understand how the female heart copes with the physiological stressors of daily living.

In this chapter, the literature on the ageing female heart will be reviewed, with a focus on the effects of the menopause. This has been organised into four sections. The first section provides an overview of the physiology of the LV, and introduces key terms and concepts relevant to this thesis. In the second section, the effects of sex and age on resting LV structure, function and mechanics will be summarised. The purpose of the third section is twofold: i) to provide an overview of the effects of the primary female sex hormones (oestrogen and progesterone) on cardiac myocytes by drawing from *in vitro* and animal work; and ii) to review the literature on the impact of the menopause on the human LV at rest. The fourth section introduces the general responses of the LV to the physiological tests that will be used in this thesis — lower body negative pressure, acute exercise and exercise training — and highlights the potential influence of the menopause on these responses. Drawing upon the literature discussed in these four sections, magnitudes of physiological "meaningfulness" for key measures of LV

function and mechanics will be consolidated. The chapter concludes with an overall summary and the aims of this thesis.

2.2 Physiology of the left ventricle

The heart has intrigued many generations—from the Egyptians who believed that it was the seat of emotions and intellect (circa 2400 BC; Faulkner et al., 2008); Aristotle who argued that it was the centre of sensation and movement (lived 384–322 BC; Gross, 1995); and Leonardo da Vinci who investigated and sketched the heart when he was over 60 years old (circa AD 1513; Figure 2.1; da Vinci et al., 1983; Keele, 1983). More than a century later — in AD 1628 — William Harvey published his theory of the circulation of blood via the heartbeat (Wright, 2013), revolutionising our understanding of the function of the heart (Levick, 2010). We now consider the heart as the primary pump that circulates blood through the entire cardiovascular system (Kenney et al., 2015). The focus of this thesis is on the LV — the most muscular chamber of the heart — which ejects oxygenated blood into the aorta and to the rest of the body (Figure 2.2; British Heart Foundation, 2007; Sherwood, 2016a). The following subsections will provide an overview of the cardiac cycle, introduce LV anatomy and mechanics, and describe the action potential underlying cardiac contraction and relaxation.

2.2.1 Left ventricular function across the cardiac cycle

A cardiac cycle refers to one heartbeat, and is made up of two distinct components: i) systole, comprising isovolumic (or "isovolumetric") contraction and ejection; and ii) diastole, comprising isovolumic relaxation and filling (Figure 2.3; Klabunde, 2012a; Sherwood, 2016a). Systole begins with the QRS complex of the electrocardiogram (ECG), which reflects ventricular depolarisation. This causes contraction of the ventricular myocardium (heart muscle; cf. Section 2.2.4). As the LV begins to contract, intra-ventricular pressure rises above left atrial pressure, and the mitral valve closes. Left ventricular pressure rises rapidly until it exceeds aortic pressure, resulting in the opening of the aortic valve. Between mitral valve closing and aortic valve opening, LV

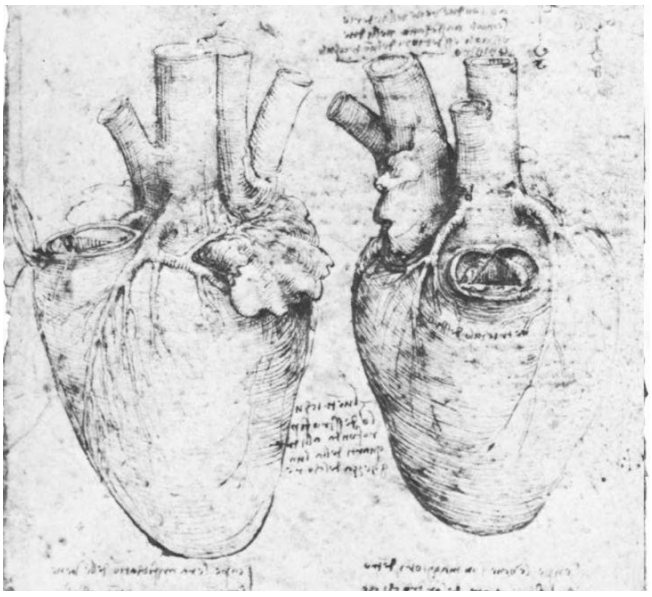


FIGURE 2.1: Drawing of the heart by Leonardo da Vinci, circa AD 1513 (lived AD 1452–1519; extracted from da Vinci et al., 1983).

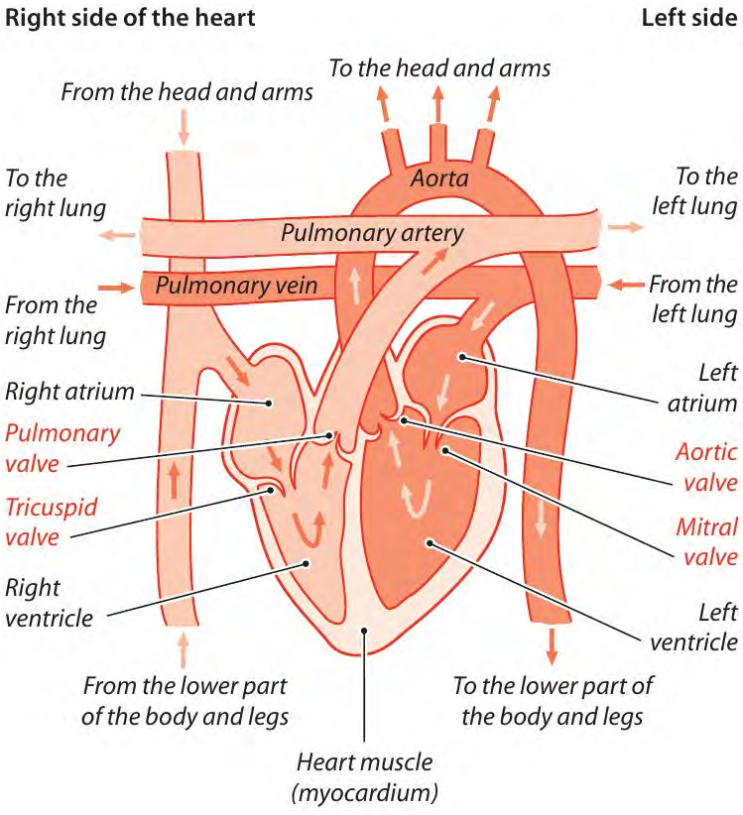


FIGURE 2.2: Direction of blood flow through the heart, as indicated by arrows (extracted from British Heart Foundation, 2007).

volume is constant — this phase of the cardiac cycle is appropriately termed "isovolumic contraction".

With the opening of the aortic valve, blood is ejected from the LV into the aorta (the systemic circulation). This phase of the cardiac cycle is known as "ejection". The total volume of blood ejected during this period is the "stroke volume" of each cardiac cycle. Stroke volume is derived from the difference between the maximum volume of blood in the ventricle after filling ("end-diastolic volume") and the residual volume of blood in the ventricle after ejection ("end-systolic volume"; Figure 2.4; Sherwood, 2016a). Accordingly, "ejection fraction" refers to the ratio of stroke volume to end-diastolic volume, expressed as a percentage.

After systole (end of ejection) and with the beginning of diastole, the LV begins to lengthen. Left ventricular pressure falls below aortic pressure, and the aortic valve closes. This marks the onset of "isovolumic relaxation" or "post-ejection isovolumic interval' '— the first phase of diastole (Buckberg, 2015). As its name implies, LV volume is constant during this phase. Left ventricular pressure declines rapidly until it falls below left atrial pressure, and the mitral valve opens. This signals the end of isovolumic relaxation.

Left ventricular filling begins after the opening of the mitral valve. Rapid passive filling first occurs, as blood flows from the left atrium (higher pressure) into the LV (lower pressure). As the LV is still relaxing at the onset of filling, intra-ventricular pressure continues to fall (despite filling). As the pressure gradient across the mitral valve declines, filling is reduced — this period is sometimes referred to as ventricular "diastasis". Atrial contraction begins with the P wave of the ECG, which represents electrical depolarisation of the left atrium, typically after LV relaxation is completed (Nishimura and Tajik, 1997). Left atrial pressure increases, and more blood flows into the LV (LV volume increases further). The two major determinants of LV filling are (i) LV relaxation, which is an energy-dependent process during which actin-myosin cross-bridges detach and sarcomeres return to their resting pre-contraction length, and (ii) effective chamber compliance, which describes the passive viscoelastic properties of the myocardium during filling (Nishimura and Tajik, 1997; Zile and Brutsaert, 2002).

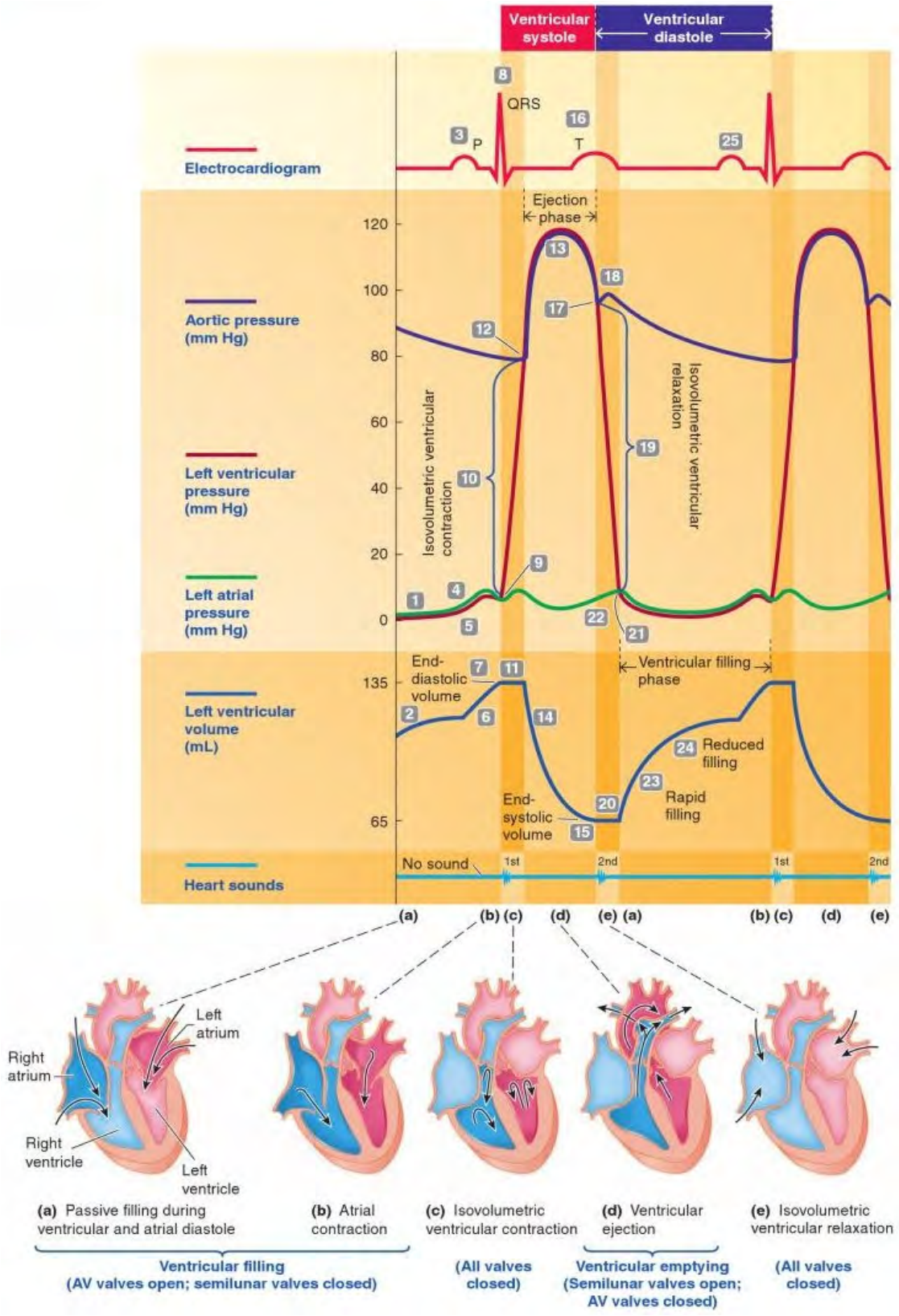
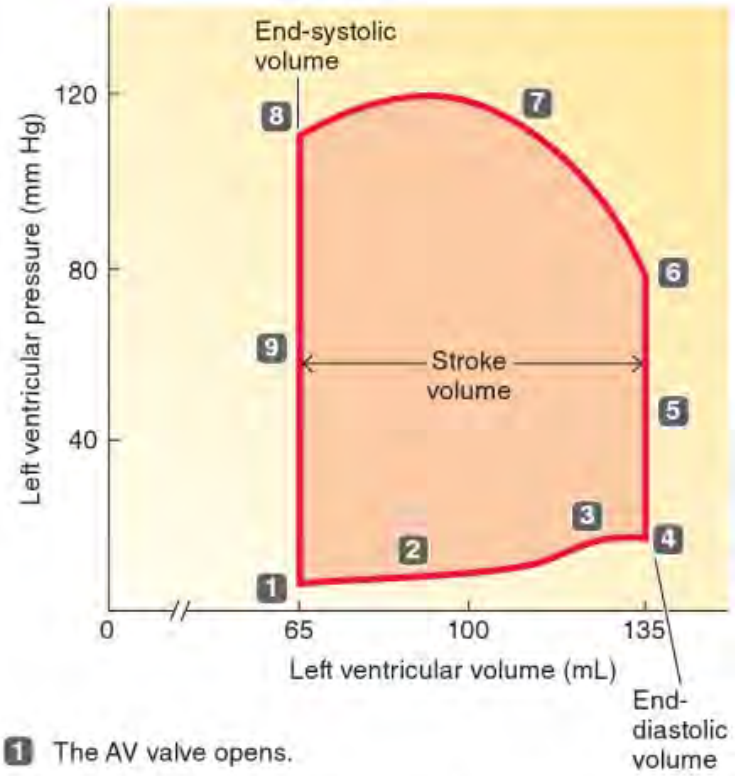


FIGURE 2.3: The cardiac cycle, illustrating the electrocardiogram, the interaction between aortic, left ventricular and left atrial pressures, and changes in left ventricular volume over systole and diastole. AV: atrioventricular (extracted from Sherwood, 2016a).



1 The AV valve opens.

2 Passive ventricular filling occurs. Volume increases considerably and pressure increases slightly as blood enters.

3 Atrial contraction completes ventricular filling. End-diastolic volume is reached at the end of this phase.

4 The AV valve closes.

5 Isovolumetric ventricular contraction occurs. Volume remains constant; pressure increases markedly.

6 The aortic valve opens.

7 A stroke volume of blood is ejected. As blood leaves, volume decreases considerably as pressure peaks, then falls more slowly until end-systolic volume is reached at the end of this phase.

8 The aortic valve closes.

9 Isovolumetric ventricular relaxation occurs. Volume remains constant; pressure falls sharply.

Back to step 1.

FIGURE 2.4: Pressure-volume loop of the left ventricle over one cardiac cycle (extracted from Sherwood, 2016a).

The end of filling coincides with the start of the next ventricular systole (i.e. isovolumic contraction), and the cardiac cycle repeats.

The number of cardiac cycles per minute is known as "heart rate", typically expressed in beats/min. With information on heart rate and stroke volume, cardiac output can be calculated — reflecting the volume of blood ejected from the ventricle every minute (typically expressed in L/min). These changes in pressures and volumes over the cardiac cycle are underpinned by a distinct helical/spiral anatomy, which optimises the ejection fraction achieved with fibre shortening (Figure 2.5; Buckberg, 2002; Buckberg et al., 2001).

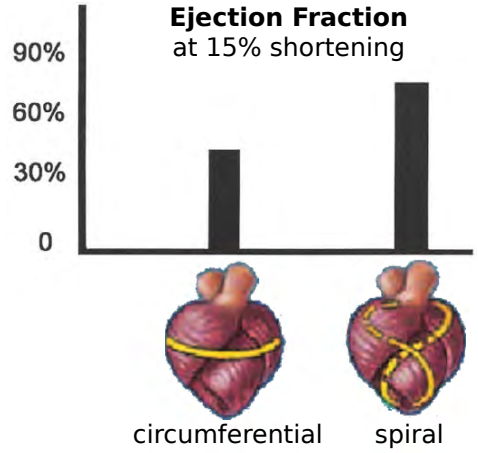


FIGURE 2.5: The impact of fibre angle on ejection fraction, based on a contractile shortening of 15%. A circumferential or transverse orientation produces an ejection fraction of only 30%; a spiral formation produces an ejection fraction of 60% (adapted from Buckberg, 2002; Buckberg et al., 2001).

2.2.2 Left ventricular anatomy

Left ventricular myocardial architecture may be depicted as a transmural continuum between two helical fibre geometries — a right-handed helix in the sub-endocardium (inner layer of the LV), circumferential fibres in the mid-wall, and a left-handed helix in the sub-epicardium (outer layer of the LV; Figure 2.6; Nakatani, 2011; Sengupta et al., 2007, 2008; Sosnovik et al., 2001; Streeter et al., 1969). The myofibre helix angle (defined as the angle between the circumferential axis and the projection of the myofibre onto the circumferential-longitudinal plane) typically ranges from +60° at the

sub-endocardium (right-handed helix set as positive) to −60° at the sub-epicardium (Chen et al., 2005; Sengupta et al., 2006b; Streeter et al., 1969). The orientation of myofibres changes across the cardiac cycle, with sub-endocardial and sub-epicardial fibres becoming more longitudinally-oriented at end-systole (Chen et al., 2005). Mathematical modelling suggests that this specific arrangement is energetically efficient, and essential in minimising differences in oxygen demand, sarcomere strain and developed stress across the ventricular wall (Beyar and Sideman, 1986; Bovendeerd et al., 1992; Sengupta et al., 2006b; Vendelin et al., 2002). A single myocardial band looped over (i.e. the helix) and wrapped around itself (i.e. the basal loop) has been suggested by Francisco Torrent-Guasp to underpin the complex spiral architecture of the heart (Figure 2.7; Buckberg, 2002; Torrent-Guasp et al., 2001).

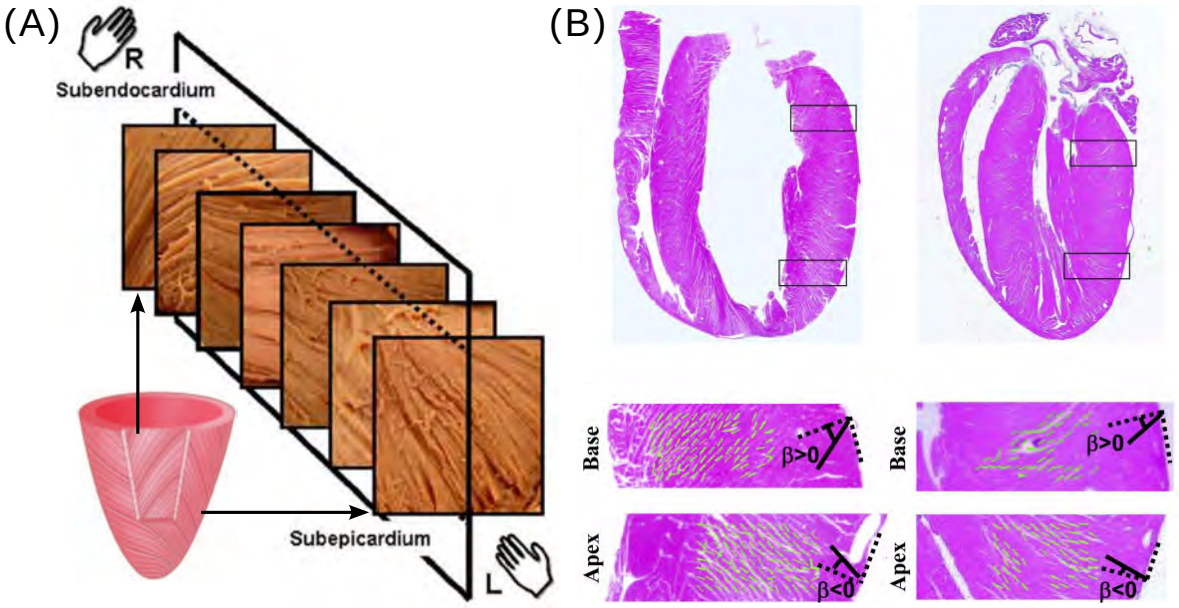


FIGURE 2.6: (A) Myocardial fibre orientation in the left ventricle changes gradually from a right-handed helix in the sub-endocardium to a left-handed helix in the sub-epicardium (adapted from Sengupta et al., 2007, 2008; Sosnovik et al., 2001). (B) Longitudinal slides of rat hearts fixed at diastole (left) and systole (right), with quantification of sheet angle in the boxed areas magnified and shown below (extracted from Chen et al., 2005).

In addition to the transmural variation in myofibre alignment in the circumferential-longitudinal plane, myocytes are organised into sheets that slide and rearrange along cleavage planes across the cardiac cycle (Chen et al., 2005; Sengupta et al., 2006b). Viewed in the longitudinal-radial plane, a sheet orientated towards the base from the

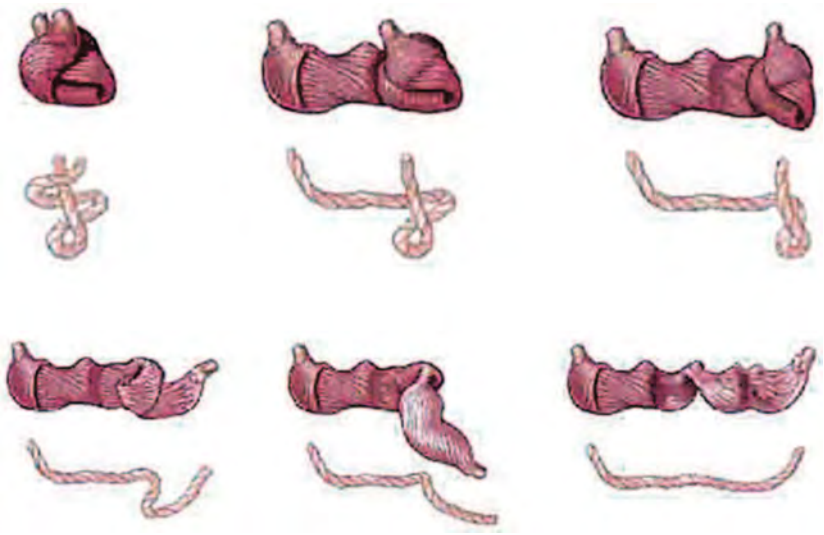


FIGURE 2.7: The unfolding of the myocardial band, with the heart likened to a coiled rope. Three components become evident: a beginning and an end; a surrounding basal loop; and a helix (extracted from Buckberg, 2002; Torrent-Guasp et al., 2001).

sub-endocardium to sub-epicardium is defined with a positive sheet angle (Figure 2.6, Chen et al., 2005). Therefore, sheet angles at the base are represented by positive values, and sheet angles at the apex by negative values. The magnitude of the sheet angle decreases during systole, which suggests a more radial orientation of the sheet that contributes to radial wall thickening (Chen et al., 2005). Taken together, the complex spiral architecture of the LV gives rise to rotation and deformation over the cardiac cycle, which in turn, contributes to healthy LV function (Doucende et al., 2010; Esch et al., 2010; Sengupta et al., 2008; Takeuchi et al., 2007; Yoneyama et al., 2012). Details on LV mechanics across the different phases of the cardiac cycle will be presented in the following section.

2.2.3 Left ventricular mechanics

In this thesis, "LV mechanics" is the umbrella term for the rotation and deformation of the LV across the cardiac cycle. Rotation is conventionally viewed from the apex, with negative values depicting clockwise rotations and positive values for anti-clockwise rotations (Sengupta et al., 2006b). Due to the right-handed helical arrangement of myocardial fibres in the sub-endocardium (Section 2.2.2), muscular contraction effects

an anti-clockwise rotation at the base and a clockwise rotation at the apex (Figure 2.8; Nakatani, 2011; Sengupta et al., 2008). Conversely, the left-handed arrangement of fibres in the sub-epicardium effects a clockwise rotation at the base, and an anti-clockwise rotation at the apex. As the radius of the sub-epicardium is greater than that of the sub-endocardium, it produces a greater torque and the rotation of the sub-epicardium dominates. The net difference in rotation between the base and the apex is termed "LV twist".

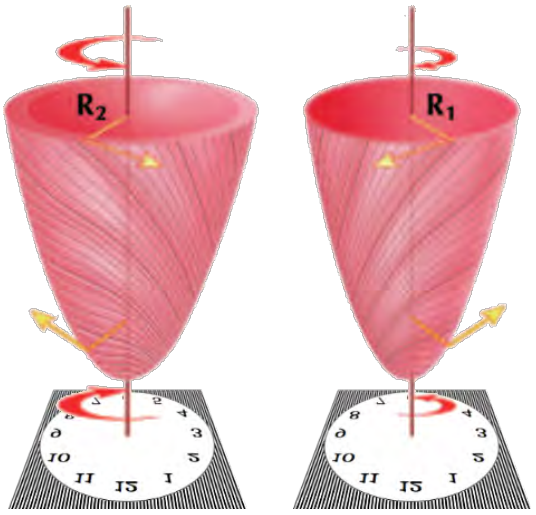


FIGURE 2.8: Diagrammatic representation of the left-handed helix of the sub-epicardium (left) and the right-handed helix of the sub-endocardium (right). When viewed from the apex (as illustrated by the clock facing downwards), contraction of the sub-epicardium produces a clockwise rotation at the base and an anti-clockwise rotation at the apex; contraction of the sub-endocardium produces an anti-clockwise rotation at the base and a clockwise rotation at the apex. As the radius of the sub-epicardium is greater than that of sub-endocardium ($R_2 > R_1$), the rotation of the sub-epicardium dominates (adapted from Sengupta et al., 2008).

Deformation — the other aspect of LV mechanics — refers to changes in length/thickness along the longitudinal, circumferential and radial coordinates of the LV (Figure 2.9; Cheung, 2012; D'hooge et al., 2000; Sengupta et al., 2006b). This is quantified by "strain", which is defined as the percentage change in length over the cardiac cycle, relative to the length at end-diastole. Therefore, thickening in the radial dimension during systole is reflected by positive values of strain, while the concomitant shortening in longitudinal and circumferential dimensions are indicated by negative values (in line with the conservation of mass). Longitudinal strain is likely dominated by the contraction of longitudinally and obliquely-oriented fibres, circumferential strain by the

contraction of obliquely-oriented fibres and radial strain by changes in sheet orientation (Chen et al., 2005).

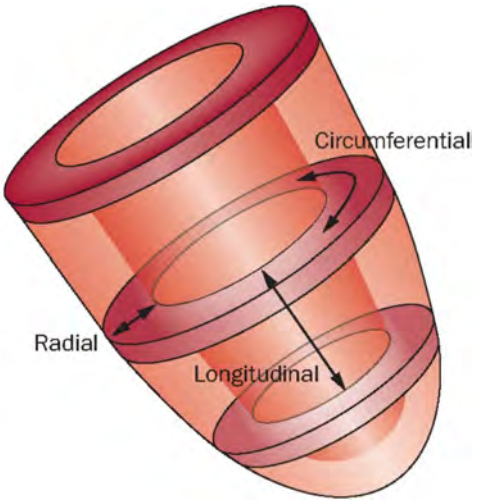


FIGURE 2.9: Diagrammatic representation of the longitudinal, circumferential and radial coordinates of the left ventricle (extracted from Cheung, 2012). During systole, left ventricular deformation comprises radial thickening, and circumferential and longitudinal shortening.

Isovolumic contraction. At the onset of systole, electrical activation begins at the apical sub-endocardium and spreads rapidly towards the base (Sengupta et al., 2006b, 2007, 2008). During isovolumic contraction, a circumferential stretch has been observed in the sub-epicardial layer, tethered by circumferential shortening in the mid-wall and sub-endocardial layer (Ashikaga et al., 2009). This shortening in one direction and stretching in another direction satisfies isovolumic mechanics (Sengupta et al., 2006b, 2007). In addition, this pre-ejection stretch has been suggested to "load" the myofibres for a Starling effect during ejection via the titin mechanism (Buckberg et al., 2008). The dominant shortening of sub-endocardial fibres during isovolumic contraction accounts for the brief decrease in LV twist observed during this period, prior to the greater torque and increase in twist caused by the shortening of sub-epicardial fibres later in systole (Figure 2.10). Sheet extension and thinning concurrent with a larger sheet angle contribute to radial thickening within the isovolumic constraint, and longitudinal shortening occurs across the myocardial wall (Ashikaga et al., 2009).

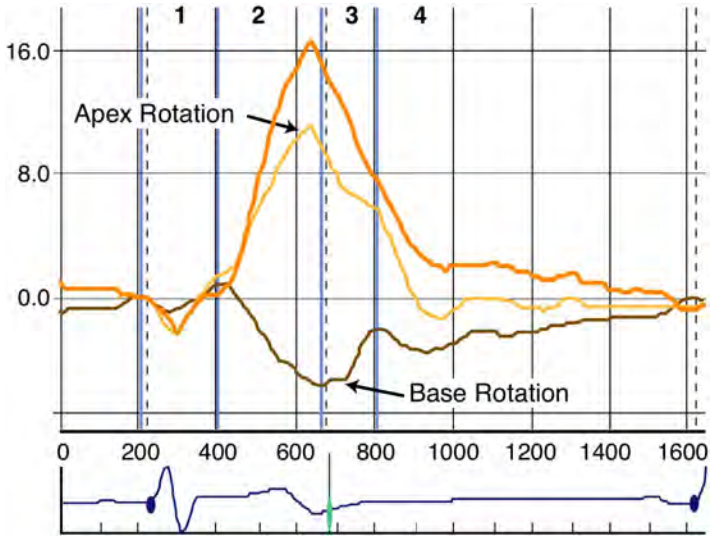


FIGURE 2.10: Left ventricular rotation at the base and apex — and net twist — over a cardiac cycle. Phase 1: isovolumic contraction; 2: ejection; 3: isovolumic relaxation, 4: filling (extracted from Sengupta et al., 2008).

Ejection. During ejection, contraction occurs across the myocardial wall and causes longitudinal and circumferential shortening (Cheung, 2012). Thickening occurs in the radial dimension, as sheet angle decreases and over-compensates for the increase in sheet angle that occurred during isovolumic contraction (Ashikaga et al., 2009; Chen et al., 2005). A pronounced anti-clockwise rotation is observed at the apex and a clockwise rotation at the base, driven by the dominant torque of the left-handed sub-epicardium. Rotations and strains are higher at the apex than the base (Buckberg et al., 2006; Sengupta et al., 2006a, 2008), and the mitral annulus moves towards the apex (Sengupta et al., 2007). Through the twisting and shearing of sub-endocardial fibres, kinetic energy is converted to potential energy in preparation for isovolumic relaxation (Buckberg et al., 2008; Sengupta et al., 2007, 2008; Waldman et al., 1988).

Isovolumic relaxation. The deformed matrix created during the ejection period may be likened to a coiled spring, which recoils and releases potential energy during isovolumic relaxation (Buckberg et al., 2008; Sengupta et al., 2007, 2008). This recoil has been attributed to the expansion of titin and collagen pathways that were compressed during ejection (Buckberg et al., 2015). Thinning of the LV wall during early relaxation occurs mainly from sheet shortening, while further wall thinning later in

diastole is driven by a return of sheet shear to baseline (Ashikaga et al., 2004; Cheng-Baron et al., 2010). Simultaneous shortening and lengthening within the LV wall during isovolumic relaxation initiates diastolic restoration without changes in LV volume (Sengupta et al., 2008). The LV cavity first expands at the apex, underpinned by the recoil and lengthening of the apical sub-endocardium, concurrent with post-systolic shortening of the basal sub-endocardium (Sengupta et al., 2006a,b). In contrast, relaxation of the sub-epicardium proceeds from base-to-apex: lengthening at the base, concurrent with post-systolic shortening at the apex. These transformations in the LV wall likely underpin a rapid decrease in intra-ventricular pressure during isovolumic relaxation (Sengupta et al., 2006a). A lower pressure in the LV relative to the left atrium, however, may not be the only stimulus for mitral valve opening (Buckberg et al., 2015). Prompted by empirical evidence of a time-lapse between mitral valve opening and the onset of flow into the LV, the resultant clockwise rotation at the apex against a fixed mitral annulus has been suggested to pull the mitral leaflet edges down (Buckberg et al., 2015). Driven by apical rotation, peak untwisting velocity typically occurs close to mitral valve opening (Doucende et al., 2010) and has been implicated as an indicator of diastolic dysfunction (Burns et al., 2009; Takeuchi et al., 2007).

Filling. Subsequent to expansion of the LV cavity at the apex during isovolumic relaxation, the base expands for early diastolic filling (Sengupta et al., 2007). Peak early diastolic basal rotational velocity and strain rates occur close to peak early diastolic filling (Doucende et al., 2010). Untwisting (recoil) occurs predominantly during isovolumic relaxation and early filling (Sengupta et al., 2008). Wall thinning (a decrease in radial strain back to baseline) is driven by a return of sheet shear to baseline (Ashikaga et al., 2004; Cheng-Baron et al., 2010). Lengthening occurs in the longitudinal and circumferential dimensions to return these strains to baseline (Cheng-Baron et al., 2010; Sengupta et al., 2006b). Peak late diastolic strain rates reflect atrial contribution to LV filling (Sengupta et al., 2006b; Weidemann et al., 2002). At the end of diastole, the LV myocardium is repolarised and the cardiac cycle repeats with the QRS complex of the ECG (depolarisation) for systole. The conversion of electrical stimuli to mechanical movements of the LV — termed cardiac excitation-contraction coupling — will be

summarised in the following subsection (Bers, 2014; Sherwood, 2016a).

2.2.4 Cardiac excitation-contraction coupling

At rest, the membrane potential of ventricular contractile cells is approximately −90 mV (Klabunde, 2012c; Sherwood, 2016a). Open leaky K^+ (potassium) channels — which allow K^+ ions to move out of the cell — maintain resting potential close to the K^+ equilibrium potential (Figure 2.11). Prior to ventricular contraction in the healthy heart (systole), an action potential (a phenomenon involving temporal changes in membrane potential) originates in the sinoatrial node, is conducted to the atrioventricular node, and travels rapidly down the septum via the bundle of His and throughout the ventricular myocardium via the Purkinje fibres (Figure 2.12; Sherwood, 2016a; Varghese, 2015). When this action potential reaches the ventricular contractile cells, these cells are rapidly depolarised to their threshold potential of approximately −70 mV and a local action potential is generated (Figure 2.11). The membrane potential is first further depolarised with rapid entry of Na^+ (sodium) into the cell, due to a transient activation of voltage-gated Na^+ channels. At peak potential (+30 mV in Figure 2.11), these voltage-gated Na^+ channels are rapidly inactivated and transient outward K^+ channels open — resulting in a small repolarisation. This initial repolarisation is followed by a plateau phase, due to a decrease in K^+ permeability and an inward diffusion of Ca^{2+} (calcium) via slow L-type Ca^{2+} channels (now open) — resulting in a maintained membrane potential near peak positive levels. Subsequent inactivation of the Ca^{2+} channels and the opening of delayed rectifier K^+ channels causes rapid repolarisation. As the cell returns to resting potential, the delayed rectifier K^+ channels close and the leaky K^+ channels open once again.

The entry of Ca^{2+} into the cell during the action potential is key to muscular contraction (Bers, 2014; Klabunde, 2012b). Whilst only a relatively small amount of Ca^{2+} enters the cell during depolarisation, it triggers the release of a large amount of Ca^{2+} from the sarcoplasmic reticulum (known as Ca^{2+}-induced Ca^{2+} release). This cytosolic Ca^{2+} binds to the troponin-tropomyosin complex, exposing the myosin-binding site on the actin molecule. Binding of a myosin head to actin results in ATP (adenosine

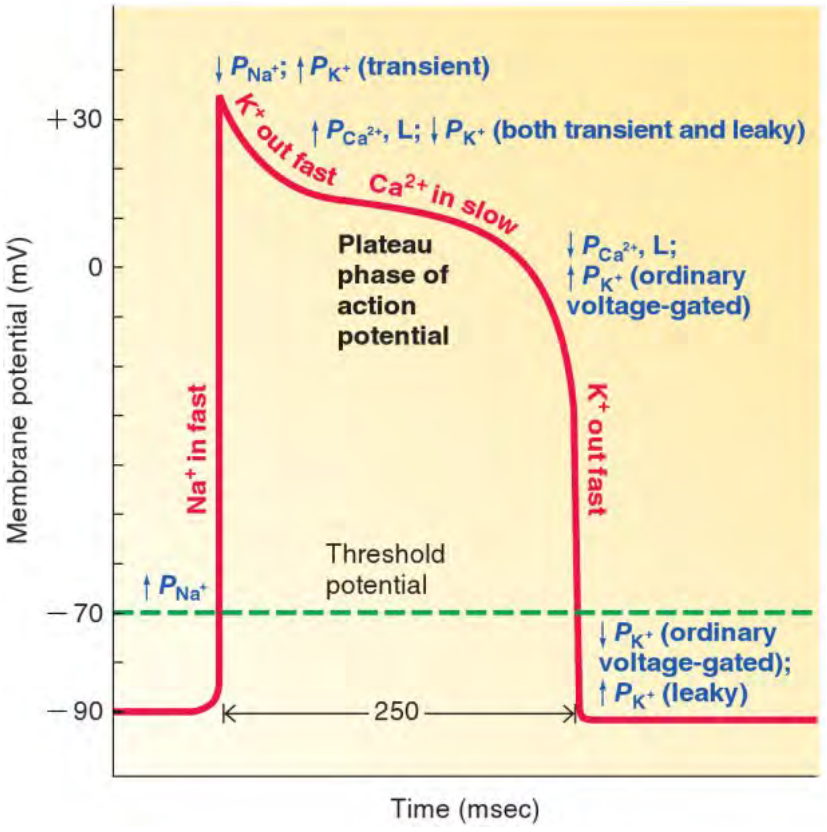


FIGURE 2.11: The action potential in left ventricular contractile cells (see Section 2.2.4 for details; extracted from Sherwood, 2016a).

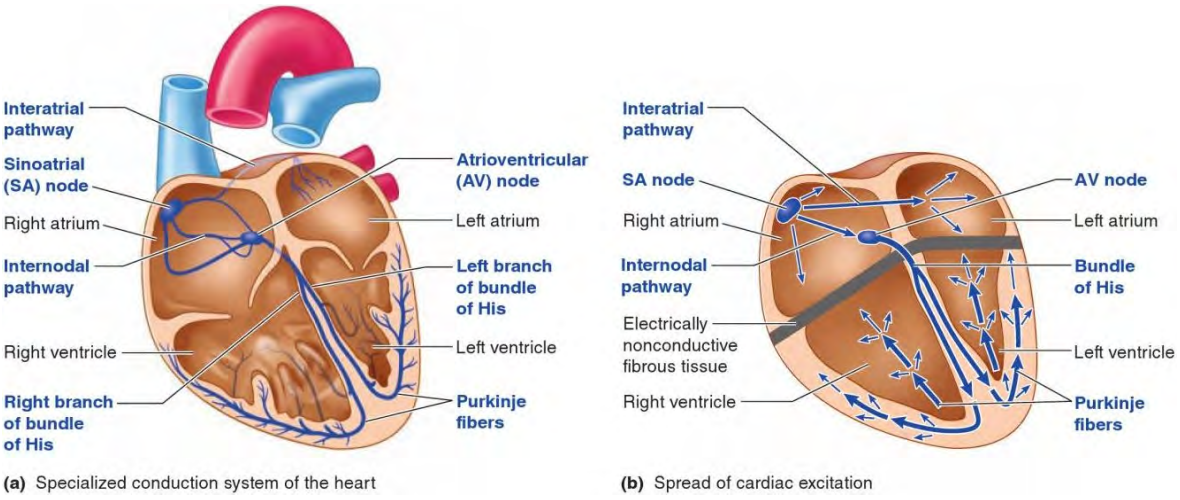


FIGURE 2.12: (a) Anatomy of the conduction system of the heart, and (b) spread of cardiac excitation (extracted from Sherwood, 2016a). An action potential initiated at the sinoatrial (SA) node is conducted to the atrioventricular (AV) node, from which it spreads rapidly throughout the ventricles via the Bundle of His and Purkinje fibres.

triphosphate) hydrolysis, which supplies energy for contraction. As Ca^{2+} is removed from the cytosol — mainly via sarcoendoplasmic reticulum calcium ATPase (SERCA) and sodium/calcium exchangers (NCX) — contraction ceases and the myocardium relaxes.

Sex hormones and the autonomic nervous system affect the cardiac excitation-contraction coupling pathway, and therefore regulate changes in cardiac contractility (inotropy) and relaxation (lusitropy), and heart rate (chronotropy) (Parks and Howlett, 2013). Parasympathetic drive dominates at rest, while sympathetic activity increases with physiological stress. By investigating cardiac function in response to various physiological stressors, the work in this thesis will provide insight into the balance of parasympathetic and sympathetic stimulation on the heart. Prior to a review of the literature on LV structure, function and mechanics in response to lower body negative pressure, acute exercise and exercise training (cf. Section 2.5), however, it is necessary to first consider the default resting state of the heart. Previous work suggests that resting LV structure, function and mechanics may differ between men and women, and between younger and older adults. This will first be reviewed in the following section.

2.3 The human left ventricle at rest

Scientists have identified differences between male and female hearts, and between those of younger and older adults. Measures of cardiac structure and function—such as LV mass, ejection fraction and cardiac output — have been reported more widely than those describing LV mechanics (i.e. rotation and strain across the cardiac cycle). The standalone effects of sex and ageing have also received more scientific attention than whether the heart ages differently in men and women (in statistical terms, the interaction effect between sex and ageing). To highlight these gaps in our knowledge base, the following subsections have been organised to review the existing literature investigating the effects of i) sex, ii) ageing and iii) the interaction between sex and ageing on LV structure, function and mechanics.

2.3.1 Male versus female hearts

Cardiac physiology

Sex differences in cardiac physiology exist throughout the human lifespan. For example, pre-pubertal boys have a higher risk of arrhythmic events than girls, but this inverts after puberty and adult women have a higher risk than men (for a consensus paper on sex differences in cardiac arrhythmia see Linde et al., 2018). Adult men typically have larger hearts than women, greater maximal aerobic capacities and maximal cardiac outputs, and may rely more on systolic contraction than diastolic filling to achieve stroke volume during exercise (Nio et al., 2015). Compared with men of the same age, women are relatively protected against cardiovascular disease before the menopause, but this gap narrows after the menopause (Garcia et al., 2016).

Sex differences also exist in cardiac pathophysiology. For example, women are twice as likely to develop heart failure with preserved ejection fraction than men (Scantlebury and Borlaug, 2011) and also twice as likely to have non-obstructive coronary artery disease (Pepine et al., 2015). Women older than 55 years are five times as likely to develop Takotsubo syndrome than younger women, and ten times as likely than men (Ghadri et al., 2018). A better understanding of sex differences and the effects of the menopause on healthy cardiac physiology will likely provide insight into the different predispositions to disease between these groups. The following sections will focus on cardiac physiology in health.

Cardiac structure

When comparing cardiac structure between men and women, it is essential to remember that a greater body size is typically associated with a larger heart (Celentano et al., 2003; Gardin et al., 1995; Kaku et al., 2011; Nio et al., 2015; Sandstede et al., 2000). Accordingly, many studies have reported smaller absolute LV mass and wall dimensions in women than men (Celentano et al., 2003; Gardin et al., 1995; Grandi et al., 1992; Hutchinson et al., 1991; Wilhelm et al., 2011; cf. Figure 2.17), and sex-specific absolute

reference ranges are employed when assessing cardiac pathology (Lang et al., 2006). In an attempt to account for the effect of body size, previous authors have often assumed a directly proportional relationship between cardiac parameters and body surface area (i.e. ratiometric scaling; Dewey et al., 2008). Although these results will be reviewed in this chapter and inform the current knowledge base, it is important to note that normalising to body surface area may not fully eliminate the impact of body size (Dewey et al., 2008; cf. Methods Section 3.3.3). Previous findings therefore need to be interpreted with caution, and current conclusions verified using more appropriate allometric scaling techniques (Batterham et al., 1997; Dewey et al., 2008).

While acknowledging the issues related to ratiometric scaling, sex differences in LV mass have been shown to persist after indexing to body surface area (Celentano et al., 2003; de Simone et al., 1991; Grandi et al., 1992; Hutchinson et al., 1991; Sandstede et al., 2000; Wilhelm et al., 2011). In one large study of young adults, differences in LV mass were still present after adjusting for body composition and size, blood pressure, alcohol consumption, pulmonary function, smoking history, physical activity, total cholesterol and family history of hypertension (Gardin et al., 1995). Beyond a single measure of mass and considering the more complex dimension of shape (Gonzalez et al., 2015), the LV appears to exhibit a greater ellipsoid geometry at end-diastole in young adult men compared with women (Kaku et al., 2011). Taken together, these findings indicate that differences in gross cardiac structure between men and women may not be fully explained by body habitus.

Cardiac function

In addition to LV mass, LV volumes at end-diastole and end-systole, and the resultant stroke volume are larger in men than women (Cain et al., 2009; Celentano et al., 2003; Hutchinson et al., 1991; Kaku et al., 2011; Lang et al., 2006; Lynn et al., 2007; Sandstede et al., 2000). As inappropriate scaling approaches have also been applied in these comparisons, it is unclear if differences between men and women are simply due to body size (Cain et al., 2009; Celentano et al., 2003; Hutchinson et al., 1991; Kaku et al., 2011; Lang et al., 2006; Sandstede et al., 2000). However, if men do have a

relatively larger stroke volume, this may help to explain their lower resting heart rate observed in some studies (Best et al., 2014; Celentano et al., 2003; Fleg et al., 1995; Hanley et al., 1989). To complicate matters, other studies have reported similar resting heart rates in men and women (Grandi et al., 1992; Lynn et al., 2007; Sandstede et al., 2000). Combining heart rate and stroke volume, most studies have found similar resting cardiac outputs in men and women after indexing to body surface area (Carlsson et al., 2012; Hossack and Bruce, 1982; Sandstede et al., 2000; Sullivan et al., 1991; Yilmaz et al., 2013). Despite the ambiguity regarding differences in resting heart rate between the sexes, it has been shown that women have greater sympathetic (Mitoff et al., 2011) as well as parasympathetic (Ryan et al., 1994) activation of the heart. It is therefore highly probable that resting heart rate is modulated differently in men and women, even if the same number of beats are achieved per minute.

Whilst there is a lack of consensus on resting heart rate and relative LV volumes between the sexes, one consistent difference emerges: adult women have a higher ejection fraction than age-matched men (Figure 2.13; Cain et al., 2009; Fleg et al., 1995; Hanley et al., 1989; Kaku et al., 2011; Sandstede et al., 2000), even after controlling for heart rate, body surface area, body mass index or fat-free mass (Bella et al., 2006; Celentano et al., 2003). These sex differences in cardiac structure, autonomic regulation and ejection fraction, therefore, provide the impetus for future studies to closely examine systolic and diastolic function (e.g. trans-mitral filling velocities and myocardial tissue velocities), and the underlying mechanics of the LV. Such efforts, combined with continued work to clarify the conflicting results in the existing literature, will ultimately improve our understanding of differences (and similarities) between male and female hearts.

2.3.2 Cardiac ageing in humans

Cardiac structure

Structural changes in the heart with ageing occur at both microscopic and macroscopic levels (Figure 2.14; for a review see Keller and Howlett, 2016). Microscopic changes

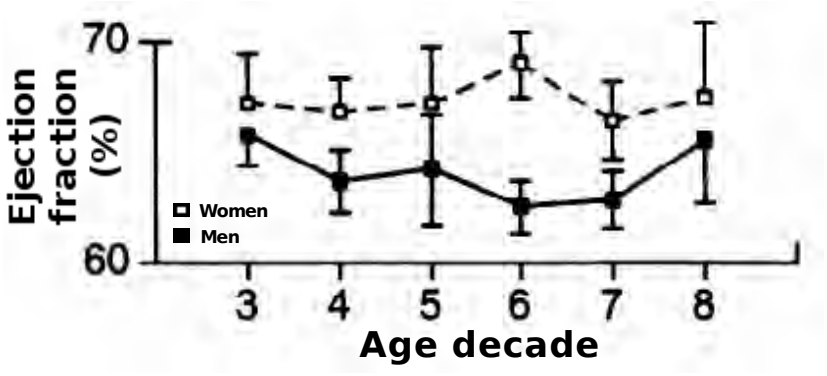


FIGURE 2.13: Ejection fraction is consistently higher in women than men across age decades. Age decade 3 refers to participants in their 20s (Baltimore Longitudinal Study of Aging; adapted from Fleg et al., 1995).

include a loss of cardiomyocytes and compensatory hypertrophy of the surviving cells — a change that may be more prominent in men than women (Olivetti et al., 1995; cf. Section 2.3.3) — and a marked proliferation of cardiac fibroblasts, leading to increased fibrosis and stiffness (Keller and Howlett, 2016). Macroscopic changes include epicardial fat deposition, calcification of the aortic valve, atrial dilation and hypertrophy, and LV hypertrophy (Keller and Howlett, 2016).

