The maternal cardiovascular system at rest and in response to functional haemodynamic testing during healthy pregnancy

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Submitted in accordance with the requirements for the Degree of

Doctor of Philosophy

Department of Physiology and Health

Cardiff Metropolitan University

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Abstract

Healthy pregnancy results in significant maternal cardiac adaptation to match the increased circulatory demands of the developing fetoplacental unit. Specifically, cardiac output, heart rate (HR), stroke volume (SV) and end-diastolic volume (EDV) increase, whereas mean arterial pressure and systemic vascular resistance decrease. Despite a large body of research, there is a lack of consensus over the magnitude and timing of these adaptations in pregnancy. Additionally, previous studies have reported reduced systolic function in the late stages of pregnancy, indicating that gestation may negatively influence left ventricular pumping capacity. Testing the ability of the maternal heart to respond to additional physiological challenge may elucidate how cardiac function is affected by healthy pregnancy. This thesis investigated cardiovascular adaptation and functional responses before, during and after healthy pregnancy. Firstly, a series of meta-analyses were completed to characterise global cardiac function across healthy gestation. These analyses showed that resting cardiac output is elevated during pregnancy, peaking late in the third trimester but reducing towards term. Secondly, a comprehensive assessment of cardiac structure and function was completed in healthy non-pregnant, pregnant and postpartum females at rest. The significantly greater cardiac output in pregnant females was result of significantly higher HR and SV. The greater SV was result of significantly higher EDV and systolic functional parameters (longitudinal and circumferential left ventricular strain), the latter of which may be linked to greater sympathetic activity. Finally, the functional cardiovascular responses of the aforementioned groups to sustained isometric handhold and submaximal aerobic exercise were tested. During both challenges, systolic function of pregnant females remained significantly greater. In conclusion, healthy pregnancy alters the function of the maternal heart through lower afterload, greater preload and enhanced systolic function. Additionally, healthy pregnant females in the late second trimester have adequate functional responses to increased demand and altered haemodynamic load.

Preface

All studies within this thesis (Chapters 3, 5 and 6) were approved by the Cardiff Metropolitan University ethics board. Data collection for experimental Chapters 5 and 6 was completed at the Physiology and Health laboratory, Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, United Kingdom.

A version of the study presented in Chapter 3 has been published: **Meah VL,** Cockcroft JR, Backx K, Shave R, Stöhr EJ. (2016). Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. *Heart.* doi:10.1136/heartjnl-2015-308476. Dr. Eric Stöhr, Prof. John Cockcroft, Prof. Rob Shave and myself were responsible for the conception of the study. I was responsible for conducting the search and screening of articles. Dr. Eric Stöhr and myself agreed the final inclusion of datasets, after which I extracted and analysed all data. Dr. Eric Stöhr, Dr. Karianne Backx, Prof. Rob Shave and I interpreted the data. I was primarily responsible for drafting the manuscript, which Dr. Eric Stöhr, Dr. Karianne Backx, Prof. Rob Shave and Prof. John Cockcroft critically reviewed the manuscript. I also received statistical advise from Dr. Steve Cooper and Professor Yoav Ben-Shlomo.

The idea for the experimental study presented in Chapters 5 and 6 was conceived by Dr. Eric Stöhr, Prof. John Cockcroft, Prof. Rob Shave and myself. I was primarily responsible for study design, participant recruitment, data collection and analysis, statistical analysis and interpretation of data. I was responsible for the writing the chapters with critical review from Prof. Rob Shave, Dr. Eric Stöhr and Dr. Karianne Backx. Data from these chapters were presented at American College of Sports Medicine Annual Meeting 2016 (Boston, USA), Okanagan Cardiovascular and Respiratory Symposium 2016 (British Columbia, Canada), The Physiological Society Annual General Meeting 2017 (London, UK), ARTERY 2017 (Pisa, Italy), and the Second International Congress on Maternal Haemodynamics 2016 (Rome, Italy).