At first glance, a number of studies investigating cardiac structure across the lifespan suggest an increase in LV mass with ageing (Lindroos et al., 1994; Savage et al., 1990). Such an increase would be in line with the wear and tear theory of ageing, which proposes that repetitive pulsations cause fatigue and damage to central arteries, resulting in dilation and increased arterial stiffness with age (for a review see O'Rourke and Hashimoto, 2007). To compensate for this increased arterial stiffness, the sarcomeric protein titin[1] may shift from its N2BA to N2B isoform to increase contractility (e.g. in heart failure with preserved ejection fraction) but at the cost of increasing LV stiffness (Frenneaux and Williams, 2007; Hamdani et al., 2013; Linke, 2008). LV hypertrophy would occur in response to the increased ventricular afterload, and this could explain

[1]Titin is a giant elastic protein that spans half the sarcomere from the Z-disk to the M-band (Frenneaux and Williams, 2007; Linke, 2008). When titin is stretched, extension of folded immunoglobin domains (Ig-domains) first occurs at low stretch-forces, followed by extension of long unique-sequence insertions at higher forces (Linke, 2008). The N2BA isoform is more compliant because it has more folds in its Ig-domains, and thus less passive force is generated when it is stretched. Overall passive stiffness depends on the proportion of compliant N2BA and stiffer N2B titin isoforms expressed in the sarcomere (N2BA:N2B expression ratio).

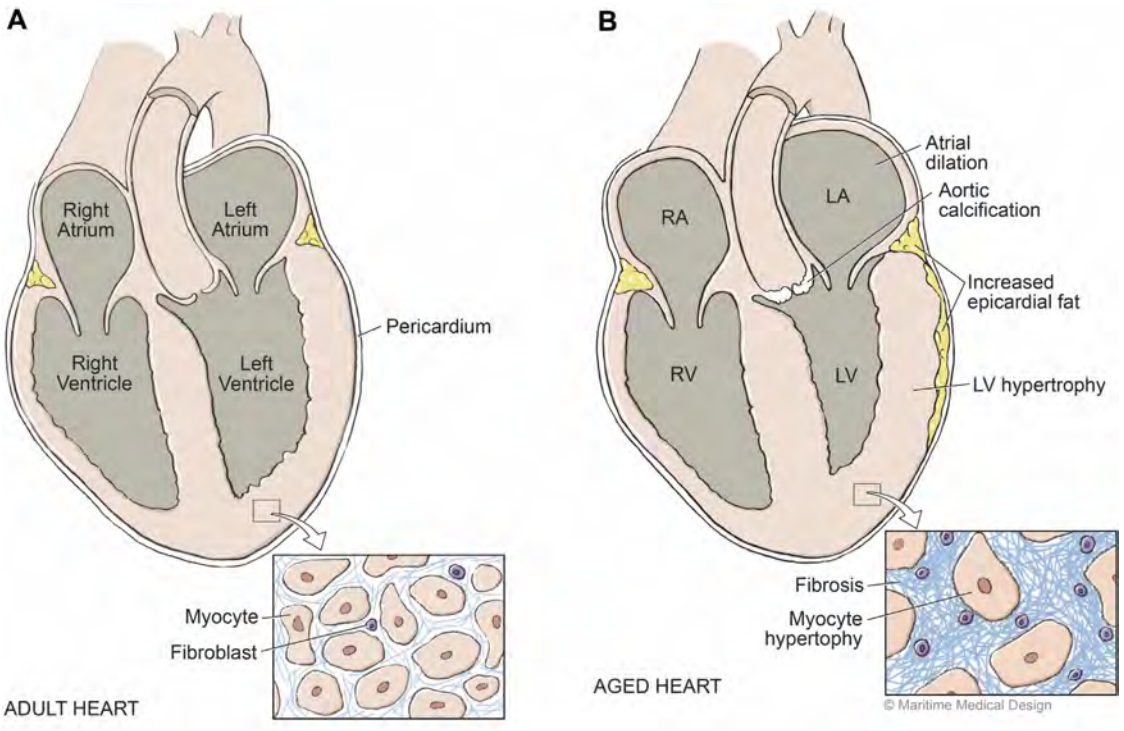


FIGURE 2.14: Illustration of the major changes in the structure of the heart with ageing. (A) Young adult and (B) aged heart at macroscopic and microscopic levels (extracted from Keller and Howlett, 2016).

the observed increase in LV mass. Importantly, an increased aortic pressure may not be reflected in brachial cuff pressures (Figure 2.15), and such individuals would escape the clinical diagnosis of hypertension and be considered healthy.

In conflict with the wear and tear theory of arterial ageing and subsequent LV hypertrophy, the marked increase in LV mass mentioned earlier was found to be significantly reduced or eliminated when only healthy individuals — who were normotensive, non-obese and had no evidence of cardiovascular disease — were examined (Figure 2.16; Dannenberg et al., 1989; Devereux et al., 1984; Gardin et al., 1979; Kaku et al., 2011). Based on these empirical findings, it is probable that a substantial increase in LV mass is in fact indicative of pathology, and not healthy ageing. Studies describing a marked increase in LV mass with ageing could have been driven by the inclusion of study participants with underlying medical issues. Further work is necessary to resolve this discrepancy in the published literature. Additionally, changes in LV mass may differ between men and women, and will be discussed in the following subsection (Olivetti et al., 1995; Shub et al., 1994; Section 2.3.3).

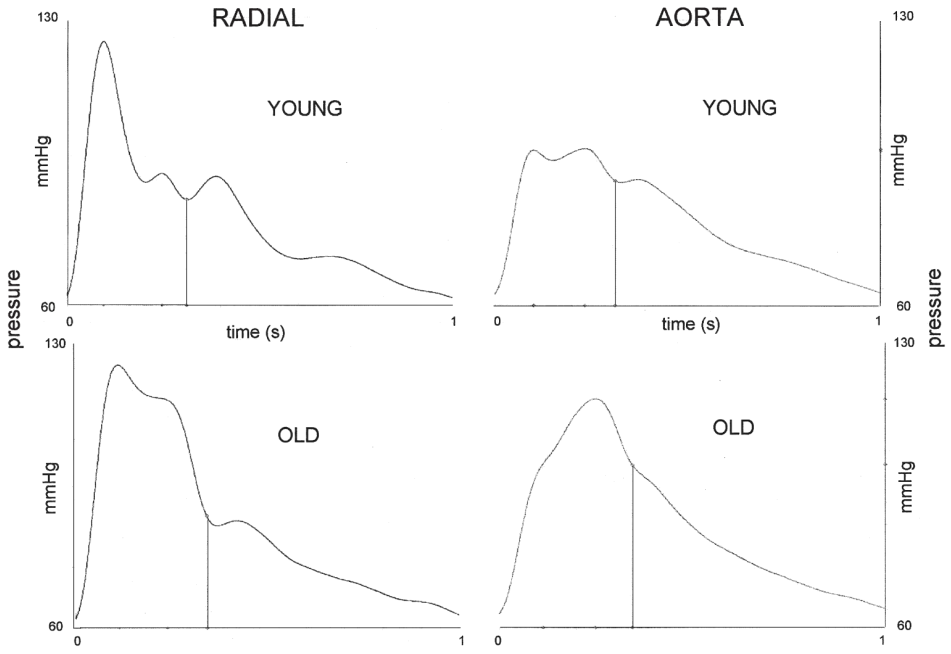


FIGURE 2.15: Radial (left) and aortic (right) pressure waves in a 36 year old man (top) and his 68 year old father (bottom) with identical brachial cuff pressures. For the same brachial systolic pressure, waveform differences were responsible for a 17 mmHg higher aortic pressure in the older man (extracted from O'Rourke and Hashimoto, 2007).

Cardiac function

Amongst the various descriptors of cardiac function, it is likely that age-related changes in heart rate (which is maintained with ageing) and ejection fraction (which either increases or is maintained with ageing) are similar in men and women (Table 2.1). Beneath a healthy ejection fraction, however, vascular stiffening with ageing likely contributes to an increased blood pressure and resistance in both pulmonary and systemic vascular beds (Davidson and Fee, 1990; Haddad et al., 2008; Lam et al., 2009). An elevated pressure in the veins, in turn, maintains a filling gradient between the veins and the right atrium, and enables cardiac filling to continue with ageing (Berlin and Bakker, 2014). Interestingly, it has been suggested that pulmonary vascular resistance increases more with ageing than mean pulmonary pressure (Davidson and Fee, 1990). One proposed explanation is a lower cardiac output with ageing, but this finding has not been consistent across studies (Table 2.1). Whilst it is unclear if cardiac output is maintained or decreases with ageing, it is overall unlikely to increase.

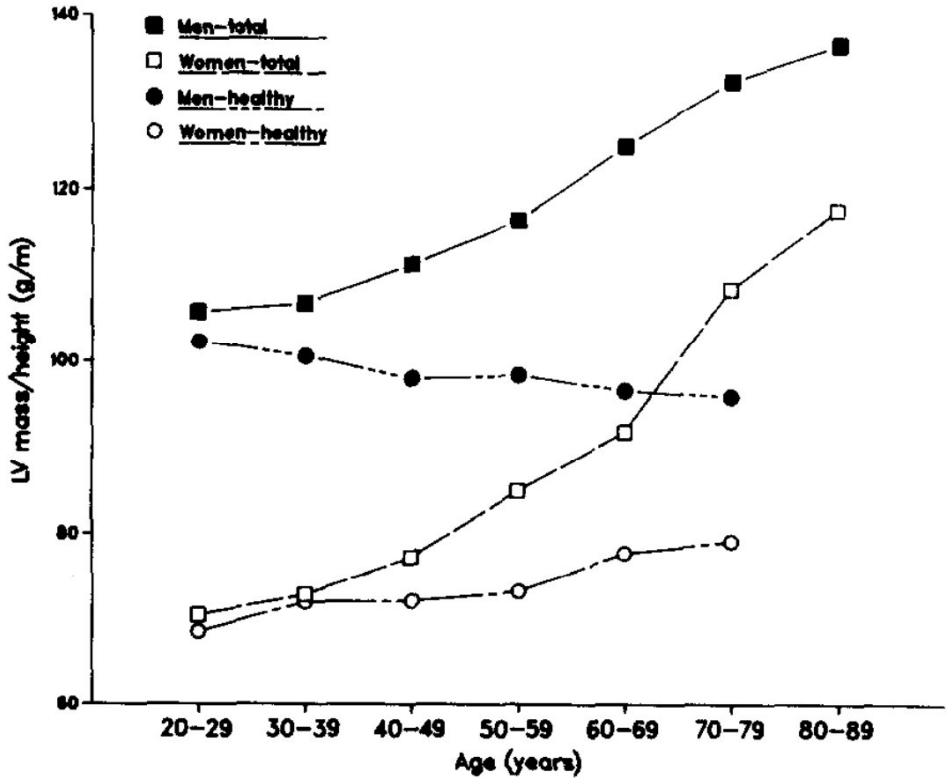


FIGURE 2.16: A marked increase in left ventricular (LV) mass with ageing was observed in the total study group of the Framingham Study Cohort Examination 16 and Offspring Cycle 2 (1979–1983: 2226 men and 2746 women). However, this increase was significantly reduced in women (n=517), and eliminated in men (n=345), when only the healthy individuals were considered (extracted from Dannenberg et al., 1989).

Imperative to cardiac output and ejection fraction, LV volumes (end-diastolic volume, end-systolic volume and stroke volume) are unlikely to increase with ageing, but whether they are maintained or decrease is unclear (Table 2.1). Diastolic dysfunction that occurs with ageing is characterised by an impaired LV relaxation and increased dependence on LV compliance to achieve end-diastolic volume (Keller and Howlett, 2016). This interaction is reflected in a decreasing ratio of peak trans-mitral early-to-late filling velocities with ageing (E/A ratio; cf. Figure 2.19). An impaired LV relaxation reduces early diastolic filling and increases diastolic filling pressures, leading to atrial dilation and hypertrophy (Keller and Howlett, 2016). An increased atrial contribution to LV filling in late diastole acts to compensate for this reduced early filling, and filling becomes increasingly dependent on LV compliance. However, LV compliance is reduced with ageing due to an increased LV stiffness, and clinical diastolic dysfunction may

TABLE 2.1: Selected articles to depict age-related changes in cardiac function in men and women.

Reference	Participants		$\dot{Q}$		HR		SV		EDV		ESV		EF	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Brandfonbrener et al. (1955)	67		↓		↓		↓							
Ogawa et al. (1992)	56	54	↔	↓	↔	↔	↔	↑/↓						
Lakatta (1993)	95	50	↔	↓	↓	↓	↑	↔	↑	↔	↔	↓	↔	↔
Stratton et al. (1994)	24		↓		↔		↓		↓		↔		↔	
Fleg et al. (1995)	121	79	↔	↓	↓	↓	↑	↔	↑	↔	↑	↔	↔	↔
Best et al. (2014)	35	35	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↑	↑
Rodeheffer et al. (1984)	47	14	↔		↔		↔		↔		↔		↔	
van Dalen et al. (2008a)	31	30			↔				↔		↔		↔	
Cheng et al. (2009)	2363	2641					↓		↓		↓		↑	
Kaku et al. (2011)	137	143			↔		↓		↓		↓		↔	

Studies that have reported data for men and women separately (top), and as a combined group (bottom) have been included. $\dot{Q}$: cardiac output. HR: heart rate. SV: stroke volume. EDV: end-diastolic volume. ESV: end-systolic volume. EF: ejection fraction. ↓: decreased with ageing. ↔: maintained with ageing. ↑: increased with ageing. The grey background (e.g. ↑) indicates that the respective changes with ageing were also dependent on participants' training status/fitness levels.

eventually develop (e.g. heart failure with preserved ejection fraction). These changes in diastolic function with ageing may be underpinned by: (i) an increased LV fibrosis, (ii) isoform shifts in cardiac titin from the more compliant N2BA isoform to the stiffer N2B isoform (Frenneaux and Williams, 2007; Hamdani et al., 2013; Linke, 2008), and (iii) a slower calcium transient decay (due to reduced expression and activity of the SERCA pump; cf. Section 2.2.4) leading to a persistent activation of contractile filaments and delayed active ventricular relaxation (Keller and Howlett, 2016).

Drawing upon the free radical theory of ageing, which is a non-programmed error theory of ageing, reactive oxygen species (ROS) generation and mitochondrial damage have been suggested to contribute to cardiac ageing. However, some studies suggest that ROS may also have beneficial effects in ageing and therefore the role of ROS accumulation in ageing is still ambiguous (reviewed by Keller and Howlett, 2016). Moreover, cardiac ageing also differs between men and women, and across individuals of different physical activity levels (e.g. sedentary vs. >3 h of exercise training/week; cf. Section 2.5.3). In the following subsection, existing information on sex differences

in cardiac ageing will be presented, and the discrepancies and gaps in our empirical knowledge base will be highlighted.

2.3.3 Sex-specific cardiac ageing

Cardiac structure

With different prevalences of cardiovascular disease in men and women (Mozaffarian et al., 2015; Nichols et al., 2014), it is intuitive that their hearts likely change differently with healthy ageing. A landmark study involving 106 human hearts from autopsies described a loss of nearly 1 g of myocardium/year in the male heart, but in contrast, preserved ventricular mass in the female heart with ageing (Figure 2.17; Olivetti et al., 1995). These trends persisted after ratiometric scaling to body surface area. Similarly, the number of myocytes decreased in male hearts with ageing, but was maintained in female hearts. Mean cell volume, interestingly, increased in male hearts with ageing — indicating a hypertrophic response to mitigate the decline in LV mass with the progressive death of cardiomyocytes. With supporting evidence from *in vivo* studies of the human heart (Dannenberg et al., 1989; Hees et al., 2002; Natori et al., 2006), it is tempting to conclude that LV mass declines more rapidly in males than females with healthy ageing. Conflicting evidence, however, from another large-scale study involving 700 apparently healthy volunteers — depicting age-related increases in LV mass in both men and women (Daimon et al., 2011; Grandi et al., 1992) — make this conclusion still premature.

Beyond LV mass, higher LV mass/volume ratios have been reported in women aged $\geq$60 years compared with similar-aged men, but no sex differences were observed between younger men and women (Figure 2.18; Kaku et al., 2011). This suggests a more advanced stage of LV concentric remodelling in older women than men, which may have been triggered by the menopause (cf. Section 2.4.3). Concurrent with a lower total systemic arterial compliance (calculated as stroke volume index/pulse pressure) in older women than men, the greater concentric remodelling in women may partly be a physiologic cardiac response to an increased afterload caused by arterial stiffening.

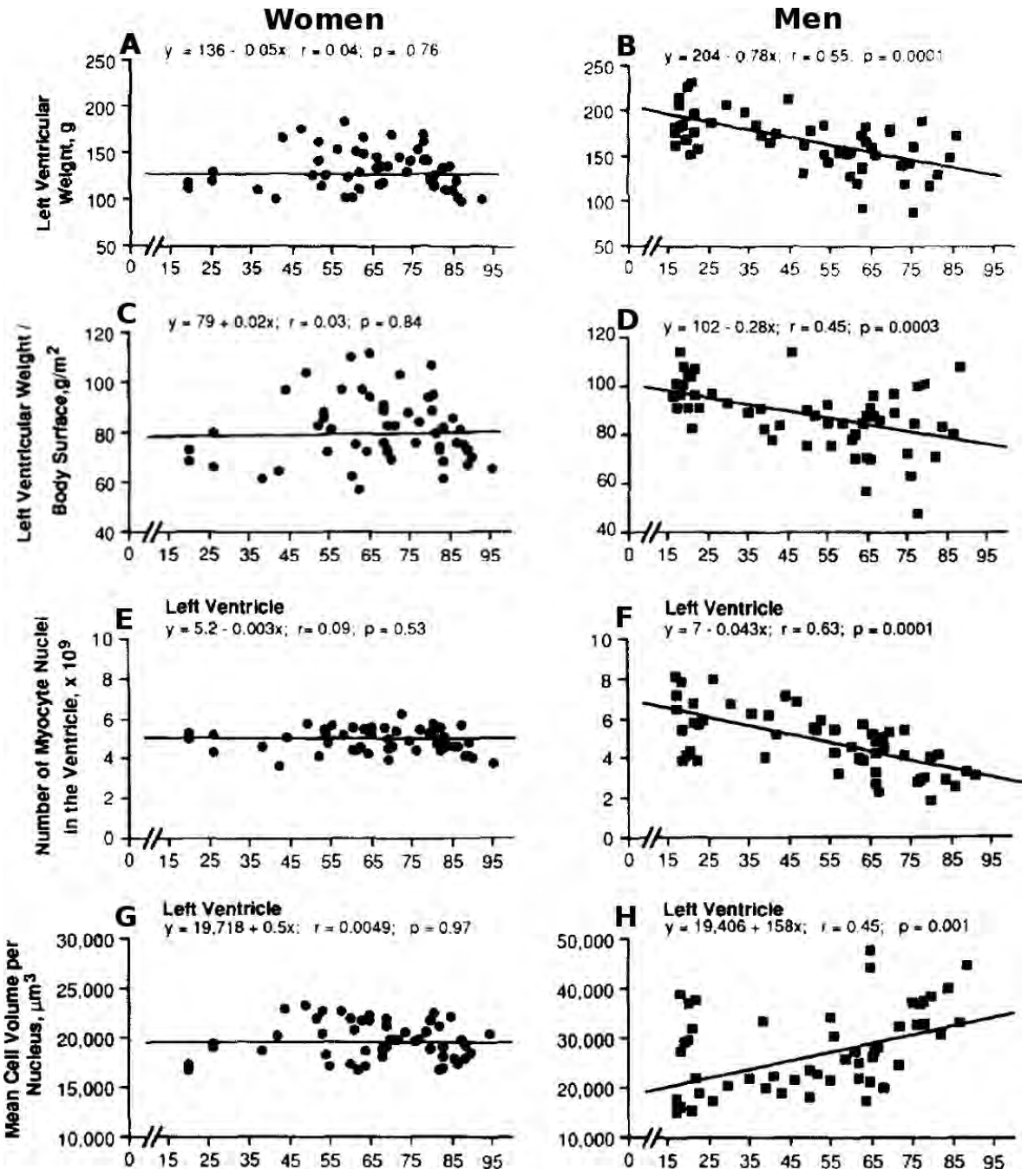


FIGURE 2.17: Effect of ageing on left ventricular mass, absolute (A–B) and indexed (C–D), number of myocyte nuclei (E–F) and mean cell volume (G–H) in men (right panel) and women (left panel; adapted from Olivetti et al., 1995).

Although the exact details still need to be clarified, it seems likely that changes in cardiac structure with ageing differ between men and women. The evidence for age-related sex differences in cardiac function will be reviewed next.

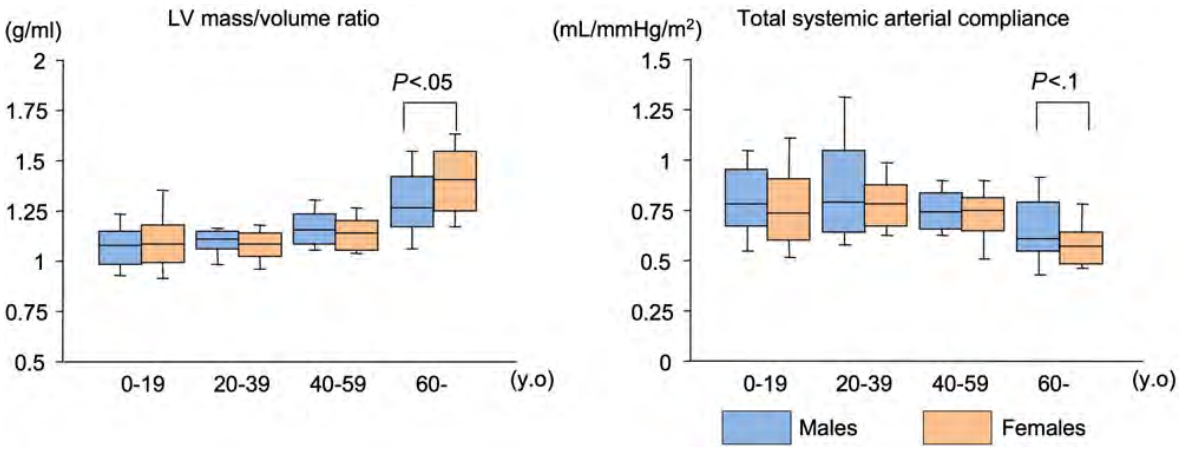


FIGURE 2.18: Boxplot of left ventricular (LV) mass/volume ratio and total systemic arterial compliance in men and women with ageing (extracted from Kaku et al., 2011).

Cardiac function

Although cardiac output and LV volumes are unlikely to increase with advancing age (cf. Section 2.3.2), whether they are maintained or decrease could differ between men and women (Table 2.1). Limited direct sex comparisons and mixed results from single-sex studies, however, make it difficult to postulate on the likely direction of changes — and differences — in men and women with ageing. In addition, participants' training status (which reflects their level of regular physical activity) is another confounding variable in these comparisons. The impact of training status on the heart will be revisited later in this chapter (cf. Section 2.5.3).

Despite the ambiguity in cardiac output and LV volumes in men and women with ageing, there is evidence supporting a greater decrease in systolic function in men, and in contrast a greater decrease in diastolic function in women (Keller and Howlett, 2016; Luczak and Leinwand, 2009; Nio et al., 2015). Ejection fraction aside (which appears to be similar in men and women with age; cf. Section 2.3.2), other markers of systolic

function such as fractional shortening and peak shortening rates of the LV appear comparable between men and women aged 18–50 years, but lower in men than women >50 years (Bella et al., 2006; Celentano et al., 2003; de Simone et al., 1991; Grandi et al., 1992). Studies in animals and *in vitro* further support this, with ventricular myocytes from male rodents showing a greater decrease in contractile function with age compared with those from female rodents (for a review see Keller and Howlett, 2016). Markers of diastolic function, such as the ratio of peak early-to-late (E/A) trans-mitral filling velocity and peak early diastolic myocardial velocity (E′), suggest a superior diastology in women than men ≤50 years (Figure 2.19; Daimon et al., 2011; Grandi et al., 1992; Okura et al., 2009). With further ageing (>50 years), however, these measures of diastolic function appear to worsen to a greater extent in women than men (Daimon et al., 2011; Grandi et al., 1992; Okura et al., 2009). These sex differences in cardiac ageing may subsequently predispose men and women toward different cardiovascular diseases. For example, the greater decrease in systolic function in men could help explain why they are more likely to suffer from heart failure with reduced ejection fraction (HFrEF; systolic heart failure), while the greater decrease in diastolic function in women could similarly relate to their higher risk of heart failure with preserved ejection fraction (HFpEF; diastolic heart failure; Maas et al., 2011). Whilst the menopause was not directly investigated in the ageing studies referenced above, it is known to occur at an average age of 51 years (Kato et al., 1998), which coincides neatly with the manifestation of differences in male and female ageing. Many researchers have therefore postulated that the changes in sex hormones with the menopause (the drop in circulating concentrations of oestrogen and progesterone; Harlow et al., 2012; Soules et al., 2001) may help to explain the distinct profiles of cardiac ageing between men and women (cf. Section 2.4.3).

Left ventricular mechanics

Very few studies have directly compared age-related changes in LV mechanics between men and women. Where results from men and women were reported separately, the effects of sex and age were still only investigated independently. For example, torsion — defined as twist per unit length of the LV — was found to be higher in women

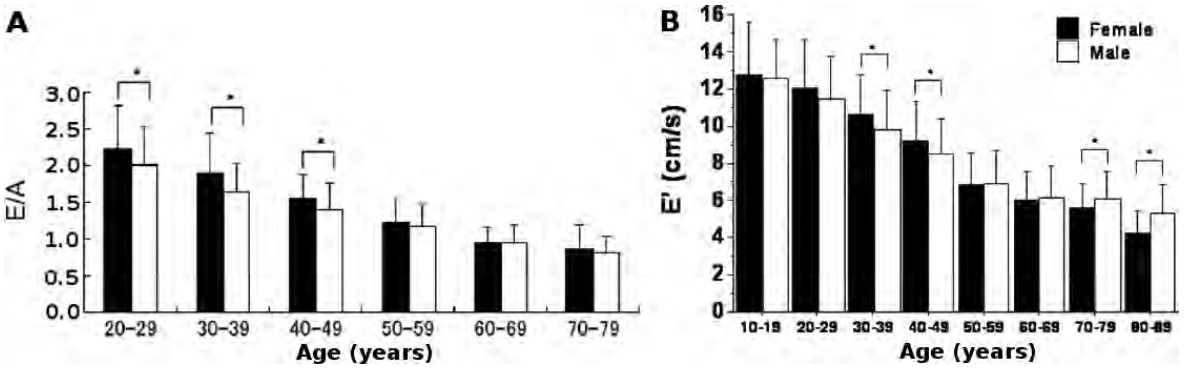


FIGURE 2.19: (A) Ratio of peak early-to-late (E/A) trans-mitral filling velocity (adapted from Daimon et al., 2011) and (B) peak early diastolic septal myocardial velocity at the level of the mitral annulus (E′; adapted from Okura et al., 2009) in men and women over 10-year age ranges. Values are mean ± standard deviation. $^*P < 0.05$ between men and women.

than men, and also in older than younger adults, but the extent of these age-related changes were not compared between men and women (Figure 2.20; Multi-Ethnic Study of Atherosclerosis involving 1478 participants; Yoneyama et al., 2012). Notwithstanding, an overall increase in LV twist with healthy ageing has been established (Lumens et al., 2006; Maharaj et al., 2013; Notomi et al., 2006; Takeuchi et al., 2006; van Dalen et al., 2008a; Zhang et al., 2010). This increased LV twist has been suggested to arise from a larger age-related decrease in sub-endocardial than sub-epicardial contractile function (Lumens et al., 2006), resulting in less opposition to the dominant rotation of the sub-epicardium (van Dalen et al., 2008a; Yoneyama et al., 2012; cf. Section 2.2.3). Such changes in sub-endocardial function could be caused by sub-clinical deteriorations in sub-endocardial coronary perfusion (Van der Toorn et al., 2002) or sub-endocardial fibrosis (Anversa and Capasso, 1991). Drawing upon the clinical literature, patients with mild diastolic dysfunction have been found to show higher LV twist and untwisting velocity relative to healthy controls (Park et al., 2008). These increased values could therefore reflect a compensatory mechanism for reduced myocardial relaxation, which begins during usual ageing. In patients with more severe diastolic dysfunction, LV twist and untwisting velocity values were normalised or reduced (Park et al., 2008), reflecting the sensitivity of LV mechanics as a potential non-invasive tool to monitor the progression of disease. Extrapolating from the greater age-related decline in diastolic function in women that was previously discussed, it is possible that healthy women

may exhibit a greater increase in LV twist and untwisting velocity with usual ageing compared with men.

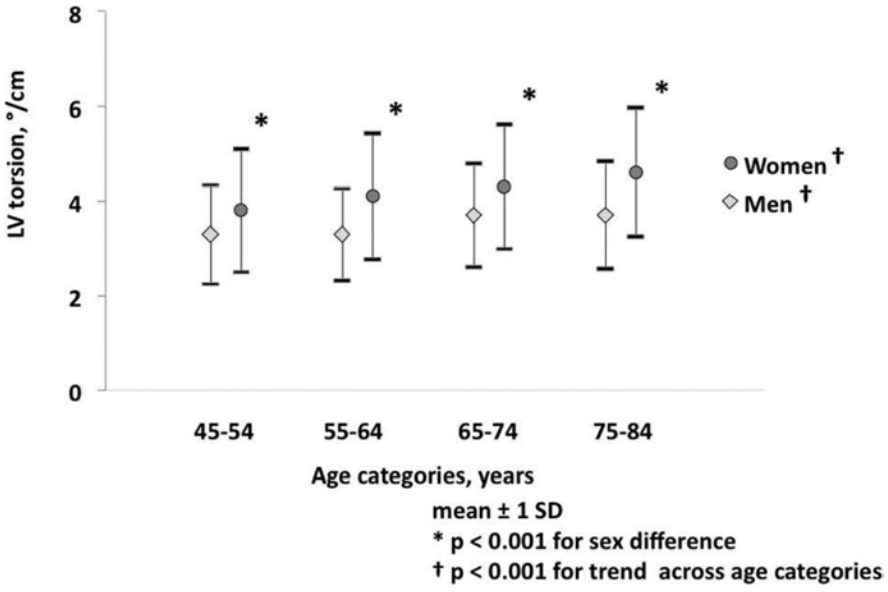


FIGURE 2.20: Left ventricular (LV) torsion was higher in women than men, and also higher in older than younger adults (extracted from Yoneyama et al., 2012). The extent of the age-related increase between men and women was not directly investigated.

Most studies of LV deformation report a decrease in strain and strain rates with ageing (Figure 2.21; Cheng et al., 2009; Maharaj et al., 2013; Oxenham et al., 2003), and suggest that women have higher values than men (Cheng et al., 2013; Dalen et al., 2009; Sun et al., 2013; Venkatesh et al., 2014; Yoneyama et al., 2012). Similar to the existing work on LV rotational mechanics, however, direct comparisons of LV deformation in men versus women with ageing are scarce. Moreover, circumferential and radial strains have been less commonly assessed than longitudinal strain (Yingchoncharoen et al., 2013), but are nonetheless relevant in characterising LV deformation. As diastolic strain rates have been found to be major determinants of early diastolic filling (Doucende et al., 2010), women may be expected to show a greater age-related decrease in early diastolic strain rates compared with men. This speculation extends from the greater decrease in diastolic function with ageing in women that was previously discussed.

Building upon the age-related differences in LV structure and function between men and women, the potential for data on LV mechanics to supplement the existing knowledge

base has been highlighted. At a molecular level, differences in circulating sex hormone concentrations in men and women are inevitably implicated in explaining sex-specific cardiac ageing. It is thus timely to next review the impact of sex hormones on the heart.

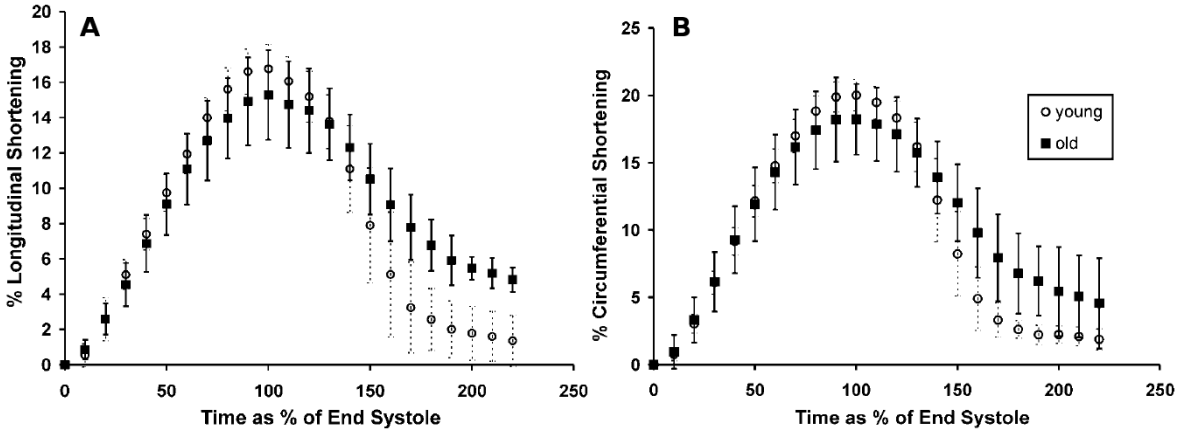


FIGURE 2.21: (A) Longitudinal and (B) circumferential shortening (strain) in younger (11 men, 4 women) and older adults (10 men, 6 women) across a cardiac cycle. Values were expressed relative to the time to end-systole to normalise for inter-individual differences in heart rate (adapted from Oxenham et al., 2003).

2.4 Impact of sex hormones on the heart

Sex hormones are likely responsible, at least in part, for differences in cardiac ageing between men and women. The impact of the primary female sex hormones — oestrogen, and progesterone — on the heart will be presented in this section. Data from *in vitro* work and animal studies will first be reviewed to provide mechanistic insight into the effects of these sex hormones on the heart. The section will conclude with a focus on *in vivo* studies that have examined the impact of the menopause on the female heart, where the decline in oestrogen and progesterone levels with the menopause are likely to have influenced cardiac ageing in women.

2.4.1 Oestrogen — insights from *in vitro* and animal research

Distinct oestrogen concentrations in men and women are likely to be key in explaining sex differences in cardiac ageing, which were discussed previously (cf. Section 2.3.3). Specifically, circulating oestrogen levels decrease steadily with ageing in men, but drop substantially with the menopause in women (Figure 2.22; Khosla et al., 1998; Leifke et al., 2000). On average — and noting the variations across the menstrual cycle (Sherwood, 2016b) — pre-menopausal women have higher levels of oestrogen compared with men, and post-menopausal women have lower levels than men. The potent influence of oestrogen on the heart will be summarised in the following paragraph.

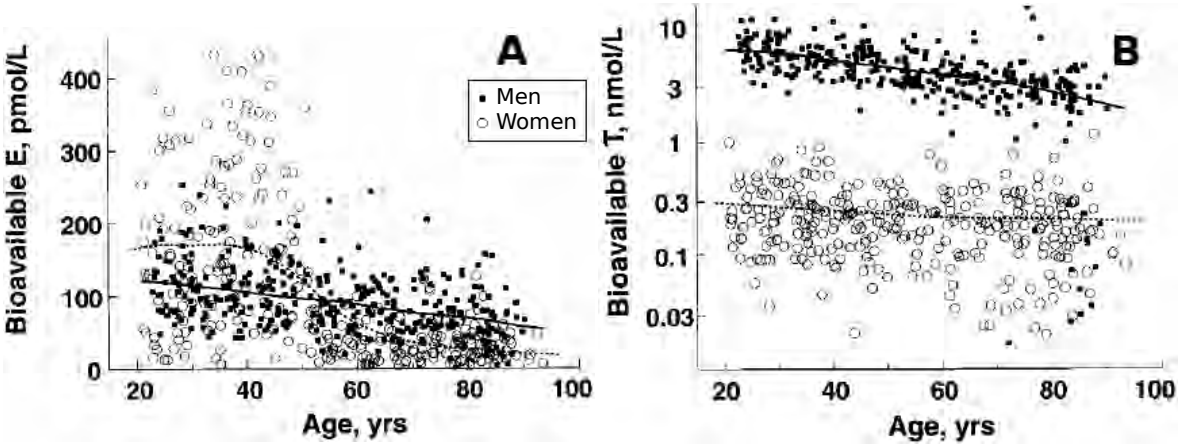


FIGURE 2.22: Bioavailable oestrogen (A) and testosterone (B) in serum samples from 346 men and 304 women, aged 21–94 years. Mean values with ageing are indicated separately for men (solid lines) and women (dashed lines; adapted from Khosla et al., 1998).

Oestrogen has been shown to exert anti-hypertrophic effects in the diseased heart, but its role in the healthy heart is less studied (for a review see Regitz-Zagrosek and Kararigas, 2017). Notwithstanding, oestrogen is generally considered to be cardioprotective, and exerts its effects via genomic and non-genomic pathways. Accordingly, oestrogen receptors have been identified in the hearts of humans (Taylor and Al-Azzawi, 2000) and other species (Grohé et al., 1997; Meyer et al., 1998; Saunders et al., 1997; Yang et al., 2012), and the expression of oestrogen receptors increases in the presence of oestrogen (Grohé et al., 1997). Drawing upon *in vitro* and animal studies, oestrogen has been shown to: i) reduce cardiomyocyte apoptosis (programmed cell death; Kim et al., 2006; Ma et al., 2009; Patten et al., 2004); ii) decrease cardiac contractility (Jiang et al.,

1992; Patterson et al., 1998; Sitzler et al., 1996); iii) influence calcium and potassium handling (Chen et al., 2011; Parks and Howlett, 2013; Tanabe et al., 1999; Yang et al., 2012); and iv) increase myocardial expression of nitric oxide synthases (signalling proteins that may contribute to cardioprotection; Murphy et al., 2011; Nuedling et al., 1999, 2001; Umar and van der Laarse, 2010; Ziolo et al., 2008). A note of caution on interpreting the existing literature, however, is that the effects of oestrogen on the heart may differ between supra-physiological and physiological concentrations (Parks and Howlett, 2013). Further work towards elucidating the effects of oestrogen are still ongoing.

Of particular relevance to this thesis, researchers working on rabbit cardiomyocytes have recently reported different responses to oestrogen in cells isolated from male compared with female hosts (Figure 2.23; Chen et al., 2011; Yang et al., 2012). These authors further demonstrate a regional effect, where changes in calcium handling upon incubation with oestrogen were only evident in cardiomyocytes isolated from the basal epicardium of the female rabbit LV, but not from the basal endocardium nor from the apex (Figure 2.24; Yang et al., 2012). It is therefore likely that comparing basal and apical LV mechanics *in vivo* between men and women, and between pre- and post-menopausal women, may complement contemporary *in vitro* and animal studies.

2.4.2 Progesterone — insights from *in vitro* and animal research

Progesterone levels decrease with ageing in men and women (Waddell et al., 2001), and with the menopause in women (Lavi et al., 2007; Longcope et al., 1986; Soules et al., 2001; Ukkola et al., 2001). Mean progesterone levels (averaged across the menstrual cycle) are higher in pre-menopausal women compared with age-matched men (Strott et al., 1969; Tea et al., 1975), and are likely similar in post-menopausal women and age-matched men (Lavi et al., 2007; Tea et al., 1975; Ukkola et al., 2001). Similar to oestrogen, progesterone may exert protective effects on the heart, but has received less scientific attention than oestrogen (Morrissy et al., 2010; Wittnich et al., 2013). If cardioprotective effects do exist, the decrease in circulating progesterone concentrations

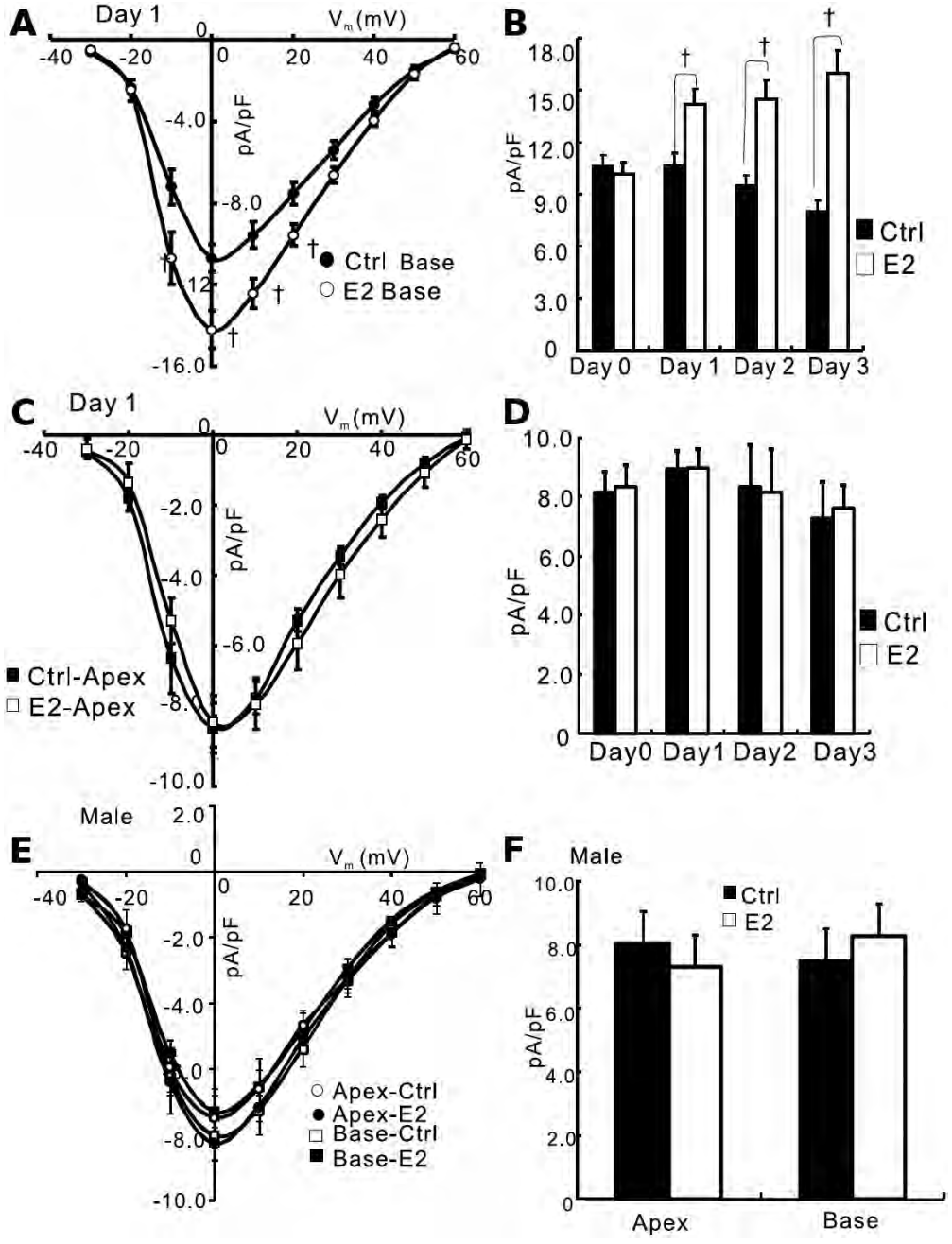


FIGURE 2.23: Effects of oestrogen (E2) on ventricular myocytes isolated from adult rabbits: (A–B) female base, (C–D) female apex, and (E–F) male base and apex. Left column: current density-to-voltage (I–V) relationship for the L-type Ca^{2+} current ($I_{Ca,L}$) in the respective myocytes incubated without (Ctrl) or with E2 (1 nM). Right column: mean $I_{Ca,L} \pm$ standard error at 0 mV in the respective myocytes. $\dagger P < 0.05$ compared with Ctrl (adapted from Yang et al., 2012).

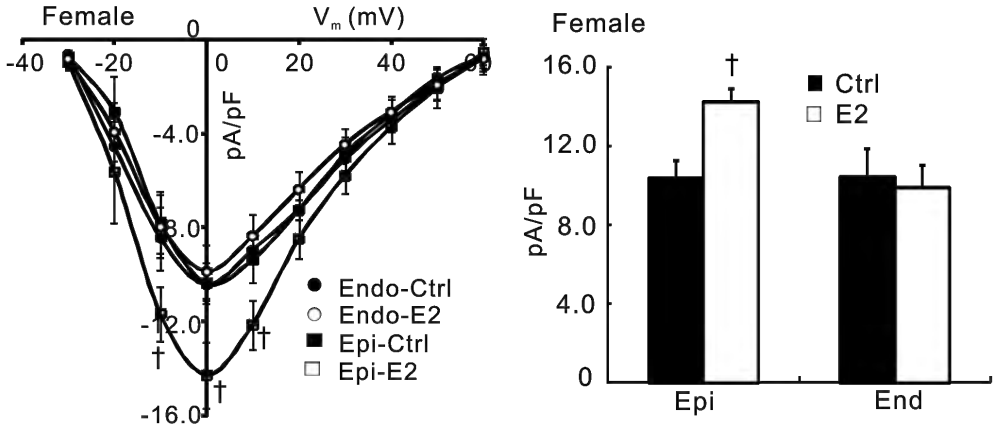


FIGURE 2.24: Effect of oestrogen (E2) on L-type Ca^{2+} current ($I_{Ca,L}$) density of ventricular myocytes isolated from the epicardium (Epi) and endocardium (Endo) at the base of female rabbit hearts. Left: current density-to-voltage (I–V) relationship for $I_{Ca,L}$ in the absence (Ctrl) or presence of E2 (1 nM) for 1 day. Right: mean $I_{Ca,L}$ $\pm$ standard error at 0 mV in the absence (Ctrl) or presence of E2 (1 nM) for 1 day. $\dagger P < 0.05$ compared with Ctrl (extracted from Yang et al., 2012).

with ageing and the menopause will therefore influence LV structure, function and mechanics. In this section, the existing literature relating to the effects of progesterone on the heart will be summarised.

Receptors for progesterone have been identified in the hearts of humans (Ingegno et al., 1988) and other species (Goldstein et al., 2004; Grohé et al., 1997; Lin et al., 1982; Morrissy et al., 2010). Although data is limited, progesterone has been suggested to act via genomic and non-genomic pathways (Goldstein et al., 2004; Morrissy et al., 2010; Nakamura et al., 2007). Some of its effects could be similar to those of oestrogen, as progesterone has been suggested to: i) reduce cardiomyocyte apoptosis (Morrissy et al., 2010); ii) decrease cardiac contractility (Mendoza and De Mello, 1974; Raddino et al., 1989); iii) down-regulate calcium handling (Mendoza and De Mello, 1974; Nakamura et al., 2007; Raddino et al., 1989); and iv) increase myocardial expression of nitric oxide synthases (Nakamura et al., 2007). Further work is imperative to confirm these effects, however, as conflicting results have also been reported (Sitzler et al., 1996; Wittnich et al., 2013; Yang et al., 2012) and effects may differ between supra-physiological and physiological concentrations (Parks and Howlett, 2013). In contrast to oestrogen, progesterone may increase cardiac protein synthesis — an anabolic effect that could, in fact, be inhibited by oestrogen (Goldstein et al., 2004). Whilst it is currently difficult

to conclude on the specific effects of progesterone on the heart, these are likely to be less potent than the effects of oestrogen. Therefore, changes in progesterone concentrations with ageing and the menopause may play a smaller (but still significant) role in explaining the differences in cardiac ageing between men and women.

2.4.3 Impact of the menopause on the heart

Amongst the changes in sex hormones over the lifespan in men and women, those that occur with the menopause in women are particularly drastic: circulating concentrations of oestrogen and progesterone decline sharply with the menopause in women and do not increase again in later life (Harlow et al., 2012; Soules et al., 2001). As sex hormones exert a significant influence on the heart (discussed above), these changes likely translate into differences in cardiac physiology between pre- and post-menopausal women. Studies investigating the impact of the menopause on the heart, however, are limited (Hayward et al., 2000). The limitations of existing studies, as well as the gaps in our current knowledge, will be discussed in this subsection. An overview of the physiology and endocrinology of the menopause will first be presented, followed by a review of the literature on the impact of the menopause on LV structure, function and mechanics. In contrast to the preceding subsections, the focus here will be on *in vivo* studies that have examined the impact of the menopause on the female human heart, instead of on *in vitro* and animal studies.

Physiology and endocrinology of the menopause

Menopause refers to the permanent cessation of menses, and is a milestone in the female lifespan signalling the end of reproductive function (Burger et al., 2007). The median age of the menopause is 51 years (Kato et al., 1998) — usually between 48–52 years, but with a range of 35–58 years (Burger et al., 2007). To characterise reproductive ageing in women independently from chronological age, a staging system was conceived at the Stages of Reproductive Aging Workshop (STRAW) in 2001 (Soules et al., 2001), and updated in 2011 (STRAW + 10; Figure 2.25; Harlow et al., 2012). The STRAW + 10

staging system is applicable to most women, regardless of age, ethnicity, body size or lifestyle characteristics (e.g. smoking). It comprises three broad phases — reproductive, the menopausal transition and post-menopause — centered around the final menstrual period (Stage 0), which is determined retrospectively after 12 consecutive months of amenorrhoea.

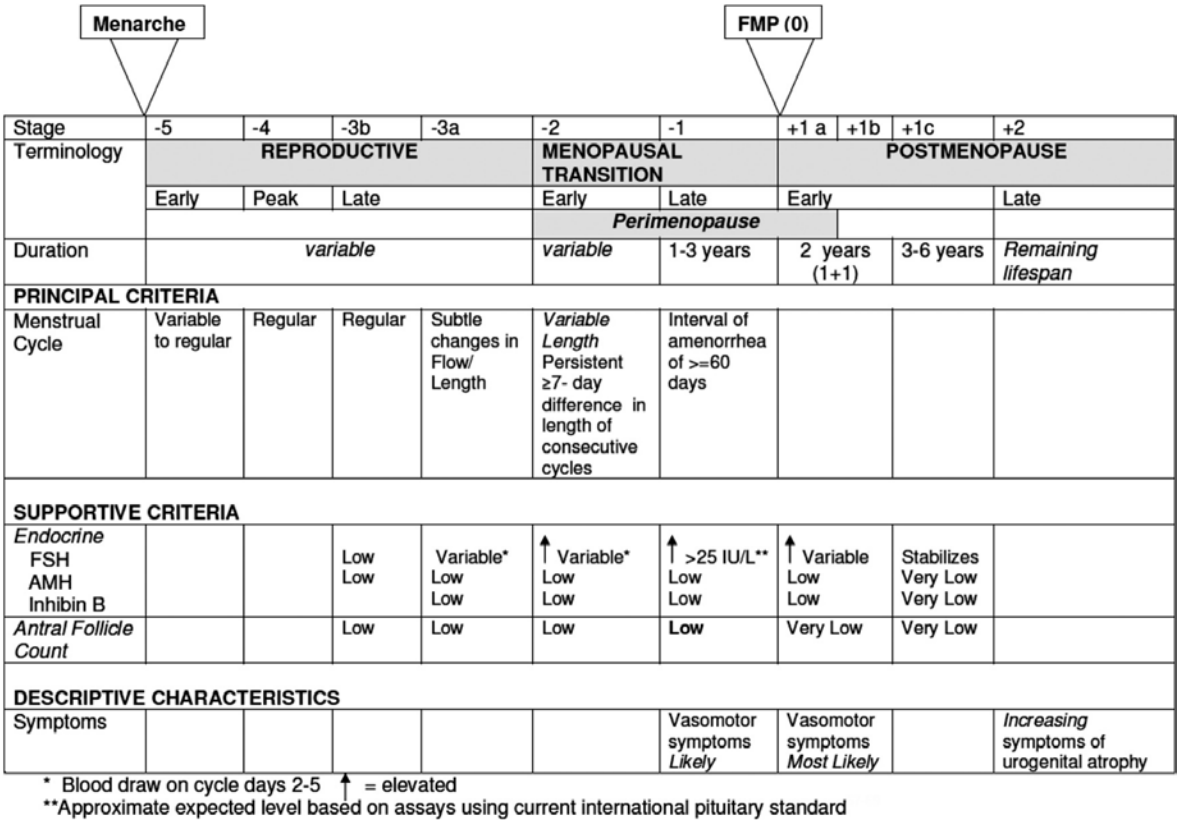


FIGURE 2.25: The STRAW + 10 (Stages of Reproductive Ageing Workshop + 10) staging system for reproductive ageing in women, conceptualised in 2011 (extracted from Harlow et al., 2012). FMP: final menstrual period. FSH: follicle-stimulating hormone. AMH: anti-müllerian hormone.

Estradiol produced by the ovaries is the major circulating oestrogen in pre-menopausal women (Figure 2.26). The start of the menopausal transition is marked by increased variability in menstrual cycle length (Stage −2), while the late menopausal transition is marked by at least 60 days of amenorrhoea (Stage −1; Figure 2.25; Harlow et al., 2012). At the time of final menses, follicle-stimulating hormone (FSH) levels are elevated and reach about 50% of their final post-menopausal concentrations, while estradiol levels are reduced and approximately 50% of reproductive-age concentrations (Figure 2.27; Burger et al., 2007; Burger, 2006; Harlow et al., 2012). In early post-menopause, FSH

levels continue to increase and estradiol levels continue to decrease, before stabilising approximately two years after the final menstrual period. Estrone becomes the major circulating oestrogen — instead of estradiol (Figure 2.26) — and is derived mainly from aromatisation of adrenally-secreted androstenedione in adipose tissue (Burger, 2006). The early post-menopause lasts 5–8 years (Stage +1; Figure 2.25; Harlow et al., 2012), and is particularly relevant to the investigations in this thesis (descriptive of the middle-aged post-menopausal women recruited for the experimental studies). In the following paragraphs, the impact of the menopause on LV structure, function and mechanics will be reviewed.

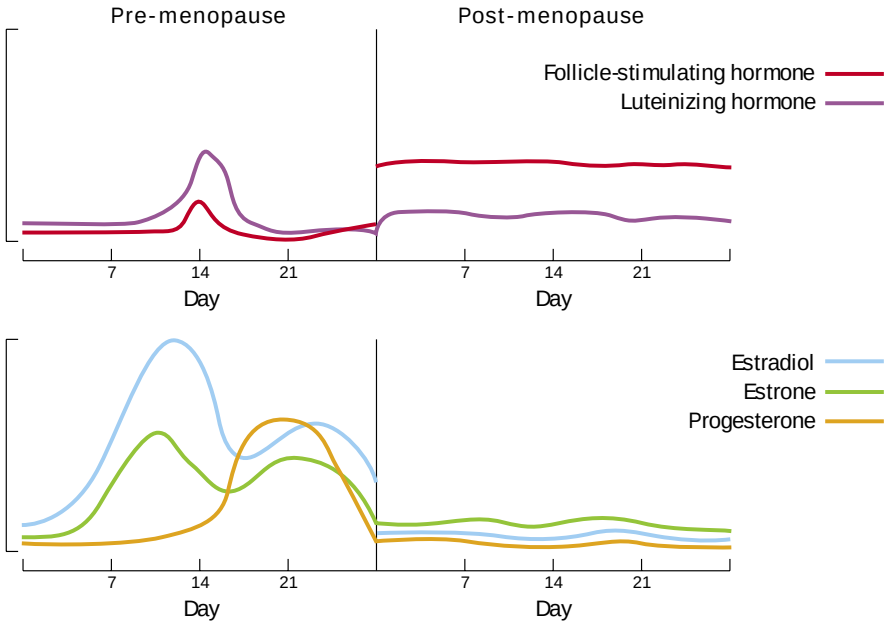


FIGURE 2.26: Contrasting endocrine features across the menstrual cycle (pre-menopause) and after the menopause (adapted from Burger, 2006).

Cardiac structure

With regards to cardiac structure, the menopause has been associated with a concentric remodelling of the LV. Post-menopausal women display greater LV mass and wall thicknesses than pre-menopausal women, concurrent with similar end-diastolic volumes (Düzenli et al., 2007; Hinderliter et al., 2002; Pines et al., 1993; Schillaci et al., 1998). This concentric remodelling could be due to the direct effects of sex hormones on the myocardium, as introduced earlier. In particular, oestrogen has been suggested to

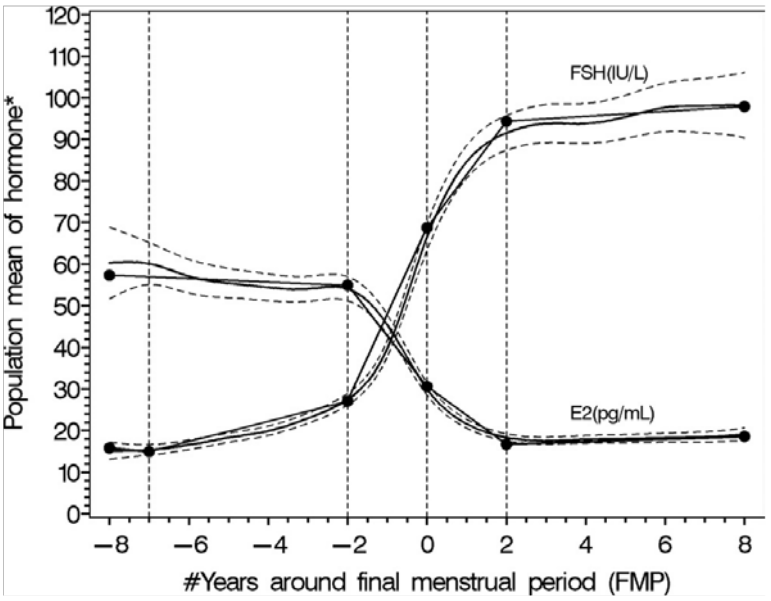


FIGURE 2.27: Adjusted population means (95% confidence interval) for follicle-stimulating hormone (FSH) and estradiol (E2) around the final menstrual period (FMP; extracted from Harlow et al., 2012). *Units for hormone concentrations are marked on the corresponding curves.

abolish the concentric remodelling induced by dihydrotestosterone — a potent androgen that is formed when testosterone is metabolised within cardiovascular tissues (Tivesten et al., 2006). Reduced levels of oestrogen concurrent with relatively maintained levels of testosterone after the menopause (Figure 2.22) may therefore, leave the cardiac effects of androgens unopposed and result in concentric remodelling (Tivesten et al., 2006). The protective effects of oestrogen against cardiac hypertrophy likely occur via multiple molecular pathways, including oestrogen receptor β signalling (Muka et al., 2016b; Skavdahl et al., 2004) — fuelled by increased oestrogen receptor expression in the presence of oestrogen (Grohé et al., 1997) — and the downregulation of cardiac androgen receptor mRNA (Tivesten et al., 2006).

In addition to the direct effects of sex hormones on the myocardium, a greater vascular resistance in post-menopausal women may also be partly responsible for this concentric remodelling (Hinderliter et al., 2002; Schillaci et al., 1998). Differences in vascular tone, which is defined as the degree of constriction of a blood vessel relative to its maximally dilated state, are more likely related to oestrogen than testosterone (for a review see

Orshal and Khalil, 2004). Specifically, oestrogen has been suggested to increase oestrogen receptor α expression and nitric oxide (NO) production in the endothelium, and therefore induce endothelial-dependent vasodilation and relaxation of vascular smooth muscle (Gavin et al., 2009; Lieberman et al., 1994; Mendelsohn and Karas, 1999; Orshal and Khalil, 2004; Vitale et al., 2010). In addition, oestrogen has been suggested to act directly on vascular smooth muscle, at least in part by affecting calcium handling mechanisms crucial for contraction (Orshal and Khalil, 2004). The effects of oestrogen extend beyond vasodilation to intrinsic arterial stiffness, where it has been shown to increase the elastin/collagen ratio and therefore increase distensibility (Natoli et al., 2005; Rossi et al., 2011). Taken together, the withdrawal of oestrogen and its potent effects on the vasculature after the menopause results in an increased arterial stiffness and therefore, a greater ventricular afterload, which likely contributes to the early concentric LV remodelling associated with post-menopausal women. This theory is further supported by reports of decreased vascular resistance and LV relative wall thickness following oestrogen replacement therapy in post-menopausal women (Light et al., 2001).

Cardiac function

Indicators of cardiac function are generally lower in post-menopausal women compared with their pre-menopausal counterparts (Table 2.2). For example, researchers have observed lower values for cardiac output, ejection fraction, and specialised measures of systolic and diastolic function (e.g. fractional shortening, peak filling velocities and myocardial performance index) in post-menopausal women compared with pre-menopausal women. In fact, the menopause is regarded as a risk factor for cardiovascular disease (Maas et al., 2011), and these changes may describe the early stages of cardiac decline after the menopause. There are still unresolved discrepancies between studies, however, which may be due to the inclusion of women with surgically-induced menopause (Düzenli et al., 2007), smokers (Kangro et al., 1995; Schillaci et al., 1998) and different levels of physical fitness (Düzenli et al., 2007). With only a limited volume of work in this area, further work is necessary to establish the effects of the menopause on cardiac function.

TABLE 2.2: Summary of studies that have compared cardiac function between pre- (Pre-M) and post-menopausal women (Post-M).

	Pre-M		Post-M		Difference after the menopause				
Reference	n	Age	n	Age	$\dot{Q}$	HR	EF	Systolic	Diastolic
Prelevic and Beljic (1994)	20	39 (11)	34	48 (7)		↔	↓		
Kangro et al. (1995)	43	50 (0)	78	50 (0)		↔		↔	↓
Schillaci et al. (1998)	30	49 (3)	30	50 (3)	↓	↔		↓	
Hinderliter et al. (2002)	64	49 (1)	54	51 (2)	↓	↔	↓		
Düzenli et al. (2007)	71	47 (3)	72	47 (3)	↔	↔	↔	↓	↓
Sherwood et al. (2010)	45	49 (1)	45	51 (2)	↓	↔			
Keskin Kurt et al. (2014)	40	47 (2)	40	48 (2)	↔	↔	↔	↔	↔

Participants' ages are expressed as mean (standard deviation) in years. $\dot{Q}$: cardiac output. HR: heart rate. EF: ejection fraction. Systolic (function): complementary to ejection fraction, this column includes comparisons of fractional shortening and peak systolic wall velocity. Diastolic (function): includes peak filling velocities and diastolic wall velocities of the left ventricle. ↓: lower in Post-M than Pre-M (decreases after the menopause). ↔: similar in Pre-M and Post-M. The comparisons against a grey background (e.g. ↔) were not explicitly described, but were instead inferred from other reported data in that particular study — typically from heart rate, and end-diastolic and end-systolic dimensions.

The suggested decline in systolic function and contractility in women after the menopause is, interestingly, contrary to an expected increase based on *in vitro* and animal studies on the effects of oestrogen (cf. Section 2.4.1). As oestrogen has been reported to exert a negative inotropic effect on isolated cardiomyocytes from guinea pigs (Jiang et al., 1992) and in ovariectomised rabbits (Patterson et al., 1998), its withdrawal after the menopause could be postulated to remove its inhibitory effects and in doing so, effect an increase in cardiac contractility. This discrepancy therefore cautions against relying solely on *in vitro* and animal models to understand human physiology. Possible explanations could be: the use of supra-physiological concentrations in *in vitro* studies (Jiang et al., 1992; Parks and Howlett, 2013) that do not reflect concentrations *in vivo*, regional effects of oestrogen that were previously unknown and not controlled for (Yang et al., 2012), interference from other hormones like progesterone and testosterone affecting the heart, and/or the influence of a vascular bed only in *in vivo* studies.

The likely decline in diastolic function after the menopause also manifests as a downward shift in the distribution of these values from pre- to post-menopausal women (Figure 2.28; Kangro et al., 1995). For example, the ratio of peak early-to-late diastolic filling velocities (E/A ratio) has been observed to be <1.00 in 7% of pre-menopausal women, but in 29% of post-menopausal women (Kangro et al., 1995). Lower oestrogen concentrations after the menopause likely contribute to this decline in diastolic function, potentially through reduced nitric oxide availability and activation of the renin-angiotensin-aldosterone system (RAAS; for a review see Zhao et al., 2014). In addition, rodent models employing ovariectomy suggest that the effects of oestrogen in preserving diastolic function act in part via the membrane G protein-coupled receptor 30 (GPR30, also called G protein-coupled oestrogen receptor 1).

Building upon the lower cardiac function in post-menopausal women compared with their pre-menopausal counterparts, it is unsurprising that scientists and clinicians are interested in identifying interventions that may mitigate this gradual decline. One obvious option that has received substantial scientific attention is hormone therapy (previously known as hormone replacement therapy), where post-menopausal women are prescribed exogenous doses of oestrogen and/or progestogen (Boardman et al., 2015; De Villiers et al., 2013). Despite the higher diastolic function that has been observed with hormone therapy (Duzenli et al., 2010; Özdemir et al., 2004), this treatment has contraindications as it may also increase the risk of stroke or venous thromboembolic events (Boardman et al., 2015; Harman, 2014). Another potential option to mitigate the decline in cardiac function with the menopause is to exercise regularly. This is a widely recommended non-pharmacological intervention to maintain and promote health in the general population. The effects of exercise training on the heart will be summarised later in this chapter (cf. Section 2.5.3), and an exercise training intervention will be used in this thesis.

Cardiac mechanics

A literature search suggests that only one study has compared LV mechanics between pre- and post-menopausal women (Keskin Kurt et al., 2014). In line with the decrease

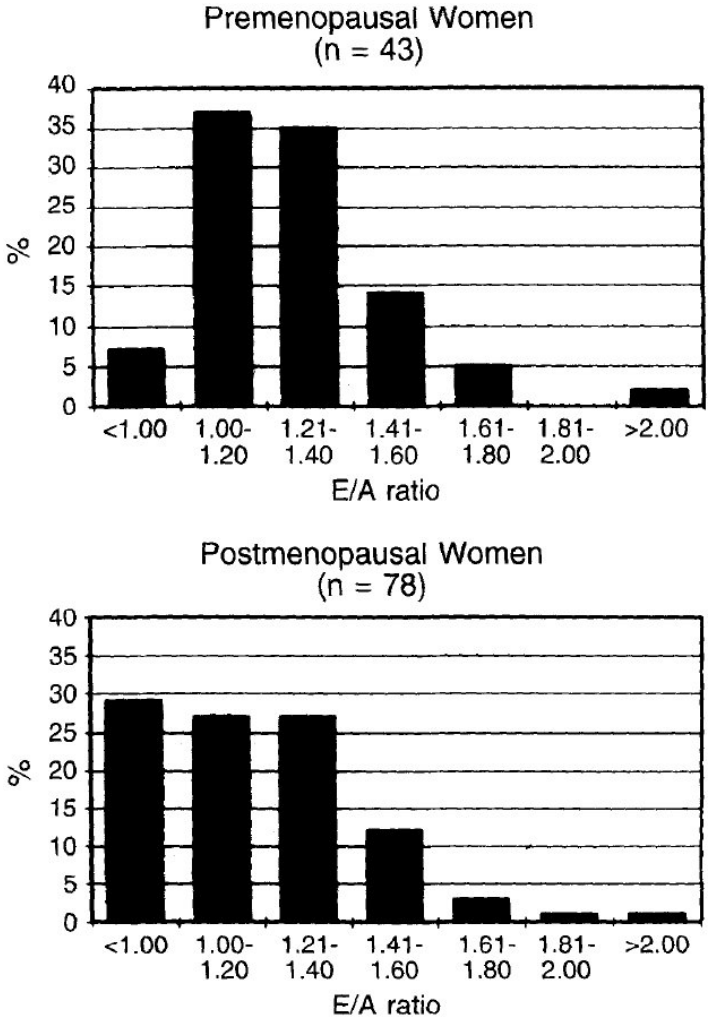


FIGURE 2.28: Distribution of peak early-to-late diastolic filling velocities in the left ventricle (E/A ratio) in pre- and post-menopausal women (extracted from Kangro et al., 1995).

in cardiac function with the menopause, Keskin Kurt and colleagues observed lower values of longitudinal strain in post-menopausal women, compared with pre-menopausal women. Their focus, however, was on longitudinal strain, and data on basal and apical rotation and deformation have not been captured. Drawing upon their findings and other animal studies, it is possible that circumferential and radial strains may similarly be lower in post-menopausal women than pre-menopausal women, but that regional differences could additionally exist (Yang et al., 2012).

It is difficult to predict the changes in LV rotational mechanics with the menopause. A lower peak LV twist and untwisting velocity in post-menopausal women is possible, based on a probable concentric remodelling and lower ejection fraction compared with

pre-menopausal women, and by drawing upon data from patients with mild LV hypertrophy (Takeuchi et al., 2007) and cardiomyopathies with varying ejection fractions (Pacileo et al., 2011), respectively. A higher peak LV twist and untwisting velocity, on the other hand, could represent a compensatory mechanism for lower diastolic function and increased afterload from increased arterial stiffness, as found in patients with mild diastolic dysfunction (Park et al., 2008) and hypertension (Yoneyama et al., 2012). In addition, oestrogen has been found to exert effects localised to epicardial cardiomyocytes from the base of female rabbit hearts, and not on cardiomyocytes isolated from the endocardium, the apex, nor male rabbit hearts (Yang et al., 2012; cf. Section 2.4.1). Whilst the withdrawal of oestrogen due to the menopause may have a localised effect on the basal epicardium, compensatory responses may nonetheless ensue across the entire organ. Empirical data on regional LV mechanics in pre- and post-menopausal women are therefore necessary to understand the impact of the menopause on the heart, and may additionally contribute to bridging the gap between mechanistic *in vitro* findings and their effects *in vivo*.

2.4.4 Summary

Circulating concentrations of the primary female sex hormones oestrogen and progesterone differ between men and women, and change with age. These hormones directly affect the heart, and are likely to play a role in the sex differences observed in cardiac ageing (discussed in Section 2.3.3). Over the course of healthy ageing, a sharp decline in oestrogen and progesterone concentrations occurs in women with the menopause. In turn, the menopause has been associated with a concentric remodelling of the LV and lower cardiac function. The effects of the menopause on regional LV mechanics have not been investigated, and may help to build the link between mechanistic *in vitro* findings and their effects on *in vivo* function.

2.5 Cardiovascular responses to physiological stress

All of the data that have been presented thus far have focused on the human heart under resting conditions. Such results only partially represent our daily lives, as measures investigating the functional capacity of the heart are lacking in this model (Goldspink, 2005). Accordingly, this section will present the responses of the heart to three cardiovascular challenges — lower body negative pressure, acute exercise and exercise training — that have been successfully applied in experimental studies involving human participants. Building upon the effects of the menopause on the female heart at rest (cf. Section 2.4.3), it is likely that the menopause also affects cardiac responses to physiological stress. The latter two research studies in this thesis will investigate the effects of the menopause on cardiac responses to physiological stress (cf. Chapter 5 and 6).

2.5.1 Lower body negative pressure

What is lower body negative pressure?

Lower body negative pressure is an experimental technique that gained popularity in the early 1960s, and is still extensively used today (Esch et al., 2007; Wolthuis et al., 1974). It involves positioning the participant's lower body within an airtight chamber sealed at the level of the iliac crest, and subsequently reducing the pressure of the air within the chamber with a vacuum pump (Esch et al., 2007; Levine et al., 1991a). The reduced atmospheric pressure around the lower body causes blood to pool in the legs, thereby reducing central venous pressure and venous return — leading to reduced cardiac preload and stroke volume (Figure 2.29; Crystal and Salem, 2015). Baroreceptors detect the resultant lower blood pressure and act to rectify it by increasing sympathetic drive and decreasing parasympathetic drive, which leads to peripheral vasoconstriction and an increase in heart rate and myocardial contractility. Intense levels of lower body negative pressure have been used to simulate acute haemorrhage (Cooke et al., 2004) and assess orthostatic tolerance (Fu et al., 2004; Levine et al., 1991a). Milder levels

may be applied to simulate seated or standing postures, while maintaining the participant in a controlled supine position for physiological measurements (Frey et al., 1986, 1994; Wolthuis et al., 1974).

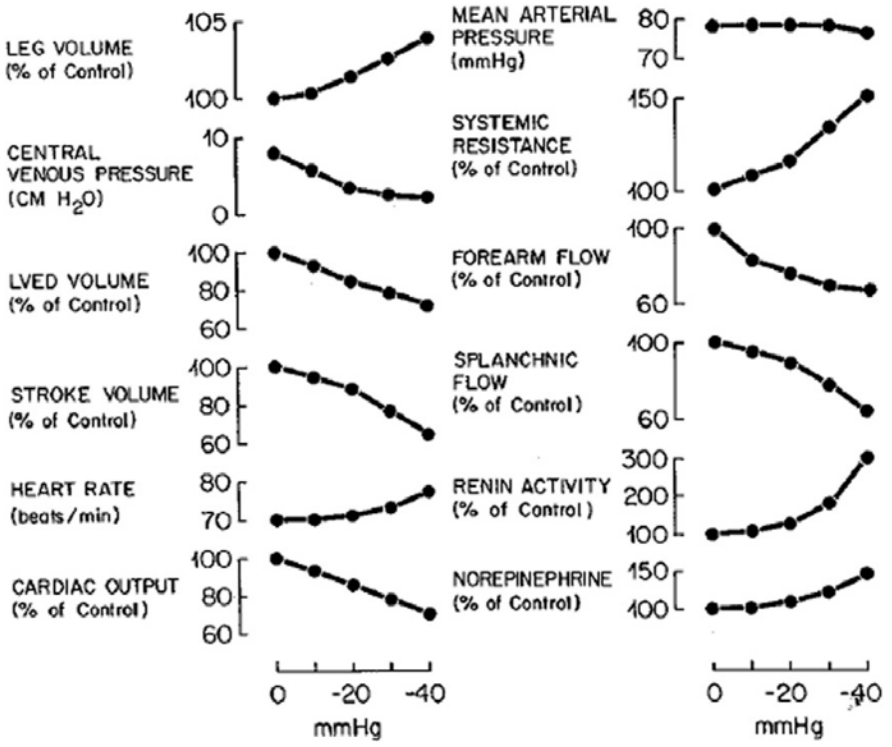


FIGURE 2.29: Principal cardiovascular responses to progressive lower body negative pressure (extracted from Crystal and Salem, 2015). LVED volume: left ventricular end-diastolic volume.

Potential role of the menopause on responses to orthostatic stress

In general, orthostatic tolerance is lower in women than men (Fu et al., 2004), but may improve in women with healthy ageing (EI-Bedawi and Hainsworth, 1994). In fact, it is possible that the menopause plays a key role in these changes in women. This postulation is founded on a better orthostatic tolerance observed in women over 50 years, compared with those up to 50 years (EI-Bedawi and Hainsworth, 1994), while recalling that the median age of the menopause is 51 years (Kato et al., 1998). The influence of the menopause on orthostatic tolerance, however, has not been directly investigated.

In response to moderate orthostatic stress, pre-menopausal women appear to evoke higher heart rates compared with post-menopausal women (Figure 2.30; Edgell et al., 2012; Harvey et al., 2005) and age-matched men (Williams et al., 2016). This reflects a greater vagal withdrawal in pre-menopausal women to cope with orthostatic stress (Williams et al., 2016). Whether their higher heart rate results in a smaller decrease in cardiac output with increasing orthostatic stress is unclear (cf. cardiac output = heart rate × stroke volume; Edgell et al., 2012; Williams et al., 2016), and previous studies comparing pre- and post-menopausal women have been limited by mean age differences of ≥26 years (Edgell et al., 2012; Harvey et al., 2005). In addition, whilst LV mechanics have not been compared between pre- and post-menopausal women, it is possible that pre-menopausal women may show higher peak LV twist and untwisting velocity during orthostatic stress, driven by greater rotations at the apex. This hypothesis is founded upon the greater LV rotational mechanics observed in pre-menopausal women during lower body negative pressure compared with age-matched men (Williams et al., 2016).

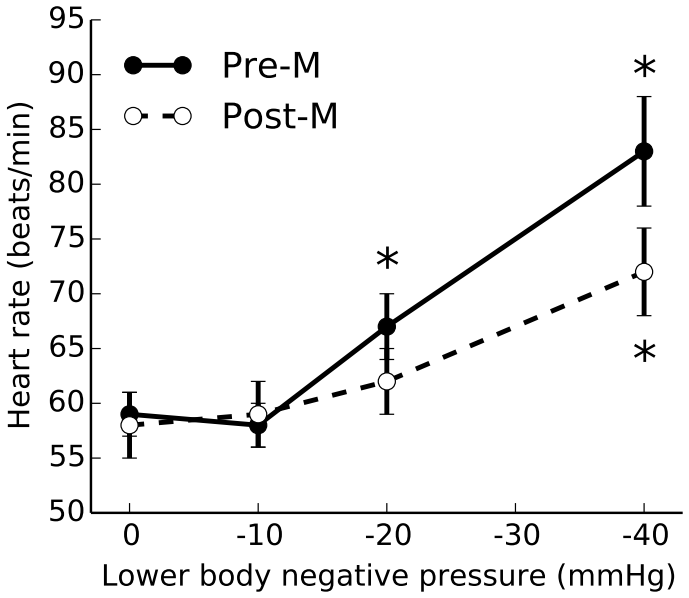


FIGURE 2.30: Heart rates in pre-menopausal (Pre-M; mean age 28 years) and post-menopausal women (Post-M; mean age 54 years) in response to lower body negative pressure. Values are mean ± standard error. $^*P < 0.05$ compared with baseline value at 0 mmHg (data from Harvey et al., 2005).

Overview of cardiac responses to lower body negative pressure

Studies that have employed lower body negative pressure as a physiological stressor demonstrate a general increase in heart rate, and decreases in cardiac output and stroke volume with increased orthostatic stress (Figure 2.29; Carrick-Ranson et al., 2012; Fu et al., 2004; Johnson et al., 2014; Levine et al., 1991a; Nixon et al., 1982; Raven et al., 1984). In line with a decreased cardiac preload, end-diastolic volume and indicators of LV filling (e.g. peak diastolic filling velocities) also decrease with lower body negative pressure (Carrick-Ranson et al., 2012; Nixon et al., 1982; Pelà et al., 2004; Prasad et al., 2007). Blood pressures typically do not fall below resting levels during mild orthostatic stress, as unloading of the cardiopulmonary volume receptors activates the sympathetic nervous system to cause peripheral vasoconstriction, in an effort to maintain homeostasis despite reduced cardiac output (Ahmad et al., 1977; Fu et al., 2004; Raven et al., 1984). In particular, vasoconstriction within the splanchnic organs, kidney and skeletal muscle helps to ensure adequate perfusion of the brain and heart at the expense of non-critical organs (Crystal and Salem, 2015). At higher levels of orthostatic stress, further decreases in stroke volume and cardiac output lead to decreases in arterial pressure. This triggers the arterial baroreceptor reflex and causes vagal withdrawal, eliciting a further increase in systemic vascular resistance and compensatory increases in heart rate and myocardial contractility to maintain blood pressure.

Data on cardiac mechanics in response to lower body negative pressure are less prevalent than those describing cardiac function, but agree on an increase in LV twist and untwisting velocity in normally-active healthy males (Esch et al., 2010; Hodt et al., 2011, 2015). These changes have been postulated to play a role in mitigating the decline in LV filling and stroke volume with reduced cardiac preload. Interestingly, opposite responses — i.e. decreases in twist and untwisting velocity with orthostatic stress — have instead been observed in endurance-trained athletes (Esch et al., 2010). This may help explain the lower orthostatic tolerance of endurance athletes compared with adults who are less trained (Esch et al., 2010; Levine et al., 1991a,b). It is important to clarify here that the negative impact of training on orthostatic tolerance

is likely to only manifest in highly-trained athletes; a moderate increase in levels of regular physical activity is likely to in fact improve orthostatic tolerance (Figure 2.31; Convertino, 1993; Levine, 1993, cf. Section 2.5.3).

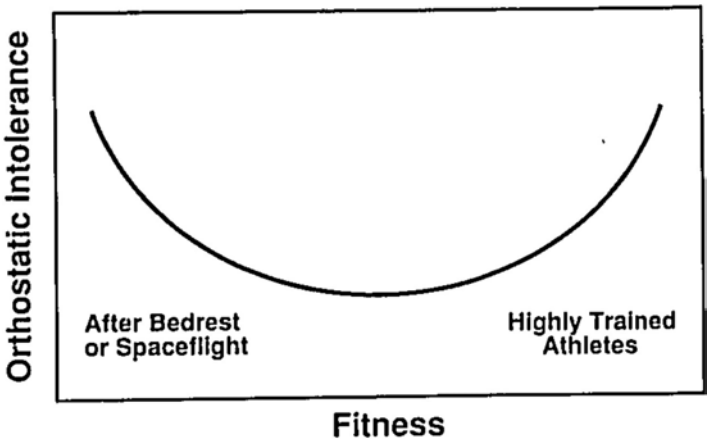


FIGURE 2.31: Hypothetical curve relating orthostatic intolerance with physical fitness. The incidence of orthostatic intolerance is higher in the very fit (e.g. endurance athletes) and the very unfit (e.g. after bed rest or space flight; extracted from Levine, 1993).

2.5.2 Acute exercise

Why use acute exercise in the laboratory?

Acute exercise — in a laboratory setting — may be employed to simulate the varying intensities of physical activity experienced in daily life. Exercise is generally recommended as a clinical tool instead of pharmacological interventions, if the patient is capable of performing an exercise test (Pellikka et al., 2007). Of specific relevance to the heart and to this thesis, the assessment of cardiac function and mechanics during exercise has shown promise in identifying cardiac dysfunction that may not be evident at rest (Ha et al., 2007; Soullier et al., 2012; Tan et al., 2010). Acute exercise is therefore a valuable physiological stressor in the laboratory and clinic. Whether menopausal status influences the cardiac responses to acute exercise, however, is unclear.

Potential role of the menopause on responses during exercise

At relative submaximal (i.e. as a percentage of the individual's maximum capacity) and maximal aerobic exercise, post-menopausal women have been observed to display lower oxygen uptakes, heart rates and cardiac outputs compared with pre-menopausal women (Green et al., 2002; Ridout et al., 2010; Wells et al., 1992; Wiebe et al., 1999; Yoshioka et al., 2003). It is important to recognise, however, that these existing studies were confounded by age differences — of at least 12 years — between pre- and post-menopausal groups. In fact, a statistical manipulation (covariance analysis to adjust for the effects of age) has suggested that these differences are due to age and not menopausal status per se (Green et al., 2002). Instead, it is possible that pre- and post-menopausal women achieve similar "global" measures of cardiac function (i.e. oxygen uptake, heart rate and cardiac output) via different regulating mechanisms (Yoshioka et al., 2003). Whilst it is difficult to decouple the effects of ageing and menopausal status, future work with smaller age gaps between pre- and post-menopausal women will necessarily complement our existing knowledge base.

Few studies have examined cardiac function beyond a global level in pre- and post-menopausal women during acute exercise, and none have additionally examined the underlying LV mechanics in these groups. The limited data available nonetheless suggest that stroke volumes at relative submaximal and maximal exercise are similar in pre- and post-menopausal women (Green et al., 2002; Ridout et al., 2010). However, post-menopausal women may generate stroke volume via a smaller increase in ejection fraction during exercise, compared with pre-menopausal women (Yoshioka et al., 2003). Recognising the potential regional effects of sex hormones (cf. Section 2.4.1), the relevance of data on cardiac mechanics during exercise is twofold: i) to help explain how cardiac function is achieved differently in pre- and post-menopausal women; and ii) to provide insight into the combined direct effects of sex hormones and elevated sympathetic drive on the myocardium (Davis et al., 2000; Lyon et al., 2008).

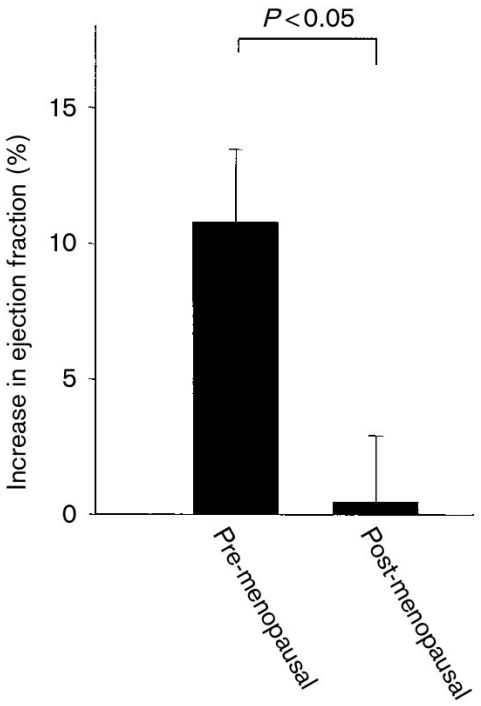


FIGURE 2.32: Percentage increase in ejection fraction from rest to peak exercise in 13 pre-menopausal (mean age 45 years) and 33 post-menopausal women (mean age 63 years; adapted from Yoshioka et al., 2003).

Overview of cardiac responses to acute exercise

It has been widely established that blood pressures, heart rate, cardiac output and stroke volume increase during aerobic exercise (Fleg et al., 1995; Fu and Levine, 2005; Hanley et al., 1989; Ogawa et al., 1992; Stöhr et al., 2011; Vella and Robergs, 2005; Wiebe et al., 1998). Although cardiac mechanics during exercise has been less extensively investigated (than measures of cardiac function), there is consensus within the literature that measures of LV rotation and strain increase in adults below the age of 40 years (Doucende et al., 2010; Drury et al., 2012; Stöhr et al., 2011). Increases in peak LV twist and untwisting velocity during exercise are driven by greater apical rotation and rotational velocity, respectively (Doucende et al., 2010; Stöhr et al., 2011). Despite higher heart rates and shorter isovolumic relaxation times during exercise, peak untwisting velocity has been observed to continue to occur before or simultaneously with mitral valve opening in healthy adults (Doucende et al., 2010; Stöhr et al., 2011; Tan et al., 2010). In addition, increases in peak diastolic strain rates may be major determinants of early diastolic filling during exercise (Doucende et al., 2010). Less

consistent data are available for adults aged >40 years, and therefore further work is imperative to establish the underlying LV mechanics in response to exercise in the older population (Drury et al., 2012).

In addition to age, the responses of the heart to acute exercise are also influenced by training status (Esch and Warburton, 2009; Green et al., 2002; Ogawa et al., 1992; Spina et al., 1993; Vella and Robergs, 2005). For example, endurance athletes have been suggested to exhibit a progressive increase in stroke volume to maximal capacity, in contrast to the historical perspective of a plateau from approximately 40% of maximal capacity (Vella and Robergs, 2005). The following section will provide an overview of the cardiac adaptations to exercise training.

2.5.3 Exercise training

Why investigate responses to exercise training?

Regular physical activity is a non-pharmacological lifestyle intervention that promotes health and reduces the risk of multiple diseases, including cardiovascular disease (Mansikkamäki et al., 2015; McTiernan et al., 2003; Pearson et al., 2002; Warburton et al., 2006). Adults are advised to perform moderate-intensity aerobic physical activity for at least 30 min on five days each week, vigorous-intensity activity for 20 min on three days, or a combination of these amounts and intensities (Fogelholm et al., 2005; Haskell et al., 2007; Nelson et al., 2007). These recommendations, however, are met by less women than men (in the developed world), with physical activity levels declining with increasing age in both sexes (Mozaffarian et al., 2015; Townsend et al., 2015). Within the middle-aged female population, changes in hormonal milieu with the menopause may also influence cardiac adaptations to regular physical activity (Parker et al., 2010). This will be explored in the subsequent paragraphs.

Potential role of the menopause on adaptations to exercise training

Based on the larger datasets in the literature — with supporting data from smaller studies — oestrogen concentrations are likely lower with exercise training in post-menopausal women (Chan et al., 2007; Friedenreich et al., 2010; McTiernan et al., 2004b, 2006; Monninkhof et al., 2009), but not in pre-menopausal women (Rinaldi et al., 2014; Smith et al., 2011). On the other hand, testosterone concentrations are likely lower in both pre- and post-menopausal women with exercise training (Chan et al., 2007; McTiernan et al., 2004a; Monninkhof et al., 2009; Rinaldi et al., 2014; Tworoger et al., 2007). As sex hormones are known to have a significant influence on the heart (cf. Section 2.4), these changes may translate into distinct cardiac adaptations to exercise training between pre- and post-menopausal women. In support of this postulation, comparisons of ovariectomised rats (as a surrogate for the post-menopausal female) with sham-operated rats have found different cardiac adaptations to exercise training between the two groups — for example, an improved calcium handling back to control levels was observed in ovariectomised rats after exercise training, but no change was observed in sham-operated rats (Bupha-Intr et al., 2009; Bupha-Intr and Wattanapermpool, 2004; Silveira et al., 2011).

Studies involving pre- and post-menopausal women indicate that both groups are able to adapt and benefit from exercise training — often assessed by increases in maximal aerobic capacity (Asikainen et al., 2004; Egelund et al., 2017; Green et al., 2002; Katyal et al., 2003; Kilbom, 1971; Murias et al., 2010a). The central and peripheral adaptations underlying these increases may, however, differ between pre- and post-menopausal women (Katyal et al., 2003; Murias et al., 2010a). For example, pre-menopausal women have been suggested to rely equally on increases in cardiac output (central) and arteriovenous oxygen difference (peripheral) to increase their maximal aerobic capacity after 12 weeks of exercise training (Murias et al., 2010a). Post-menopausal women, on the other hand, were suggested in that study to rely more on a widened arteriovenous oxygen difference, which explained approximately two-thirds of their improvement in maximal aerobic capacity (Murias et al., 2010a). As age has been associated with a decline in maximal aerobic capacity (American College of Sports Medicine, 2014;

Fitzgerald et al., 1997; Wiebe et al., 1999), it is a confounding variable in exercise training studies as post-menopausal women are usually older than pre-menopausal women. Most comparisons of pre- and post-menopausal women in response to exercise training have been limited by age differences of at least 20 years (Green et al., 2002; Katyal et al., 2003; Murias et al., 2010a), except for the recent Copenhagen Women Study with a mean age difference of 4 years (Egelund et al., 2017). In addition, studies examining central adaptations to exercise training between pre- and post-menopausal women have typically only assessed global indicators of cardiac function (e.g. heart rate, cardiac output and stroke volume; Green et al., 2002; Murias et al., 2010a), and the mechanics of the LV underlying these global cardiac adaptations are unknown.

Overview of cardiac adaptations to exercise training

The majority of studies investigating the impact of exercise training on the heart have employed cross-sectional designs to compare individuals with varying levels of regular physical activity (Baggish et al., 2010; Ferguson et al., 2001; Levine et al., 1991a). These have the advantage of assessing the chronic effects of exercise training, and indicate that elite athletes have larger hearts (Baggish et al., 2010; Pelliccia et al., 1996; Pluim et al., 2000) and greater maximal capacities (Ferguson et al., 2001; Levine et al., 1991a; Wilmore, 2005). Such studies, however, lack information on the time course of adaptations to exercise training and cannot account for any baseline pre-training differences between individuals. In light of these limitations, the research in this thesis will include a longitudinal 12-week exercise training intervention to assess cardiac adaptations between pre- and post-menopausal women. Drawing upon the limited number of longitudinal studies in the literature, the current knowledge on the time course of cardiac adaptations to exercise training will be summarised in the following paragraph.

When sedentary and recreationally-active individuals begin to follow a structured exercise training programme, their maximal aerobic capacities increase as early as 2 weeks after the onset of training (Burgomaster et al., 2008; Dart et al., 1992; Murias et al., 2010a,b; Talanian et al., 2007). Drawing upon the Fick principle — where oxygen uptake is equal to the product of cardiac output and arteriovenous oxygen difference —

both central and peripheral adaptations have been implicated in this increase in aerobic capacity (Green et al., 2002; Murias et al., 2010a,b). It is likely that changes in cardiac function precede a structural remodelling (Dart et al., 1992; Esfandiari et al., 2014; Goodman et al., 2005; Wolfe et al., 1979), with increases in LV mass only evident after at least 8 weeks of training (Arbab-Zadeh et al., 2014; Baggish et al., 2008a,b; Slørdahl et al., 2004; Spence et al., 2011). Cardiac adaptations continue for months, shifting towards but still not matching the hearts of elite athletes even after a year of exercise training (Arbab-Zadeh et al., 2014). Data on cardiac mechanics would complement the current understanding of functional and structural adaptations to exercise training, but are scarce. Only three longitudinal studies have examined cardiac mechanics in response to exercise training, and all of the data have been collected under resting conditions with no information on functional capacity (Baggish et al., 2008b; Spence et al., 2011; Weiner et al., 2010a). Notwithstanding, it appears that resting measures of LV rotation increase after three months of training (Weiner et al., 2010a), but decrease with longer training periods, as evidenced by cross-sectional studies (Nottin et al., 2008; Stöhr et al., 2012; Zocalo et al., 2007). Further work is required to continue to elucidate the time course and extent of cardiac adaptations to exercise training.

2.5.4 Summary

A review of the literature on the cardiac responses to lower body negative pressure, acute exercise and exercise training has been presented in this section. Applying these physiological stressors in experimental studies enables insight into the functional capacity of the human heart; of how the heart meets the demands of the activities in daily living. Building upon the existing evidence that the menopause affects LV structure, function and mechanics at rest (cf. Section 2.4.3), it is likely that the menopause additionally affects LV responses to physiological stress. However, the effects of the menopause on functional cardiac responses are unclear and further research is necessary.

2.6 Physiological relevance

Drawing upon the literature discussed above, it is possible to summarise physiological meaningfulness based on changes in magnitude for LV volumes, function and mechanics (Table 2.3). The references in Table 2.3 were selected if the comparison of interest had been found to be statistically significant and judged to be physiologically meaningful by the authors of the study. The 95% confidence interval of the mean difference for each comparison was estimated (Equation 2.1). In addition, the magnitude of change corresponding to small, medium and large effect sizes using Cohen's d were calculated using the suggested values of 0.2, 0.5 and 0.8, respectively, to provide a reference backdrop for each comparison (Equation 2.2; Cohen, 1988; Ellis, 2010; Pace, 2012). With Cohen's d, effect sizes are calculated relative to the standard deviation and therefore, for example, $d = 1$ would indicate that the two investigated group means were 1 standard deviation apart.

$$(\bar{x}_1 - \bar{x}_2) \pm 1.96 \times s_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}} \tag{2.1}$$

where s_p is the pooled standard deviation:

$$s_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

and numerical subscripts indicate values from different samples; $\bar{x}$: sample mean; s: sample standard deviation; n: sample size.

$$\Delta\bar{x} = d \times s_p \tag{2.2}$$

where d: Cohen's d as an indicator of effect size.

TABLE 2.3: Magnitude of physiologically relevant mean differences and arbitrary small, medium and large effect sizes based on published data, for key parameters of left ventricular volumes, function and mechanics.

				Effect size		
Reference	Comparison	Parameter	Difference (95% CI)	$d = 0.2$	$d = 0.5$	$d = 0.8$
Kaku et al. 2011	Younger vs. older	EDV (mL)	−14 (−24, −3)	4	11	17
Kaku et al. 2011	Younger vs. older	ESV (mL)	−6 (−11, 0)	2	5	9
Kaku et al. 2011	Younger vs. older	SV (mL)	−8 (−14, −2)	2	6	9
Prasad et al. 2007	Younger vs. older	IVRT (ms)	46 (34, 58)	3	8	12
Düzenli et al. 2007	Pre-M vs. Post-M	E′ (cm/s)	−1.6 (−2.3, −0.9)	0.4	1.1	1.8
Stöhr et al. 2011	0 vs. 30% exercise	Basal rot (deg)	1.8 (−0.3, 3.9)	0.5	1.2	1.8
Cheng et al. 2013	Men vs. women	Mid circ strain (%)	−2.4 (−3.2, −1.6)	1.0	2.5	4.1
Cheng et al. 2013	Men vs. women	Mid rad strain (%)	2.0 (−0.4, 4.4)	3.2	7.9	12.6
van Dalen et al. 2008a	Younger vs. older	Apical rot (deg)	1.0 (−0.4, 2.4)	0.5	1.2	1.9

References were selected based on physiological relevance ascribed by the authors of those studies, and on the relevance of comparisons to the research questions on the menopause and ageing in this thesis. Mean differences, 95% confidence intervals (CI; Equation 2.1) and magnitudes corresponding to arbitrary small, moderate and large effect sizes using Cohen's d (Ellis, 2010; Equation 2.2) were estimated using published data. Measures of left ventricular mechanics refer to systolic peaks, and the directional component for rotations (rot) has been excluded for simplicity. Pre-M: pre-menopausal women. Post-M: post-menopausal women. EDV: end-diastolic volume. ESV: end-systolic volume. SV: stroke volume. IVRT: isovolumic relaxation time. E′: peak septal wall velocity at the level of the mitral annulus in early diastole. Circumferential (circ) and radial (rad) strains were at at the mid-ventricular level.