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List of key terminology, associated acronyms and definitions

Term	Acronym	Definition
Afterload		The ventricular wall stress experienced during ejection
Apex		Inferior portion of the left ventricle, distal to the papillary muscles
Arterial blood pressure (mmHg)	BP	Pressure of blood within the arterial tree
Base		Superior portion of the left ventricle, between the papillary muscle and mitral annulus
Cardiac output ($L \cdot min^{-1}$)		Volume of blood ejected by the left ventricle in one minute
Circumferential strain (%)		Percentage deformation of the myocardial wall from diastole to systole in a short-axis plane, at basal and apical levels
Contractility		Neurohormonal control of myocardial contraction
Diastole	d	Relaxation phase of the cardiac cycle between aortic valve closure and mitral valve closure
Early filling septal velocity ($m \cdot s^{-1}$)	E'	Peak velocity of the septum following mitral valve opening
Early filling velocity ($m \cdot s^{-1}$)	E	Peak velocity of blood flow from atria to ventricle following opening of the mitral valve
Echocardiography		Ultrasound imaging of the heart in which structure and function of the heart and great vessels can be visualised
Ejection fraction (%)	EF	Amount of blood ejected with each contraction expressed as a percentage
Endocardium		Inner layer of left ventricular myocardium
End-diastolic volume (ml)	EDV	Volume of blood within the left ventricle at the end of the diastolic period
End-systolic volume (ml)	ESV	Volume of blood within the left ventricle at the end of the systolic period
End-systolic wall stress ($kilodyne \cdot cm^2$)	ESWS	The tension within the wall of the left ventricle at end-systole
Epicardium		Outer layer of left ventricular myocardium
Gestation		Period of foetal development from conception to birth, to be used interchangeably with 'pregnancy'
Gestational age		Measured in weeks, this describes the age of the pregnancy from the females last menstrual cycle.
Heart rate ($beats \cdot min^{-1}$)	HR	Number of myocardial contraction per minute
Hypertrophy (cardiac)		Enlargement of left ventricle muscle mass
Late filling septal velocity ($m \cdot s^{-1}$)	A'	Peak velocity of the septum during late diastole corresponding to atrial contraction
Late filling velocity ($m \cdot s^{-1}$)	A	Peak velocity of blood flow from atria to ventricle following atrial contraction

Left ventricular	LV	Myocardial chamber responsible for output to the systemic circulation
Longitudinal strain (%)		Percentage deformation of the myocardial wall from diastole to systole in a long-axis plane
Mechanics		Collective term for left ventricular deformation parameters including twist, untwist, rotation and strain
Nulliparous		Female who has never been pregnant
Parturition		Process of giving birth to offspring
Peak systolic septal velocity ($m \cdot s^{-1}$)	S'	Peak velocity of the septum during contraction
Postpartum		Period following childbirth
Pregnancy		See 'gestation'
Preload		Degree of myocardial distension as a result of ventricular filling prior to contraction
Primiparous		Female who is pregnant for the first time or has borne only one child
Renin-angiotensin-aldosterone system	RAAS	Neurohormonal system involved in the regulation of blood volume and arterial pressure
Rotation (°)		Degrees of rotation of either the base or apex of the left ventricle during systole
Speckle-tracking echocardiography	STE	Ultrasound analysis technique allowing for the characterisation of deformation by tracking the movement of naturally occurring 'speckles' in myocardial tissue across the cardiac cycle
Sphericity index		Ratio of the left ventricular long-axis length divided by short-axis length that identifies more spherical or more ellipsoid chamber shape
Strain rate ($\%/sec^{-1}$)	SR	Rate at which deformation occurs
Stroke volume (ml)	SV	Volume of blood ejected from the left ventricle in one contraction
Sympathetic nervous system		Part of the autonomic nervous system resulting in stimulatory excitation of the cardiovascular system
Systemic vascular resistance ($dyne \cdot s \cdot cm^{-6}$)	SVR	Resistance to blood flow exerted by the systemic vasculature
Systole	s	Contraction phase of the cardiac cycle between mitral valve closure and aortic valve closure
Tissue Doppler imaging	TDI	Ultrasound methodology allowing for measurement of velocity of the myocardium
Trimester		Any of three periods of approximately 12 weeks into which a human pregnancy is divided.
Twist (°)		Sum of apical and basal rotation during systole
Two-dimensional echocardiography	2D	Standard ultrasound acquisition mode allowing structures to be imaged in a cross-sectional view
Untwist velocity (°/s)		Velocity of myocardial recoil during diastolic relaxation

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Chapter 1. General Introduction

Pregnancy induces significant changes in female anatomy and physiology in order to support the developing foetus (Macdonald et al., 2011). Adaptive processes start at conception, progress over the course of gestation and are reversed in the postpartum period (Wallenburg, 1990a). Most prominently, the development of a low resistance high flow fetoplacental circulation results in significantly increased metabolic demand and changes in haemodynamic load (May, 2015, Wang and Zhao, 2010, Lof et al., 2005). The maternal heart must therefore respond, and adapt, to meet these additional cardiovascular demands to ensure a successful pregnancy (Wallenburg, 1990a).