2.7 Overall summary

The menopause has been associated with lower LV function and mechanics, and is likely to explain, at least in part, differences in cardiac ageing between men and women. Investigating LV function and mechanics in response to physiological stress is likely to provide additional insight into the effects of the menopause on the heart, complementing the existing evidence base that consists largely of cardiac measures assessed under resting conditions. Regional LV mechanics, in particular, may be affected by the regional effects of oestrogen withdrawal after the menopause.

2.8 Thesis aims

Building upon this literature review, the aim of the series of research studies in this thesis will be to examine the effects of the menopause on LV structure, function and mechanics at rest and in response to physiological stress. In the first research study, the effects of the menopause on LV structure, function and mechanics will be investigated within the context of a cross-sectional ageing study of young adult and middle-aged men and women (cf. Chapter 4). The specific study aims are: (a) to investigate age-related sex differences in LV structure, function and mechanics in young adult and middle-aged men and women; and (b) to compare LV structure, function and mechanics between middle-aged pre- and post-menopausal women. In the second research study, the effects of the menopause on LV function and mechanics will be investigated in response to lower body negative pressure and acute exercise (supine cycling) after 12 weeks of exercise training (cf. Chapter 5). This will be achieved using a longitudinal study design to compare LV function and mechanics between middle-aged pre- and post-menopausal women. In the third research study, the potential for exercise training to mitigate the effects of the menopause on LV function and mechanics in post-menopausal women will be investigated (cf. Chapter 6). The Bayes factor will be used to complement classical P-values to investigate the strength of evidence for similar LV function and mechanics between post-menopausal women before and after exercise training, relative to untrained pre-menopausal women as a reference group. The methods used in this thesis will be detailed in the following chapter (Chapter 3).

Chapter 3

Methods

3.1 Introduction

The effects of the menopause on left ventricular (LV) structure, function and mechanics were investigated in this thesis in three research studies. In the first study, resting LV structure, function and mechanics were compared between pre- and post-menopausal women in a cross-sectional study involving young adult and middle-aged men and women. In the second study, LV function and mechanics during lower body negative pressure (LBNP) and submaximal supine cycling were compared between pre- and post-menopausal women after 12 weeks of exercise training. To determine whether exercise training mitigates the effects of the menopause on LV function and mechanics, the Bayes factor was used to evaluate the strength of evidence for similarities between post-menopausal women before and after exercise training, relative to a pre-menopausal reference group. Therefore, the latter two research studies in this thesis involved longitudinal data acquired before and after a 12-week exercise training intervention. The experimental procedures used in these research studies will be presented in detail in this chapter.

The chapter will begin with a section on research design (Section 3.2), which will include information on participant enrolment (Section 3.2.1), the study participants recruited (Section 3.2.2) and the pre-test instructions for participants (Section 3.2.3).

Details on LBNP (Section 3.2.4), exercise testing (Section 3.2.5) and exercise training (Section 3.2.6) will be presented next. There will be a section dedicated to echocardiography, which will describe the key methods used to assess LV structure, function and mechanics central to this thesis (Section 3.3). Fat free mass (FFM; Section 3.4.1), blood pressure (Section 3.4.2) and blood volume (Section 3.4.3) were assessed as complementary measures to LV parameters in this thesis, and will be presented before the final section on statistical methods (Section 3.5).

3.2 Research design

Study 1 – A cross-sectional study. To investigate the effects of the menopause on the heart within the context of ageing, the first study in this thesis compared resting LV structure, function and mechanics between young and middle-aged adult women, with reference to age-matched men (i.e. the age × sex interaction in statistical terms; Chapter 4). The middle-aged women were further categorised into pre- or post-menopausal groups to investigate the effects of the menopause on these same parameters of LV structure, function and mechanics. To supplement the cardiac data, FFM (Section 3.4.1), maximal aerobic capacity on an upright cycle ergometer (Section 3.2.5) and blood pressure (Section 3.4.2) were also assessed.

Study 2 – A longitudinal study. Complementary to the resting measures in the cross-sectional study above, the second study in this thesis compared LV function and mechanics between pre- and post-menopausal women in response to LBNP and supine cycling, before and after a 12-week exercise training intervention (Figure 3.1; Chapter 5). Blood pressure was recorded simultaneously with LV data (Section 3.4.2). In addition, FFM (Section 3.4.1), maximal aerobic capacity on both upright and supine cycle ergometers (Section 3.2.5), blood volume and total haemoglobin mass (Section 3.4.3) were measured before and after exercise training.

Study 3 – Bayesian statistics. To determine whether exercise training mitigates the effects of the menopause on resting LV function and mechanics in post-menopausal

women, the Bayes factor was used to assess the evidence for similarities between post-menopausal women before and after exercise training, relative to an untrained pre-menopausal reference group (Chapter 6). The data in this study were a combination of the post-menopausal women who completed the 12-week exercise training intervention in the longitudinal study, and the pre-menopausal women in the cross-sectional study. Details on participant enrolment, the study participants recruited and their pre-test instructions will be provided in the following subsections.

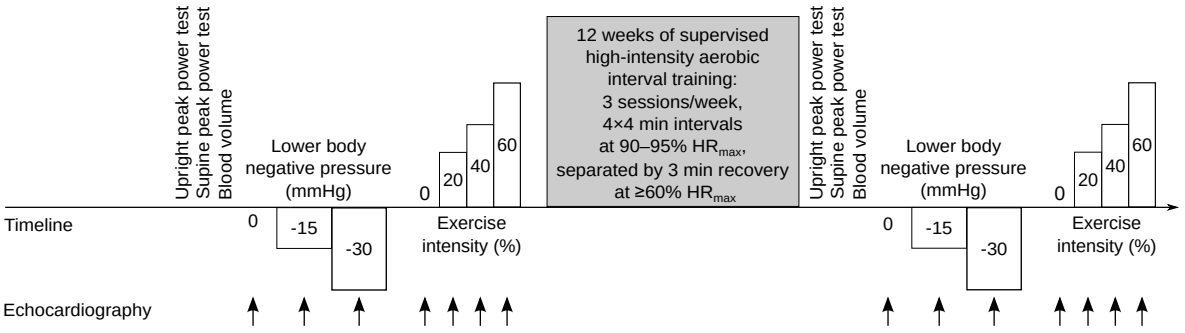


FIGURE 3.1: Schematic representation of the experimental timeline for the longitudinal study (Chapter 5). A series of physiological tests were conducted on four separate days before and after 12 weeks of exercise training. Day 1: Peak power test on an upright cycle ergometer. Day 2: Peak power test on a supine cycle ergometer. Day 3: Blood volume assessment. Day 4: Echocardiography for left ventricular function and mechanics during lower body negative pressure and submaximal supine cycling. HR_{max}: maximum heart rate.

3.2.1 Participant enrolment

All experimental procedures were approved by the Cardiff Metropolitan University's School of Sport Research Ethics Committee (Appendix A) and conformed to the ethical principles in the Declaration of Helsinki. Only non-smoking, non-diabetic (self-reported) and normotensive (resting systolic/diastolic blood pressure <140/90 mmHg) healthy volunteers not taking any cardiovascular or lipid-lowering medications were recruited. Prior to the start of any experimental procedures, the study was explained verbally and participants were encouraged to ask questions at any time. Verbal and written informed consent were obtained from all participants.

3.2.2 Study participants

Study 1

Young adult (age 19–32 years) and middle-aged (age 45–58 years) men and women were recruited from the university population and the general community for a cross-sectional study examining age-related sex differences in LV structure, function and in particular, mechanics (15 young women, 34 middle-aged women, 14 young men, 19 middle-aged men; Figure 3.2). In addition, to investigate the effects of the menopause on LV structure, function and mechanics, the recruitment of middle-aged women was targeted to include only distinctly pre- or post-menopausal women (15 pre-menopausal, 19 post-menopausal); by design peri-menopausal women were not recruited. The middle-aged pre-menopausal women were characterised as having regular menstrual cycles ranging from 21–35 days in length without a persistent difference of more than seven days between consecutive cycles (Harlow et al., 2012; Moreau et al., 2012), and had not used oral contraceptives in the preceding four months. Menstrual cycle phase was not examined as a covariate in this thesis. Post-menopausal women were identified by at least 12 consecutive months of amenorrhoea (Harlow et al., 2012), which had not been induced by surgery (e.g. hysterectomy). None of the post-menopausal women had used hormone replacement therapy (HRT) in the preceding six months.

Study 2

The middle-aged pre- and post-menopausal women from Study 1 were invited to undertake 12 weeks of exercise training and physiological testing in a longitudinal study (Chapter 5; Figure 3.2). Participants were sedentary or recreationally-active (<3 days vigorous exercise/week; Haskell et al., 2007) prior to the exercise training intervention. Women with asthma, anaemia and diseases that affect erythrocytes (e.g. thalassaemia, sicklemia and malaria) were excluded from this study, to minimise the risk of harm from the 2-min CO-rebreathing procedure for measuring blood volume (Section 3.4.3).

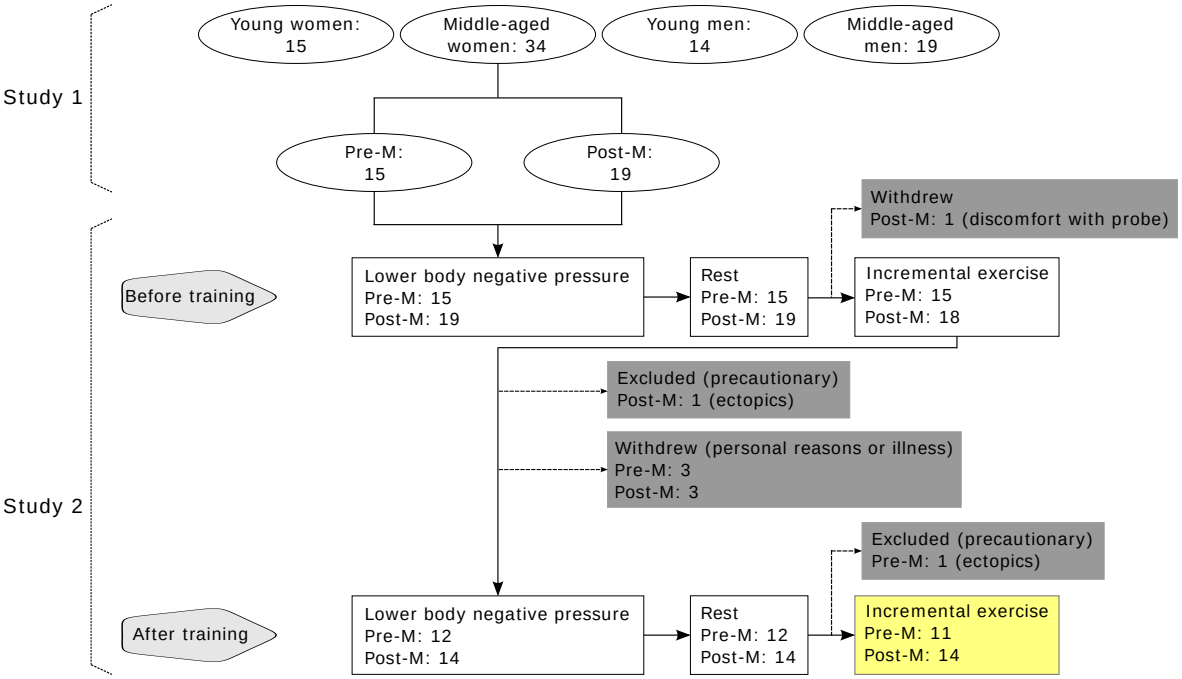


FIGURE 3.2: Flowchart of participant numbers through the studies in this thesis. Two participants were excluded from subsequent tests upon observation of ectopics during echocardiography. Twenty-five participants completed Study 2 (74% retention). Pre-M: middle-aged pre-menopausal women. Post-M: middle-aged post-menopausal women. NB: Study 3 included 15 Pre-M from Study 1 and 14 Post-M from Study 2 who completed the exercise training intervention.

In addition, women with a history of regular syncope, hernia and other cardiovascular pathologies were also excluded as a safety precaution, as LBNP was used as a physiological stressor in this study (Section 3.2.4; Esch et al., 2007).

Only 74% of the enrolled participants completed the entire study (i.e. 9 out of 34 did not complete; Figure 3.2). One participant withdrew from the study because of discomfort during echocardiography, four withdrew citing personal reasons and commitments, and a further two after the onset of illnesses not related to the study. Two participants were referred to a cardiologist upon observation of ectopics, and were excluded from further tests as a precautionary measure. The 11 pre-menopausal and 14 post-menopausal women who completed the exercise training intervention and all of the laboratory tests have been included in the statistical analyses and results in Chapter 5.

Study 3

The third research study used a subset of the data from both of the previous studies to investigate if exercise training may be used to mitigate the effects of the menopause on LV function and mechanics (Chapter 6). Resting data from the 14 post-menopausal women who completed the exercise training intervention in Study 2 were compared with the 15 pre-menopausal women in Study 1 as a reference group. These participants were selected to maximise the available sample size in this study for Bayesian statistical testing.

3.2.3 Pre-test instructions

Prior to all laboratory tests, participants were asked to abstain from caffeine, alcohol and strenuous exercise in the 24 h before arrival. In addition, participants were advised to drink a large glass of water (approximately 500 mL) 90 min before arrival. These instructions ensured that participants were euhydrated and well-rested before each test.

3.2.4 Lower body negative pressure

In Study 2, mild LBNP was used to simulate the reduced cardiac filling typical of orthostasis (i.e. the upright posture; Levine et al., 1991a). The basis of LBNP was detailed previously in the Literature review Section 2.5.1. Briefly, lower body negative pressure involves the application of a reduced atmospheric pressure to the lower body, distal to the iliac crest (Esch et al., 2007; Levine et al., 1991a; Wolthuis et al., 1974). This results in a fluid shift away from the upper body, which is still exposed to room air pressure, towards the legs (Esch et al., 2007; Wolthuis et al., 1974). In this way, the cardiovascular system can be manipulated and observed in a well-controlled manner (Esch et al., 2007; Wolthuis et al., 1974). The LBNP protocol employed in this thesis will be described here, followed by information on the precautions that were enforced to minimise risk to participants.

Experimental procedure

Participants were positioned at a 30° left lateral tilt with a neoprene kayak skirt on their iliac crest, and their lower body in an LBNP box (built in-house; length 126 cm, width 55 cm, height 90 cm; Esch et al., 2007; Figure 3.3). A seat in the LBNP box minimised any shift in position when a reduced pressure was applied. Two consecutive 10-min stages at −15 and −30 mmHg LBNP were applied (cf. Figure 3.1). A variable transformer (CMV 5E-1, Carroll & Meynell Transformers Ltd, Stockton-On-Tees, UK) connected to a vacuum pump (Henry HVR200A, Numatic International Ltd, Chard, England) was used to achieve the desired negative pressure within the box. A differential pressure meter (Testo AG, Lenzkirch, Germany) with a sensor connected to the inside of the LBNP box, and another sensor at room pressure, enabled the monitoring of reduced pressure within the box. Participants were instructed to minimise any muscular movement during LBNP, as this would counteract the desired fluid shift away from the heart. Echocardiographic images (Section 3.3) and blood pressure (Section 3.4.2) were recorded at rest and after 5 min of exposure to each stage of LBNP (Fujimoto et al., 2012; Levine et al., 1991a). Upon completion of −30 mmHg LBNP, the pressure within the box was slowly returned to atmospheric pressure over 90 s to allow for physiological compensations, to minimise the risk of inducing bradycardia and asystole (Esch et al., 2007). A 30-min recovery period was enforced between the end of LBNP and the baseline measures for supine cycling (Section 3.2.5; Fujimoto et al., 2012; Levine et al., 1991a; cf. Figure 3.1).

Precautions

As a general risk mitigation strategy, volunteers with a history of regular syncope, hernia, high blood pressure and other cardiovascular pathologies were not allowed to undergo LBNP (Esch et al., 2007; Wolthuis et al., 1974; cf. Appendix B). If a participant experienced unintended syncope, the established plan-of-action was to immediately release the reduced pressure in the LBNP box (James Pearson, personal communication, 23 October 2012). Quick release valves were fitted on both sides of the box to enable

FIGURE 3.3: Participant lying in the lower body negative pressure box. A researcher can be seen acquiring echocardiographic images (Section 3.3) in the background, to the left of the participant. A finger cuff and upper arm cuff on the participant's right arm was used for the continuous monitoring of blood pressure (FinometerPRO; Section 3.4.2).

a prompt return to room pressure (Esch et al., 2007). The participant would feel better immediately (James Pearson, personal communication, 23 October 2012). Up to and including −40 mmHg LBNP, however, the incidence of unintended syncope is low (Wolthuis et al., 1974). Lower body negative pressures of −15 and −30 mmHg have been frequently applied to both men and women, of various ages (Aratow et al., 1993; Arbab-Zadeh et al., 2004; Fujimoto et al., 2012, 2010).

Another potential side effect of LBNP is hyperventilation, as inspiration decreases right atrial pressure and increases venous return (Convertino et al., 2009; Moren et al., 1967). To minimise the likelihood of hyperventilation, participants were advised and reminded to breathe normally during LBNP. They were also informed that the tingling of hands and feet, a symptom of hyperventilation, was common during LBNP. As an additional safety measure, two researchers were always present during LBNP tests. At

least one researcher was trained in cardiopulmonary resuscitation (CPR) and in the use of the automated external defibrillator (AED). Throughout the LBNP protocol, the participant's blood pressure, heart rate (HR) and electrocardiogram (ECG) were closely monitored (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands; PowerLab 16/30, ML880, ADInstruments Pty Ltd, New South Wales, Australia; cf. Section 3.4.2). The indications for immediate test termination included unintended syncope, severe hyperventilation, a sudden fall in mean arterial pressure or HR, a systolic/diastolic blood pressure of <80/50, or the participant's decision to stop the test.

3.2.5 Exercise testing

Upon completion of LBNP, participants rested for 30 min to ensure a return to baseline (Fujimoto et al., 2012; Levine et al., 1991a; cf. Figure 3.1). Resting echocardiographic images (Section 3.3) and blood pressure (Section 3.4.2) were recorded again, with the participant lying on a supine cycle ergometer at a 30° left lateral tilt (Angio 2003, Lode, Groningen, Netherlands). Following these resting measures, participants performed three consecutive 5-min stages of exercise at 20, 40 and 60% of their supine peak power output (details on the supine peak power test are below). After 2 min of supine cycling at each stage, echocardiographic images and blood pressure were recorded during steady-state exercise. More than 24 h before this incremental exercise protocol, participants were familiarised with the supine cycle ergometer and completed peak power tests on upright and supine cycle ergometers.

Upright peak power test

All participants in the studies in this thesis completed a continuous ramp test to volitional exhaustion on an upright cycle ergometer (Corival, Lode, Groningen, The Netherlands). The peak power output (W_{peak}) for each participant was estimated according to Equation 3.1 (Wasserman et al., 2005). Each test started at 0 W and was

individualised to reach the participant's predicted W_{peak} in 10 min (Buchfuhrer et al., 1983).

$$\begin{aligned}\text{Estimated upright } W_{peak} = \big[&(\text{height in cm} - \text{age in years}) \\ &\times (20 \text{ for men or } 14 \text{ for women}) \\ &- 150 + (6 \times \text{body mass in kg})\big] \Big/ 10 \end{aligned} \tag{3.1}$$

To analyse expired air samples during exercise, participants wore a facemask connected to a metabolic cart (Oxycon Pro, Viasys Healthcare, Basingstoke, UK). Heart rate was monitored via telemetry (RS400, Polar Electro, Kempele, Finland). Peak oxygen uptake ($\dot{V}O_{2peak}$) was identified as the highest five-breath average during exercise. The corresponding five-breath average for respiratory exchange ratio (RER_{peak}) was calculated. For pre- and post-menopausal women who continued to participate in Study 2, their supine W_{peak} was estimated as 80% of their measured upright W_{peak} (unpublished results; Doucende et al., 2010), and used to calculate their familiarisation workloads below.

Two familiarisations on the supine cycle ergometer

Supine cycling was selected to maximise the quality of echocardiographic images recorded during exercise. Prior to the assessment of LV function and mechanics during incremental exercise, participants were familiarised with supine cycling. The first familiarisation consisted of a 5 min rest at a 30–45° lateral tilt, followed by five 2-min intervals at 10, 20, 30, 40, and 50% of estimated supine W_{peak} (80% of measured upright W_{peak}). Each interval was separated by 2 min of rest, during which the supine cycle ergometer was returned from a lateral tilt to level ground (0°). The first familiarisation was completed at least a day before the supine peak power test. The second familiarisation again included 5 min of rest at a lateral tilt, but was followed by three 5-min stages of supine cycling at 20, 30, and 40% of supine peak power. This was completed at least

a day before echocardiographic imaging, and accustomed participants to the duration of supine cycling required for echocardiographic data collection.

Supine peak power test

On a separate day after the upright peak power test, the pre- and post-menopausal women in Study 2 completed another continuous ramp test to volitional exhaustion on a supine cycle ergometer (Figure 3.4). As with the upright peak power test, each test started at 0 W and was individualised to reach the the participant's estimated supine W_{peak} in 10 min (Buchfuhrer et al., 1983). Expired air samples were analysed and HR was monitored.

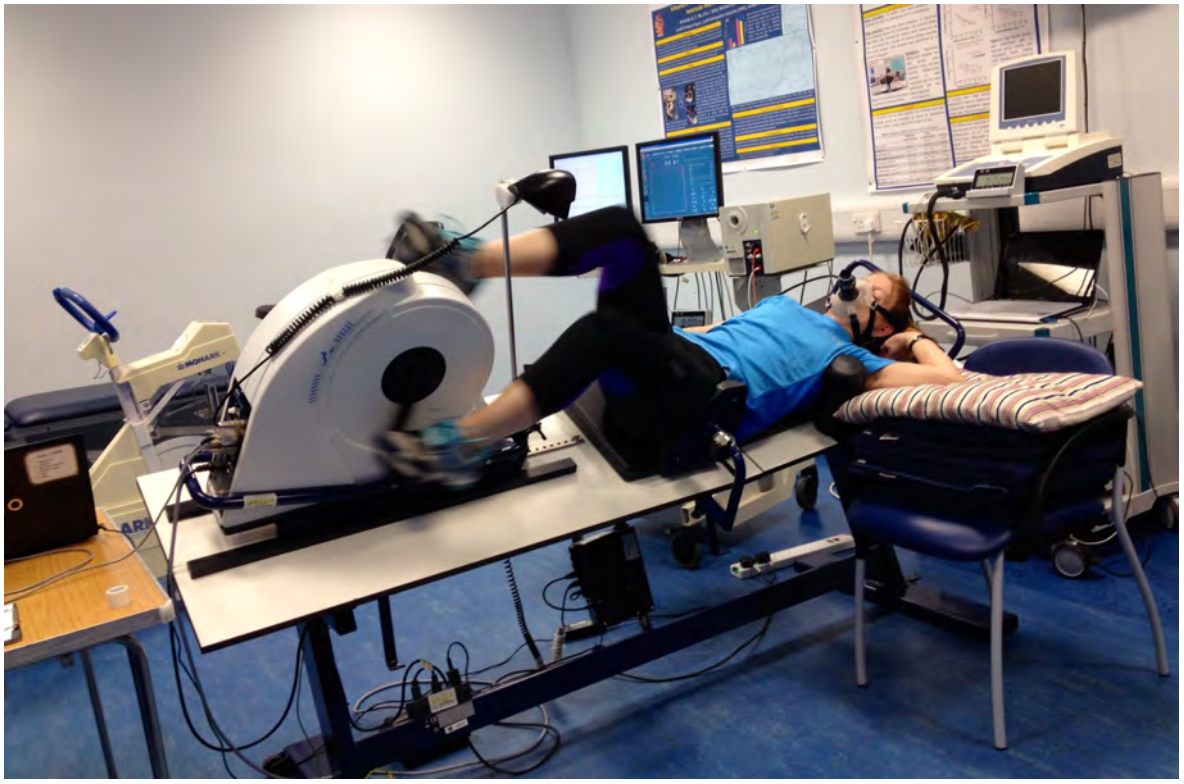


FIGURE 3.4: Participant performing a supine peak power test. Expired air samples were analysed continuously by wearing a facemask connected to a metabolic cart.

3.2.6 Exercise training

After completing their baseline physiological tests (cf. Figure 3.1), participants commenced 12 weeks of supervised, high-intensity, aerobic interval training for Study 2 (Kessler et al., 2012). Participants were asked to attend three cycling (Monark 824E, Varberg, Sweden) sessions of <1 h every week. Each exercise session consisted of a 10-min warm-up, 4 × 4-min intervals at 90–95% HR_{max} (maximum heart rate), separated by 3-min of active recovery at >60% HR_{max}, and a 5-min cool-down (Wisløff et al., 2009). The researcher on-site encouraged participants to reach 90% HR_{max} within the first 2 min of each 4-min interval. Heart rate was monitored continuously by short-range telemetry during each exercise session.

Rationale for high-intensity interval training. High-intensity aerobic intervals were selected for the exercise training intervention to maximise the likelihood of cardiorespiratory adaptations (Slørdahl et al., 2004; Wisløff et al., 2007). This was based on reports of at least equal, or greater, increases in maximal oxygen uptake following high-intensity aerobic interval training, compared with continuous moderate-intensity training (for a review see Kessler et al., 2012). An increase in maximal oxygen uptake not only indicates an improved cardiovascular fitness, but has clinical implications associated with lower likelihoods of cardiovascular disease and all-cause mortality (Kodama et al., 2009). Beyond maximal oxygen uptake, high-intensity aerobic interval training may potentially bring about greater increases in maximal cardiac output and maximal stroke volume compared with continuous moderate-intensity training (Daussin et al., 2008; Helgerud et al., 2007), along with improved left ventricular function (Kemi et al., 2005; Wisløff et al., 2009, 2007). Although sprint-interval training (supra-maximal efforts over 30 s) has shown promise in improving skeletal muscle oxidative capacity and exercise performance (Burgomaster et al., 2008, 2005; Gibala et al., 2006), such supra-maximal efforts may be less palatable to the older and sedentary populations (Little et al., 2010). The high-intensity aerobic interval stimulus was deemed likely to be well-received by the untrained middle-aged female demographic, as it has been successfully applied to middle-aged men and women with metabolic syndrome (Tjønna

et al., 2008), sedentary young adult women (Slørdahl et al., 2004) and patients with stable post-infarction heart failure (Wisløff et al., 2007).

After 12 weeks, participants were considered to have successfully completed the exercise training intervention if they had undertook at least 70% of the total number of sessions (26/36 sessions; equivalent to >8 weeks of exercise training). These participants were reassessed in the exercise physiology laboratory (cf. Figure 3.1).

Estimation of peak power output after exercise training. Participants repeated the upright and supine peak power tests, and familiarisations with the supine cycle ergometer after 12 weeks of exercise training (Section 3.2.5). An 18% improvement in $\dot{V}O_{2peak}$ was used to estimate upright W_{peak} for the peak power test after exercise training (Slørdahl et al., 2004). Individualised increments in work rate for an ideal test duration of 10 min were recalculated using this higher estimate of W_{peak} (Buchfuhrer et al., 1983).

Maintenance of exercise training adaptations. After both the upright and supine peak power tests in the laboratory, participants also completed 2 × 2-min intervals at 90–95% HR_{max}, separated by 3-min of active recovery at >60% HR_{max}. This ensured that training adaptations were maintained until all laboratory tests were completed.

3.2.7 Summary

This section has provided an overview of the research design for this thesis. Study 1 is a cross-sectional resting study (Chapter 4), Study 2 is a longitudinal exercise training study (Chapter 5), and Study 3 includes resting longitudinal data from Study 2 and cross-sectional data from Study 1 (Chapter 6). Details on participant enrolment and characteristics, and of the physiological stressors used in this thesis were presented. At rest and in response to physiological stress, ultrasound imaging was used to assess LV structure, function and mechanics. The echocardiographic techniques used in this thesis will be presented in the next section.

3.3 Echocardiography

Echocardiography is the examination of the heart and its great vessels using ultrasound (Oxborough, 2008). Among cardiac tests, echocardiography has been suggested to rank second to resting electrocardiography (ECG; Feigenbaum, 1996). In this thesis, echocardiography was used to assess LV structure, function and mechanics at rest and in response to physiological stress. In addition to conventional two-dimensional (2D) grey-scale ultrasound images, imaging modalities such as M-mode, pulsed-wave and continuous-wave Doppler, and speckle tracking (Armstrong and Ryan, 2010b; Nagueh et al., 2009) can be applied and combined to enhance the sensitivity and specificity of echocardiography (Oxborough, 2008). Details on image acquisition and analysis, including the application of additional imaging modalities to conventional grey-scale ultrasound images, will be presented in the following subsections.

3.3.1 Image acquisition and analysis

Echocardiographic images were acquired at end-expiration to examine LV structure, function and mechanics, in accordance with guidelines at the start of data collection in 2011 (Lang et al., 2006; Nagueh et al., 2009). Phased array transducers (1.5–3.6 MHz, M4S-RS; 1.7–3.3 MHz, 4V) were used on commercially available ultrasound systems (Vivid q, GE Medical Systems, Israel Ltd, Israel; Vivid E9, GE Vingmed Ultrasound AS, Horten, Norway, respectively) and images were analysed offline (EchoPAC, Version 112, GE Healthcare, Horten, Norway). The frequencies for cardiac harmonic imaging in this thesis were selected based on manufacturer recommendations, and kept consistent for examinations on each ultrasound system and for within-individual repeated measures. Harmonic imaging reduces near-field clutter and imaging artifacts that plague fundamental frequency imaging, by exploiting non-linear interactions of the propagating ultrasound wave with tissue (Armstrong and Ryan, 2010a). Signal-to-noise ratio and image quality were therefore enhanced with harmonic imaging. The ultrasound transmit frequency reflects the balance between penetration and spatial resolution: a higher ultrasound frequency would identify small structures more precisely

(i.e. better spatial resolution), but would penetrate less than lower frequencies (i.e. more attenuation; Armstrong and Ryan, 2010a). Overall gain and time gain compensation settings were adjusted during image acquisition to optimise myocardial definition. Images for the cross-sectional resting study were collected on two ultrasound systems due to equipment constraints and upgrades; images during physiological stress for the exercise training study were acquired on the Vivid E9.

Participants were supine at a 30–45° left lateral tilt for image acquisition. Imaging depth, sector width and focus position for each image were optimised for each participant at their first echocardiographic assessment. For repeated measures, these settings were noted and used for subsequent scans to standardise frame rates within each participant. Three consecutive cardiac cycles were assessed for each variable. In addition, to enhance standardisation of echocardiographic images during physiological stress (Chapter 5), transducer positions during resting measurements were temporarily marked on the participant's chest to assist the rapid relocation of similar acoustic windows during LBNP and supine cycling, and images were further confirmed with anatomic landmarks.

Left ventricular structure

Left ventricular dimensions were assessed directly from the 2D parasternal long-axis image acquired at rest. Specifically, (i) thickness of the interventricular septum (IVSd), (ii) thickness of the left ventricular posterior wall (LVPWd), and (iii) internal diameter of the LV (LVIDd) were determined during diastole (Figure 3.5; Lang et al., 2006). Left ventricular mass was estimated according to American Society of Echocardiography (ASE) recommendations, where the LV was modelled as a prolate ellipse of revolution (Devereux et al., 1986; Lang et al., 2006):

$$\text{LV mass} = 0.8 \times \Big(1.04 \times \big[(\text{IVSd} + \text{LVIDd} + \text{LVPWd})^3 - (\text{LVIDd})^3\big]\Big) + 0.6 \text{ g} \quad (3.2)$$

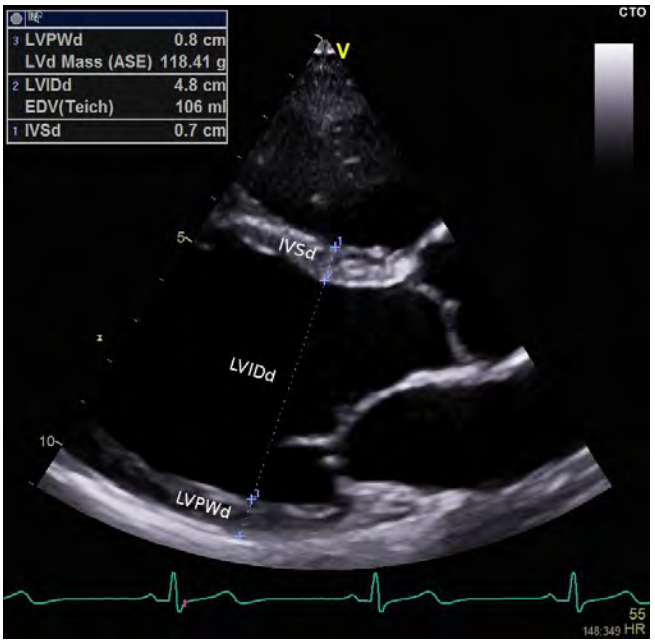


FIGURE 3.5: Assessment of left ventricular (LV) dimensions during diastole. IVSd: interventricular septum; LVIDd: LV internal diameter; LVPWd: LV posterior wall; LVd Mass (ASE): software calculation of LV mass as recommended by the American Society of Echocardiography.

Left ventricular function

End-diastolic and end-systolic volumes were determined from separate 2D recordings of apical four- and two-chamber views in the cross-sectional study (Chapter 4), and simultaneous triplane 2D recordings of apical four-, two- and three-chamber (long-axis) views in the latter two studies (Chapter 5 and 6). Two different echocardiographic methods were used to assess LV volumes in this thesis because of equipment upgrades between the start of data collection for the cross-sectional and longitudinal studies. Whilst echocardiography is known to underestimate LV volumes relative to the gold standard of magnetic resonance imaging (MRI), this systematic error has been found to be smaller with the triplane than the biplane method (Malm et al., 2006). At the start of data collection for the cross-sectional study, the Vivid E9 and 4V transducer were not yet available for simultaneous triplane recordings. When it became available in the laboratory, triplane LV volumes were adopted as the preferred method for the longitudinal study. Images from pre- and post-menopausal women were therefore analysed twice to maintain consistency of the LV volume calculation within each study:

using the biplane method for Chapter 4 and the triplane method for Chapter 5 and 6.

Analyses of end-diastolic and end-systolic volumes were performed offline by manually tracing the endocardial border, leaving the papillary muscles within the LV cavity (Figure 3.6; Lang et al., 2006). In the triplane images, the position of the LV apex was automatically positioned in the two remaining apical views after its identification in one apical view — to guide endocardial tracing in the remaining views and to reduce measurement error due to foreshortening (Nucifora et al., 2009). Following endocardial tracing, inbuilt algorithms in EchoPAC determined LV volumes using the biplane method of discs in the cross-sectional study (modified Simpson's rule; Lang et al., 2006), and using surface triangulation and summation of all triangles (by the divergence theorem) applied to an interpolated 3D triangular mesh in the longitudinal study (Malm et al., 2006; Nucifora et al., 2009). The default inter-plane angles of 60° were used to acquire triplane images, and simultaneous real-time display of three apical planes provided immediate feedback to the sonographer to optimise myocardial definition and minimise apical foreshortening during image acquisition. The spatial and temporal resolution of images acquired in triplane scanning mode using the 4V matrix array transducer are similar to that of conventional 2D images (Lang et al., 2012).

Left ventricular length was calculated as the mean of the diastolic LV lengths from the biplane images for the cross-sectional study (Chapter 4), and from the triplane images for the longitudinal studies (Chapter 5 and 6). Heart rate was determined from the ECG inherent to the ultrasound. Stroke volume (end-diastolic volume − end-systolic volume), ejection fraction ([stroke volume/end-diastolic volume] × 100) and cardiac output (HR × stroke volume) were calculated.

Pulsed-wave Doppler. Trans-mitral peak early (E) and late (A) filling velocities were determined with pulsed-wave Doppler in the apical four-chamber view (Nagueh et al., 2009; Figure 3.7). The sample volume was positioned between the mitral leaflet tips during diastole to obtain this velocity profile. The E/A ratio was calculated as an additional indicator of diastolic function (Fujimoto et al., 2010; Nagueh et al., 2009).

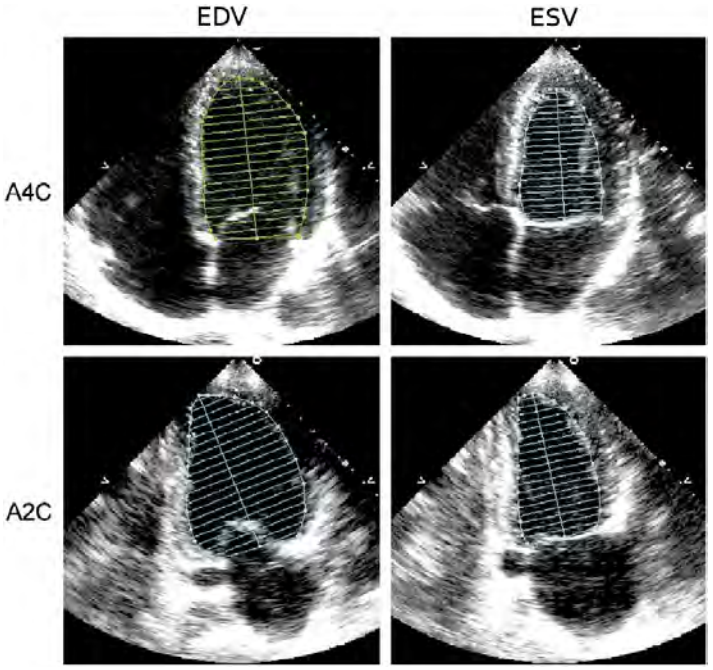


FIGURE 3.6: Analysis of apical four-chamber (A4C) and two-chamber (A2C) images for left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) using the biplane method of discs (modified Simpson's rule; extracted from Lang et al., 2006).

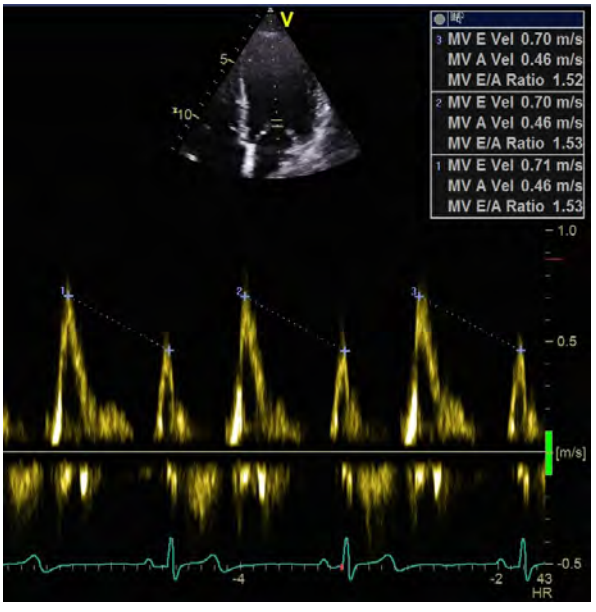


FIGURE 3.7: Trans-mitral peak early (E) and late (A) filling velocities determined by pulsed-wave Doppler in the apical four-chamber view. The E/A ratio was calculated. Measurements from three consecutive cardiac cycles are shown.

Pulsed-wave tissue Doppler imaging. Mitral annular velocities were assessed by pulsed-wave tissue Doppler imaging (TDI) in the apical four-chamber view (Nagueh et al., 2009). The sample volume was positioned at the septal insertion site of the mitral leaflet, and adjusted to cover the longitudinal excursion of the mitral annulus over the cardiac cycle. Peak septal wall velocity at the level of the mitral annulus was assessed during systole (S′), and early (E′) and late (A′) diastole (Figure 3.8; Stöhr et al., 2011). Isovolumic relaxation time (IVRT) was measured as the time between the deceleration of the S′ wave to 0 cm/s, and the onset of the E′ wave. The values of IVRT determined in this thesis, using pulsed-wave TDI, are likely to differ from those determined by continuous-wave Doppler (Nagueh et al., 2016) and this has been indicated later as a limitation of this thesis (cf. Section 7.5 Limitations). Mitral valve opening was estimated as aortic valve closure (obtained with the analysis of LV mechanics) + IVRT. Whilst the E/E′ ratio has prognostic value in patients with depressed ejection fractions and in patients with normal ejection fractions and myocardial disease, it does not accurately predict LV filling pressures in healthy volunteers (Nagueh et al., 2016) and therefore was not calculated in this thesis.

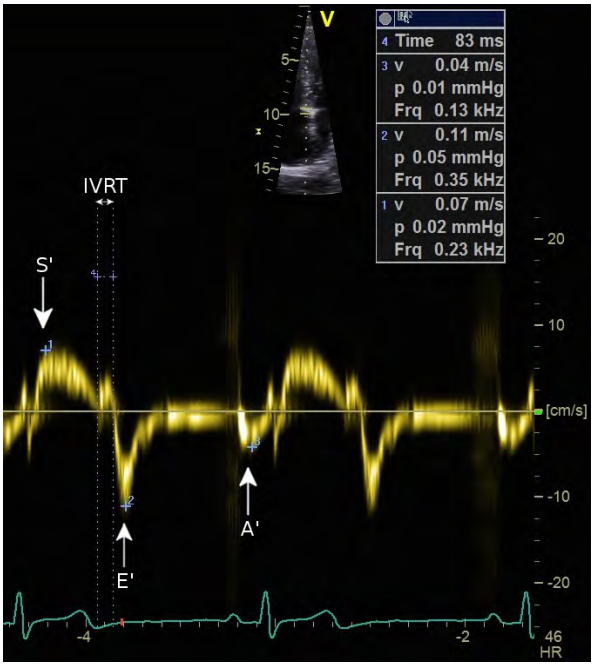


FIGURE 3.8: Pulsed-wave tissue Doppler imaging (TDI) to measure peak septal wall velocity at the level of the mitral annulus during systole (S′: 1), and early (E′: 2) and late (A′: 3) diastole, and isovolumic relaxation time (IVRT: 4).

Left ventricular mechanics

Technical background. When an ultrasound beam crosses a boundary between two tissues in the body, part of the energy is reflected, another portion is refracted, and some continues in a relatively straight line (Figure 3.9). When the target is large relative to the transmitted ultrasound wavelength, a specular reflection occurs (Figure 3.9B). The amount of energy reflected depends on the angle and impedence of the tissue. Scattering occurs when targets are small relative to the transmitted ultrasound wavelength. A small portion of energy is returned, resulting in "speckle" and the observed texture in tissues in grey-scale echocardiographic images (Helle-Valle et al., 2005). The speckles in a small region of interest are fairly consistent over the cardiac cycle, and a region within the myocardium can therefore be followed throughout a cardiac cycle. This technique is known as speckle tracking (Dandel et al., 2009; Helle-Valle et al., 2005; Kawagishi, 2008; Notomi et al., 2005; Sengupta et al., 2006b) and is key to assessing LV mechanics in this thesis ("speckle tracking echocardiography"; cf. Figure 3.10).

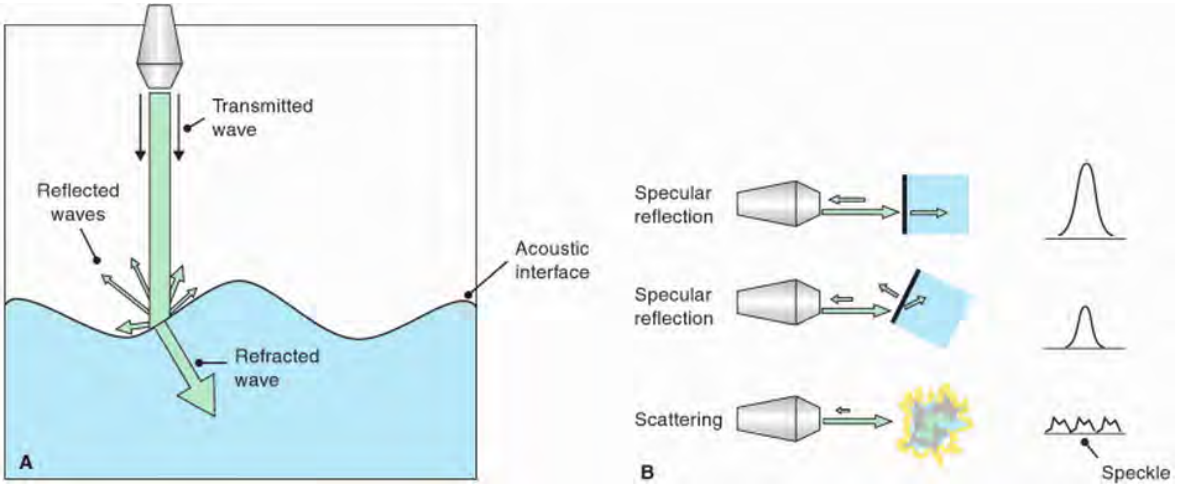


FIGURE 3.9: (A) A transmitted ultrasound wave interacts with an acoustic interface, resulting in reflection and refraction. (B) Relative to the transmitted ultrasound wavelength, a large target causes specular reflection, and small targets result in scattering. "Speckle tracking" is possible when scattering returns a small portion of energy and a "speckle" is observed (extracted from Armstrong and Ryan, 2010a).

Left ventricular mechanics were assessed using 2D speckle tracking of the myocardium

in the parasternal short-axis image at the base (mitral valve) and at the apex (just proximal to end-systolic luminal obliteration; Oxborough, 2008; van Dalen et al., 2008b). Clockwise rotations were defined as negative and anti-clockwise rotations as positive in line with conventions in the literature (Sengupta et al., 2006b; cf. Literature review Section 2.2.3). Using commercially-available software (EchoPAC, Version 112, GE Healthcare, Horten, Norway), the endocardial border was manually traced, starting and ending in the anterior wall (top-middle segment in the greyscale ultrasound image; Figure 3.10). A region of interest was generated automatically, and its width was adjusted to include the full myocardial area. Speckle tracking across the cardiac cycle was performed automatically using inbuilt algorithms in EchoPAC based on this region of interest. The software provided feedback on tracking adequacy, and tracking was further verified visually. When the tracking did not agree with visual impression of wall motion, the region of interest was manually adjusted to improve tracking quality. Images with persistent poor tracking following further adjustments to the region of interest were excluded. Data for LV mechanics at the base and apex were downloaded and transferred to in-house software for cubic spline interpolation (2D Strain Analysis Tool 1.0β14; Stuttgart, Germany). Timings of systole and diastole were extracted from these speckle tracking datasets generated by EchoPAC, and averaged across three cardiac cycles and across basal and apical images. The time at end-systole was used as a surrogate for time of aortic valve closure.

To account for inter- and intra-individual differences in heart rate and imaging frame rate, raw data for LV mechanics were smoothed with cubic spline interpolation: 1200 data points were generated in total, with 600 each for systole and diastole. Systolic and diastolic phases were normalised as equal proportions of the cardiac cycle, instead of as their proportions in time across the full duration of the cardiac cycle. Using this method, the start of a cardiac cycle was normalised and denoted by 0%, end-systole by 100%, and end-diastole by 200%. The cubic spline interpolation fits a third-degree polynomial to each pair of data points, such that the slopes (first derivative) and curvatures (second derivative) are continuous at each data point (Hoffman, 2001). Twist and twisting velocity curves were calculated by subtracting interpolated time-aligned basal

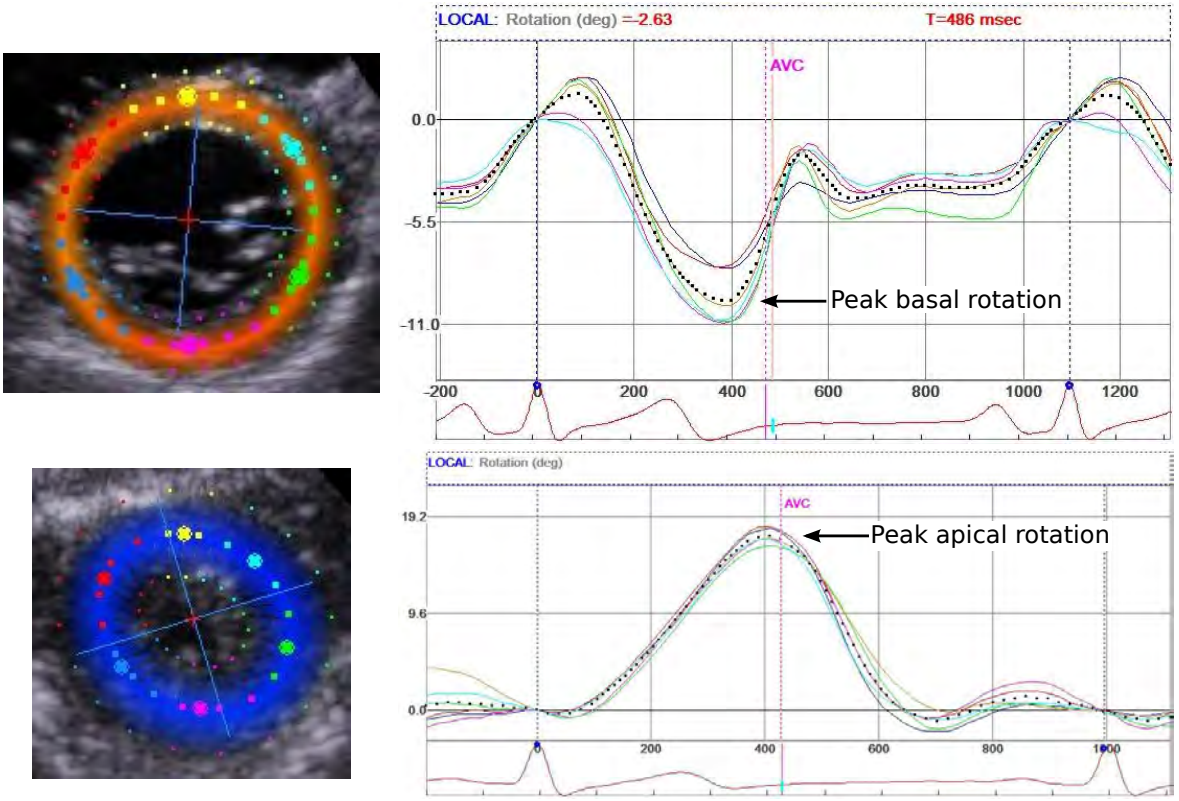


FIGURE 3.10: Examples of 2D speckle tracking and segmental rotation graphs at the left ventricular (LV) base (top) and apex (bottom) across one cardiac cycle (edited screenshots from EchoPAC). Clockwise rotations are represented by negative values and anti-clockwise rotations by positive values. AVC: aortic valve closure.

data from apical data, and peak values for circumferential strain and strain rate, rotation, rotational velocity, twist and twisting velocity were extracted from interpolated curves. Radial strain and strain rate were excluded due to their poor reproducibility (Oxborough et al., 2012; cf. Section 3.3.2). Torsion was determined as: peak twist/LV length during diastole. Time to peak untwisting velocity, and to peak diastolic basal and apical rotational velocities were derived from interpolated curves (Burns et al., 2009). Mean curves for LV mechanics across individuals within comparison groups were generated from interpolated curves, and presented over normalised systolic and diastolic phases.

3.3.2 Reliability

To minimise the error in echocardiographic measurements, images were acquired via a systematic approach (Oxborough, 2008) and according to international recommendations (Lang et al., 2006; Nagueh et al., 2009). Echocardiographic images in this thesis were all acquired and analysed by the same sonographer (the doctoral candidate). The reliability of the sonographer was assessed in 10 volunteers, consisting of both males and females (age range 20–55 years). Volunteers rested in a supine position at a 30–45° left lateral tilt for 10 min prior to image acquisition. The full sequence of echocardiographic images was recorded once, and then repeated. The coefficient of variation was calculated (Table 3.1; Atkinson and Nevill, 1998) and found to be comparable with that reported previously (Kearney, 2014; Oxborough et al., 2012).

Table 3.1: Reliability of echocardiographic parameters.

Parameter	Test	Retest	CV (%)
End-diastolic volume (mL)	130 (22)	129 (24)	3.4
End-systolic volume (mL)	56 (12)	55 (12)	4.3
Stroke volume (mL)	74 (12)	74 (14)	4.4
Isovolumic relaxation time (ms)	79 (13)	83 (12)	4.0
Peak septal wall velocity during early diastole (m/s)	0.14 (0.03)	0.14 (0.02)	2.6
Basal rotation (deg)	-4.6 (2.0)	-4.8 (2.1)	25.7
Basal circumferential strain (%)	-16 (3)	-17 (3)	6.1
Basal radial strain (%)	34 (11)	37 (9)	16.3
Apical rotation (deg)	12.5 (3.7)	10.4 (2.8)	15.0
Apical circumferential strain (%)	-22 (4)	-20 (3)	10.0
Apical radial strain (%)	23 (7)	18 (7)	31.4

Values are mean (standard deviation). CV: coefficient of variation.

3.3.3 Scaling of cardiac parameters for body size

In the cross-sectional study presented in Chapter 4, measures of cardiovascular structure and function were allometrically scaled to FFM (cf. Section 3.4.1) to enable comparisons independent of body size (Dewey et al., 2008; George et al., 1998). Variability in body size in the adult population is evident when comparing the average Chinese female, the average US male and an NBA player (Figure 3.11; Dewey et al., 2008). Such differences in body size account for up to 50% of the variability in adult LV size (Dewey

et al., 2008; Pelliccia et al., 1999). Scaling cardiovascular measurements, however, is poorly performed in adult clinical cardiology (Dewey et al., 2008). Linear relationships between the cardiovascular variable and body size variable are empirically erroneous, but often assumed (Batterham et al., 1999; Dewey et al., 2008; George et al., 2001), i.e.:

$$\text{ratiometrically-scaled variable} = \frac{\text{cardiovascular variable}}{\text{body size variable}} \tag{3.3}$$

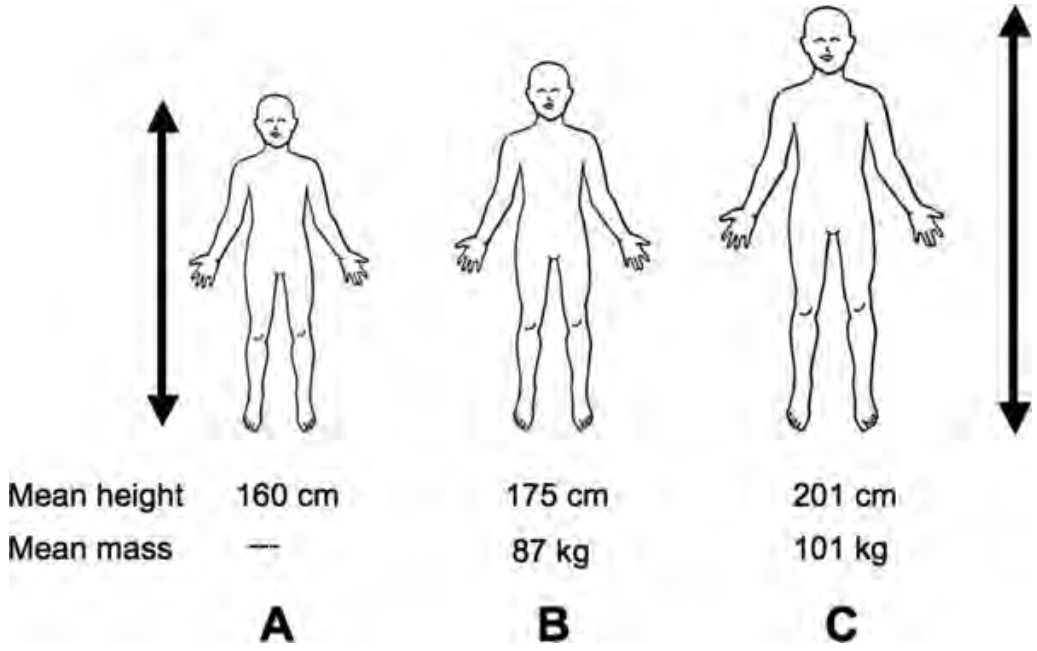


FIGURE 3.11: Relative body size of an (A) average Chinese woman, (B) average 20- to 29-year old US man, and (C) NBA player (extracted from Dewey et al., 2008).

Mathematically, Tanner's Special Circumstance is typically violated and ratiometric scaling may lead to spurious conclusions (Batterham et al., 1997; George et al., 2001; Tanner, 1949). Tanner's Special Circumstance is only met if the regression line between the body size variable and the cardiovascular variable passes through the origin (Tanner, 1949). This may be simplified to the specific condition where the coefficient of variation for the body size variable (FFM in this thesis) divided by the coefficient of variation for cardiovascular variable equals the Pearson product moment correlation between the two variables, i.e.:

$$\frac{\mathrm{CV}_{\mathrm{FFM}}}{\mathrm{CV}_{y}} \;=\; r_{\mathrm{FFM},y} \tag{3.4}$$

where $\mathrm{CV}_{\mathrm{FFM}}$ denotes the coefficient of variation for fat free mass (standard deviation/mean); y is the cardiovascular variable of interest; and $r_{\mathrm{FFM},y}$ is the Pearson's product moment correlation between FFM and y.

Ratiometric scaling is also theoretically inappropriate according to dimensionality theory, where relative geometries underpin the relationships between variables (Dewey et al., 2008; George et al., 1998; Schmidt-Nielsen, 1984). Applying this theory, LV mass (a three-dimensional variable) would be related to body surface area (two-dimensional) raised to the power of 1.5. In another example, LV wall thicknesses (one-dimensional measurements) would be proportional to $\mathrm{FFM}^{1/3}$ (three-dimensional).

Given the empirical, mathematical and theoretical flaws of simple ratiometric scaling, experts recommend allometric scaling instead (Batterham and George, 1998; Batterham et al., 1999; Dewey et al., 2008). In contrast to ratiometric scaling, allometric scaling assumes an exponential relationship between the cardiovascular variable and measure of body size (Equation 3.5). Fat free mass is the preferred measure of body size in scaling approaches, as it is of high metabolic potential (Batterham and George, 1998; Batterham et al., 1999; Dewey et al., 2008). Theoretically, this is founded on the evolution of the cardiovascular system to efficiently distribute substrates to metabolically-active tissues (Dewey et al., 2008).

$$\text{allometrically-scaled variable} = \frac{\text{cardiovascular variable}}{\text{body size variable}^{\text{scaling exponent}}} \tag{3.5}$$

$$\text{i.e.} \qquad a = \frac{y}{[\mathrm{FFM}]^{b}}$$

$$y = a[\mathrm{FFM}]^{b} \tag{3.6}$$

$$\log\ y = b\ \times\ \log\ [\mathrm{FFM}]\ +\ \log\ a \tag{3.7}$$

Allometric scaling procedure. In the cross-sectional study in Chapter 4, measures of LV structure and function (i.e. LV mass, dimensions, volumes and cardiac output) were allometrically-scaled to FFM (Equation 3.6; cf. Section 3.4.1). Firstly, ratiometric scaling was confirmed to be mathematically inappropriate (Batterham et al., 1997; Tanner, 1949; Equation 3.4; Table 3.2). Secondly, the scaling exponent (b) for allometric scaling was determined using a log transformation (Equation 3.7). The similarity of b exponents between groups (i.e. young men, young women, middle-aged men and middle-aged women) was assessed using an analysis of covariance (ANCOVA; dependent variable: log y; fixed factor: group; covariate: log [FFM]; Batterham et al., 1997). Individual b exponents were verified as similar by a non-significant interaction term ($P > 0.05$ for group $\times$ log [FFM]); any variables with a significant interaction term were examined visually to identify the source of this interaction. Finally, the groups were combined to derive a "best compromise" b exponent (Batterham et al., 1997). Although LV posterior wall thickness during diastole (LVPWd) showed a significant interaction term ($P = 0.023$), this was attributed to discrete-like measurements (0.6–1.1 cm in 0.1 cm increments) across the study sample. The suitability of allometrically-scaling LVPWd was further supported by a derived b exponent of 0.3, agreeing with the value of 0.33 according to dimensionality theory. Applying these "best compromise" b exponents therefore enabled size-independent comparisons of LV structure and volumes in the cross-sectional study in Chapter 4.

3.4 Other physiological measures

Complementary to the measures of LV structure, function and mechanics assessed using echocardiography, other physiologically relevant measures were assessed in this thesis. Specifically, fat free mass was assessed via skinfolds, blood pressure via a finger cuff connected to a non-invasive haemodynamic monitor, and total haemoglobin mass and blood volume via the 2-min CO-rebreathing technique. These methods will be elaborated upon in the following subsections.

TABLE 3.2: Allometric scaling calculations for left ventricular (LV) structure and volumes in the cross-sectional study of young adult and middle-aged men and women (cf. Chapter 4).

Parameter	$\frac{CV_{FFM}}{CV_y}$	$r_{FFM,y}$	Tanner's	ANCOVA P-value	b exponent
Cardiac output (L/min)	0.94	0.66	No	0.227	0.68
IVSd (cm)	2.10	0.53	No	0.215	0.26
LVPWd (cm)	1.82	0.54	No	0.023	0.30
LV mass (g)	0.89	0.82	No	0.098	0.90
Stroke volume (mL)	1.00	0.74	No	0.844	0.74
End-diastolic volume (mL)	0.87	0.79	No	0.607	0.92
End-systolic volume (mL)	0.64	0.76	No	0.444	1.21

CV_{FFM}: coefficient of variation for fat free mass. y: cardiovascular variable of interest. $r_{FFM,y}$: Pearson's product moment correlation between FFM and y. Tanner's: was Tanner's Special Circumstance met? ANCOVA p-value for the group × log [FFM] interaction term. b exponent: allometric scaling exponent (cf. Equation 3.6). IVSd: inter-ventricular septum thickness during diastole. LVPWd: LV posterior wall thickness during diastole.

3.4.1 Anthropometry

Participants' standing height was measured to the nearest 0.1 cm (wall-mounted stadiometer, Seca, Germany), and body mass to 0.1 kg (Model 770, Seca, Hamburg, Germany). Skinfold thicknesses were measured in duplicate at the biceps, triceps, subscapular and suprailiac on the right-side of the body (Harpenden Skinfold Calliper, Baty International, West Sussex, UK). The mean value at each site was used to calculate total skinfolds. Body density was calculated according to the age- and sex-specific equations derived by Durnin and Womersley (1974), and percent body fat was estimated using Equation 3.8 from Siri (1961). Fat free mass (FFM) was calculated as (1 − percent body fat/100) × body mass.

$$\text{Body fat (\%)} = [(4.95/\text{body density}) - 4.5] \times 100 \tag{3.8}$$

3.4.2 Continuous blood pressure monitoring

Concurrent with the echocardiographic imaging described in the previous section (Section 3.3), blood pressure was monitored continuously. In this subsection, a technical background on the non-invasive commercial system used in this thesis will be presented. Experimental procedures to record beat-by-beat blood pressure will then be detailed.

Technical background

The FinometerPRO (FMS, Finapres Measurement Systems, Arnhem, Netherlands; Figure 3.12) was used to measure blood pressure in this thesis. This is a commercially-available non-invasive haemodynamic monitor that reconstructs the brachial artery pressure waveform from continuous measurements of finger arterial pressure (Bos et al., 1996; FMS, 2002; Guelen et al., 2008). Finger arterial pressure is non-invasively measured using the volume-clamp method of Peňáz (1973), where the external pressure of a cuff worn on the finger adjusts simultaneously with intra-arterial pressure (Boehmer, 1987). Blood volume under the finger cuff is measured with a built-in photoelectric plethysmograph (Imholz et al., 1998). When this blood volume is constant, the pressure of the cuff equals the intra-arterial pressure (Imholz et al., 1998). Blood pressure recordings are interrupted at regular time intervals for an automatic check and adjustment, if necessary, of the setpoint of blood volume under the finger cuff (Imholz et al., 1998). This Physiocal criteria developed by Wesseling et al. (1995) ensures that the cuff pressure continually reflects arterial blood pressure (FMS, 2002; Imholz et al., 1998).

Brachial artery pressure is reconstructed from the measured finger arterial pressure via: (i) waveform filtering; (ii) a level correction formula; and (iii) a return-to-flow calibration (FMS, 2002; Guelen et al., 2008). First, the finger waveform is filtered to a brachial waveform (Gizdulich and Wasseling, 1990; Guelen et al., 2008). Next, the level correction formula compensates for differences in hydrostatic pressure between the height of the heart and finger (Guelen et al., 2008; Imholz et al., 1993). Finally, a return-to-flow calibration detects the first returning pulse in the finger as an upper

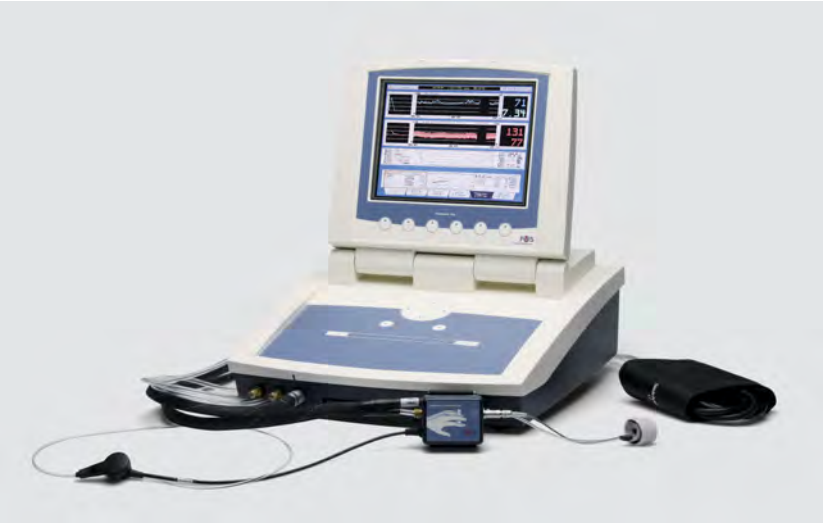


FIGURE 3.12: The FinometerPRO (FMS, Finapres Measurement Systems, Arnhem, Netherlands): a commercially-available non-invasive haemodynamic monitor, which was used to measure beat-by-beat blood pressure in this thesis (figure from Smart Medical, 2015).

arm cuff is deflated, and corrects for individual pressure gradients (Bos et al., 1996; Guelen et al., 2008). These reconstructed blood pressures (i.e. systolic, diastolic and mean) have been validated against direct measurements of brachial artery pressure (Bos et al., 1996; Guelen et al., 2003, 2008; Schutte et al., 2004). The differences between reconstructed and direct measurements were found to be less than 5 $\pm$ 8 mmHg (mean $\pm$ standard deviation) — within clinically acceptable limits for accuracy and precision (Bos et al., 1996; Guelen et al., 2003, 2008; Schutte et al., 2004).

Experimental procedure

During echocardiographic imaging, blood pressure was monitored beat-by-beat with the FinometerPRO. A finger cuff and upper arm cuff were fitted on the participant's right arm, and a return-to-flow calibration was performed after at least 10 min of supine rest. Reconstructed brachial artery pressure was recorded continuously (PowerLab 16/30, ML880, ADInstruments Pty Ltd, New South Wales, Australia) and analysed off-line (LabChart 7, Version 7.2, ADInstruments Pty Ltd, New South Wales, Australia). Systemic vascular resistance was calculated as mean arterial pressure/cardiac output.

3.4.3 Total haemoglobin mass and blood volume

The 2-min carbon monoxide (CO)-rebreathing technique (Prommer and Schmidt, 2007; Schmidt and Prommer, 2005) was used to measure total haemoglobin mass and blood volume before and after exercise training (cf. Figure 3.1). The following subsections will (a) provide a technical background to this method, (b) describe the experimental procedure employed in this thesis, (c) present information on the researcher's (the doctoral candidate's) measurement reliability, and (d) elaborate on the precautions enforced for the safety of participants and researchers.

Technical background

Haemoglobin is present in red blood cells (erythrocytes) and consists of four iron-containing pigments called hemes and a protein called globin (Plowman and Smith, 2007). Each iron atom can bind reversibly with one molecule of oxygen and accordingly, each molecule of haemoglobin can transport four molecules of oxygen (Plowman and Smith, 2007). The total mass of haemoglobin, instead of its concentration in the blood, has a significant impact on maximal oxygen uptake (Kanstrup and Ekblom, 1984; Schmidt and Prommer, 2010a). For instance, a change of 1 g of haemoglobin has been related to a change of approximately 4 mL/min in maximal oxygen uptake (Schmidt and Prommer, 2010a). In endurance athletes, a greater total haemoglobin mass is concomitant with a larger blood volume (Heinicke et al., 2001; Malczewska-Lenczowska et al., 2013). Differences in blood volume influence cardiac preload and thereby affect cardiac function (Prasad et al., 2007; Weiner et al., 2010b).

The International Committee for Standardization in Haematology (1980) recommends the measurement of total haemoglobin mass using sodium radiochromate (Cr-51) or sodium pertechnetate (Tc-99m) as a red blood cell label (El-Hemaidi et al., 1997). In addition, the recommended technique for measuring plasma volume is with radioiodine-labelled human serum albumin as a plasma label (El-Hemaidi et al., 1997; International Committee for Standardization in Haematology, 1980). A key disadvantage of these

methods are that they involve participant exposure to radioactive isotopes. To counteract this disadvantage, other dilution techniques using Evans blue dye (Gibson and Evans, 1937), hydroxyethyl starch (Tschaikowsky et al., 1997), indocyanine green (He et al., 1998), and carbon monoxide (Courtice and Gunton, 1949; Thomsen et al., 1991) have been developed (Ertl et al., 2007).

Carbon monoxide was first used for the determination of blood volume in humans by Haldane and Smith (1900). It is a useful marker because it has a 250-fold greater affinity for haemoglobin, compared with oxygen (Powell and Schochet, 2003). Recent optimisation has reduced the duration of carbon monoxide exposure to a 2-min rebreathing period (Prommer and Schmidt, 2007; Schmidt and Prommer, 2005) — a significant improvement from previous 10-min (Burge and Skinner, 1995) and 15-min (Heinicke et al., 2001) protocols. In addition, the optimised 2-min rebreathing protocol permits the use of capillary blood samples (Prommer and Schmidt, 2007; Schmidt and Prommer, 2005), which reduces the discomfort to participants compared with venous blood samples (Burge and Skinner, 1995; Heinicke et al., 2001). By measuring the changes in carbon monoxide bound to haemoglobin after inhaling a defined volume of gas, total haemoglobin mass and blood volume can be calculated accurately (Blood tec GbR, Bayreuth, Germany; Durussel et al., 2013; Gore et al., 2005; Schmidt and Prommer, 2010b). The 2-min CO-rebreathing method has been successfully applied to unfit individuals, and professional, national, regional and leisure athletes (Garvican et al., 2010b; Gore et al., 2006; Prommer et al., 2008; Robertson et al., 2010).

Experimental procedure

In this thesis, participants underwent the optimised 2-min CO-rebreathing procedure (Figure 3.13) to assess total haemoglobin mass and blood volume before and after 12 weeks of exercise training (cf. Figure 3.1). The carbon monoxide bolus (99.9% certified Carbon Monoxide CP, BOC Limited, Guildford, Surrey, UK) that was administered was individualised as 0.7 and 0.8 mL/kg body mass for unfit and aerobically fit female participants, respectively (Gore et al., 2006; Schmidt and Prommer, 2010b; Turner et al., 2014). For participants with a BMI above 25, a hypothetical body mass was

calculated from their measured height and a fixed BMI of 25 to limit the dose of carbon monoxide administered. This hypothetical body mass was used, instead of measured body mass, to derive the dose of carbon monoxide to be administered (Schmidt and Prommer, 2010b). Participants were familiarised with the protocol and equipment before starting the procedure.

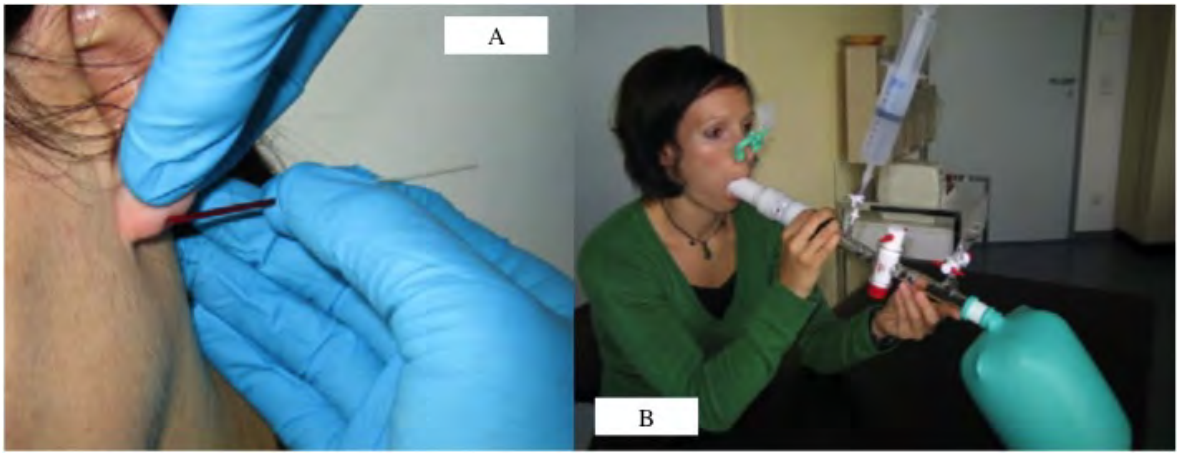


FIGURE 3.13: Procedures involved in the 2-min carbon monoxide (CO)-rebreathing method for measuring total haemoglobin mass (extracted from Schmidt and Prommer, 2010b). (A) Collection of a capillary blood sample from the participant's ear. (B) Participant rebreathing a mixture of carbon monoxide and oxygen via a spirometer.

After 15 min of seated rest, a venous blood sample (4 mL; K_3EDTA, Vacuette, Greiner Bio-One Ltd., Stonehouse, UK) was collected from an antecubital vein by a trained phlebotomist for the analysis of haemoglobin concentration (ABL80 FLEX, Radiometer, Radiometer Medical ApS, Brønshøj, Denmark) and haematocrit (Micro-haematocrit Centrifuge, Hawksley, England). These analyses were completed within 4 h of blood collection. The collection, handling and storage of blood samples complied with the Human Tissue Act and the Cardiff School of Sport Code of Practice. Capillary blood samples were collected for the immediate analysis of baseline percentage carboxyhaemoglobin (HbCO%; ABL80 FLEX, Radiometer, Radiometer Medical ApS, Brønshøj, Denmark; Figure 3.13A). Study participants wore a mouthpiece connected to a spirometer filled with 3–4 L of oxygen (SpiCO®, Blood tec, Bayreuth, Germany; Figure 3.13B). After maximal exhalation, they were instructed to fully inhale the administered carbon monoxide bolus and hold their breath for 10 s. Subsequent normal breathing via the spirometer was continued for 1 min 50 s. Capillary blood samples

Table 3.3: Reliability of the 2-min carbon monoxide (CO)-rebreathing method for measuring total haemoglobin mass and blood volume in the longitudinal study.

Parameter	Test	Retest	% Typical error
Total haemoglobin mass (g)	682 (132)	681 (133)	1.0
Erythrocyte volume (mL)	2054 (297)	2022 (394)	1.2
Plasma volume (mL)	3406 (482)	3320 (473)	2.1
Blood volume (mL)	5460 (851)	5342 (845)	1.3

Values are mean (standard deviation).

were collected 6 and 8 min after carbon monoxide inhalation to measure HbCO%. The volume of gas left in the spirometer was measured with a calibrated syringe. Remaining carbon monoxide concentrations in the lung and spirometer were measured using a carbon monoxide analyser (Dräger Pac® 7000, Drägerwerk AG & Co. KGaA, Lübeck, Germany). Ambient temperature and barometric pressure were recorded. Total haemoglobin mass, and erthyrocyte, plasma and blood volume were calculated using dedicated software (Blood Volume Measurement SpiCO, Blood tec, Bayreuth, Germany).

Reliability

Errors in measurement when using the 2-min CO-rebreathing protocol include gas leaks in the spirometer or at the mouthpiece or noseclip by the volunteer, and the reliability and precision of the HbCO% measurement (Gore et al., 2005). Careful adherence to the examination instructions is therefore necessary to achieve accurate and reproducible results (Schmidt and Prommer, 2010b). The reliability of the 2-min carbon monoxide-rebreathing protocol was assessed in 10 volunteers (3 men and 7 women; age 20–54 years; height 163.0–181.3 cm; body mass 58.6–77.9 kg; Table 3.3). Volunteers underwent the 2-min CO-rebreathing protocol on two separate days. Percentage typical error for the measurement of total haemoglobin mass was 1.0%, within the acceptable error of 2.1% (Gore et al., 2006; Prommer and Schmidt, 2007; Schmidt and Prommer, 2005, 2010b; Turner et al., 2014).

Precautions

Carbon monoxide is an odourless, colourless, tasteless, non-irritant gas that is produced when carbon-based fuels burn without enough air (Bucholtz, 2015; Powell and Schochet, 2003). At high levels, it results in tissue deprivation of oxygen and can cause sudden collapse and death. The preferential affinity of carbon monoxide for haemoglobin — the same reason for its usefulness as an erythrocyte marker — is the root of its toxicity. As such, special precautions were enforced for the safe use of the 2-min CO-rebreathing method.

The gas cylinder containing carbon monoxide was stored in a locked open-air container specially designed for gas cylinder storage. A 100-mL syringe was used to measure and introduce the exact volume of carbon monoxide required for each participant. This meant that the maximum volume of carbon monoxide that could be physically administered was 100 mL, which would increase percent carboxyhaemoglobin by less than 7.5% (as observed after a 112-mL dose; Garvican et al., 2010a). With the individualised dose per body mass used in this study, the typical maximum percent carboxyhaemoglobin expected was 6% (Gore et al., 2006). This level would be less than that observed after work in non-smoking garage employees, who were found to have values of 7.3–8.7% (Andrecs et al., 1979; Ramsey, 1967; Raub, 1999a). The 2-min acute exposure to carbon monoxide in our participants was therefore expected to be less than that encountered occupationally. In a report published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals, levels below 10% are usually not associated with symptoms (Raub, 1999b).

Once the 2-min CO-rebreathing is terminated, carbon monoxide will be removed from the circulation via exhalation, and basal percent carboxyhaemoglobin levels will be nearly reached after 6 h (Schmidt and Prommer, 2005). Exercise tests were only conducted after a time lapse of at least 12 h to prevent any effect of residual carboxyhaemoglobin levels on exercise performance (Schmidt and Prommer, 2005). As an overall risk mitigation strategy — for the laboratory and not unique to this thesis —

all pregnant or breastfeeding women, asthma patients, and participants with anaemia, acute blood loss, or diseases that affect erythrocytes (e.g. thalassaemia, sicklemia and malaria) were not allowed to undergo the 2-min CO-rebreathing procedure.

3.5 Statistical analysis

The statistical analyses in this thesis were performed in R — a language and environment for statistical computing and graphics (R Core Team, 2015). The rationale for using $\alpha = 0.1$ in the cross-sectional study will be presented here. Details on the statistical analyses differ across the three research studies and will be presented in their respective chapters.

3.5.1 Rationale for $\alpha = 0.1$

In the cross-sectional study presented in Chapter 4, α was set at 0.1 *a priori*. α is the probability of rejecting the null hypothesis when it is in fact true, and is known as Type I error (Table 3.4; Comrey and Lee, 2007; McKillup, 2012; Riegelman, 2013). When the null hypothesis is in reality false, however, β is the probability of failing to reject it, and is known as Type II error. Statistical power $(1 - \beta)$ is the probability of correctly rejecting the null hypothesis when it is false. A statistical power of at least 0.8 is generally advised, meaning at least an 80% probability that the null hypothesis is correctly rejected. It is important to note here that power decreases as sample size decreases. Quoting from Riegelman (2013): "Without actually stating it, investigators who use relatively small samples may be accepting a 30%, 40% or even greater probability that they will fail to demonstrate a statistically significant difference when a true difference exists in the larger population."

Statistical power was calculated using G*Power (Version 3.1.9.2; Faul et al., 2007). A sample size of 15 in each comparison group was used in the power calculations, to reflect the likely sample size that could be recruited for this thesis. Based on published data, adhering to the conventional α level of 0.05 would substantially limit the power

Table 3.4: Errors in statistical significance testing.

Statistical conclusion	Null hypothesis is true	Null hypothesis is false
Reject null hypothesis	Type I error (α)	Correct decision ($1 - \beta$)
Do not reject null hypothesis	Correct decision ($1 - \alpha$)	Type II error (β)

α: probability of rejecting the null hypothesis when it is true. β: probability of failing to reject the null hypothesis when it is false. Adapted from Comrey and Lee (2007) and Riegelman (2013).

of the tests performed (Table 3.5). Increasing α to 0.1 would mean a 10% probability of Type I errors (i.e. instead of 5%), but this compromise would increase power and reduce the probability of Type II errors (cf. examples in Table 3.5). Within the resource constraints for the work in this thesis, an α of 0.1 would result in the best trade-off between false positives (Type I error: rejecting the null hypothesis when it is true) and false negatives (Type II error: not rejecting the null hypothesis when it is false). By balancing and minimising possible errors in statistical interpretation, this approach maximised confidence in interpreting the data collected (Nio et al., 2017). Nonetheless, in acknowledging that the alpha level is an arbitrary cut-off, actual *P*-values will be presented to a precision of 2 decimal places so that no reader has to rely solely on the 0.1 cut-off to interpret the results. In addition, the limitation of a relatively small sample size will be acknowledged in the discussion of these results (cf. Section 4.4.4).

Table 3.5: Power calculations based on published data, adjusted for the estimated sample size ($n = 15$ in each comparison group) in this thesis given $\alpha = 0.05$ and 0.1.

				Computed power with $n = 15$	
Reference	Comparison	Parameter	*P*-value	$\alpha = 0.05$	$\alpha = 0.1$
Oxenham et al. (2003)	Younger vs. older	Stroke volume	0.018	0.67	0.78
van Dalen et al. (2008a)	Younger vs. older	Apical rotation	<0.050	0.57	0.70
Okura et al. (2009)	Younger vs. older	E DT	<0.001	0.14	0.22
Okura et al. (2009)	Men vs. women	E′	<0.010	0.20	0.30
Duzenli et al. (2010)	Pre-M vs. Post-M	E	0.009	0.22	0.32
Yoneyama et al. (2012)	Men vs. women	Torsion	<0.001	0.34	0.46
Keskin Kurt et al. (2014)	Pre-M vs. Post-M	Longitudinal strain	0.000	0.66	0.78

Power: probability of rejecting the null hypothesis when it is false; computed using G*Power (Version 3.1.9.2; Faul et al., 2007). n: sample size. *P*-value: from the comparison in the reference study. α: probability of rejecting the null hypothesis when it is true. Pre-M: pre-menopausal women. Post-M: post-menopausal women. During early diastole, peak trans-mitral filling velocity (E), E deceleration time (DT), and peak septal wall velocity at the level of the mitral annulus (E′).

3.6 Summary

In this chapter, the research design to investigate the effects of the menopause on LV structure, function and mechanics at rest and in response to physiological stress has been described. Details on the study participants and experimental procedures have been provided, and specialised methods have been accompanied by a technical background and safety precautions. The following chapters will present the results of the three experimental studies.

Chapter 4

Age-related differences in left ventricular structure and function between healthy men and women: A cross-sectional study

A version of this chapter has been published: Nio AQX, Stöhr EJ and Shave RE (2017). Age-related differences in left ventricular structure and function between healthy men and women. *Climacteric*, 20(5):476–483.

4.1 Introduction

Ageing is associated with a general decline in cardiovascular function (Merz and Cheng, 2016; Nio et al., 2015). Whilst recent reviews have suggested a different pattern of age-related changes in men compared with women (Merz and Cheng, 2016; Nio et al., 2015), conflicting data in the literature — such as that on left ventricular (LV) mass and diastolic function (Carvalho et al., 2013; Daimon et al., 2011; Dannenberg et al., 1989; Grandi et al., 1992; Luczak and Leinwand, 2009; Natori et al., 2006; Okura et al., 2009; Olivetti et al., 1995; Redfield et al., 2005) — highlight the need for more empirical evidence. Owing to the chronic exposure to different levels of testosterone, oestrogen, progesterone and epinephrine, it seems reasonable to expect that age may have a differential impact on LV structure and function between men and women (Khosla et al., 1998; Tea et al., 1975; Zouhal et al., 2008). Specifically, these hormones have been implicated in myocardial apoptosis (Luczak and Leinwand, 2009), contractility (Luczak and Leinwand, 2009; Lyon et al., 2008; Paur et al., 2012; Yang et al., 2012) and stiffness (Brouri et al., 2004; Dubey et al., 1998; Jalil et al., 1989; Nagueh et al., 2009). The drop in endogenous oestrogen and progesterone concentrations following the menopause (Harlow et al., 2012) likely contributes to the reduced systolic and diastolic function (Düzenli et al., 2007; Kangro et al., 1995; Schillaci et al., 1998) observed in post-menopausal women, yet menopausal status has rarely been accounted for within large-scale ageing studies. In studies focused on comparing pre- and post-menopausal women alone, study groups have often been limited by large age differences (e.g. a mean age difference of 24 years in Green et al., 2002), or by a lack of distinction between women of natural and surgically-induced menopause (Düzenli et al., 2007). Accordingly, a detailed characterisation of the impact of the natural menopause on LV function and mechanics in a relatively age-matched cohort will help clarify the age-related decline in cardiac function in men and women.

To first investigate sex differences in early cardiac ageing, we examined the interaction between age and sex on LV structure, function and mechanics (rotation and deformation of the LV base and apex) in a cross-sectional sample of young and middle-aged men and women. Additionally, in the middle-aged female cohort, we hypothesised that

indicators of systolic and diastolic function, as well as measures of LV mechanics, would be lower in post-menopausal women.

4.2 Methods

4.2.1 Ethical approval

All experimental procedures were approved by the Cardiff Metropolitan University's School of Sport Research Ethics Committee and conformed to the ethical principles in the Declaration of Helsinki. Prior to the start of any experimental procedures all participants provided written and verbal informed consent.

4.2.2 Study design

Young adult (age 19–32 years) and middle-aged (age 45–58 years) men and women were recruited from the university population and the general community for a cross-sectional study examining the interaction of sex and age on LV structure, function and in particular, mechanics (15 young women, 34 middle-aged women, 14 young men, 19 middle-aged men; Table 4.1). Only non-smoking, non-diabetic (self-reported) and normotensive healthy volunteers not taking any cardiovascular or lipid-lowering medications were recruited. In addition, to examine the impact of menopausal status on LV structure, function and mechanics, our recruitment of middle-aged women was targeted to include only distinctly pre- or post-menopausal women (15 pre-menopausal, 19 post-menopausal; Table 4.2; Figure 4.1); by design we did not recruit peri-menopausal women. The middle-aged pre-menopausal women were characterised as having regular menstrual cycles ranging from 21–35 days in length without a persistent difference of more than seven days between consecutive cycles (Harlow et al., 2012; Moreau et al., 2012), and had not used oral contraceptives in the preceding four months. Post-menopausal women were identified by at least 12 consecutive months of amenorrhoea (Harlow et al., 2012), which had not been induced by surgery (e.g. hysterectomy). None

of the post-menopausal women had used hormone replacement therapy (HRT) in the preceding six months.

TABLE 4.1: Demographics and aerobic capacity of young adult and middle-aged (older) men and women.

	Female		Male		*P*		
Parameter	Younger	Older	Younger	Older	Sex	Age	Sex × Age
Age (years)	23 (4)	52 (4)	24 (4)	52 (4)	0.60	**<0.01**	0.70
Height (cm)	165.9 (5.7)	163.5 (5.8)	179.0 (6.6)	178.4 (7.8)	**<0.01**	0.36	0.58
Body mass (kg)	65.9 (9.1)	64.0 (9.2)	81.0 (8.5)	83.2 (12.6)	**<0.01**	0.93	0.39
Body fat (%)	30 (4)	34 (5)	17 (5)	24 (4)	**<0.01**	**<0.01**	0.24
FFM (kg)	45.9 (5.8)	41.8 (5.0)	66.7 (6.6)	63.2 (8.9)	**<0.01**	**0.02**	0.87
Upright peak power test							
W_{peak} (W)	191 (34)	146 (26)	297 (31)	254 (46)	**<0.01**	**<0.01**	0.85
$\dot{V}O_{2peak}$ (mL/min/kg)	36 (6)	29 (5)	44 (7)	36 (8)	**<0.01**	**<0.01**	0.97
Predicted $\dot{V}O_{2max}$ (mL/min/kg)	39 (3)	28 (3)	48 (2)	36 (4)	**<0.01**	**<0.01**	0.53
HR_{max} (beats/min)	181 (8)	169 (11)	181 (5)	166 (9)	0.47	**<0.01**	0.47
Test duration (min)	8.45 (1.16)	8.11 (1.21)	8.64 (0.75)	8.83 (1.14)	**0.08**	0.77	0.30

Values are in mean (SD). FFM: fat-free mass. W_{peak}: Peak power output. $\dot{V}O_{2peak}$: Peak oxygen uptake. Predicted $\dot{V}O_{2max}$: Maximal oxygen uptake predicted using the FRIEND equation (Myers et al., 2017). HR_{max}: Maximum heart rate. ANOVA effects with $P < 0.1$ (White-adjusted for heteroscedasticity) are in **bold**.

TABLE 4.2: Demographics and aerobic capacity of middle-aged pre- and post-menopausal women.

	Middle-aged female		*P*
Parameter	Pre-menopausal	Post-menopausal	Menopause
Height (cm)	162.3 (6.8)	164.5 (4.8)	0.27
Body mass (kg)	65.3 (10.5)	63.0 (8.3)	0.49
Body fat (%)	32 (4)	36 (5)	**0.03**
FFM (kg)	43.8 (5.9)	40.1 (3.4)	**0.03**
Upright peak power test			
W_{peak} (W)	150 (27)	142 (25)	0.40
$\dot{V}O_{2peak}$ (mL/min/kg)	29 (4)	29 (5)	0.74
Predicted $\dot{V}O_{2max}$ (mL/min/kg)	29 (3)	27 (3)	0.10
HR_{max} (beats/min)	169 (10)	168 (11)	0.70
Test duration (min)	8.17 (1.11)	8.07 (1.32)	0.81

FFM: fat-free mass. W_{peak}: Peak power output. $\dot{V}O_{2peak}$: Peak oxygen uptake. Predicted $\dot{V}O_{2max}$: Maximal oxygen uptake predicted using the FRIEND equation (Myers et al., 2017). HR_{max}: Maximum heart rate. T-tests with $P < 0.1$ are in **bold**.

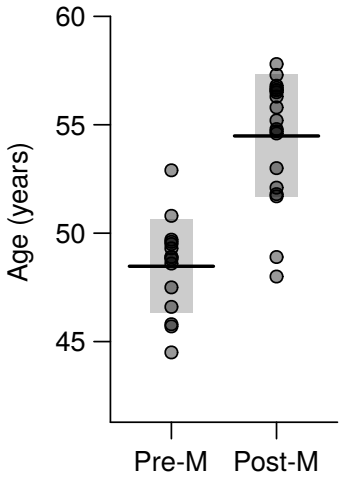


FIGURE 4.1: Age distribution of pre- (Pre-M) and post-menopausal (Post-M) women. —— Mean and ■ standard deviation within each group.

4.2.3 Aerobic capacity test

To ensure that participants were euhydrated and well-rested before any measurements, they were asked to abstain from caffeine, alcohol and strenuous exercise for 24 h, and to drink 500 ml of water 90 min before arrival at the laboratory. Participants' height and body mass (Model 770, Seca, Hamburg, Germany) were assessed (Table 4.1; Table 4.2), and skinfolds measured at the biceps, triceps, subscapular and suprailiac (Harpenden Skinfold Calliper, Baty International, West Sussex, UK) in order to estimate percentage body fat and fat-free mass (FFM; Durnin and Womersley, 1974; Siri, 1961). All participants completed a continuous ramp test to volitional exhaustion on an upright cycle ergometer (Corival, Lode, Groningen, The Netherlands) to determine peak aerobic capacity ($\dot{V}O_{2peak}$). Each test started at 0 W, and the subsequent increase in intensity was individualised using age, height and body mass (Wasserman et al., 2005; cf. Equation 3.1) to achieve peak power output in approximately 10 min. Respiratory gas exchange (Oxycon Pro, Viasys Healthcare, Basingstoke, UK) and heart rate (RS400, Polar Electro, Kempele, Finland) were monitored and recorded throughout the test. Measured $\dot{V}O_{2peak}$ was not statistically different from predicted maximal oxygen uptake (Myers et al., 2017) within each age-sex group ($P > 0.05$ with Holm-Bonferroni correction).

4.2.4 Resting cardiovascular function

Resting cardiovascular function was assessed either prior to the exercise test, or on a separate day. Following 10 minutes of rest, blood pressure (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands) and echocardiographic images were recorded with the participant lying supine at a 30–45° left lateral tilt (Angio 2003, Lode, Groningen, Netherlands). In accordance with current guidelines, echocardiographic images were acquired at end-expiration by the same trained sonographer (Lang et al., 2006; Nagueh et al., 2009; cf. Section 3.3). Phased array transducers (M4S-RS, 1.5–3.6 MHz; 4V 1.7–3.3 MHz) were used on commercially available ultrasound systems (Vivid q, GE Medical Systems, Israel Ltd, Israel; Vivid E9, GE Vingmed Ultrasound AS, Horten, Norway, respectively), and images were analysed offline for LV structure, function and mechanics (EchoPAC, Version 112, GE Healthcare, Horten, Norway). Three consecutive cardiac cycles were analysed for each variable and the mean was used for statistical analyses.

Left ventricular structure and function. Left ventricular dimensions were determined directly from two-dimensional (2D) parasternal long-axis images (Lang et al., 2006). Left ventricular mass was estimated according to the American Society of Echocardiography recommendations (Lang et al., 2006). End-diastolic and end-systolic volumes (EDV and ESV, respectively) were determined using the biplane method of discs ("modified Simpson's rule"; Lang et al., 2006). Left ventricular length was calculated as the mean of the diastolic LV lengths from the biplane images. Left ventricular mass, dimensions, volumes and cardiac output were allometrically-scaled to FFM to enable cross-sectional comparisons independent of body size, as recommended (Dewey et al., 2008). A "best compromise" scaling exponent was calculated and applied to each measure of LV size (Batterham et al., 1997). Heart rate was determined from the ECG inherent to the ultrasound. Stroke volume (SV = EDV − ESV), ejection fraction ([SV/EDV] × 100), cardiac output (heart rate × SV) and systemic vascular resistance (mean arterial pressure/cardiac output) were then calculated. Trans-mitral peak filling velocities were measured using pulsed-wave Doppler in the apical four-chamber view

(Nagueh et al., 2009). Isovolumic relaxation time (IVRT) and peak septal wall velocities at the level of the mitral annulus were assessed using pulsed-wave tissue Doppler imaging (TDI) in the apical four-chamber view (Nagueh et al., 2009; Okura et al., 2009).

Left ventricular mechanics. Left ventricular mechanics was assessed using 2D speckle tracking of the myocardium in the parasternal short-axis images at the LV base and apex, in line with previous methodology (Stöhr et al., 2015) and as described in Section 3.3. Circumferential strain and strain rate, rotation and rotational velocity at the base and apex of the LV were analysed offline using EchoPAC. Building upon the potential effects of age, sex and the menopause on basal and apical mechanics suggested by the literature (van Dalen et al., 2008a; Yang et al., 2012; Yoneyama et al., 2012), longitudinal strain was not assessed in this study, as it has been found to underestimate apical contribution (Stöhr et al., 2015). To account for differences in heart rate, raw data were normalised to percentages of systole and diastole (2D Strain Analysis Tool 1.0β14, Stuttgart, Germany; Stöhr et al., 2015). Twist and twisting velocity curves were calculated by subtracting time-aligned basal data from apical data, and peak values for all parameters were extracted from interpolated curves. Similarly, time to peak untwisting velocity, and to peak diastolic basal and apical rotational velocities were derived from interpolated curves (Burns et al., 2009). Torsion was calculated as LV twist/end-diastolic LV length. Due to poor image quality, data on LV mechanics could not be obtained from one middle-aged male participant.

4.2.5 Statistical analysis

Statistical analyses were performed with R (R Core Team, 2015). Reasonable normality of residuals was confirmed with the Shapiro-Francia test for normality and Normal quantile-quantile (Q-Q) plots. As Levene's test for homogeneity of variances revealed unequal variances in some of our parameters, the two-way analysis of variance (ANOVA; factors: sex and age) with White-adjusted P-values for heteroscedasticity was used to compare all variables between young adult and middle-aged men and women. For

variables where the sex × age interaction effect was significant, Student's t-test for independent samples was used *post hoc* to identify differences between groups. In our secondary analysis, Student's t-test for independent samples was used to compare all variables between middle-aged pre- and post-menopausal women, and age was added as a covariate to verify our findings. Alpha was set at 0.1 *a priori* for the best possible trade-off between false positives and negatives (based on power calculations using published data and the available sample size for this study; cf. Section 3.5.1 and Table 3.5). Data are presented as mean and standard deviation (SD) unless stated otherwise.