Cardiac output is closely related to overall metabolic demand (Klabunde, 2005), and it is well established that resting maternal cardiac output increases above non-pregnant levels across gestation. At present, however, there is a lack of consensus regarding the timing and magnitude of this increase across the trimesters of healthy pregnancy (van Oppen et al., 1996, Melchiorre et al., 2012a). Unlike cardiac output, the resting heart rate (HR) response to pregnancy is well understood. Over gestation, HR progressively increases reaching a peak at term (Clapp and Capeless, 1997, Mahendru et al., 2014). Accordingly, the lack of consensus regarding cardiac output is due to the lack of clarity regarding the potential change in stroke volume (SV) across gestation (Melchiorre et al., 2012a). The generation of SV is influenced by the volume of blood in the left ventricle (LV) at end diastole (preload), the contractile state of the myocardium, and the pressure against which the LV needs to work to eject the blood during systole (afterload) (Levick, 2010) all of which are altered during pregnancy (Melchiorre et al., 2012a). In healthy adults, increased preload, increased contractility or reduced afterload (as experienced during pregnancy) would induce positive changes in measures of cardiac function, however there is some evidence to suggest that both systolic contraction and diastolic relaxation in the latter half of pregnancy are reduced

(Zentner et al., 2009, Kametas et al., 2001, Cong et al., 2015, Estensen et al., 2013, Melchiorre et al., 2016, Bamfo et al., 2007), although these findings have also been challenged (Valensise et al., 2000, Savu et al., 2012, Geva et al., 1997, Mesa et al., 1999, Yoon et al., 2011, Papadopoulou et al., 2013).

The lack of clarity regarding systolic and diastolic function across pregnancy may be the result of the traditional echocardiographic measures that have been used (*e.g.* ejection fraction, trans-mitral filling patterns and myocardial tissue displacement velocities). These measures oversimplify the complex multi-directional movement of tissue and blood across the cardiac cycle (Sengupta et al., 2017), and do not account for the mechanical movements that underpin overall systolic or diastolic function. Therefore, assessing LV myocardial deformation, also termed LV mechanics, may provide greater insight into cardiac function than traditional measures. Additionally, LV mechanics have been suggested to be more sensitive to subtle changes in cardiac function (Feigenbaum et al., 2012), such as those previously noted during the late stages of healthy pregnancy (Zentner et al., 2009, Kametas et al., 2001, Cong et al., 2015, Estensen et al., 2013, Melchiorre et al., 2016, Bamfo et al., 2007). Therefore, combining the assessment of traditional echocardiographic techniques with measures of LV mechanics may further our understanding of functional adaptations to healthy pregnancy, and help to discern whether there are subtle changes in cardiac function.

In addition to the assessment of cardiac function at rest with both traditional and more novel methods, physiological challenges may also be useful to improve our understanding of the functional capacity of the maternal heart. Cardiovascular reserve describes the ability of the heart to match output to increased demand whilst coping with changes in haemodynamic load (Koelwyn et al., 2012). Physiological challenges test this reserve and may help to elucidate if maternal cardiac function is reduced, maintained or enhanced during healthy pregnancy

(Ohuchi et al., 2013). Although previous research has shown that healthy non-pregnant and pregnant females have similar HR, cardiac output and blood pressure responses to physiologic challenges (May, 2015, Finkelstein et al., 2011, Sady et al., 1989, Heenan et al., 2001, Veille et al., 1992, Petrov Fieril et al., 2016, Avery et al., 1999), the underpinning systolic and diastolic responses of the healthy maternal heart are not known. It is possible that the altered global cardiac function at rest during pregnancy reduces functional reserve capacity. Consequently, there may be a reduced or lack of response in systolic and diastolic function during physiological challenges.