4.3 Results

4.3.1 Sex differences in LV structure, function and mechanics

Left ventricular mass, wall thicknesses, volumes and cardiac output were all smaller in women than men ($P < 0.01$; Table 4.3). Once scaled to FFM, however, these parameters were no longer significantly different between the sexes ($P > 0.1$). Diastolic function was greater in women than men, as indicated by greater early diastolic velocities (E, E/A and E′; $P < 0.1$) and peak diastolic basal circumferential strain rate ($P < 0.001$; Table 4.4).

4.3.2 Age-related differences in LV structure, function and mechanics

Left ventricular volumes, mass and cardiac output were smaller in middle-aged participants compared with the young adults ($P < 0.05$; Table 4.3). After normalising for differences in FFM, LV mass was no longer statistically different between young and middle-aged adults ($P = 0.23$), while the effect of age on LV volumes and cardiac output was still statistically significant ($P < 0.05$).

Peak LV twist, torsion, twisting velocity, and basal and apical rotation were greater in middle-aged participants compared with the young adults ($P < 0.1$; Table 4.4;

TABLE 4.3: General haemodynamics, and left ventricular (LV) structure and function in young adult and middle-aged (older) men and women at rest.

	Female		Male		*P*		
Parameter	Younger	Older	Younger	Older	Sex	Age	Sex × Age
General haemodynamics							
SBP (mmHg)	114 (7) †*	131 (14) ‡*	121 (12)	128 (13)	0.33	**<0.01**	**0.08**
SVR (mmHg·min/L)	26.0 (4.0)	32.3 (5.2)	19.4 (5.0)	25.3 (5.5)	**<0.01**	**<0.01**	0.84
Heart rate (beats/min)	57 (6)	56 (7)	54 (11)	55 (7)	0.40	0.93	0.57
$\dot{Q}$ (L/min)	3.25 (0.45)	2.89 (0.45)	4.51 (1.05)	3.69 (0.79)	**<0.01**	**<0.01**	0.23
$\dot{Q}$ (L/min/kg FFM$^{0.68}$)	0.25 (0.03)	0.23 (0.04)	0.26 (0.06)	0.22 (0.04)	0.67	**0.02**	0.21
LV structure							
IVSd (cm)	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.9 (0.1)	**<0.01**	0.25	0.83
LVPWd (cm)	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.8 (0.1)	**<0.01**	0.60	0.92
LV mass (g)	114 (24)	107 (18)	173 (30)	148 (31)	**<0.01**	**0.01**	0.18
SV (mL)	58 (8) †*	52 (9) †*‡	84 (12)	67 (11) ‡*	**<0.01**	**<0.01**	**0.02**
EDV (mL)	97 (14) †*	78 (13) †*‡*	146 (17)	109 (15) ‡*	**<0.01**	**<0.01**	**0.02**
ESV (mL)	39 (7) †*	26 (7) †*‡*	62 (8)	43 (7) ‡*	**<0.01**	**<0.01**	**0.08**
LVLd (cm)	8.0 (0.5)	7.5 (0.7)	9.3 (0.5)	8.7 (0.5)	**<0.01**	**<0.01**	0.75
Allometrically-scaled							
IVSd (cm/kg FFM$^{0.26}$)	0.30 (0.03)	0.30 (0.04)	0.30 (0.02)	0.30 (0.02)	0.92	0.69	0.96
LVPWd (cm/kg FFM$^{0.30}$)	0.25 (0.03)	0.25 (0.03)	0.25 (0.03)	0.25 (0.02)	0.78	0.80	0.77
LV mass (g/kg FFM$^{0.90}$)	3.71 (0.49)	3.81 (0.66)	4.03 (0.58)	3.60 (0.53) ‡*	0.70	0.23	**0.05**
SV (mL/kg FFM$^{0.74}$)	3.34 (0.36) †*	3.27 (0.59)	3.68 (0.51)	3.06 (0.43) ‡*	0.58	**<0.01**	**0.02**
EDV (mL/kg FFM$^{0.92}$)	2.89 (0.31) †	2.57 (0.45) ‡*	3.10 (0.34)	2.46 (0.31) ‡*	0.56	**<0.01**	**0.05**
ESV (mL/kg FFM$^{1.21}$)	0.38 (0.05)	0.29 (0.08)	0.39 (0.05)	0.29 (0.06)	0.91	**<0.01**	0.81
Systolic function							
Ejection fraction (%)	60 (2) †	67 (6) †*‡*	57 (4)	61 (5) ‡*	**<0.01**	**<0.01**	**0.05**
S′ (m/s)	0.07 (0.01)	0.07 (0.01)	0.08 (0.01)	0.08 (0.01)	**0.02**	0.52	0.51
Diastolic function							
IVRT (ms)	75 (8)	92 (16)	78 (14)	93 (14)	0.59	**<0.01**	0.70
IVRT (%)	112 (3)	114 (3)	111 (4)	114 (3)	0.73	**<0.01**	0.69
E (m/s)	0.80 (0.07)	0.70 (0.13)	0.74 (0.10)	0.59 (0.10)	**<0.01**	**<0.01**	0.25
E′ (m/s)	0.14 (0.02)	0.10 (0.02)	0.14 (0.02)	0.09 (0.02)	**0.06**	**<0.01**	0.40
A (m/s)	0.38 (0.07)	0.54 (0.10) ‡*	0.42 (0.08)	0.51 (0.08) ‡*	0.93	**<0.01**	**0.10**
A′ (m/s)	0.07 (0.01)	0.09 (0.01)	0.07 (0.01)	0.09 (0.01)	0.17	**<0.01**	0.63
E/A	2.14 (0.39)	1.33 (0.27)	1.82 (0.32)	1.19 (0.26)	**<0.01**	**<0.01**	0.26

SBP: systolic blood pressure. SVR: systemic vascular resistance. $\dot{Q}$: cardiac output. FFM: fat-free mass. IVSd: inter-ventricular septum thickness during diastole. LVPWd: LV posterior wall thickness during diastole. SV: stroke volume. EDV: end-diastolic volume. ESV: end-systolic volume. LVLd: LV length during diastole. Peak septal wall velocity at the level of the mitral annulus during systole (S′), and early (E′) and late diastole (A′). IVRT: isovolumic relaxation time in ms and in % diastole, where the period of systole is defined as 0–100% and diastole as 100–200%. Peak trans-mitral filling velocity during early (E) and late diastole (A). †$P < 0.1$ compared with age-matched men. †*$P < 0.05$ compared with age-matched men. ‡$P < 0.1$ compared with younger counterparts. ‡*$P < 0.05$ compared with younger counterparts. ANOVA effects with $P < 0.1$ (White-adjusted for heteroscedasticity) are in **bold**.

TABLE 4.4: Peak left ventricular (LV) mechanics during systole and diastole in young adult and middle-aged (older) men and women at rest.

	Female			Male			*P*		
Parameter	Younger	Older		Younger	Older		Sex	Age	Sex × Age
Systolic duration (ms)	413 (27)	442 (26)	†*‡*	400 (37)	395 (29)		**<0.01**	0.12	**0.03**
Systolic peaks									
Twist (deg)	12.7 (4.0)	16.8 (4.6)		12.4 (3.3)	18.7 (5.2)		0.43	**<0.01**	0.31
Torsion (deg/cm)	1.6 (0.5)	2.3 (0.6)		1.3 (0.3)	2.2 (0.7)		0.16	**<0.01**	0.54
Twisting vel (deg/s)	85 (14)	91 (16)		91 (10)	104 (32)		**0.06**	**0.04**	0.44
LV base									
Rotation (deg)	-3.5 (2.5)	-5.6 (3.1)		-4.4 (2.1)	-4.7 (2.6)		0.95	**0.06**	0.16
Rotational vel (deg/s)	-55 (11)	-49 (15)		-55 (13)	-45 (16)		0.51	**0.01**	0.65
Circ strain (%)	-18 (3)	-18 (4)		-16 (2)	-15 (3)		**<0.01**	0.82	0.40
Circ strain rate (1/s)	-1.0 (0.2)	-1.0 (0.2)		-1.0 (0.2)	-0.9 (0.1)		0.82	0.51	0.22
LV apex									
Rotation (deg)	9.7 (2.7)	11.8 (3.8)	†*‡	8.7 (2.4)	14.9 (4.2)	‡*	0.18	**<0.01**	**0.01**
Rotational vel (deg/s)	56 (21)	53 (14)	†*	53 (16)	70 (23)	‡*	0.15	0.13	**0.03**
Circ strain (%)	-22 (4)	-20 (4)	†*‡	-22 (4)	-24 (5)		0.12	0.67	**0.10**
Circ strain rate (1/s)	-1.4 (0.2)	-1.1 (0.2)	†*‡*	-1.4 (0.3)	-1.4 (0.3)		**<0.01**	**0.01**	**0.02**
Diastolic duration (ms)	657 (107)	664 (127)		742 (170)	659 (131)		0.24	0.26	0.18
Diastolic peaks									
Untwisting vel (deg/s)	-104 (33)	-93 (28)		-101 (27)	-91 (25)		0.70	0.13	0.94
Time to untwisting vel (%)	105 (6)	109 (7)	†*‡	106 (4)	116 (8)	‡*	**<0.01**	**<0.01**	**0.03**
LV base									
Rotational vel (deg/s)	55 (20)	50 (16)		49 (22)	45 (13)		0.24	0.35	0.88
Time to rotational vel (%)	105 (7)	104 (7)	†*	104 (5)	111 (10)	‡*	0.17	**0.10**	**0.03**
Circ strain rate (1/s)	1.6 (0.3)	1.4 (0.4)		1.2 (0.3)	1.1 (0.3)		**<0.01**	0.10	0.55
LV apex									
Rotational vel (deg/s)	-69 (29)	-58 (22)		-62 (22)	-68 (21)		0.84	0.71	0.14
Time to rotational vel (%)	110 (8)	113 (10)	†*	107 (6)	120 (10)	‡*	0.33	**<0.01**	**0.01**
Circ strain rate (1/s)	2.2 (0.6)	1.6 (0.5)		2.1 (0.7)	1.7 (0.6)		0.98	**<0.01**	0.47

Vel: velocity. Circ: circumferential. Time to peak untwisting vel and rotational vel in % diastole, where the period of systole is defined as 0–100% and diastole as 100–200%. †$P < 0.1$ compared with age-matched men. †*$P < 0.05$ compared with age-matched men. ‡$P < 0.1$ compared with younger counterparts. ‡*$P < 0.05$ compared with younger counterparts. ANOVA effects with $P < 0.1$ (White-adjusted for heteroscedasticity) are in **bold**.

Figure 4.2). Diastolic function was lower in middle-aged participants, as evidenced by longer isovolumic relaxation times and slower early diastolic velocities (E and E′), with faster late diastolic velocities (A and A′) to compensate (lower E/A; all $P < 0.01$; Table 4.3). In addition, middle-aged participants achieved peak untwisting velocities later in the cardiac cycle, and had lower peak diastolic apical circumferential strain rates compared with the young adults ($P < 0.001$; Table 4.4; Figure 4.2).

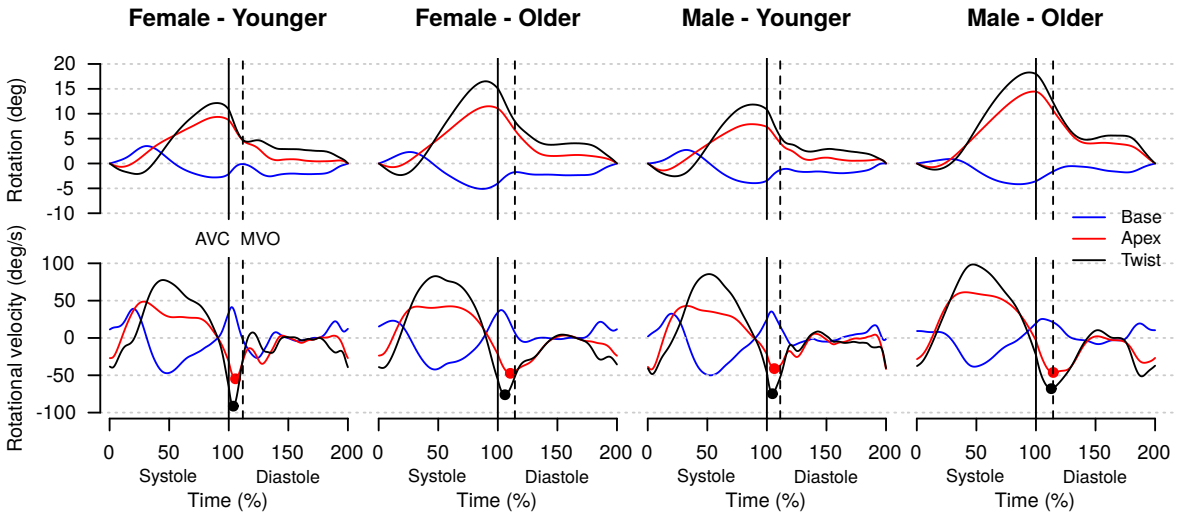


FIGURE 4.2: Interpolated rotation (top) and rotational velocity (bottom) curves at the base (blue) and apex (red), and the resultant twist/twisting velocity (black) across the cardiac cycle. Time at end-systole is defined as 100%, and end-diastole as 200%. AVC: aortic valve closure (solid vertical line). MVO: mitral valve opening (dashed vertical line). • peak untwisting velocity. • peak apical rotational velocity during diastole.

4.3.3 Age-related sex differences in LV structure, function and mechanics

Normalised LV mass, SV and EDV were smaller in middle-aged than young men; but LV mass and SV were similar in middle-aged and young women, and the difference in EDV between female groups was smaller than that between male groups ($P < 0.06$; Table 4.3). Measures of peak systolic apical mechanics were similar in young men and women, but yet were all larger in middle-aged men than middle-aged women ($P < 0.1$; Table 4.4; Figure 4.2–4.3). Middle-aged men achieved peak diastolic apical and basal rotational velocities, and untwisting velocity later in the cardiac cycle than the young men, but this age-related difference was smaller in women ($P < 0.05$).

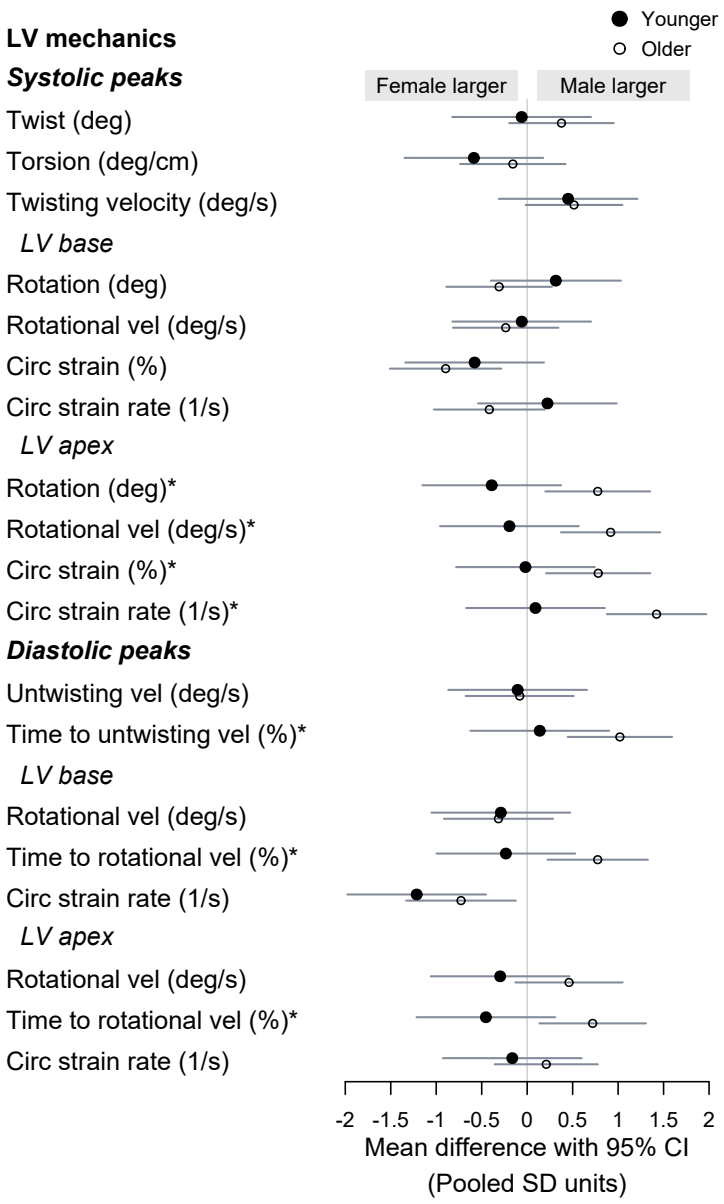


FIGURE 4.3: Forest plot of the mean difference and 95% confidence interval (CI) in left ventricular (LV) mechanics between young adult and middle-aged (older) men and women, normalised to pooled standard deviation (SD) units. $^*P < 0.1$ for the sex × age interaction effect (cf. Table 4.4).

4.3.4 Impact of the menopause on general haemodynamics, and LV structure, function and mechanics

General haemodynamics and LV mass, dimensions and volumes were all similar in middle-aged pre- and post-menopausal women (Table 4.5). Normalising LV structure and volumes to FFM, however, revealed a greater relative LV mass, ESV and EDV in post-menopausal women ($P < 0.1$). Peak LV torsion, twisting velocity and systolic apical circumferential strain rate were lower in post-menopausal women compared with the pre-menopausal women ($P < 0.1$; Table 4.6; Figure 4.4). In line with their slower early diastolic myocardial velocity (E′; $P = 0.06$), post-menopausal women also had lower peak diastolic apical circumferential strain rate ($P = 0.01$). Our findings did not change when age was added as a covariate.

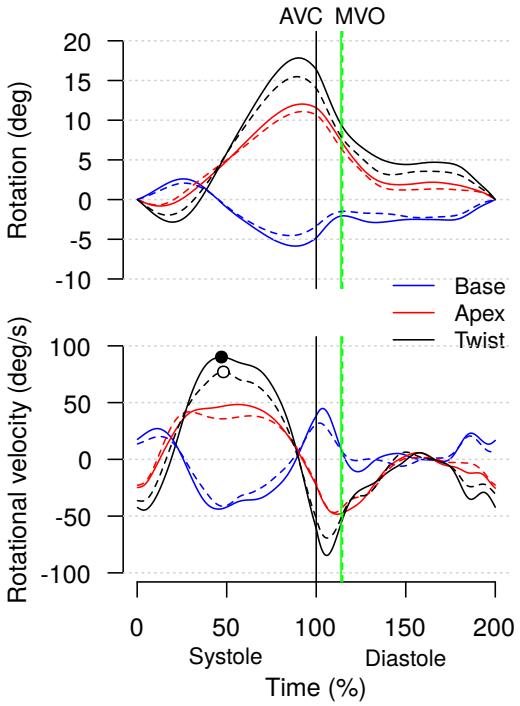


FIGURE 4.4: Interpolated rotation (top) and rotational velocity (bottom) curves at the base (blue) and apex (red), and the resultant twist/twisting velocity (black) across the cardiac cycle in middle-aged pre- (solid lines) and post-menopausal (dashed lines) women. Time at end-systole is defined as 100%, and end-diastole as 200%. Peak twisting velocity in • pre-menopausal and ∘ post-menopausal women. AVC: aortic valve closure (solid black vertical line). MVO: mitral valve opening (green vertical line).

TABLE 4.5: General haemodynamics, and left ventricular (LV) structure and function in middle-aged pre- and post-menopausal women at rest.

	Middle-aged female		*P*
Parameter	Pre-menopausal	Post-menopausal	Menopause
General haemodynamics			
Systolic blood pressure (mmHg)	128 (14)	132 (15)	0.42
SVR (mmHg·min/L)	32.3 (6.9)	32.3 (3.6)	0.99
Heart rate (beats/min)	57 (7)	55 (7)	0.63
$\dot{Q}$ (L/min)	2.89 (0.57)	2.89 (0.35)	0.99
$\dot{Q}$ (L/min/kg FFM$^{0.68}$)	0.23 (0.04)	0.24 (0.03)	0.30
LV structure			
IVSd (cm)	0.8 (0.1)	0.8 (0.1)	0.51
LVPWd (cm)	0.8 (0.1)	0.8 (0.1)	0.45
LV mass (g)	104 (17)	109 (19)	0.44
SV (mL)	52 (11)	53 (9)	0.79
EDV (mL)	76 (14)	80 (13)	0.36
ESV (mL)	24 (6)	28 (7)	0.13
LVLd (cm)	7.4 (0.5)	7.5 (0.8)	0.45
Allometrically-scaled			
IVSd (cm/kg FFM$^{0.26}$)	0.29 (0.04)	0.31 (0.03)	0.27
LVPWd (cm/kg FFM$^{0.30}$)	0.25 (0.03)	0.25 (0.03)	0.94
LV mass (g/kg FFM$^{0.90}$)	3.55 (0.50)	4.02 (0.71)	**0.04**
SV (mL/kg FFM$^{0.74}$)	3.12 (0.60)	3.39 (0.58)	0.19
EDV (mL/kg FFM$^{0.92}$)	2.38 (0.39)	2.72 (0.45)	**0.03**
ESV (mL/kg FFM$^{1.21}$)	0.25 (0.06)	0.32 (0.07)	**0.01**
Systolic function			
Ejection fraction (%)	68 (6)	66 (5)	0.27
S′ (m/s)	0.08 (0.01)	0.07 (0.01)	0.73
Diastolic function			
IVRT (ms)	89 (17)	95 (15)	0.25
IVRT (%)	114 (3)	115 (4)	0.47
E (m/s)	0.72 (0.12)	0.68 (0.13)	0.40
E′ (m/s)	0.11 (0.02)	0.09 (0.02)	**0.06**
A (m/s)	0.54 (0.09)	0.54 (0.11)	0.88
A′ (m/s)	0.09 (0.02)	0.09 (0.01)	0.47
E/A	1.37 (0.28)	1.29 (0.26)	0.41

SVR: systemic vascular resistance. $\dot{Q}$: cardiac output. FFM: fat-free mass. IVSd: inter-ventricular septum thickness during diastole. LVPWd: LV posterior wall thickness during diastole. SV: stroke volume. EDV: end-diastolic volume. ESV: end-systolic volume. LVLd: LV length during diastole. Peak septal wall velocity at the level of the mitral annulus during systole (S′), and early (E′) and late diastole (A′). IVRT: isovolumic relaxation time in ms and in % diastole, where the period of systole is defined as 0–100% and diastole as 100–200%. Peak trans-mitral filling velocity during early (E) and late diastole (A). T-tests with $P < 0.1$ are in **bold**.

TABLE 4.6: Peak left ventricular (LV) mechanics in middle-aged pre- and post-menopausal women at rest.

	Middle-aged female		*P*
Parameter	Pre-menopausal	Post-menopausal	Menopause
Systolic duration (ms)	439 (25)	444 (27)	0.56
Systolic peaks			
Twist (deg)	18.1 (3.6)	15.8 (5.2)	0.15
Torsion (deg/cm)	2.5 (0.5)	2.1 (0.6)	**0.07**
Twisting vel (deg/s)	98 (13)	86 (17)	**0.03**
LV base			
Rotation (deg)	-6.2 (3.2)	-5.1 (2.9)	0.32
Rotational vel (deg/s)	-51 (16)	-47 (15)	0.43
Circ strain (%)	-19 (4)	-17 (4)	0.33
Circ strain rate (1/s)	-1.0 (0.2)	-1.0 (0.2)	0.19
LV apex			
Rotation (deg)	12.3 (3.6)	11.4 (4.0)	0.53
Rotational vel (deg/s)	55 (14)	51 (14)	0.39
Circ strain (%)	-21 (3)	-19 (4)	0.13
Circ strain rate (1/s)	-1.1 (0.2)	-1.0 (0.2)	**0.05**
Diastolic duration (ms)	657 (127)	669 (131)	0.79
Diastolic peaks			
Untwisting vel (deg/s)	-98 (26)	-89 (29)	0.37
Time to untwisting vel (%)	108 (6)	109 (7)	0.80
LV base			
Rotational vel (deg/s)	54 (14)	46 (17)	0.17
Time to rotational vel (%)	105 (5)	104 (8)	0.82
Circ strain rate (1/s)	1.5 (0.4)	1.4 (0.5)	0.58
LV apex			
Rotational vel (deg/s)	-60 (24)	-57 (21)	0.67
Time to rotational vel (%)	114 (11)	112 (9)	0.56
Circ strain rate (1/s)	1.8 (0.6)	1.4 (0.3)	**0.01**

Vel: velocity. Circ: circumferential. Time to peak untwisting vel and rotational vel in % diastole, where the period of systole is defined as 0–100% and diastole as 100–200%. T-tests with $P < 0.1$ are in **bold**.

4.4 Discussion

In this study, LV structure, function and mechanics were assessed in a cross-sectional sample of young adult and middle-aged men and women. The results revealed a greater age-related difference in LV mass, SV and EDV in men compared with women, coincident with greater peak systolic apical mechanics and later peak diastolic rotational velocities over the cardiac cycle in middle-aged men compared with middle-aged women. These findings suggest that sex differences in early cardiac ageing may be related to changes at the apex. In addition, we observed that post-menopausal women had lower peak LV mechanics (torsion, twisting velocity and apical circumferential strain rates)

compared with their middle-aged pre-menopausal counterparts. This may indicate an initial reduction in myocardial function after the menopause.

4.4.1 Age-related differences in LV structure and function between men and women

We found that LV mass and SV were lower in middle-aged men than young adult men, but were similar in middle-aged and young adult women. In addition, EDV was lower in the middle-aged groups relative to the young adult groups, but this difference was greater among men than women. This supports previous work showing that a significant loss of cardiomyocytes in response to early ageing occurs only in men (Olivetti et al., 1995). The associated lower EDV in middle-aged men could be underpinned, at least in part, by a greater sub-clinical impairment in LV relaxation in men than women with early ageing, as suggested by longer times to peak diastolic rotational velocities (Burns et al., 2009) in our study. Although we did not measure hormone concentrations in this study, it is helpful to consider our findings in the context of previous research. It is unclear if differences in oestrogen and progesterone concentrations contribute to the age-related differences in LV mass and volumes observed here, as these parameters have been found to be similar in pre- and post-menopausal women who typically experience contrasting levels of oestrogen and progesterone (Harlow et al., 2012; Olivetti et al., 1995; Schillaci et al., 1998). Higher levels of testosterone and/or epinephrine in men compared with women (Khosla et al., 1998; Zouhal et al., 2008) may however explain the age-related differences in LV structure and volumes, as these hormones have been shown to stimulate apoptosis and fibrosis, which could thus decrease LV mass and increase LV stiffness (Brouri et al., 2004; Jalil et al., 1989; Luczak and Leinwand, 2009; Nagueh et al., 2009; Papamitsou et al., 2011). Notwithstanding, it is important to acknowledge that our structural data conflict with a number of previous studies. Contradictory findings to ours — such as an age-related increase in LV mass (Daimon et al., 2011; Grandi et al., 1992; Redfield et al., 2005) — may have arisen from the inclusion of individuals with cardiovascular risk factors (Dannenberg et al., 1989), overlapping

but different levels of cardiorespiratory fitness and age groups perused (Redfield et al., 2005), and/or different scaling methods in previous studies (Dewey et al., 2008).

4.4.2 Sex differences in apical mechanics with early ageing

Interestingly, we found that the differences in peak LV mechanics between young and middle-aged men compared with women were localised at the apex, with males showing a greater systolic rotation and rotational velocity. Beyond the previously discussed loss of functional myocytes in men, a potential explanation for these differences is their higher epinephrine concentrations compared with women (Zouhal et al., 2008), which coupled with a greater β-adrenergic receptor density in males (Vizgirda et al., 2002), may influence LV mechanics. Epinephrine has been shown to exert a dominant effect on the LV apex compared with the base (Lyon et al., 2008), while catecholamine administration in animal studies has been shown to induce myocardial fibrosis especially at the apex (Brouri et al., 2004). We thus speculate that men may experience — sub-clinically — a greater sub-endocardial fibrosis (Anversa and Capasso, 1991) at the apex with ageing compared with women, induced by higher circulating epinephrine concentrations (Brouri et al., 2004). If true, this could explain the higher peak apical rotation and rotational velocity that we observed in the middle-aged men compared with women due to a more dominant apical sub-epicardium (Lumens et al., 2006; van Dalen et al., 2008a; Yoneyama et al., 2012).

Sex differences in arterial stiffness with ageing could further explain the localised apical differences that we observed (Fernandes et al., 2008; Redfield et al., 2005). In the Multi-Ethnic Study of Atherosclerosis (MESA), regression analyses detected a significant relationship between arterial stiffness and circumferential strain rate at the apex but not the base (Fernandes et al., 2008). The lower peak apical circumferential strain and strain rate observed in middle-aged women in this cross-sectional study may thus reflect a greater increase in arterial stiffness with early ageing in women than men (Fernandes et al., 2008). Whilst our measures of brachial blood pressure and calculated systemic vascular resistance did not indicate sex differences with age, these are poor surrogates for central pressure and arterial stiffness (Laurent et al., 2006). Future investigations

focused on delineating age-related differences in vascular properties between men and women, and on the influence of differing levels of epinephrine on regional LV function would help further interpretation of these findings.

4.4.3 Impact of the menopause on LV structure, function and mechanics

Given that the menopause has been associated with decreases in vascular function (Moreau et al., 2012), it is important to also consider this influence within the context of ageing studies examining the heart. Counter to previous reports of early concentric remodelling in women following the menopause (Hinderliter et al., 2002; Lang et al., 2006; Schillaci et al., 1998), similar LV mass, dimensions and volumes were observed in pre- and post-menopausal women in this study. This discrepancy may be due to the inclusion of women with surgically-induced menopause in earlier work (Schillaci et al., 1998), and/or different cardiovascular risk factors and cardiorespiratory fitness levels relative to our study (Hinderliter et al., 2002). The greater relative LV mass observed in post-menopausal women may, in fact, reflect a maintenance of LV mass despite the known menopausal-related decline in FFM. A longitudinal study following middle-aged pre-menopausal women through the menopause is needed to clarify the effects of the menopause on LV mass.

Irrespective of LV structure, an earlier study by Keskin Kurt et al. (2014) has suggested that post-menopausal women may have lower LV mechanics than age-matched pre-menopausal women, as evidenced by lower longitudinal systolic strain and diastolic strain rate. In this study, we have further identified lower torsion, twisting velocity and circumferential strain rates in post-menopausal women compared with pre-menopausal women. These lower LV mechanics — albeit from a cross-sectional study — may reflect an initial reduction in myocardial function following the menopause, due to withdrawal of the positive effects of oestrogen and progesterone on apoptosis (Luczak and Leinwand, 2009), contractility (Luczak and Leinwand, 2009; Yang et al., 2012) and/or stiffness (Dubey et al., 1998). It is possible that these changes in the underpinning cardiac mechanics occur prior to differences in global measures of function, such as

cardiac output or ejection fraction (Keskin Kurt et al., 2014). Interestingly, the findings do not indicate a localised effect of the menopause on either the LV base or apex. This suggests that the menopause is unlikely to explain the age-related sex differences at the LV apex discussed earlier, and additionally, appears to contradict the effects of oestrogen specific to the LV base that have been identified through animal research (Yang et al., 2012). These findings highlight the greater need for detailed longitudinal studies across the menopause and life-course of females in order to better understand the complex biological ageing in this population.

4.4.4 Limitations, implications and future directions

A limitation of this study was that circulating concentrations of catecholamines and sex hormones were not measured. Pre- and post-menopausal women were, however, carefully recruited based on menstrual history to ensure that circulating female sex hormone concentrations were chronically lower in the post-menopausal group. Menstrual cycle criteria has been recommended as the most important criteria for staging reproductive ageing in women by the Stages of Reproductive Aging Workshop + 10 (STRAW + 10), because of the known limitations in standardisation, cost and invasiveness of biomarker assays (Harlow et al., 2012). Hence, the women in the two study groups have been appropriately categorised as pre- and post-menopausal according to current recommendations. Future work delineating the effects of cyclical variations in female sex hormone concentrations (e.g. comparing early-follicular, late-follicular and mid-luteal menstrual cycle phases) from chronically lower concentrations (e.g. after the menopause) would provide further insight into the potent effects of female sex hormones on the heart.

Additional limitations of this study are its relatively small sample size and cross-sectional design. The small sample size reflects the limited research resources, but also the primary focus on LV mechanics, which have been suggested to be more sensitive than global indicators of cardiac function (e.g. heart rate and cardiac output). To reduce the likelihood of committing a type II error due a small sample size, the level of statistical significance was set *a priori* at 0.1, and the resultant trade-off between type

I and type II errors was acknowledged. The cross sectional design limits the predictive inferences of cause and effect between ageing and the menopause on the LV, but is a relatively easy approach to generate hypotheses for future longitudinal studies. To that end, we found mechanical differences localised to the apical region of the LV, which could inform future studies investigating sex differences with ageing.

Previous studies and the literature review have discussed the difficulties in separating the effects of the menopause from those of ageing on the female heart (Merz and Cheng, 2016; Nio et al., 2015), and the present study is another example of this. Despite including only middle-aged women in the secondary analysis, naturally post-menopausal women were, on average, six years older than the pre-menopausal women. Including age as a covariate, however, did not change the study results, and accordingly confirmed a significant impact of menopause on the LV. Notwithstanding, longitudinal ageing studies from young adulthood and through the menopausal transition will likely provide further insight into female cardiovascular ageing. Of particular relevance to women's health in mid-life, future work should investigate whether lifestyle interventions (e.g. exercise training and dietary modifications) may be used to mitigate the decline in myocardial function associated with the menopause.

4.5 Conclusion

In conclusion, the findings of this cross-sectional study suggest that changes in LV structure and function from young adulthood to middle-age differ between men and women: normalised LV mass, SV and EDV were lower in middle-aged men compared with their younger counterparts, but this difference was markedly less in women. Peak systolic apical mechanics were greater in middle-aged men than middle-aged women, but not between younger men and women or at the base. During middle-age, post-menopausal women may have altered LV mechanics (lower peak torsion, twisting velocity and apical circumferential strain rates) compared with pre-menopausal women. The results of this study provide new insight into the regional cardiac changes that may occur

with healthy ageing, and set the evidence needed to shape future longitudinal studies investigating this life stage.

Chapter 5

The menopause alters aerobic adaptations to high-intensity interval training

A version of this chapter has been published: Nio AQX, Rogers S, Mynors-Wallis R, Meah VL, Black JM, Stembridge M and Stöhr EJ (2020). The menopause alters aerobic adaptations to high-intensity interval training. *Medicine and Science in Sports and Exercise*, 52(10):2096–2106.

5.1 Introduction

Menopause is a normal part of a woman's lifespan (Harlow et al., 2012), and has been associated with a decline in resting cardiovascular function (Nio et al., 2015). These menopause-related effects include a concentric remodelling of the left ventricle (LV), lower diastolic function and higher blood pressure (Düzenli et al., 2007; Hart et al., 2012; Kangro et al., 1995; Nio et al., 2017; Schillaci et al., 1998). However, there has been no evidence in the existing literature that the menopause influences cardiovascular *capacity*, such as that during maximal exercise (Egelund et al., 2017; Green et al., 2002). It is therefore probable that pre- and post-menopausal women achieve similar cardiac outputs during daily activities that depend on cardiovascular capacity, but that they do so via different underlying cardiac function. This may be underpinned by a menopause-related surge in cardiac sympathetic nerve activity (Sakata et al., 2009) interacting with a greater density of sympathetic nerve endings at the base of the LV than at the apex (Kawano et al., 2003). Such differences may in turn result in different cardiac adaptations to exercise training in pre- and post-menopausal women, but these effects remain to be elucidated.

Traditionally, assessments of cardiac function have focused on heart rate, cardiac output and Doppler-derived indices of loading. However, the regional effects of sympathetic drive on the LV (Kawano et al., 2003; Pianca et al., 2019) suggest that differences in cardiac function between pre- and post-menopausal women may potentially manifest as differences in regional LV muscle function ("LV mechanics"). In the LV, myofibre alignment varies transmurally from a right-handed helix in the endocardium to a left-handed helix in the epicardium (Sengupta et al., 2007). This complex spiral architecture gives rise to opposing rotations at the LV base and apex during systole and diastole, enabling the *in vivo* measurement of LV mechanics. In addition to the influence of sympathetic drive on cardiac function, the withdrawal of oestrogen after the menopause may also specifically affect regional LV muscle function. For example, in female rabbits, oestrogen has been shown to selectively increase the L-type calcium current and the sodium-calcium exchange current in epicardial myocytes excised from the base of the LV, but not in endocardial myocytes excised from the base, nor from the apex (Chen

et al., 2011; Yang et al., 2012). Since these calcium currents influence the plateau phase of the cardiac action potential (Chen et al., 2011), it is likely that the menopause influences both contraction and relaxation of the *basal epicardium*, but the effects *in vivo* are not known. These previously proposed effects of the menopause on regional LV muscle function may therefore manifest *in vivo* as differences in rotation at the base but not at the apex.

To determine the functional relevance of altered regional LV muscle function due to the menopause, detailed physiological tests that investigate cardiovascular function and capacity are required. In this study, lower body negative pressure (LBNP) and supine cycling were used as physiological tests to investigate the effects of exercise training on LV function and mechanics in pre- and post-menopausal women. Mild LBNP was used to simulate orthostatic stress due to gravity by reducing cardiac preload, and low to moderate intensity supine cycling was used to simulate the physical exertion associated with daily activities. Peak aerobic capacity and blood volume were assessed to demonstrate conventional adaptations to exercise training. We hypothesised that pre- and post-menopausal women would show similar increases in peak aerobic capacity after exercise training, but with differences in underlying regional LV muscle function.

5.2 Methods

5.2.1 Ethical approval

All experimental procedures were approved by the Cardiff Metropolitan University's School of Sport Research Ethics Committee and conformed to the ethical principles in the Declaration of Helsinki. Prior to the start of any experimental procedures, all participants provided written and verbal informed consent.

5.2.2 Study design

Twenty-five healthy untrained middle-aged (age 45–58 years) women completed a longitudinal study to investigate the effects of the menopause on LV adaptations to exercise training. Only non-smoking, non-diabetic (self-reported) and normotensive healthy volunteers who were not taking any cardiovascular or lipid-lowering medications were recruited. These study participants were a subset sample of the previous chapter investigating age-related differences in resting LV structure, function and mechanics in healthy men and women (Nio et al., 2017).

Only distinctly pre- or post-menopausal women were included in this study (11 pre-menopausal, 14 post-menopausal), and peri-menopausal women were excluded. In line with recommendations by the Stages of Reproductive Aging Workshop + 10 (STRAW + 10), menstrual cycle criteria rather than sex hormone concentrations was used to categorise the pre- and post-menopausal women, because of current limitations in standardisation, cost and invasiveness of biomarker assays (Harlow et al., 2012). Women were characterised as pre-menopausal if they had regular menstrual cycles ranging from 21–35 days in length without a persistent difference of more than seven days between consecutive cycles (Harlow et al., 2012), and had not used oral contraceptives in the preceding four months. Post-menopausal women were identified by at least 12 consecutive months of amenorrhoea (Harlow et al., 2012), which had not been induced by surgery (e.g. hysterectomy). None of the post-menopausal women had used hormone replacement therapy (HRT) in the preceding six months. Post-menopausal women were, on average, 6 years older than the pre-menopausal women (Table 5.1), and thus we adjusted for age in our statistical analyses (by using age as a covariate).

Participants visited the laboratory for a series of physiological tests before and after 12 weeks of high-intensity aerobic interval training (Figure 5.1). Separated by at least 24 h, these laboratory tests consisted of (i) an aerobic capacity test on an upright cycle ergometer, (ii) an aerobic capacity test on a supine cycle ergometer, (iii) the measurement of total haemoglobin mass and blood volume using the 2-min carbon monoxide (CO)-rebreathing method, and (iv) echocardiographic images for LV function

TABLE 5.1: Demographics, aerobic capacity and haematological parameters in pre- (Pre-M) and post-menopausal (Post-M) women before and after exercise training (Trg).

	Pre-M ($n = 11$)		Post-M ($n = 14$)		P		
Parameter	Before	After	Before	After	M	Trg	M × Trg
Age (years)*	49 (2)	-	55 (2)	-	**<0.01**	-	-
Height (cm)*	161.1 (6.2)	-	163.3 (3.6)	-	0.27	-	-
Body mass (kg)	63.4 (10.5)	62.4 (9.9)	61.8 (8.4)	61.4 (8.3)	0.19	**0.02**	0.51
Systolic blood pressure (mmHg)†	117 (10)	121 (11)	122 (14)	123 (16)	0.42	0.14	0.84
Diastolic blood pressure (mmHg)†	65 (5)	68 (5)	65 (9)	66 (8)	0.45	0.08	0.79
Aerobic capacity							
Upright peak power test							
W_{peak} (W)	147 (29)	179 (28) ¶	145 (26)	169 (24) ¶	0.20	**<0.01**	**0.02**
$\dot{V}O_{2peak}$ (L/min)	1.84 (0.31)	2.27 (0.31) ¶	1.80 (0.34)	2.08 (0.29)¶	0.44	**<0.01**	**<0.01**
$\dot{V}O_{2peak}$ (mL/min/kg)	29 (5)	37 (5) ¶	29 (6)	34 (5) ¶	**0.04**	**<0.01**	**0.02**
HR_{max} (beats/min)	169 (10)	171 (9)	168 (12)	166 (9)	0.36	0.78	0.31
Supine peak power test							
W_{peak} (W)	125 (32)	162 (22) ¶	126 (23)	148 (20) ¶	0.07	**<0.01**	**0.01**
$\dot{V}O_{2peak}$ (L/min)	1.77 (0.33)	2.03 (0.27) ¶	1.72 (0.35)	1.90 (0.34)¶	0.12	**<0.01**	**0.04**
$\dot{V}O_{2peak}$ (mL/min/kg)	29 (6)	33 (6) ¶	28 (6)	31 (6) ¶	**0.01**	**<0.01**	**0.05**
HR_{max} (beats/min)	160 (17)	160 (11)	155 (13)	154 (13)	0.25	0.94	0.35
Haematological parameters							
tHb mass (g)	535 (108)	541 (105)	526 (56)	534 (61)	0.61	0.07	**0.02**
Blood volume (mL)	4401 (858)	4601 (846) ¶	4294 (445)	4367 (390)	0.97	**<0.01**	**<0.01**

Values are in mean (SD). n: sample size. W_{peak}: Peak power output. $\dot{V}O_{2peak}$: Peak oxygen uptake. HR_{max}: Maximum heart rate. tHb mass: total haemoglobin mass. *Student's t-tests to compare age and height in pre- and post-menopausal women before exercise training. †Blood pressures correspond to data at rest with participants lying supine at a 30° left lateral tilt with their lower body in a lower body negative pressure box at room pressure (i.e. 0 mmHg). ¶$P < 0.05$ compared with values before training. Statistical effects with $P < 0.05$ are highlighted in **bold**.

and mechanics at rest, during −15 and −30 mmHg LBNP, and during 20, 40 and 60% peak supine cycling.

5.2.3 Exercise training intervention

High-intensity aerobic intervals on an upright cycle ergometer (Monark 824E, Varberg, Sweden) were used in this study, to optimise the likelihood of cardiorespiratory adaptations to exercise training (Slørdahl et al., 2004; Wisløff et al., 2009). The exercise training intervention was supervised by a schedule of trained exercise researchers. Each exercise session consisted of a 10-min warm-up, 4×4-min intervals at 90–95% maximum heart rate (HR_{max}; RS400, Polar Electro, Kempele, Finland) separated by 3-min active

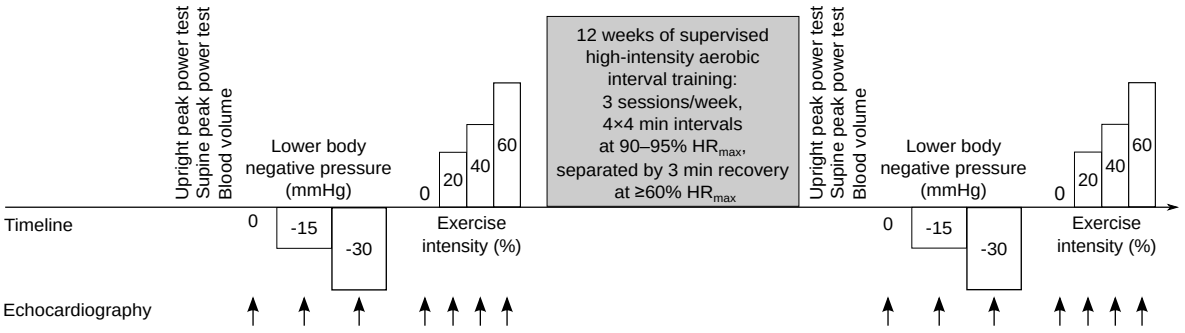


FIGURE 5.1: Schematic representation of the experimental timeline (repeat of Figure 3.1 from the General Methods for easy reference). A series of physiological tests were conducted on four separate days before and after 12 weeks of exercise training. Day 1: Peak power test on an upright cycle ergometer. Day 2: Peak power test on a supine cycle ergometer. Day 3: Blood volume assessment. Day 4: Echocardiography for left ventricular function and mechanics during lower body negative pressure and submaximal supine cycling. HR_{max}: maximum heart rate.

recovery at >60% HR_{max}, and a 5-min cool-down (total duration 40 min) (Wisløff et al., 2009). The researcher on-site encouraged participants to reach 90% HR_{max} within the first 2 min of each 4-min interval. There were 1–6 participants in each exercise session. Three exercise sessions per week were strongly recommended, over a consecutive period of 12 weeks. All participants undertook at least 70% of the total number of sessions, equivalent to at least 8 weeks of exercise training to improve aerobic fitness (Kessler et al., 2012) (time ≥90% HR_{max} per session: Pre-M 9.2 ± 1.7 min vs. Post-M 8.3 ± 1.5 min, *t*-test $P = 0.14$). The exercise training intervention was generally well-tolerated with no adverse events.

5.2.4 Aerobic capacity tests

To ensure that participants were euhydrated and well-rested for all of the physiological tests, they were asked to abstain from caffeine, alcohol and strenuous exercise for 24 h, and to drink 500 mL of water 90 min before arrival at the laboratory. Participants' height and body mass (Model 770, Seca, Hamburg, Germany) were measured (Table 5.1). Participants completed continuous ramp tests to volitional exhaustion on upright (Corival, Lode, Groningen, The Netherlands) and supine cycle ergometers

(Angio 2003, Lode, Groningen, The Netherlands) on separate days to determine peak aerobic capacity ($\dot{V}O_{2peak}$) and peak power output (W_{peak}).

The aerobic capacity test on the upright cycle ergometer was individualised using age, height and body mass (Wasserman et al., 2005), with the test workload programmed to increase from 0 W to predicted W_{peak} in 10 min. Respiratory gas exchange (Oxycon Pro, Viasys Healthcare, Basingstoke, UK) and heart rate were monitored and recorded throughout the test. Following a self-selected recovery period, participants were familiarised with the supine cycle ergometer. On a separate day, participants completed another aerobic capacity test, but on the supine cycle ergometer. The test workload on the supine cycle ergometer was programmed to increase from 0 W to 80% of each individual's measured upright W_{peak} in 10 min.

After 12 weeks of exercise training, participants' $\dot{V}O_{2peak}$ were reassessed on both upright and supine cycle ergometers. The increments in workload during the aerobic capacity tests were increased so that participants would still achieve their W_{peak} in approximately 10 min, based on an expected 18% improvement in $\dot{V}O_{2peak}$ after exercise training (Slørdahl et al., 2004).

5.2.5 Total haemoglobin mass and blood volume

After 15 min of seated rest, total haemoglobin mass and blood volume were measured using the optimised 2-min CO-rebreathing technique (SpiCO®, Blood tec GbR, Bayreuth, Germany). Participants were familiarised with the protocol and equipment before starting the procedure. A technical background on the 2-min CO-rebreathing technique can be found in the Methods Chapter (Section 3.4.3).

5.2.6 Measures of cardiovascular function

Blood pressure (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands) and echocardiographic images were recorded at 0, −15 and −30 mmHg LBNP, and at 0, 20, 40 and 60% peak supine cycling, with 30 min of rest between the end

of LBNP and the start of supine cycling. These tests were conducted at least 24 h after any of the aerobic capacity tests or an interval training session. Participants lay supine at a 30° left lateral tilt for all measurements. Echocardiographic images were acquired in accordance with guidelines at the start of data collection for this study (January 2013), at end-expiration and by the same trained sonographer (i.e. the doctoral candidate; Lang et al., 2006; Nagueh et al., 2009). A phased array transducer (4V 1.7–3.3 MHz) was used on a commercially-available ultrasound system (Vivid E9, GE Vingmed Ultrasound AS, Horten, Norway), and images were analysed offline for LV function and mechanics (EchoPAC, Version 112, GE Healthcare, Horten, Norway). Transducer positions during resting measurements were temporarily marked on the participant's chest to assist the rapid relocation of similar acoustic windows during LBNP and supine cycling, during which images were further optimised and confirmed with anatomic landmarks. Three consecutive cardiac cycles were analysed for each variable and the mean was used for statistical analyses.

Left ventricular structure and function. End-diastolic and end-systolic volumes (EDV and ESV, respectively) were determined from triplane images of the same heartbeats. Heart rate was determined from the ECG inherent to the ultrasound. Stroke volume (SV = EDV − ESV), ejection fraction $\left(\frac{\text{SV}}{\text{EDV}} \times 100\%\right)$, cardiac output (HR × SV) and systemic vascular resistance (mean arterial pressure/cardiac output) were then calculated.

Left ventricular mechanics. Rotation and rotational velocity were assessed using 2D speckle tracking of the myocardium in the parasternal short-axis images at the LV base and apex, in line with previous methodology (Stöhr et al., 2015). To account for differences in heart rate between and within participants, raw data were smoothed with cubic spline interpolation to generate 1200 data points, with 600 points each for systole and diastole (2D Strain Analysis Tool 1.0β14, Stuttgart, Germany) (Stöhr et al., 2015). Twist and twisting velocity curves were calculated by subtracting time-aligned basal data from apical data, and peak values in systole and early diastole were extracted from interpolated curves. Due to poor image quality in some participants, data on LV

mechanics during LBNP are reported for 9 pre-menopausal and 10 post-menopausal women, and data during supine cycling for 8 pre-menopausal and 10 post-menopausal women.

5.2.7 Physiological tests

Lower body negative pressure. Mild LBNP was used to simulate the reduced cardiac filling typical of the upright posture due to gravity (Levine et al., 1991a). Participants were positioned with a neoprene kayak skirt on their iliac crest, and with their lower body in an LBNP box (built in-house; length 126 cm, width 55 cm, height 90 cm). Two consecutive 10-min stages at −15 and −30 mmHg LBNP were applied. A variable transformer (CMV 5E-1, Carroll & Meynell Transformers Ltd, Stockton-On-Tees, UK) connected to a vacuum pump (Henry HVR200A, Numatic International Ltd, Chard, England) was used to achieve the desired negative pressure within the box, which was monitored continuously using a differential pressure meter (Testo AG, Lenzkirch, Germany). Blood pressure and echocardiographic images were recorded at rest and after 5-min exposure to each stage of LBNP (Levine et al., 1991a).

Supine cycling. Upon completion of LBNP, participants relaxed for 30 min to ensure a return to a resting physiological state (Levine et al., 1991a). Participants then completed three consecutive 5-min stages of supine cycling at 20, 40 and 60% supine W_{peak}. Supine cycling was used to simulate the typical physical exertion from performing activities of daily living. Blood pressure and echocardiographic images were recorded at rest with the participant lying on the supine cycle ergometer at a 30° left lateral tilt, and during the final 3 min at each exercise intensity.

5.2.8 Statistical analysis

Statistical analyses were performed with R (R Core Team, 2015). The two-way repeated-measures analysis of variance (ANOVA) with age as a covariate was used to examine the effects of exercise training on aerobic capacity, total haemoglobin mass and blood

volume in post-menopausal women compared with pre-menopausal women. For variables with a significant menopause × training interaction effect, *post hoc* Student's *t*-tests were used to identify differences between groups.

The three-way repeated-measures ANOVA with age as a covariate was used to examine the impact of the menopause, exercise training and the physiological tests on LV function and mechanics. Figure 5.2 shows the flowchart for interpreting the three-way ANOVA, with a focus on the effects of the menopause as the key research question. This approach integrated all data within one statistical test and avoided the reuse of data in multiple disparate ANOVAs. For variables with a statistically significant three-way interaction effect, individual differences with exercise training were calculated *post hoc* and Student's *t*-tests were used to identify differences between pre- and post-menopausal women at each LBNP and exercise stage. For variables with statistically significant two-way interaction effects from the three-way ANOVA, data were grouped *post hoc* across the non-significant factor to reduce complexity, and to enable interpretation of the two-way interaction effects. The Holm-Bonferroni correction was used to adjust for multiple comparisons across LBNP and supine cycling stages.

To examine whether the effects of the menopause on LV function and mechanics following exercise training could be detected at rest (i.e. without requiring the physiological tests), *post hoc* two-way ANOVAs were used to compare resting data if any of the menopause or training effects in the three-way ANOVA were statistically significant. Alpha was set at 0.05. Data are presented as mean and standard deviation (SD) unless stated otherwise.

5.3 Results

5.3.1 Menopause-related effects on peak aerobic capacity and LV function under resting conditions

Exercise training elicited smaller increases in peak aerobic capacity and blood volume in post-menopausal women than pre-menopausal women ($P < 0.05$; Table 5.1). However,

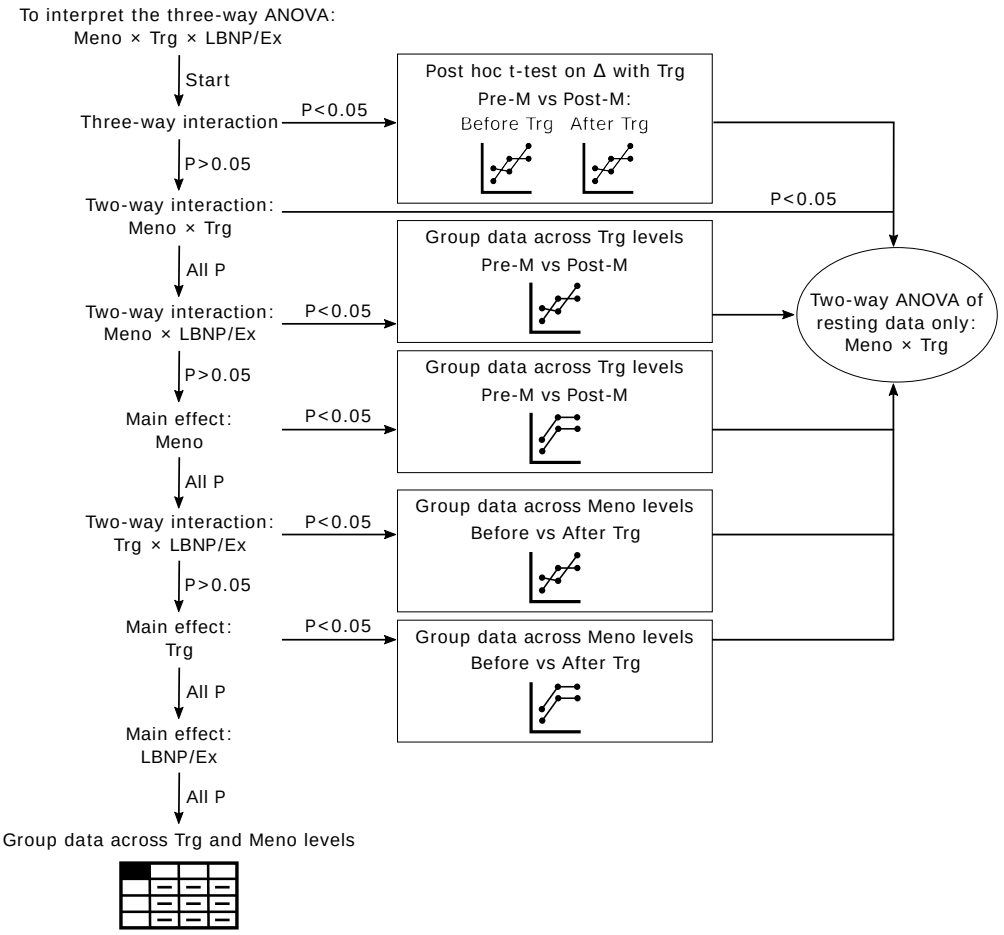


FIGURE 5.2: Flowchart to interpret the three-way ANOVA. The interaction and main effects of the three-way ANOVA were addressed based on their importance to our research question, which was to investigate the impact of the menopause (M/Meno) on left ventricular function and mechanics. Therefore, all of the three-way ANOVA outputs that included the menopause were addressed first. Data were grouped across non-significant factors (i.e. if $P > 0.05$) to reduce complexity and aid interpretation. Graphs were used to visualise the data and to identify the source of statistically significant differences. Trg: exercise training. LBNP: lower body negative pressure. Ex: supine cycling.

there was no evidence of differences in LV function between pre- and post-menopausal women at rest before and after exercise training ($P > 0.05$).

5.3.2 Menopause-related effects on LV function during lower body negative pressure

In pre- and post-menopausal women, cardiac output, end-diastolic volume and stroke volume decreased in response to LBNP, concomitant with an increase in heart rate and systemic vascular resistance ($P < 0.001$; Figure 5.3). There was no evidence of differences in general haemodynamics and LV volumes during LBNP between pre- and post-menopausal women ($P > 0.05$). However, exercise training elicited a significant difference in peak diastolic basal rotational velocity during LBNP between pre- and post-menopausal women ($P = 0.04$) — specifically, peak diastolic basal rotational velocity was maintained at resting values during LBNP after exercise training in pre-menopausal women, but decreased during LBNP in post-menopausal women (Figure 5.4). These distinct responses in pre- and post-menopausal women were not apparent before exercise training. There was no evidence of differences in apical mechanics between pre- and post-menopausal women during LBNP ($P > 0.05$), nor of any other changes in LV mechanics in response to LBNP ($P > 0.05$; Table 5.2).

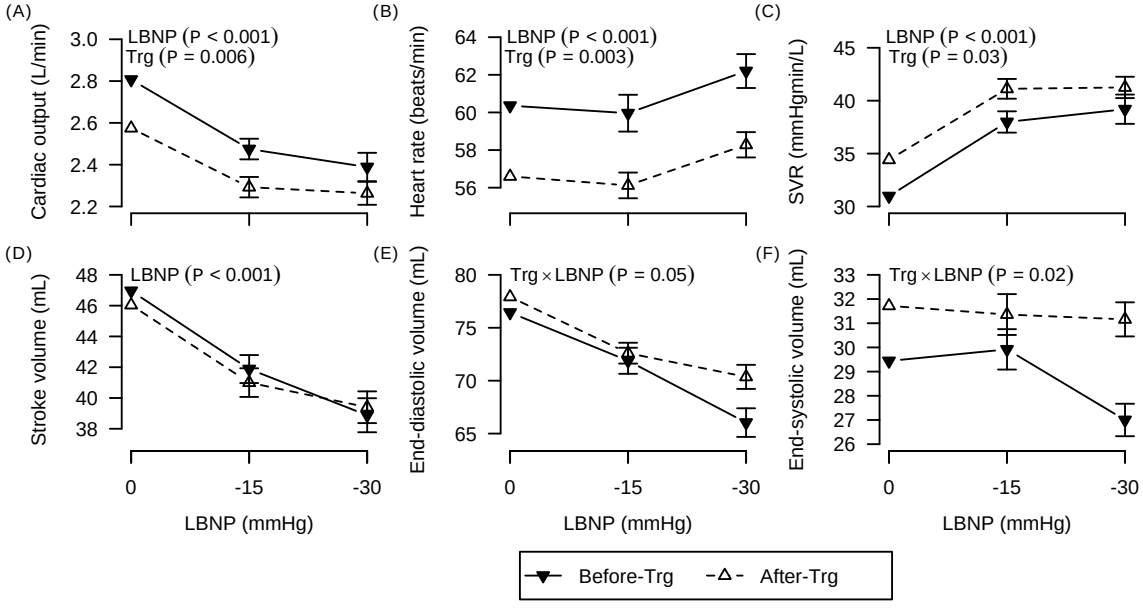


FIGURE 5.3: Left ventricular function and systemic vascular resistance (SVR) in pre- and post-menopausal women in response to lower body negative pressure (LBNP) before and after exercise training (Trg). Data in pre- and post-menopausal women were not statistically different (menopause effects $P > 0.05$) and were grouped for clarity. Values are mean ± standard error of the change from rest.

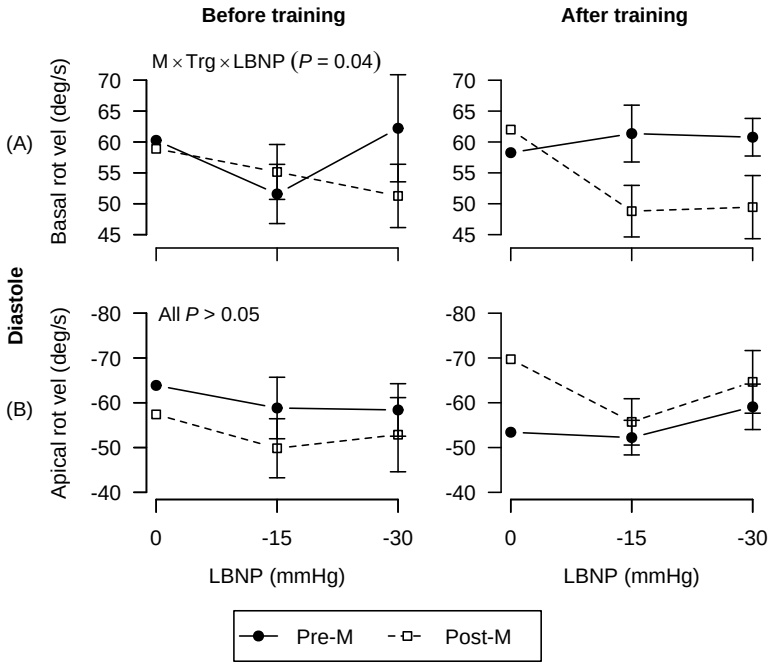


FIGURE 5.4: Peak diastolic basal and apical rotational velocities (rot vel) in response to lower body negative pressure (LBNP) in pre- and post-menopausal (M) women before and after exercise training (Trg). Values are mean ± standard error of the change from rest.

TABLE 5.2: Peak left ventricular (LV) mechanics during lower body negative pressure (LBNP) and supine cycling.

	LBNP (mmHg)			Exercise intensity (%)			
LV mechanics	0	-15	-30	0	20	40	60
Systolic peaks							
Twist (deg)	17.9 (4.5)	16.6 (4.5)	17.0 (4.0)	16.0 (4.7)	20.2 (4.8) †	23.3 (6.2) †‡	24.9 (6.7) †‡
Twisting velocity (deg/s)	99 (20)	98 (20)	104 (19)	88 (16)	112 (26) †	143 (37) †‡	185 (44) †‡§
Basal mechanics							
Rotation (deg)	-6.2 (2.7)	-5.6 (3.3)	-5.5 (2.7)	-5.6 (2.4)	-6.2 (3.8)	-7.6 (4.2) †	-9.1 (4.4) †‡
Rotational velocity (deg/s)	-54 (17)	-51 (14)	-53 (16)	-46 (11)	-62 (17) †	-84 (33) †‡	-118 (38) †‡§
Apical mechanics							
Rotation (deg)	12.4 (4.5)	11.8 (4.8)	12.5 (4.5)	11.0 (3.9)	14.7 (5.0) †	16.6 (5.3) †‡	17.0 (5.4) †‡
Rotational velocity (deg/s)	60 (20)	57 (22)	63 (17)	49 (13)	79 (26) †	105 (37) †‡	127 (37) †‡§
Diastolic peaks							
Untwisting velocity (deg/s)	-102 (33)	-91 (26)	-88 (22)	-93 (28)	-143 (34) †	-181 (54) †‡	-223 (65) †‡§
Basal mechanics							
Rotational velocity (deg/s)	60 (19)	54 (18)	56 (19)	55 (16)	67 (23) †	86 (23) †‡	103 (36) †‡§
Apical mechanics							
Rotational velocity (deg/s)	-61 (26)	-54 (21)	-59 (23)	-55 (23)	-92 (31) †	-116 (42) †‡	-145 (50) †‡§

Values are in mean (standard deviation). Data in pre- and post-menopausal women before and after exercise training were grouped together to show the main effects of LBNP (effective $n = 38$) and supine cycling (effective $n = 36$). †$P < 0.05$ compared with 0% exercise intensity. ‡$P < 0.05$ compared with 20% exercise intensity. §$P < 0.5$ compared with 40% exercise intensity.

5.3.3 Menopause-related effects on LV function during supine cycling

Heart rate, cardiac output and stroke volume increased during supine cycling in both pre- and post-menopausal women, along with a decrease in systemic vascular resistance and end-systolic volume ($P < 0.001$; Figure 5.5). All indices of peak LV mechanics increased in response to incremental exercise ($P < 0.001$; Table 5.2). Similar to the effects of LBNP, there was no evidence of differences in general haemodynamics and LV volumes between pre- and post-menopausal women during supine cycling ($P > 0.05$). However, and in line with the differences in regional LV muscle function during LBNP, exercise training elicited a significant difference in peak systolic basal rotation between pre- and post-menopausal women during supine cycling ($P = 0.02$; Figure 5.6). Although peak basal rotation increased during supine cycling across all groups and conditions, a plateau became apparent at 40% peak exercise after exercise training in pre-menopausal women, but not in post-menopausal women. There was no evidence of differences in apical mechanics between pre- and post-menopausal women during supine cycling ($P > 0.05$).

5.3.4 Impact of exercise training on LV function during supine cycling

In line with a greater peak workload after exercise training, the increase in cardiac output and heart rate from rest to 60% peak supine cycling was greater after exercise training in pre- and post-menopausal women, concomitant with a greater decrease in systemic vascular resistance ($P < 0.05$; Figure 5.5). End-systolic volume during supine cycling was lower after exercise training across all exercise intensities in both groups ($P = 0.04$), but end-diastolic volume was lower only at 40% peak supine cycling ($P = 0.04$; Figure 5.5). There was no evidence that exercise training influenced the stroke volume response to supine cycling in either pre- or post-menopausal women ($P > 0.05$). In addition to the plateau in peak basal rotation at 40% peak supine cycling observed in pre-menopausal women after exercise training, peak diastolic apical

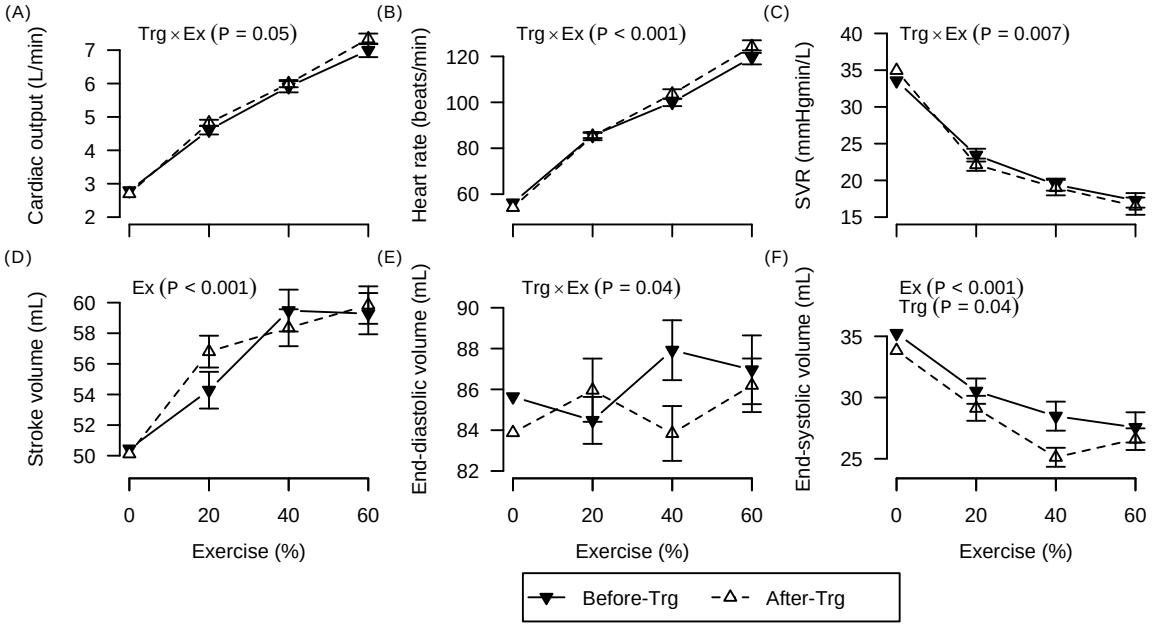


FIGURE 5.5: Left ventricular function and systemic vascular resistance (SVR) in pre- and post-menopausal women in response to supine cycling (Ex) before and after exercise training (Trg). Data from pre- and post-menopausal women were not statistically different (menopause effects $P > 0.05$) and have been grouped for clarity. Values are mean ± standard error of the change from rest.

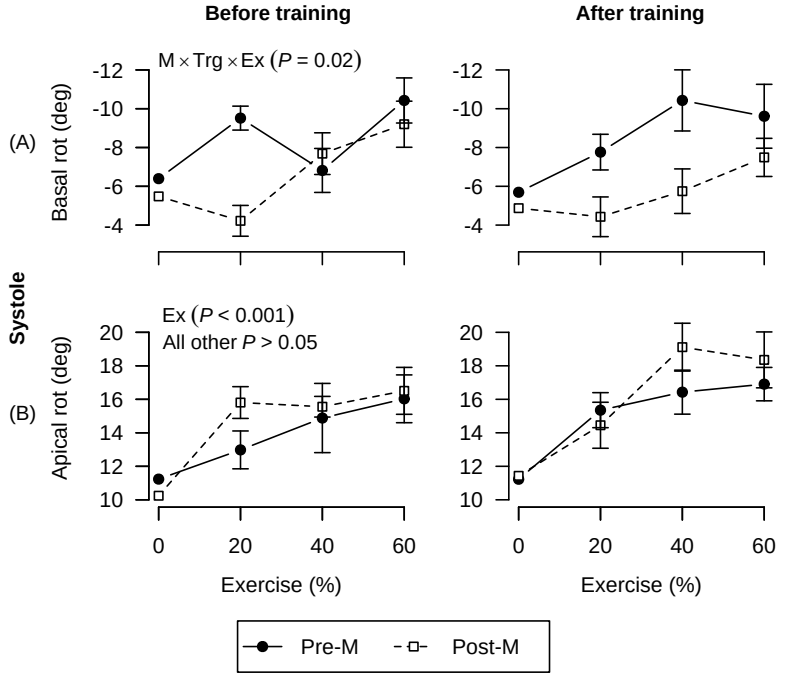


FIGURE 5.6: Peak systolic basal and apical rotation (rot) in response to supine cycling (Ex) in pre- and post-menopausal (M) women before and after exercise training (Trg). Values are mean ± standard error of the change from rest.

rotational velocity at 60% peak supine cycling was greater after exercise training in both pre- and post-menopausal women ($P = 0.007$), while peak systolic twisting velocity was greater at 40% peak supine cycling ($P < 0.05$; Figure 5.7).

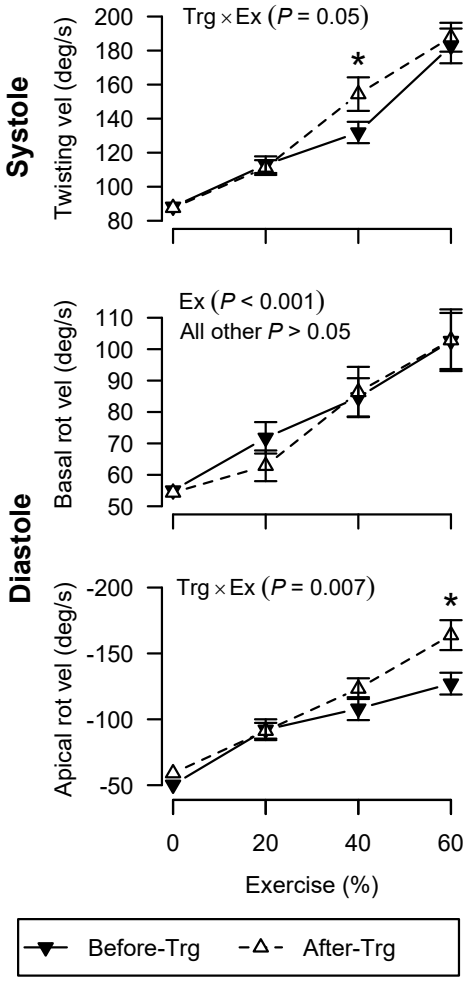


FIGURE 5.7: (A) Peak twisting velocity (vel), and peak diastolic (B) basal and (C) apical rotational velocity (rot vel) in response to supine cycling (Ex) before and after exercise training (Trg). Data from pre- and post-menopausal women were not statistically different (menopause effects $P > 0.05$) and have been grouped for clarity. Values are mean ± standard error of the change from rest. $^*P < 0.05$ following exercise training.

5.4 Discussion

This is the first study to determine the effects of the menopause on regional LV muscle function underpinning the increase in cardiovascular capacity after 12-weeks of exercise training. In this study, high-intensity aerobic interval training elicited a smaller increase in peak aerobic capacity and blood volume in post-menopausal than pre-menopausal women. In addition, physiological testing revealed that post-menopausal women had lower basal mechanics during LBNP and supine cycling after exercise training, compared with pre-menopausal women. This is the first study to suggest that the menopause may reduce aerobic adaptability to exercise training. Furthermore, the findings of this study suggest that the limitation to aerobic adaptability in post-menopausal women is likely due to peripheral (arterial, skeletal muscle and/or blood volume distribution) rather than central (cardiac) factors, as there was no evidence of differences in cardiac output between pre- and post-menopausal women. Nonetheless, cardiac output during physiological testing was underpinned by differences in regional LV muscle function between pre- and post-menopausal women, as hypothesised, confirming for the first time *in vivo* the previously reported regional LV differences from *in vitro* studies.

5.4.1 Post-menopausal women may have lower aerobic adaptability to high-intensity aerobic interval training

In line with previous studies (Egelund et al., 2017; Green et al., 2002; Murias et al., 2010a), 12 weeks of exercise training evoked an increase in peak aerobic capacity in pre- and post-menopausal women in this study. However, post-menopausal women had a smaller increase in peak aerobic capacity than pre-menopausal women, concomitant with a smaller increase in blood volume. This finding refutes the *a priori* hypothesis of similar increases in peak aerobic capacity in pre- and post-menopausal women after exercise training and contradicts previous results from other research groups. For example, the multi-centre HERITAGE Family Study found no evidence that the increase in peak aerobic capacity after exercise training differed between pre- and post-menopausal women, after using statistical methods to adjust for a mean age difference

of >20 years (Green et al., 2002). It is possible that the smaller age difference in the present study (6 years) influenced the response to exercise training. More recently, the Copenhagen Women Study found that cardiorespiratory fitness increased similarly between pre- and post-menopausal women after exercise training (mean age difference of 4 years; Egelund et al., 2017). Interestingly, the mean percentage increase in maximal/peak oxygen uptake (in L/min) across pre- and post-menopausal groups was higher in this study (16–23%) than in the Copenhagen Women Study (9–10%). This may reflect a more intense exercise training intervention in this study compared with the Copenhagen Women Study (which used a spinning exercise training intervention with gradually increasing intensities across the weeks). In addition, high-intensity aerobic interval training has been suggested to elicit greater improvements in maximal aerobic capacity and LV function compared with traditional moderate continuous exercise training (Wisløff et al., 2009), which may have contributed to the differences observed between pre- and post-menopausal women in this study. Although a comparison between high-intensity interval training and moderate continuous training was beyond the scope of this study, future work may want to focus on a direct comparison to assess differences in cardiovascular outcomes.

Considering the results of this study in the context of previous work, it is possible that post-menopausal women are able to match the improvement in cardiorespiratory fitness in pre-menopausal women up to 10–16%, but that there may be a ceiling effect with further improvements limited by the menopause. Exercise training studies of a longer duration, such as those conducted by Howden and colleagues (Howden et al., 2018), will be required to determine the presence of such a ceiling effect.

Despite a smaller increase in peak aerobic capacity and blood volume in post-menopausal women after exercise training, there was no evidence that cardiac output, heart rate or LV volumes were different between pre- and post-menopausal women, whether at rest or during the physiological tests. Thus, the greater blood volume (with a similar total haemoglobin mass) in pre-menopausal women after exercise training may have instead improved thermoregulation during exercise, via increased body fluid for sweating and heat dissipation (Convertino, 2007). Whilst body temperature or sweat responses were

not assessed, the results suggest that the contribution of cardiac output (central) adaptations to exercise training are similar in pre- and post-menopausal women, and that peripheral adaptations, such as altered blood volume distribution, arterial function or skeletal muscle capillarisation, may limit the improvement in cardiorespiratory fitness in post-menopausal women. This observation is in direct agreement with previous studies showing that older women are more dependent on a widened arterial-venous oxygen difference (indicative of a peripheral mechanism) to improve cardiorespiratory fitness, compared with younger women (Murias et al., 2010a). Collectively, the current data indicate that the menopause does not limit the cardiac output adaptation to high-intensity interval training despite different regional LV muscle function. Additionally, future studies should investigate the role of the menopause in peripheral adaptations to exercise training.

5.4.2 The menopause alters regional LV muscle function

In support of the *a priori* hypothesis that the menopause affects regional LV muscle function, this study found evidence of differences in LV rotation at the base between pre- and post-menopausal women during physiological testing. There was no evidence of differences in apical mechanics between the two study groups, despite greater apical changes typically occurring in response to both ageing (Nio et al., 2017; van Dalen et al., 2008a) and cardiovascular challenges (Stöhr et al., 2011; Williams et al., 2016), supporting a dominant effect of the menopause on regional LV muscle function as previously suggested *in vitro* (Chen et al., 2011; Yang et al., 2012). After exercise training, pre-menopausal women had greater basal mechanics during the physiological tests compared with post-menopausal women, which agrees with previous findings that young adult pre-menopausal women are more dependent on cardiac compensatory mechanisms to achieve filling and generate stroke volume compared with men (Williams et al., 2016) and older women (Murias et al., 2010a).

Drawing upon mechanistic studies conducted using animals and *in vitro* approaches, the regional effects of the menopause on basal mechanics were first hypothesised and are now confirmed in this study in humans. It is likely that higher calcium currents due

to oestrogen, which were previously observed in basal but not apical cardiomyocytes *in vitro* (Chen et al., 2011; Yang et al., 2012), underpinned the *in vivo* contraction and relaxation patterns in post-menopausal women in this study. In addition, the menopause-related surge in cardiac sympathetic nerve activity (Sakata et al., 2009) interacting with a greater density of sympathetic nerve endings at the basal epicardium than the apical endocardium (Kawano et al., 2003; Pianca et al., 2019) may have also contributed to the regional differences observed. The interaction between calcium handling and sympathetic activity on cardiomyocytes may explain why differences in basal mechanics were detected during physiological testing but not at rest. These results additionally suggest that any differences at rest are likely to be smaller than the differences during physiological testing. Although not linked with altered cardiac output in the present study, altered regional myocardial function may be linked with early afterdepolarisations originating at the base of the LV, as previously postulated (Sims et al., 2008), which may indicate a menopause-related effect on cardiac repolarization and susceptibility to arrhythmias (Yang and Clancy, 2010). Taken together, the results of this study begin to build a link between the effects of oestrogen and sympathetic activity observed in animal or *in vitro* studies and *in vivo* function. Future studies examining regional myocardial fibrosis or electrical activation patterns may further discern the true implications of the menopause.

5.4.3 Regulation of cardiac output during exercise in middle-aged women

In line with greater absolute workloads after exercise training, the increase in cardiac output from rest to 60% peak supine cycling was greater in both pre- and post-menopausal women, with no evidence of differences between groups. This response is likely not confined to submaximal exercise efforts, and may be extrapolated to a greater cardiac output at maximal exercise intensities after exercise training. A greater cardiac output is typically achieved via a greater stroke volume (Murias et al., 2010a), but interestingly, in this study it was explained by higher heart rates. Maximum heart rates, however, did not increase after exercise training in this study, and are in fact unlikely

to increase with exercise training based on the existing literature (Murias et al., 2010a). Further work is thus required to clarify the cardiac output and stroke volume response from 60–100% peak aerobic exercise in middle-aged women, which may additionally provide new insight into the regulation of cardiac output in this under-represented cohort (Vella and Robergs, 2005).

5.4.4 Regulation of cardiac output during orthostatic stress in middle-aged women

In line with previous work (Edgell et al., 2012; Williams et al., 2016), cardiac output and stroke volume decreased during LBNP in both pre- and post-menopausal women, with no evidence of differences between groups. Whilst the stroke volume response to LBNP did not differ before and after exercise training, an improved filling was evident at −30 mmHg after exercise training, as evidenced by greater end-diastolic and end-systolic volumes in both groups. Apart from basal mechanics, there was no evidence of other changes in LV mechanics in response to LBNP. In contrast, previous studies have shown an increase in peak untwisting velocity with LBNP in men and women, and a decrease in male athletes with more than 5 years of training (Esch et al., 2010; Williams et al., 2016). However, this is the first study examining LV mechanics in middle-aged women in response to LBNP. Therefore, the discrepancy between these results and previous studies may indicate that middle-aged women have different LV mechanics in response to LBNP compared with younger women, female athletes and men. In addition, the strict coupling between LV mechanical function and filling has been questioned recently, and it may be that other factors such as altered atrial function or complex geometric changes may influence preload (Samuel and Stöhr, 2017).

In this study, there was no evidence of a greater increase in heart rate in pre-menopausal compared with post-menopausal women in response to orthostatic stress, which had been described previously (Edgell et al., 2012; Harvey et al., 2005). One key difference between this study and previous studies is a smaller mean age difference of 6 years between pre- and post-menopausal women, compared with ≥26 years in previous studies (Edgell et al., 2012; Harvey et al., 2005). As age has also been shown to reduce

heart rate responsiveness to orthostatic stress (Frey and Hoffler, 1988), previous findings may be due to age more than the menopause, a hypothesis that warrants future investigation.

5.4.5 Limitations

As the same researcher (the doctoral candidate) was involved in all of the data collection procedures and in supervising the training intervention, there may be an element of outcome assessor bias in this study. Similarly, the sonographer (the doctoral candidate) was not blinded to the menopausal and training statuses of participants while analysing their images. Notwithstanding, the key parameters of regional LV muscle function in this study were derived from a speckle tracking algorithm embedded in GE software and were largely operator-independent. It is therefore unlikely that blinding would have altered the current results.

Although this study had a smaller age difference between pre- and post-menopausal women than some previous work (Edgell et al., 2012; Harvey et al., 2005), it was not possible for us to totally eliminate it. This reflects the inherent difficulty of disentangling the effects of a naturally-occurring menopause from those of chronological ageing in the female lifespan (Nio et al., 2015). To further improve confidence in the study conclusions related to the menopause, we included age as a covariate in our statistical analyses (Green et al., 2002).

Another possible limitation of this study is the lack of measurement of sex hormones in grouping women as pre- or post-menopausal, as discussed previously in Chapter 4 (Section 4.4.4). Briefly, we defined the two groups using menstrual cycle criteria instead, because of current limitations in standardisation, cost and invasiveness of biomarker assays (Harlow et al., 2012). In addition, we did not control for menstrual cycle phase in the pre-menopausal women for the physiological tests in this study, as previous work has not found conclusive evidence that the menstrual cycle affects maximum aerobic capacity, cardiac output during orthostasis (Fu et al., 2009), or plasma volume shifts during exercise (for reviews see Janse de Jonge, 2003; Oosthuyse and Bosch, 2010).

Moreover, not controlling for menstrual cycle phase allowed for a more precise matching of total exercise training volume (i.e. 12 weeks of exercise training) between pre- and post-menopausal groups. Whilst it is possible that the menstrual cycle could have mildly increased the variability of responses in pre-menopausal women, it is likely that the effects of the menstrual cycle are smaller than the effects elicited by exercise training and the physiological tests used in this study.

5.4.6 Implications and future directions

The main practical implication of this study is the smaller increase in peak aerobic capacity observed in middle-aged post-menopausal women after 12 weeks of high-intensity aerobic interval training, compared with middle-aged pre-menopausal women. As peak aerobic capacity is an important prognostic biomarker for cardiovascular disease (Kessler et al., 2012; Wisløff et al., 2009), these findings indicate that the menopause may reduce a middle-aged woman's ability to modify her risk of cardiovascular disease with exercise, or more specifically, high-intensity aerobic interval training. Building upon this work, a replication study (McLoughlin and Drummond, 2017) is strongly recommended to verify the effects of high-intensity aerobic interval training on cardiorespiratory adaptations in middle-aged pre- and post-menopausal women. A better understanding of adaptations to exercise training in middle-aged women would improve public recommendations for lifestyle interventions to improve cardiorespiratory fitness, which has implications for improving health outcomes globally in the ageing population.

Beyond the influence of traditional risk factors such as blood pressure and cholesterol on cardiovascular function (Kannel et al., 1976), the results of this study begin to delineate the early cardiac changes that occur with the menopause. The menopause has itself been identified as a risk factor for cardiovascular disease, first through the seminal Framingham Study (Kannel et al., 1976), but the underlying pathophysiology is unclear. Future work examining vascular responsiveness, LV pressures and myocardial properties (Howden et al., 2018; Xi et al., 2014) in pre- and post-menopausal women will likely provide further insight into the effects of the menopause on the heart. In

particular, alterations in regional electrical conduction and the consequences on arrhythmias are warranted given the altered regional LV muscle function observed *in vivo* in this study.

5.4.7 Conclusion

In conclusion, post-menopausal women had a smaller increase in peak aerobic capacity after 12 weeks of high-intensity aerobic interval training, compared with pre-menopausal women. Cardiac output and LV volumes during LBNP and supine cycling were not different between pre- and post-menopausal women, but were underpinned by differences in regional LV muscle function. These findings provide new insight into the effects of the menopause on aerobic fitness, cardiac adaptability and regional LV muscle function.

Chapter 6

Does exercise training mitigate the effects of the menopause on LV mechanics in post-menopausal women?

A version of this chapter has been presented at a conference: Nio AQX, Stöhr EJ, Rogers S, Mynors-Wallis R, Meah VL, Black JM, Stembridge M and Shave RE (2018). Exercise training may attenuate the cardiac changes associated with the menopause. American College of Sports Medicine, 2018 Annual Meeting, Minneapolis, MN, USA, May–June.

6.1 Introduction

The menopause refers to the cessation of menstruation and the end of the reproductive phase in the female human lifespan (Harlow et al., 2012). In addition to its permanent effects on fertility, the menopause is generally accepted to have an adverse effect on the female heart (Luczak and Leinwand, 2009; Nio et al., 2015; Salerni et al., 2015). The effects of the menopause on the heart include lower systolic and diastolic function, and lower left ventricular (LV) mechanics (Keskin Kurt et al., 2014; Nio et al., 2017; Schillaci et al., 1998). It is also recognised as a risk factor for cardiovascular disease, independent of other traditional risk factors such as hypertension, diabetes and lipids (Kannel et al., 1976; Maas et al., 2011). Using classical statistical testing, most studies (including the first two research studies in this thesis) have focused on identifying differences between pre- and post-menopausal women, rather than on quantifying the extent of the similarities between the two groups. The latter approach can be achieved using Bayesian statistical testing (Wagenmakers et al., 2018), and in contrast to traditional studies, would enable the evaluation of interventions seeking to reverse the effects of the menopause in post-menopausal women, toward pre-menopausal values.

Recommendations for improving or maintaining cardiovascular health with ageing include exercising regularly, eating a healthy diet and not smoking (Lichtenstein et al., 2006; Stampfer et al., 2000). On exercising regularly, the Nurses' Health Study found a 41% higher risk of coronary events in women who exercised for less than 1 h/wk compared with women who exercised for more than 5.5 h/wk (Stampfer et al., 2000). In addition, a scientific statement from the American Heart Association provides a review of the association between cardiorespiratory fitness and health outcomes (Ross et al., 2016), and concluded that small increases in cardiorespiratory fitness (e.g. 1–2 METs; metabolic equivalent: a multiple of the resting metabolic rate approximating 3.5 mL/min/kg) are associated with considerably (10–30%) lower adverse cardiovascular event rates. However, the interaction between the effects of physical activity and the effects of menopause on the heart has not been established.

Building upon the research in Chapter 5 that found differences in mechanics at the base of the LV between pre- and post-menopausal women during physiological tests after

exercise training, but not at rest — the aim of the final research study in this thesis was to investigate whether exercise training causes a shift in resting regional LV mechanics in post-menopausal women towards typical pre-menopausal values. Bayesian statistical testing was used to quantify the strength of evidence for similarities between pre- and post-menopausal women (also known as the null hypothesis H_0). It was hypothesised that exercise training would mitigate the effects of the menopause on regional LV mechanics in middle-aged post-menopausal women.

6.2 Methods

6.2.1 Ethical approval

All experimental procedures were approved by the Cardiff Metropolitan University's School of Sport Research Ethics Committee and conformed to the ethical principles in the Declaration of Helsinki. Prior to the start of any experimental procedures, all participants provided written and verbal informed consent.

6.2.2 Study design

Twenty-nine untrained middle-aged women (age 45–58 years) were included in this retrospective study to investigate whether exercise training mitigates the effects of the menopause on LV function and mechanics. Only non-smoking, non-diabetic (self-reported) and normotensive healthy volunteers not taking any cardiovascular or lipid-lowering medications were included in this study. Data in this study were consolidated from a mixture of the pre-menopausal women in Chapter 4 ($n = 15$; Nio et al., 2017) and the post-menopausal women in Chapter 5 who completed the exercise training intervention ($n = 14$; Nio et al., 2020). The pre-menopausal women were used as a reference group to investigate whether LV function and mechanics in post-menopausal women became more similar to values in pre-menopausal women after exercise training.

All participants completed (i) an aerobic capacity test on an upright cycle ergometer, (ii) the 2-min carbon monoxide (CO)-rebreathing protocol for assessing total haemoglobin mass and blood volume, and (iii) a resting echocardiographic scan for LV structure, function and mechanics. The post-menopausal women then undertook 12 weeks of exercise training before repeating the series of laboratory tests. Details on the classification criteria for pre- and post-menopausal women, and on the physiological measures assessed can be found in the earlier chapters and will not be reiterated in this section. Due to poor image quality in some participants, data on LV mechanics are reported for only 12 post-menopausal women (out of 14 post-menopausal women who completed the exercise training intervention).

6.2.3 Statistical analysis

Statistical analyses were performed with R (R Core Team, 2015). The Student's t-test was used to compare the ages of pre- and post-menopausal women. Despite including only middle-aged women in this study, post-menopausal women were 6.5 years older than the pre-menopausal women (Table 6.2). To adjust for the effects of age in the statistical analyses, the one-way analysis of variance (ANOVA) with age as a covariate (R package: afex) was used to compare all measured variables of haematology and cardiovascular function between pre-menopausal women (the reference group), and post-menopausal women (a) before and (b) after exercise training. Alpha was set at 0.05. Data are presented as mean and standard deviation (SD) unless stated otherwise.

Bayes factor. To supplement classical statistical testing and to investigate whether exercise training attenuates the effects of the menopause on LV function and mechanics, the Bayes factor (BF_{01}; Wagenmakers et al., 2018) was used to assess the evidence for differences between pre-menopausal women (the reference group) and post-menopausal women (a) before and (b) after exercise training (H_1; Equation 6.1). A BF_{01} of 1 indicates equal evidence for both the null (H_0) and alternative (H_1) hypotheses, while larger values indicate increasing strength of evidence for H_0 and smaller values favour H_1. BF_{01} 1–3 may be classified as merely anecdotal evidence for H_0, and BF_{01} 3–10 as

moderate evidence for H_0. Table 6.1 shows a complete heuristic classification scheme for BF_{01} (Stefan et al., 2019). The subscripts "01" in BF_{01} indicate that H_0 is in the numerator and H_1 in the denominator. Accordingly, the subscripts "10" in BF_{10} indicate the reverse (Equation 6.2). The Bayes factor therefore quantifies evidence that the data provide for two competing hypotheses (H_0 and H_1), which is not possible with the P-value in null hypothesis statistical testing (NHST). The P-value does not provide a measure of evidence in favour of the null hypothesis; it is the probability of obtaining results at least as extreme as those observed given that the null hypothesis is true. For a comparison between classical statistical inference and Bayesian inference, see Wagenmakers et al. (2018).

$$BF_{01} = \frac{p(\text{data} \mid H_0)}{p(\text{data} \mid H_1)} \tag{6.1}$$

$$BF_{10} = \frac{1}{BF_{01}} \tag{6.2}$$

where:

BF_{01} is the ratio of the likelihood of the null hypothesis (H_0) to the alternative hypothesis (H_1)

$p(\text{data} \mid H_0)$ is the likelihood of the data under H_0

$p(\text{data} \mid H_1)$ is the likelihood of the data under H_1

BF_{10} is the ratio of the likelihood of H_1 to H_0

Using the default options of the BayesFactor package in R (version 0.9.12-4.2; maintained by Richard D. Morey, https://richarddmorey.github.io/BayesFactor/), a non-informative Jeffreys prior was placed on the mean and variance. Zellner and Siow inspired g-priors were placed on effects using the default arguments of "medium" for fixed effects (rscaleFixed = 1/2) and "nuisance" for random effects (rscaleRandom = 1 = "ultrawide"). Random effects were assumed to be in the data due to variance from participants, but were not of interest in this study. These default priors have been shown to be general, broadly applicable, computationally convenient, and lead to Bayes factors that have desirable theoretical properties (for the derivation of these

TABLE 6.1: A heuristic classification scheme for Bayes factors (BF_{01}) adapted from Stefan et al. (2019).

BF_{01}	Evidence category
>100	Extreme evidence for H_0
30–100	Very strong evidence for H_0
10–30	Strong evidence for H_0
3–10	Moderate evidence for H_0
1–3	Anecdotal evidence for H_0
1	No evidence
1/3–1	Anecdotal evidence for H_1
1/10–1/3	Moderate evidence for H_1
1/30–1/10	Strong evidence for H_1
1/100–1/30	Very strong evidence for H_1
<1/100	Extreme evidence for H_1

priors see Rouder et al., 2012). To adjust for the effects of age in this study, the Bayes factor for the model including menopausal status, age and their interaction was divided by the model including only age.

6.3 Results

6.3.1 Body composition, general haemodynamics, aerobic capacity and haematological parameters

Post-menopausal women before exercise training. There was no evidence of differences in body composition, general haemodynamics, aerobic capacity and haematological parameters between post-menopausal women before exercise training and untrained pre-menopausal women ($P > 0.05$; Table 6.2). The significant P-value for relative $\dot{V}O_{2peak}$ ($P = 0.03$) was not supported by a difference in the mean values between pre- and post-menopausal women (29 ± 4 vs. 29 ± 6 mL/min/kg, respectively) nor by the Bayes factor ($BF_{01} = 2.003$), and is thus likely to be a spurious finding.

TABLE 6.2: Demographics, aerobic capacity, haematological parameters and left ventricular (LV) function in middle-aged pre-menopausal women (Pre-M; reference group), and post-menopausal women (Post-M) before and after exercise training (Trg).