The aim of this thesis was to further the understanding of cardiac adaptation and functional cardiovascular responses in healthy pregnancy. Firstly, a series of meta-analyses examining cardiac output and related haemodynamics across healthy pregnancy were completed to characterise cardiac adaptation. Secondly, an assessment of cardiac structure and function, including LV mechanics, at rest was completed in non-pregnant females, pregnant females (22-26 weeks) and postpartum females (12-16 weeks after delivery). Thirdly, the functional cardiovascular responses of non-pregnant, pregnant and postpartum females during a sustained isometric handhold and during two stages of a submaximal aerobic exercise assessment were measured. Accordingly, this thesis adopts the following structure:

- Review of previous literature (Chapter 2);
- Methods and findings of the meta-analyses (Chapter 3);
- General methodology for the experimental studies (Chapter 4);
- Comparisons of cardiac structure and function at rest in non-pregnant, pregnant and postpartum females (Chapter 5);
- Assessment of functional cardiovascular responses to physiological challenges in non-pregnant, pregnant and postpartum females (Chapter 6);

- Overall findings and key conclusions of the thesis (Chapter 7).

Associated study documents, supplementary data, abstracts and published papers relating to this thesis are included in the appendices.

Chapter 2. Review of Literature

In recent years, there has been a significant move to improve our understanding of sex dimorphism within physiology (Arnold, 2010). Significant evidence supporting the hypothesis that males and females differ in their basic physiology exists (Miller, 2014). These differences are exacerbated by reproductive events of the female lifespan, such as the menstrual cycle, pregnancy and menopause, all of which are mediated by changes in the hormonal milieu (Macdonald et al., 2011). Fluctuations in female sex hormones directly influence the cardiovascular system in women (Leinwand, 2003). For example, the luteal phase of the menstrual cycle (acute rises in oestrogen and progesterone) transiently alter blood volume and blood pressure (Chapman et al., 1997, Hayward et al., 2000), whereas menopause (low oestrogen and progesterone) is associated with a decline in cardiac function and cardiovascular health (Hayward et al., 2000). During pregnancy, the hormonal profile is altered to a far greater magnitude, with oestrogen and progesterone reaching 32 and 12 times greater than levels experienced during the menstrual cycle (O'Leary et al., 1991). Consequently, gestation induces cardiovascular remodelling and significant functional adaptation of the maternal heart (Macdonald et al., 2011).

Due to the marked changes to the prevailing haemodynamics, pregnancy has been suggested to reflect a cardiac stress model (Sattar and Greer, 2002) (Figure 1). In healthy adults, the primary purpose of the cardiovascular system is to maintain blood pressure (BP) and facilitate the exchange of gases, fluids, substrates and heat between the cells and outside environment (Klabunde, 2005). During healthy pregnancy, the maternal cardiovascular system must also transport adequate nutrients and oxygen to meet the additional needs of the developing foetus (Macdonald et al., 2011). As a result of these increased demands, progressive remodelling and functional adaptation of the cardiovascular system occurs. However, the favorable adaptations associated with a normal pregnancy

are not always evident and cardiovascular maladaptation can occur (Sattar and Greer, 2002, Melchiorre et al., 2012b, Valensise et al., 2001). Cardiovascular complications of pregnancy, such as preeclampsia or gestational hypertension, can develop in previously healthy females under the additional cardiovascular stress of pregnancy (Figure 1).