	Pre-M	Post-M ($n = 14$)					
Parameter	($n = 15$)	Before Trg	P	BF_{01}	After Trg	P	BF_{01}
Demographics							
Age (years)	48.5 (2.2)	55.0 (2.1)	**<0.001**	<0.001	-	-	-
Height (cm)	162.3 (6.8)	163.3 (3.6)	0.09	0.372	-	-	-
Body mass (kg)	65.3 (10.5)	61.8 (8.4)	0.22	3.173	61.4 (8.3)	0.21	3.015
Body fat (%)	32 (4)	36 (4)	0.42	4.814	35 (4)	0.51	3.560
General haemodynamics							
Systolic blood pressure (mmHg)	128 (14)	133 (16)	0.13	4.291	132 (16)	0.18	4.555
SVR (mmHg·min/L)	31.7 (7.2)	33.6 (4.2)	0.82	4.266	35.1 (6.5)	0.57	3.627
Heart rate (beats/min)	56 (6)	57 (7)	0.93	0.698	54 (7)	0.75	0.952
Cardiac output (L/min)	2.78 (0.50)	2.82 (0.33)	0.97	3.060	2.67 (0.31)	0.85	3.772
Aerobic capacity							
W_{peak} (W)	150 (27)	145 (26)	0.11	3.960	169 (24)	**0.01**	0.818
$\dot{V}O_{2peak}$ (L/min)	1.90 (0.30)	1.80 (0.34)	0.69	2.654	2.08 (0.29)	0.23	2.385
$\dot{V}O_{2peak}$ (mL/min/kg)	29 (4)	29 (6)	**0.03**	2.003	34 (5)	**<0.01**	0.387
HR_{max} (beats/min)	169 (10)	168 (12)	0.13	3.203	166 (9)	0.18	2.638
Haematological parameters							
Total haemoglobin mass (g)	554 (100)	526 (56)	0.39	4.950	534 (61)	0.71	3.784
Blood volume (mL)	4494 (761)	4294 (445)	0.61	4.787	4367 (390)	0.85	3.769
LV structure and function							
LV structure							
IVSd (cm)	0.8 (0.1)	0.8 (0.1)	0.13	3.168	0.8 (0.1)	0.53	2.830
LVPWd (cm)	0.8 (0.1)	0.8 (0.1)	0.57	3.012	0.8 (0.1)	0.82	3.366
LV mass (g)	104 (17)	109 (18)	0.42	3.589	115 (17)	0.36	3.094
Stroke volume (mL)	50 (11)	50 (8)	0.91	3.979	50 (7)	0.95	3.264
End-diastolic volume (mL)	83 (15)	86 (13)	0.58	2.823	84 (11)	0.74	3.060
End-systolic volume (mL)	33 (7)	36 (7)	0.22	2.030	35 (6)	0.41	3.029
Systolic function							
Ejection fraction (%)	60 (4)	59 (5)	0.26	3.530	59 (5)	0.58	4.477
S′ (m/s)	0.08 (0.01)	0.07 (0.01)	0.80	4.569	0.07 (0.01)	0.65	4.241
Diastolic function							
E (m/s)	0.72 (0.12)	0.69 (0.15)	0.75	2.558	0.74 (0.09)	0.69	2.103
E′ (m/s)	0.11 (0.02)	0.09 (0.02)	0.63	5.170	0.10 (0.02)	0.75	4.332
A (m/s)	0.54 (0.09)	0.56 (0.10)	0.82	4.332	0.60 (0.10)	0.88	3.384
A′ (m/s)	0.09 (0.02)	0.09 (0.01)	0.78	4.427	0.08 (0.01)	0.42	3.360
E/A	1.37 (0.28)	1.23 (0.21)	0.73	1.821	1.26 (0.20)	0.57	4.487

Values are in mean (SD). n: sample size. SVR: systemic vascular resistance. W_{peak}: peak power output. $\dot{V}O_{2peak}$: peak oxygen uptake. HR_{max}: maximum heart rate. IVSd: inter-ventricular septum thickness during diastole. LVPWd: LV posterior wall thickness during diastole. Peak septal wall velocity at the level of the mitral annulus during systole (S′), and early (E′) and late diastole (A′). Peak trans-mitral filling velocity during early (E) and late diastole (A). Statistically significant P-values ($P < 0.05$) are in **bold**.

Post-menopausal women after exercise training. $\dot{V}O_{2peak}$ in post-menopausal women after exercise training was higher than in untrained pre-menopausal women by 5 mL/min/kg ($P < 0.01$; Table 6.2). This was accompanied by a higher W_{peak} in post-menopausal women than untrained pre-menopausal women ($P = 0.01$). There was no evidence of any additional differences in body composition, general haemodynamics and haematological parameters between post-menopausal women after exercise training and untrained pre-menopausal women.

6.3.2 Left ventricular structure, function and mechanics

Left ventricular structure and function. There was no evidence of differences in LV structure, volumes, or Doppler indices of systolic and diastolic function between post-menopausal women and untrained pre-menopausal women, both before and after exercise training ($P > 0.05$; Table 6.2).

Left ventricular mechanics in post-menopausal women before exercise training. Peak systolic LV mechanics, namely twist, and apical rotation, rotational velocity and circumferential strain, were lower in post-menopausal women before exercise training compared with untrained pre-menopausal women ($P < 0.05$; Table 6.3). There was no evidence of differences in peak diastolic LV mechanics between post-menopausal women before exercise training and pre-menopausal women ($P > 0.05$).

Left ventricular mechanics in post-menopausal women after exercise training. After exercise training, only peak LV twist remained lower in the post-menopausal women compared with untrained pre-menopausal women ($P < 0.05$; Table 6.3). The peak systolic apical parameters that had been lower in post-menopausal women before exercise training were no longer significantly different from untrained pre-menopausal women ($P > 0.05$). Drawing upon Bayesian hypothesis testing, the probability that post-menopausal women had similar LV mechanics to pre-menopausal women increased from $65 \pm 10\%$ before exercise training, to $70 \pm 11\%$ after exercise training (Figure 6.1).

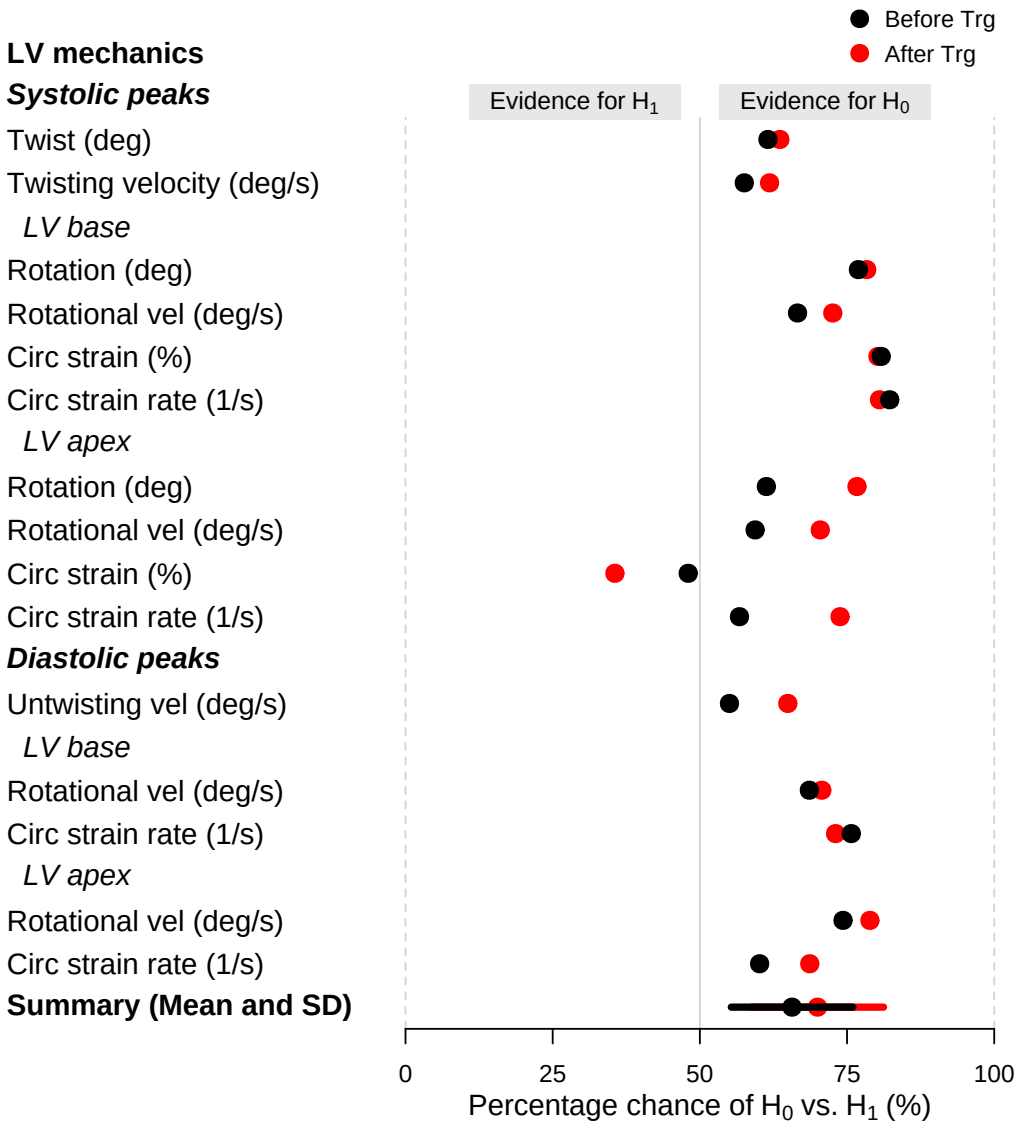


FIGURE 6.1: Effects of exercise training (Trg) on peak left ventricular (LV) mechanics in post-menopausal women, compared with untrained middle-aged pre-menopausal women. H_0: the null hypothesis that pre- and post-menopausal women have similar peak LV mechanics. H_1: the alternative hypothesis that pre- and post-menopausal women have different peak LV mechanics. 50% indicates equal evidence for the null and alternative hypotheses, i.e. BF_{01}=1. 75% indicates moderate evidence for H_0, i.e. BF_{01}=3 (Stefan et al., 2019). Probabilities toward 100% rightwards from 50% indicate increasing evidence for H_0; probabilities toward 0% leftwards from 50% indicate increasing evidence for H_1. Vel: velocity. Circ: circumferential.

TABLE 6.3: Peak left ventricular (LV) mechanics in middle-aged pre-menopausal women (Pre-M; reference group), and post-menopausal women (Post-M) before and after exercise training (Trg).

	Pre-M	Post-M ($n = 12$)					
LV mechanics	($n = 15$)	Before Trg	P	BF_{01}	After Trg	P	BF_{01}
Systolic peaks							
Twist (deg)	18.1 (3.6)	16.0 (6.0)	**0.04**	1.516	15.9 (4.9)	**<0.05**	1.754
Twisting vel (deg/s)	98 (13)	86 (17)	0.18	1.374	89 (18)	0.25	1.584
LV base							
Rotation (deg)	-6.2 (3.2)	-5.8 (2.4)	0.67	3.266	-4.5 (1.9)	0.59	3.615
Rotational vel (deg/s)	-51 (16)	-50 (7)	0.50	2.023	-44 (8)	0.82	2.632
Circ strain (%)	-19 (4)	-19 (3)	0.77	4.119	-19 (3)	0.92	4.071
Circ strain rate (1/s)	-1.0 (0.2)	-1.1 (0.1)	0.91	4.555	-1.0 (0.2)	0.58	4.262
LV apex							
Rotation (deg)	12.3 (3.6)	10.8 (4.0)	**0.02**	1.581	12.1 (4.7)	0.08	3.217
Rotational vel (deg/s)	55 (14)	48 (13)	**0.03**	1.432	54 (15)	0.08	2.435
Circ strain (%)	-21 (3)	-19 (5)	**0.01**	0.937	-19 (4)	0.05	0.558
Circ strain rate (1/s)	-1.1 (0.2)	-1.0 (0.2)	0.09	1.336	-1.0 (0.2)	0.31	2.887
Diastolic peaks							
Untwisting vel (deg/s)	-98 (26)	-94 (31)	0.53	1.230	-92 (34)	0.61	1.892
LV base							
Rotational vel (deg/s)	54 (14)	51 (11)	0.94	2.196	53 (16)	0.96	2.335
Circ strain rate (1/s)	1.5 (0.4)	1.5 (0.5)	0.71	3.201	1.6 (0.4)	0.26	2.643
LV apex							
Rotational vel (deg/s)	-60 (24)	-52 (23)	0.26	2.971	-62 (29)	0.53	3.681
Circ strain rate (1/s)	1.8 (0.6)	1.4 (0.4)	0.08	1.492	1.5 (0.4)	0.35	2.208

Values are in mean (SD). n: sample size. Vel: velocity. Circ: circumferential. Statistically significant P-values ($P < 0.05$) are in **bold**.

6.4 Discussion

This study investigated whether 12 weeks of high-intensity aerobic interval training mitigates the effects of the menopause on LV function and regional mechanics. Doppler indices of systolic and diastolic function in post-menopausal women both before and after exercise training did not differ from untrained pre-menopausal women. Peak LV twist, and apical rotation, rotational velocity and circumferential strain during systole were lower in post-menopausal women before exercise training than in pre-menopausal women. After exercise training, only peak LV twist remained statistically lower in post-menopausal women compared with untrained pre-menopausal women. Using Bayesian

statistics, the probability that post-menopausal women had similar LV mechanics to untrained pre-menopausal women increased by 5% with exercise training. These results suggest that exercise training is a potential intervention to mitigate the effects of the menopause on LV mechanics.

6.4.1 Benefits of exercise training on cardiovascular function in post-menopausal women

After the exercise training programme, the post-menopausal women had, on average, a 5 mL/min/kg higher peak aerobic capacity than their untrained pre-menopausal counterparts. This demonstrates that middle-aged post-menopausal women are able to adapt and benefit from exercise training, as discussed in the previous chapter. The magnitude of the increase in cardiorespiratory fitness observed in this study after just 12 weeks of exercise training may imply a 13% reduction in risk of all-cause mortality and a 15% reduction in risk of coronary heart disease and cardiovascular disease events (Kodama et al., 2009). These findings have practical and clinical implications for advising middle-aged post-menopausal women to start exercising regularly.

6.4.2 Exercise training mitigates the effects of the menopause on LV mechanics

Beyond the implications of an increased cardiorespiratory fitness on health, this is the first study to evaluate the strength of the evidence for similarities in LV function and regional LV mechanics between post-menopausal women after exercise training and untrained pre-menopausal women. Previous work in this thesis (Chapter 4 and 5) and in the literature have instead focused on identifying differences between pre- and post-menopausal women. In this study, a mean increase of 5 mL/min/kg in peak aerobic capacity in post-menopausal women after 12 weeks of exercise training was concurrent with a 5% increase in the probability of similar regional LV mechanics to untrained pre-menopausal women, relative to the hypothesis of different LV mechanics between the two groups. Whilst this shift towards pre-menopausal reference values was evident

for LV mechanics, such a shift was less clear for indices of LV systolic and diastolic function assessed using pulsed-wave Doppler imaging. These findings thus suggest that LV mechanics may be a more sensitive marker of changes in cardiac function than traditional Doppler indices, particularly in the context of early adaptations to exercise training. In addition, the lower peak systolic apical rotation, rotational velocity and circumferential strain in post-menopausal women before but not after exercise training, compared with untrained pre-menopausal women, appear to be in line with previous work suggesting that LV adaptations to exercise training start at the apex rather than at the base (Weiner et al., 2010a).

Taken together, the approach of using Bayes factors to supplement *P*-values in this study allowed for the classical identification of differences between pre- and post-menopausal women, and additionally enabled the evaluation of similarities between the two groups. It may be useful to consider a combination of classical and Bayesian statistical approaches in future studies, particularly where assessing the evidence for similarities is equally or possibly more important than identifying differences between groups.

6.4.3 Limitations and future directions

A plausible limitation of this study is that default priors in the BayesFactor R package were used in our statistical analyses, and that these default priors were developed by Rouder et al. (2012) with a focus on studies in experimental psychology and not exercise physiology. However, as the use of Bayesian statistics is relatively new in exercise physiology, more informed priors were not readily available for our statistical analyses. Whilst these default priors may not be the best choice across all possible studies and research fields, they are nonetheless likely to be reasonable in most circumstances (Rouder et al., 2012).

Despite non-significant *P*-values (>0.05) for LV function between pre- and post-menopausal women (whether before or after exercise training), the supplementary Bayes factors indicated at most a moderate strength of evidence for similarities between the two

groups (highest BF_{01}=5.17 for E′ between pre-menopausal women and post-menopausal women before exercise training). This upper limit of a moderate strength of evidence for similarities between pre- and post-menopausal women was also echoed for LV mechanics, despite an overall shift towards pre-menopausal reference values in post-menopausal women after exercise training. This may indicate that more data is required to increase the strength of evidence for similar LV function and mechanics between pre- and post-menopausal women, or that other confounding factors may be limiting the extent of similarities between pre- and post-menopausal women. Potential confounding factors may include: age at menopause, time from menopause, duration of reproductive lifespan (Ley et al., 2017; Muka et al., 2016a) and a history of pre-eclampsia (Wu et al., 2017). Building upon the results of this study, future work may include a larger sample size and a subset analysis to identify any confounding factors in middle-aged women that are distinct from the menopause.

6.4.4 Conclusion

In conclusion, 12 weeks of high-intensity aerobic interval training elicited a mean increase of 5 mL/min/kg in peak aerobic capacity in middle-aged post-menopausal women. This was concurrent with a 5% increase in the likelihood of similar LV mechanics between post-menopausal women and untrained pre-menopausal women, compared with the likelihood of differences in LV mechanics between the two groups. Exercise training may be a potential lifestyle intervention to mitigate the effects of the menopause on LV mechanics in middle-aged women.

Chapter 7

General discussion

7.1 Thesis aims and main findings

The aim of this thesis was to investigate the effects of the menopause on left ventricular (LV) mechanics. This was achieved through three research studies that have been detailed in the preceding chapters (Chapter 4–6). The main findings of these studies will be recapped below, accompanied by a general discussion linking the individual studies to the overall aim of the thesis. In considering all three studies together, overall limitations and implications of this work will be discussed, and future directions to build upon the work in this thesis will be indicated later in this chapter. An overall conclusion at the end of this chapter will complete this thesis.

7.1.1 Effects of the menopause on resting LV mechanics in ageing

Background and aim: The menopause has been associated with changes in LV structure, function and mechanics, but is rarely addressed in ageing studies. Therefore, the aim of the first research study in Chapter 4 (cf. Nio et al., 2017) was to investigate the effects of the menopause on resting LV structure, function and mechanics within the context of usual ageing (i.e. ageing that is characterised by an absence of overt

pathology but some decline in function; Weinert and Timiras, 2003). This was achieved by: (a) investigating age-related sex differences in LV structure, function and mechanics in a cross-sectional sample of young adult and middle-aged men and women; and (b) comparing LV structure, function and mechanics between middle-aged pre- and post-menopausal women.

Main findings on age-related sex differences: Middle-aged men had a lower LV mass, stroke volume and end-diastolic volume than young adult men (allometrically scaled to fat free mass to normalise for differences in body size between groups), but this difference was smaller between middle-aged and young adult women. This was accompanied by greater peak systolic apical LV mechanics and later peak diastolic rotational velocities in middle-aged men compared with middle-aged women, but not between young adult men and women. The predominance of apical differences that were detected suggest that apical LV mechanics may be an early identifier of distinct ageing pathways in men and women.

Main findings on menopause-related differences: Post-menopausal women had lower LV mechanics (i.e. torsion, twisting velocity and apical circumferential strain rates) than pre-menopausal women. The lower LV mechanics in post-menopausal women may reflect a decrease in myocardial function following the menopause. As menopause-related differences in LV mechanics were not specific to the apex, the menopause may only partly explain the sex differences in LV apical mechanics with ageing discussed above.

7.1.2 Effects of the menopause on LV mechanics in response to physiological stress

Background and aim: The menopause may affect cardiac adaptations to exercise training, but the underlying differences in LV function and mechanics are unknown. In addition, physiological tests may reveal menopause-related differences in LV function

and mechanics that are not evident under resting conditions, but the existing literature is limited. Therefore, the aim of the second research study in Chapter 5 was to investigate the effects of the menopause on LV adaptations to exercise training using physiological testing. This was achieved by comparing LV function and mechanics in response to lower body negative pressure and submaximal supine cycling between pre- and post-menopausal women before and after a 12-week exercise training intervention.

Main findings: The increase in relative peak aerobic capacity after 12 weeks of high-intensity aerobic interval training was 9% less in post-menopausal women than pre-menopausal women, concomitant with a smaller increase in blood volume. This is the first study to suggest that the menopause may limit aerobic adaptability, which has implications for exercise prescription in middle-aged women. However, cardiac output and LV volumes were not different between pre- and post-menopausal women despite altered regional LV muscle function, as indicated by higher basal mechanics in pre-menopausal women during the physiological tests after exercise training. These findings are the first to show altered LV mechanics specific to the base in post-menopausal women. In addition, the reduced aerobic adaptability to high-intensity aerobic interval training in post-menopausal women does not appear to be a central cardiac limitation, and may be due to altered blood volume distribution and lower peripheral adaptations.

7.1.3 Exercise training to mitigate the effects of the menopause on LV mechanics

Background and aim: Regular physical activity is widely recommended to promote and maintain cardiovascular health, but whether it can be used to reverse the effects of the menopause on the heart is unknown. Therefore, the aim of the final research study in Chapter 6 of this thesis was to investigate whether exercise training causes a shift in resting LV function and mechanics in post-menopausal women toward pre-menopausal values. Using classical statistical testing, the first two studies in this thesis (and most studies in the existing literature base) have focused on identifying differences between pre- and post-menopausal women, rather than on quantifying the extent of

the similarities between the two groups. In this study, Bayesian statistical testing was used to complement classical P-values to evaluate the strength of evidence for similar LV function and mechanics in post-menopausal women before and after 12 weeks of exercise training, relative to untrained pre-menopausal women.

Main findings: Twelve weeks of high-intensity aerobic interval training elicited a mean increase of 5 mL/min/kg in peak aerobic capacity in middle-aged post-menopausal women. This was concurrent with a 5% increase in the likelihood of similar LV mechanics between post-menopausal women and untrained pre-menopausal women, while the change in likelihood towards similar LV function was less pronounced. Exercise training may be a potential lifestyle intervention to mitigate the effects of the menopause on LV mechanics in middle-aged women.

7.1.4 Summary of research findings

The effects of the menopause on LV mechanics were investigated in this thesis. Post-menopausal women had lower resting LV mechanics than pre-menopausal women, which may partly explain the sex differences in regional LV mechanics with ageing. Physiological testing to assess adaptations to exercise training revealed higher LV basal mechanics in pre-menopausal women compared with post-menopausal women. However, differences in cardiac adaptations did not appear to explain the smaller increase in peak aerobic capacity observed in post-menopausal women after 12 weeks of high-intensity aerobic interval training. Nonetheless, exercise training increased the extent of similarities in resting LV mechanics in post-menopausal women and untrained pre-menopausal women, suggesting that exercise training may be used to reverse the effects of the menopause on LV mechanics. This thesis provides new insight into the effects of the menopause on aerobic adaptability and regional LV function.

7.2 Ageing affects the hearts of men and women differently

The findings of this thesis support the position that the hearts of men and women change differently with age: women are not simply "small men" (Dannenberg et al., 1989; Hees et al., 2002; Natori et al., 2006; Olivetti et al., 1995). Interestingly, while Doppler indices of LV relaxation (E, E′ and septal TDI-derived isovolumic relaxation time) were lower in middle-aged than younger adults in Chapter 4, these age-related differences were not statistically different between men and women (sex × age $P > 0.1$). Considered together with previous work (Daimon et al., 2011; Grandi et al., 1992; Okura et al., 2009), it is possible that the decline in diastolic relaxation with ageing is largely similar in men and women from young adulthood to middle-age, before accelerating in women in their 50s and older. The menopause may still be partly responsible for differences in LV systolic and diastolic function with ageing (Düzenli et al., 2007; Hayward et al., 2000; Kangro et al., 1995; Schillaci et al., 1998), but a longer time from menopause may be necessary before these differences become evident.

7.3 Potential age-related sex differences in LV mechanics during exercise

Aside from differences in LV mechanics between pre- and post-menopausal women during submaximal supine cycling (i.e. Chapter 5), sex differences may additionally exist (Nio et al., 2015). Building upon greater peak apical mechanics in middle-aged men than women under resting conditions observed in Chapter 4, middle-aged men may either have (i) a smaller "reserve" in LV mechanics to achieve maximal cardiac output during exercise, or (ii) consistently greater LV mechanics from rest to maximal exercise. Direct comparisons of LV function and mechanics during exercise in men and women of different ages are necessary to provide further insight into age-related sex differences and the compensatory mechanisms that stem from differences at rest.

7.4 Basal and apical mechanics as novel indices of regional LV muscle function

The assessment of LV basal and apical mechanics in this thesis has enabled new insight into the effects of ageing and the menopause on the heart, beyond the conventional global measures such as ejection fraction and cardiac output. In Chapter 4, age-related sex differences were observed mainly at the LV apex, and it appears that the menopause only partly explains these differences. Instead, physiological testing and exercise training revealed differences in LV basal rather than apical rotational mechanics between pre- and post-menopausal women in Chapter 5. These regional differences likely reflect the interacting effects of sex hormones and sympathetic drive on the heart (discussed in the respective experimental chapters). In addition, LV basal and apical mechanics appear to be more sensitive to the beneficial effects of exercise training than the traditional Doppler indices of LV function. Whilst LV basal and apical mechanics are currently not assessed routinely in the clinic, they have been shown to change with cardiovascular disease (Pacileo et al., 2011; Park et al., 2008; Takeuchi et al., 2007; Yoneyama et al., 2012) and may potentially be incorporated in echocardiography scans for disease diagnosis and prognosis. The regional LV differences observed *in vivo* in this thesis may additionally encourage future *in vitro* work on isolated cardiomyocytes from different regions on the heart (e.g. the basal and apical epicardium and endocardium), in the effort to elucidate the underlying mechanisms of sex hormones and catecholamines on myocardial function (Chen et al., 2011; Yang et al., 2012).

It is important to note that the study of LV mechanics lends itself to multiple comparisons and therefore a higher chance of detecting a spurious statistical difference. To mitigate this and increase confidence in the findings of this thesis, indices of LV mechanics were only discussed as physiologically significant if statistical significance did not occur in isolation — for example, statistically significant differences in multiple measures of LV apical mechanics in Chapter 4, at the base in Chapter 5, and across all peak regional LV mechanics in Chapter 6.

7.5 Limitations

Participant recruitment. Healthy volunteers were recruited for the studies in this thesis by email, flyers and word-of-mouth, and enrolled based on a resting blood pressure of <140/90 mmHg and no reported history of heart disease. The echocardiographic images acquired and the accompanying 3-lead ECG on the ultrasound system were examined by researchers, but were not forwarded to a clinician for screening or diagnosis. Blood cholesterol levels were not assessed and a cardiac risk score was not calculated. It is therefore possible that participants with sub-clinical disease could have been included in the studies in this thesis, although there was no indication of myocardial dyssynchrony in the echocardiographic images.

Echocardiography. The methodological limitations of using ultrasound to assess LV mechanics include poor image quality and through-plane motion of the myocardium across the cardiac cycle (Stöhr et al., 2011; Takeuchi et al., 2006; van Dalen et al., 2008b). In this thesis, adequate image quality for LV mechanics were only successfully recorded in a subset of participants (detailed in the methods in the respective experimental chapters). To enhance the repeatability of echocardiographic images during physiological testing in Chapter 5, the optimal position of the ultrasound transducer was identified under resting conditions and temporarily marked on the participant's chest. This assisted the quick relocation of optimal transducer positions during the acute cardiovascular challenges, prior to further minor adjustments based on anatomical landmarks visible during image acquisition. In addition, the configuration settings on the ultrasound system were standardised within each participant. Moreover, to minimise the error between LV mechanics assessed via echocardiography and via gold standards such as tagged magnetic resonance imaging (MRI) and sonomicrometry, the most caudal transducer position was selected for apical mechanics (Helle-Valle et al., 2005; van Dalen et al., 2008b).

An additional limitation of the echocardiographic measures in this thesis is that isovolumic relaxation time in Chapter 4 was derived from septal tissue Doppler imaging (TDI). This was used in this thesis in line with previous work (e.g. Stöhr et al., 2011),

but guidelines have been updated to recommend the use of continuous-wave Doppler in the apical long-axis or five-chamber view instead (Nagueh et al., 2016). In the latter method, the sample volume positioned in the LV outflow tract would enable the simultaneous display of the end of aortic ejection and the onset of mitral inflow (Nagueh et al., 2016). Future work should use continuous-wave Doppler in the apical long-axis view to assess isovolumic relaxation time. Another echocardiographic measure that may be included in future work is the Doppler-derived parameter of E deceleration time (Nagueh et al., 2016), which may contribute to a more complete picture of diastolic function with ageing and the menopause. This was not assessed in this thesis due to the low statistical power to detect any differences in E deceleration time with the available sample size (cf. Table 3.5), and in addition, it cannot be assessed during physiological stress when E and A peaks merge at higher heart rates.

Alpha of 0.1. A statistical limitation in Chapter 4 is the α level of 0.1, instead of the conventional value of 0.05 or lower (Benjamin et al., 2017). This means a 10% probability of incorrectly rejecting the null hypothesis when it is in fact true (Type I error; cf. Table 3.4; Comrey and Lee, 2007; McKillup, 2012; Riegelman, 2013). This adjusted α level, however, was found to result in the best possible trade-off between Type I and Type II errors, limited by the relatively small sample size that could be assessed within the research resources available (for details on the power calculations, see Section 3.5.1). To improve the clarity of the statistical findings, actual P-values were presented to a precision of 2 decimal places so that no reader would have to rely solely on the 0.1 cut-off to interpret the results. Minimising false negatives (Type II errors) was deemed to be important in this work due to the novelty of the parameters and comparisons in a non-convenience female sample in sports and exercise medicine research (Costello et al., 2014). The study in Chapter 4 was therefore specifically designed to reveal possible differences between groups for future verification. In light of the raised Type I error rate in Chapter 4, however, replication studies with larger sample sizes and lower Type I and Type II error rates are recommended to verify our findings, and the results have been interpreted cautiously (Lakens et al., 2018; McLoughlin and Drummond, 2017; Section 7.7).

Orthostatic tolerance. The maximum level of lower body negative pressure tolerable by each participant was not assessed in this thesis. Age, sex and cardiorespiratory fitness (or blood volume; Vella and Robergs, 2005) may influence orthostatic tolerance, and additional insight may be gained by charting cardiovascular responses up to each individual's limit of orthostatic tolerance. However, identifying the point of maximum orthostatic tolerance is severe physiological stress and was not regarded as ethically necessary in the study in Chapter 5. It is plausible that the differences observed between pre- and post-menopausal women at absolute levels of lower body negative pressure in Chapter 5, and in previous studies (e.g. Edgell et al., 2012; Harvey et al., 2005; Williams et al., 2016) may disappear when responses are expressed as a percentage of maximum orthostatic tolerance. Further studies involving men and women of different ages, while accounting for the menopause in women, would help determine the effects of age and sex hormones on the cardiovascular responses to orthostatic stress.

7.6 Implications

Research on ageing is especially relevant in today's socio-economic climate, where the proportion of older people in the global population is rising and projected to exceed the number of children for the first time in 2047 (i.e. population ageing; United Nations, Department of Economic and Social Affairs, Population Division, 2013). The following subsections will discuss the practical implications, physiological significance and clinical relevance of this thesis on the effects of ageing and the menopause on LV structure, function and mechanics.

7.6.1 Practical implications

The key practical implication of this thesis is the smaller increase in peak aerobic capacity in post-menopausal women after 12 weeks of high-intensity aerobic interval training, compared with middle-aged pre-menopausal women (Chapter 5). This is the first study to suggest that the menopause may limit aerobic adaptability to exercise training. In hindsight, it seems reasonable that the menopause, which reflects ovarian

ageing, may affect adaptations to exercise training independent from chronological ageing. This finding provides further evidence to encourage lifelong regular physical activity for successful ageing (i.e characterised by little or no physiological loss and no pathology; Weinert and Timiras, 2003), as adaptations to exercise training may be smaller or slower in women after the menopause. Notwithstanding, exercise training may be a viable lifestyle intervention to mitigate the effects of the menopause on the heart in middle-aged women (Chapter 6).

Irrespective of the differences between pre- and post-menopausal women, the work in this thesis adds to the growing literature demonstrating the feasibility of high-intensity aerobic interval training to improve cardiorespiratory fitness (e.g. Helgerud et al., 2007; Tjønna et al., 2008; Wisløff et al., 2007). Whilst the exercise training intervention per se was not the research focus of this thesis, the findings nonetheless raise questions regarding exercise prescription for middle-aged women. Do post-menopausal women have a lower ceiling for increases in peak oxygen uptake, or do they simply take longer to adapt to exercise training? Would a different exercise training programme, such as continuous moderate-intensity exercise or sprint-interval training (Gibala et al., 2014; Green et al., 2002), be more efficient in eliciting adaptations in post-menopausal women? Further research into different exercise training interventions and the associated cardiovascular adaptations have the potential to influence exercise prescription and recommendations, and on a wider level, health policies and public spending/investment.

7.6.2 Physiological significance

With regards to LV mechanics, the findings in this thesis agree with the existing literature on an increased twist with age (van Dalen et al., 2008a; Yoneyama et al., 2012), lower resting peak LV mechanics in post-menopausal than pre-menopausal women (Keskin Kurt et al., 2014), and increased peak LV mechanics in response to acute incremental exercise (Stöhr et al., 2011). The data during lower body negative pressure, however, differ from the existing literature. Instead of exhibiting an increase in peak LV twist during lower body negative pressure as previously reported (Hodt et al., 2011; Williams et al., 2016), twist did not change in pre- and post-menopausal women in

Chapter 5. In addition, an increase in untwisting velocity during lower body negative pressure has been suggested to compensate for a reduced stroke volume, by mitigating the decrease in early filling due to reduced cardiac preload (Esch et al., 2010; Hodt et al., 2015; Williams et al., 2016; cf. Section 2.5.1). However, no change in untwisting velocity with lower body negative pressure was observed in Chapter 5. As previous studies have largely investigated men (Esch et al., 2010; Hodt et al., 2011, 2015), with a single study by Williams et al. (2016) that included young adult women, further work is necessary to delineate the effects of sex and age on the cardiovascular response to orthostatic stress.

7.6.3 Clinical relevance

Cardiovascular disease is currently diagnosed with poorer sensitivity and specificity in women than men (Maas et al., 2011; Tan et al., 2015). Whilst this clinical outcome strongly indicates that disease affects the hearts of men and women differently, the discrepancy in diagnostic ability is evidence that the underlying mechanisms of these sex differences are incompletely understood. In Chapter 4, the cross-sectional comparisons suggest that usual ageing from young adulthood to middle-age is accompanied by a greater decrease in LV mass, stroke volume and end-diastolic volume in men than women, underpinned by sex differences at the LV apex. These findings contribute to building a definitive profile of cardiac changes with usual ageing, which is relevant as a reference for detecting changes with disease and subsequent prognosis. Future work focusing on regional LV mechanics may provide further insight into sex differences with usual ageing and disease. A better understanding of sex differences in cardiovascular function with usual ageing will likely have implications in understanding why women are more likely to suffer from heart failure with preserved ejection fraction (HFpEF) and non-obstructive coronary artery disease, while men are more likely to suffer from heart failure with reduced ejection fraction (HFrEF) and obstructive coronary artery disease (Maas et al., 2011; Shaw et al., 2009). In addition, future work investigating age-related sex differences in response to cardiovascular challenges, such as those employed in Chapter 5, may provide insight into why symptoms of heart failure, acute

coronary syndromes and ischaemic heart disease differ between men and women. Applying "functional" data to clinical practice may have implications in reducing missed diagnoses of acute coronary syndromes in women — particularly those younger than 55 years (Pope et al., 2000) — and in improving in-hospital mortality of young women with acute myocardial infarctions (Gupta et al., 2014).

Beyond the effects of advancing age, physical inactivity and the menopause have been associated with an increased risk of cardiovascular disease (Maas et al., 2011; Mozaffarian et al., 2015). Through a multi-factorial prospective longitudinal study of healthy non-athlete middle-aged women, Chapter 5 and 6 findings provide novel insight into changes in LV function and mechanics with the menopause and exercise training. Compared with pre-menopausal women, post-menopausal women had lower resting LV mechanics (i.e. longitudinal systolic strain and diastolic strain rate, torsion, twisting velocity and apical circumferential strain rates) in this study and in previous work (Keskin Kurt et al., 2014), which matches the sub-clinical systolic dysfunction proposed by Pacileo et al. (2011) in their pathophysiological cascade of heart failure in cardiomyopathies (Figure 7.1). Importantly, these changes appear to occur prior to compensatory increases in LV rotational mechanics with mild clinical diastolic dysfunction (Park et al., 2008). Early cardiac changes after the menopause, if indeed indicative of sub-clinical systolic dysfunction, could begin to uncover the mechanisms underlying menopause as a risk factor for cardiovascular disease. Sub-clinical dysfunction following the menopause may, in fact, occur at the base before the apex, as evidenced by differences in LV basal mechanics between pre- and post-menopausal women revealed with "functional" cardiovascular challenges in Chapter 5. In line with resting values, there was no evidence of clinical dysfunction during exercise between pre- and post-menopausal women in Chapter 5, as previously evidenced by lower cardiac outputs (Borlaug et al., 2010) or lower and delayed diastolic rotational velocities (Tan et al., 2009). Longitudinal studies of middle-aged women starting prior to the menopause, and through the menopausal transition into later life may provide insight into the predictive capability of changes in LV mechanics for future cardiovascular disease and clinical outcomes.

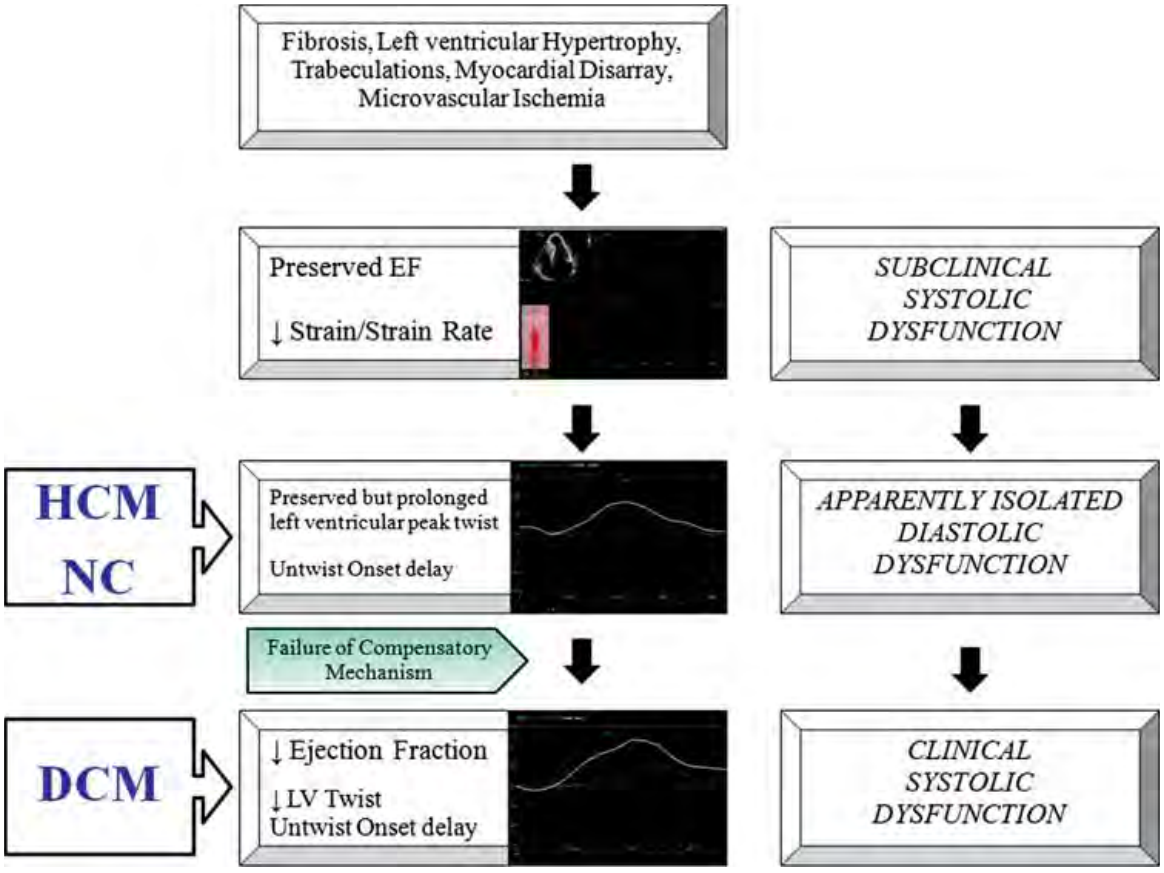


FIGURE 7.1: Pathophysiological cascade of heart failure in cardiomyopathies (extracted from Pacileo et al., 2011). EF: ejection fraction. HCM: hypertrophic cardiomyopathy. NC: non-compaction. DCM: dilated cardiomyopathy. LV: left ventricular.

7.7 Future directions

Our findings highlight the importance of including women in cardiovascular research (Costello et al., 2014), as ageing and the menopause elicit changes that likely underpin different prevalences of cardiovascular disease between men and women (Garcia et al., 2016; Maas et al., 2011; Shaw et al., 2009). Future directions toward better understanding the effects of ageing on the LV include investigating age-related sex differences under resting conditions and in response to cardiovascular challenges (such as lower body negative pressure and supine cycling used in Chapter 5). Building upon the physiological tests in this thesis, empirical data at higher intensities of physiological stress — for example, higher levels of lower body negative pressure and relative exercise intensity, and longer periods of exercise training — would extend the range

of functional responses beyond that examined in this thesis. In addition, large-scale ageing studies and longitudinal studies accounting for menopausal status in women would help delineate the effects of the menopause and "time from menopause" from chronological ageing (Harlow et al., 2012; Pines et al., 1992; cf. Literature review Section 2.3.3). Measures of sex hormone concentrations, sympathetic drive (circulating concentrations vs. direct innervation), and myocardial density of adrenoceptors and sex hormone receptors may help piece together the mechanisms underlying (i) apical sensitivity to sex differences in ageing (Chapter 4), and (ii) basal sensitivity to the menopause revealed during physiological testing (Chapter 5). Studies involving women after a hysterectomy (i.e. surgically-induced menopause) may additionally be informative in elucidating the effects of sex hormones on the cardiovascular system, but must be carefully considered before such results are extrapolated to the naturally-occurring menopause (Laughlin et al., 2000). Beyond the cardiac variables assessed in this thesis, measures of vascular function, LV filling pressures and myocardial properties (Fujimoto et al., 2010; Xi et al., 2014) will likely provide further insight into the effects of sex, age and the menopause on the heart.

Amongst the significant findings in this thesis, a replication study to investigate the effects of high-intensity aerobic interval training on cardiorespiratory adaptations in middle-aged pre- and post-menopausal women may have particularly important implications. Following *Experimental Physiology*'s guidelines for a replication study, a sample size calculation using G*Power (Version 3.1.9.2; Faul et al., 2007) with a significance level α set at 0.05 and statistical power at 90%, to detect an effect size that is 70% of that reported in our study ([2]effect size for relative peak oxygen uptake in our study, $\eta_p^2 = 0.21$), indicated that a total of 86 middle-aged pre- and post-menopausal women aged 45–58 years would be required (McLoughlin and Drummond, 2017). Whilst this

[2] η_p^2 is a measure of effect size for the ANOVA:

$$\eta_p^2 = \frac{SS_{\text{Effect}}}{SS_{\text{Effect}} + SS_{\text{Error}}} \tag{7.1}$$

where SS_{Effect}: sum of squares of the effect; SS_{Error}: sum of squares of the error. η_p^2 describes the proportion of the variance accounted for by the effect (Cohen, 1973). If $\eta_p^2 = 0.1$, 10% of the variance is explained by the effect. Suggested values for small, medium and large effect sizes are: 0.01, 0.06 and 0.14, respectively (Ellis, 2010).

is a substantial sample size for a supervised exercise training study, the subsequent implications for exercise prescription in middle-aged women arguably outweigh the cost of confirming or disproving our findings. A better understanding of adaptations to exercise training in middle-aged women would improve recommendations for lifestyle interventions toward improving cardiorespiratory fitness, which has implications for improving health outcomes in our ageing population. Future work should also investigate the potential effects of other exercise training interventions with different training intensities and/or duration on LV mechanics and aerobic adaptability between pre- and post-menopausal women.

7.8 Conclusion

In conclusion, this thesis has investigated the effects of the menopause on LV mechanics. The menopause was associated with lower resting LV mechanics and altered LV basal mechanics in response to physiological stress. This thesis is the first to provide evidence that the menopause may limit aerobic adaptability to exercise training in middle-aged women, and to demonstrate regional effects of the menopause on LV muscle function. In addition, this thesis is the first to investigate similarities in LV mechanics between pre- and post-menopausal women. Exercise training was found to shift indices of LV mechanics in post-menopausal women toward pre-menopausal values, indicating that exercise training may be used as a lifestyle intervention to mitigate the effects of the menopause on LV mechanics in middle-aged women. The menopause is shown to affect LV structure, function and mechanics, and should be considered as a contributing factor in understanding cardiac changes with usual ageing, successful ageing and disease.

Appendix A

Ethical approval

Cardiff Metropolitan University
Prifysgol Fetropolitan Caerdydd
UWIC

8th July 2013

To : Amanda Nio

The project "Impact of gender and menopause on the responses of the heart to lower body negative pressure and exercise" has been granted ethical clearance by the Cardiff School of Sport Research Ethics committee. The project code is 13/06/01R.

Yours sincerely

Peter O'Donoghue
Chair
Cardiff School of Sports, Research Ethics Committee

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Appendix B

Risk assessment for lower body negative pressure

RISK ASSESSMENT (RA99) (V3/07)	*Page 1 - (Hazards)*		
School / Unit and Area:	Sport / Physiology	**Assessment Number:**	45
Risk Assessment undertaken by: Recommended to be 2 or more people	Amanda Nio	Mike Stembridge	
	James Pearson		
Description of the work activity being assessed:	Lower body negative pressure (LBNP) to -40 mmHg		
Persons Affected:	Staff [X] Students [X] Others [X]		
Details of Others:	Participants, visitors, consultants, external clients		

	HAZARD IDENTIFICATION Please provide details of the hazards associated with the area or task. **EXAMPLES INCLUDE:** Working at height, Manual Handling, Electricity, Fire, Noise, Contact with moving parts of machinery, Dust etc	**RISK RATING - without Controls** The Risk Rating **(RR)** and Degree of Risk are determined by multiplying the Severity **(S)** of injury by the Likelihood **(L)** of occurrence. Please see UWIC Risk Rating Matrix for details			
		S	**L**	**RR**	**Degree of Risk**
1	Unintended syncope	2	3	**6**	**Moderate**
2	Aggravation of an existing hernia	3	2	**6**	**Moderate**
3	Stress and anxiety with unfamiliar equipment	1	3	**3**	**Low**
4	Dizziness when resuming the upright position	2	3	**6**	**Moderate**
5	Hyperventilation, causing tingling of hands and feet, and the accompanying anxiety	1	3	**3**	**Low**
6					
7					
8					
9					
10					
	Example - 1. Electric Shock (office)	**4**	**3**	**12**	**Unacceptable**

Once all potential hazards have been identified and a Risk Rating has been applied, please go to page 2 and provide details of the control measures required to reduce the risk to an acceptable level.

↦↕⇳ RISK ASSESSMENT (RA99) *Page 2 – (Controls)*

	CONTROLS TO BE APPLIED **Examples Include:** Elimination, Substitution for something less hazardous, Barriers or fixed guards, standard operating procedures and personnel protective equipment	Date Applied	RISK RATING - with Controls			
			S	L	RR	Degree of Risk
1	Participants with a history of regular syncope will be excluded from this test. Up to and including -40 mmHg, the incidence of unintended syncope is extremely low in healthy individuals. At least two researchers will be present during testing. Participant's heart rate, blood pressure and ECG trace will be closely monitored. Immediate test termination if researchers observe irregularities in the ECG trace, a sudden fall in mean arterial pressure or heart rate, or systolic/diastolic blood pressure <80/50, or if the participant requests to stop. The negative pressure will be released immediately by switching off the vacuum pump and opening all three valves attached to the LBNP box. Participants will start to feel better immediately upon termination of the test.	23/10/12	2	2	**4**	**Moderate**
2	Participants with a history of hernia will be excluded from this test	23/10/12	3	1	**3**	**Low**
3	Demonstration, instruction and familiarisation with associated equipment.	23/10/12	1	2	**2**	**Low**
4	Participants will be monitored for 5 min in the supine position after termination of LBNP to ensure that blood pressure returns to baseline levels. Instructions to stand up slowly will be given to mitigate the risk of dizziness. If the participant feels faint, they will resume a supine position with legs elevated.	23/10/12	2	1	**2**	**Low**
5	At the severity proposed here, LBNP may be accompanied by hyperventilation. This can cause a small drop in partial pressures of CO_2 in the blood stream and subsequent sensations of tingling in the hands and feet. Prior to the test, participants will be informed that tingling of hands and feet is not unusual and they will be advised and reminded to breathe normally. The test will be terminated if severe hyperventilation occurs. Participants are free to stop the test at any point.	23/10/12	1	3	**3**	**Low**

Date of First Assessment:	23/10/12	Review Date of overall Assessment:	

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