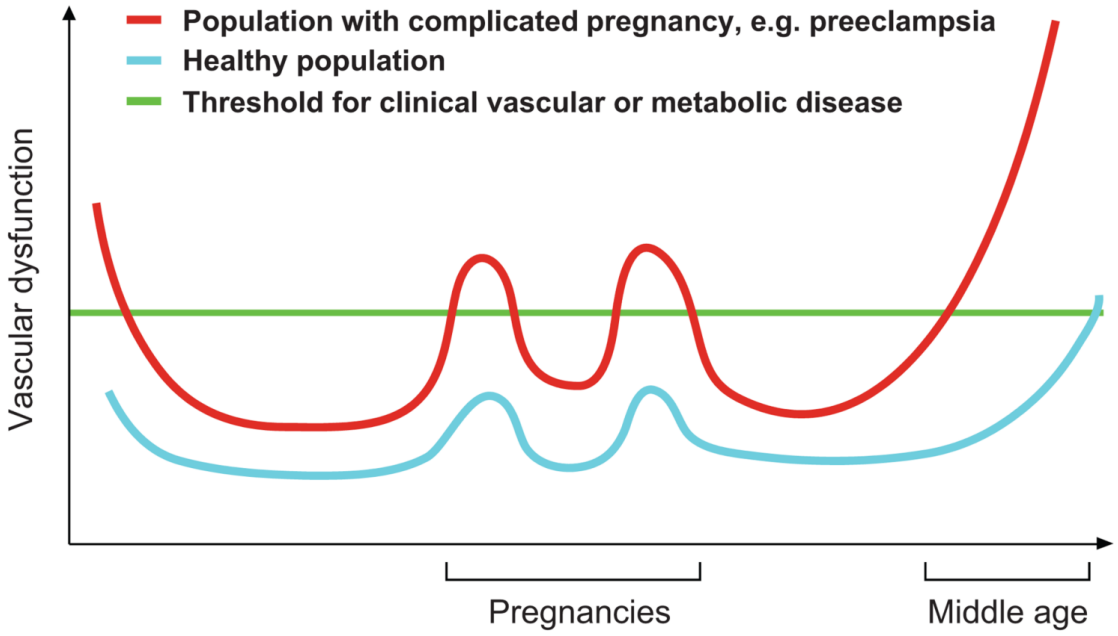


Figure 1. Pregnancy is considered a cardiovascular stress test. Females that have a healthy pregnancy experience an increase in cardiovascular stress, however this does not reach the threshold for clinical disease during gestation. In females at risk of future cardiovascular disease, pregnancy may constitute a stressor at which point subclinical risk becomes overt disease. Females who develop pregnancy complications have a significantly increased risk of cardiovascular disease at middle age. Original figure from Sattar and Greer (2002), adapted by Rich-Edwards et al. (2010).

Cardiovascular complications of pregnancy are the leading cause of maternal and foetal non-obstetric morbidity and mortality (Khan et al., 2006) and their development has significant long-term implications for health (Rich-Edwards et al., 2010), however the understanding of their aetiology is limited. This is confounded by a lack of consensus in the normal adaptation to healthy pregnancy. The reported magnitude and timing of peak cardiac adaptation varies across the literature, and there is a lack of clarity surrounding whether cardiac function is enhanced, unchanged or reduced across gestation (Melchiorre et al., 2012a). As a

result, there are no normative ranges for cardiovascular adaptation to healthy pregnancy and this hinders the identification of maladaptation (Melchiorre and Thilaganathan, 2011). This lack of certainty in cardiovascular adaptation to healthy pregnancy needs to be addressed in order to further develop the understanding of gestational complications. As such, this thesis seeks to characterise and clarify healthy maternal adaptation to pregnancy.

This review provides an overview of the current understanding of adaptations to the maternal heart during and following healthy pregnancy, and will highlight the gaps in knowledge. It is important to note that, many changes in other maternal physiological systems also occur during pregnancy, however, only those that directly contribute to cardiovascular structure and function are discussed where relevant. To aid the flow of the thesis and improve brevity there is an assumption that the reader has an understanding of basic cardiovascular physiology, accordingly in-depth explanation of underpinning cardiovascular principles has been avoided.

2.1. Global cardiac function during healthy pregnancy

Cardiac output, a fundamental parameter of cardiac function rises proportionally in line with metabolic demand (Levick, 2010). During gestation, resting cardiac output is increased by approximately 30% from non-pregnant values in response to the progressively increasing foetal and uteroplacental metabolic demand (Melchiorre et al., 2012a, Clapp and Capeless, 1997, Mahendru et al., 2014, Poppas et al., 1997, Sengupta et al., 2017). However, the temporal pattern of this change in cardiac output is inconsistently reported within the literature with either a peak at term (Desai et al., 2004, Clapp and Capeless, 1997, Savu et al., 2012, Poppas et al., 1997), peak and then plateau until term (Robson et al., 1989, Ogueh et al., 2009, Estensen et al., 2013) or peak and then decline towards term (Mahendru et

al., 2014, Mone et al., 1996, Easterling et al., 1990). Furthermore, the proportional contribution of heart rate (HR) and stroke volume (SV) to the elevated cardiac output across pregnancy is also not understood. A progressive increase in HR with a peak at term is well established (Clapp and Capeless, 1997, Mahendru et al., 2014, Melchiorre et al., 2012a) and is attributed to greater sympathetic tone during pregnancy (May, 2015). This is supported by data showing an elevated muscle sympathetic nerve activity at rest in pregnant females when compared to non-pregnant females (Usselman et al., 2015a, Jarvis et al., 2012). In contrast to the consistent HR response, the contribution of SV to cardiac output across gestation is less clear. Previous research has reported an increase in SV into the second trimester before plateauing (Poppas et al., 1997, Sengupta et al., 2017, Savu et al., 2012) or an initial increase followed by a decline towards term (Mahendru et al., 2014, Mone et al., 1996, Sanghavi and Rutherford, 2014).

Changes in preload, inotropy and afterload during pregnancy

The increase in SV early during pregnancy is primarily attributed to blood volume expansion (Figure 2) mediated by the renin-angiotensin-aldosterone system (RAAS). Upregulation of the RAAS during gestation is stimulated by reduced afferent arteriole pressure due to hormonally mediated systemic vasodilation (Levick, 2010) and increased ovarian production of renin (Sanghavi and Rutherford, 2014, Charkoudian et al., 2017). Increased circulating renin causes the conversion of angiotensinogen to angiotensin I, that in turn is converted to angiotensin II by angiotensin converting enzyme (Levick, 2010). Angiotensin II stimulates the release of aldosterone, which in turn induces sodium and fluid retention within the maternal kidneys, leading to a 45-50% increase in plasma volume (Lumbers and Pringle, 2014, Picciano, 2003). Total blood volume increases by approximately 35–40% from non-pregnant values, with a

proportionally lower increase in erythrocytes leading to a physiologic anaemia (Horowitz et al., 2013, Sanghavi and Rutherford, 2014). The volume load experienced by the maternal heart is therefore increased above the non-pregnant state. Increased circulating blood volume causes a greater volume of blood at end-diastole (EDV) and thus preload, and may contribute to greater SV via the Frank-Starling mechanism (Reynolds et al., 2010, Thornburg and Louey, 2013).

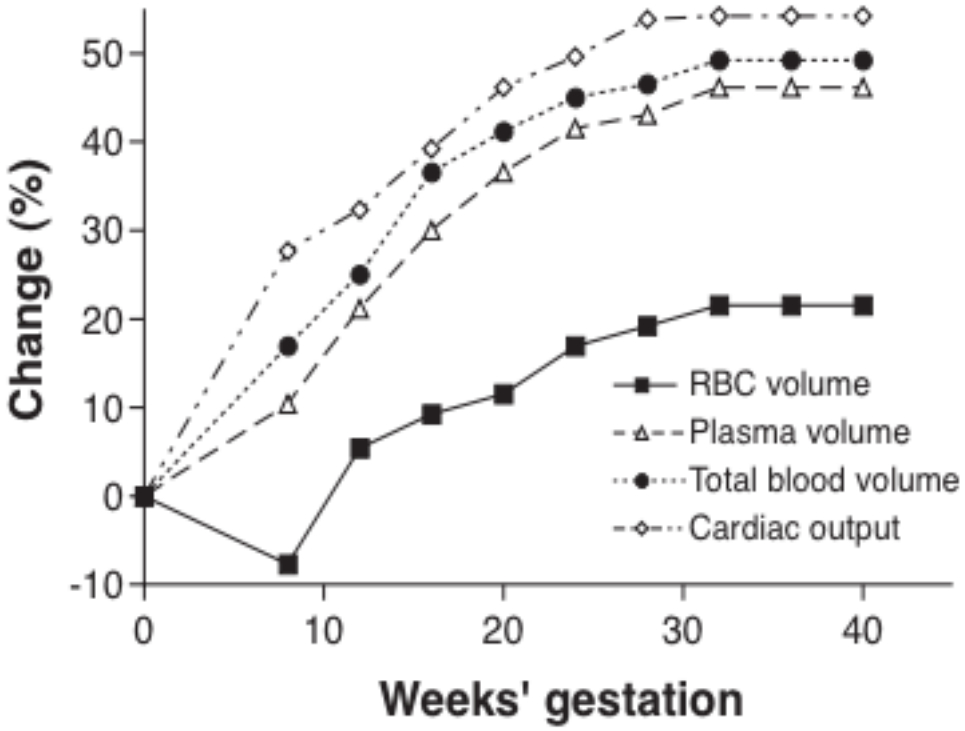


Figure 2. Blood volume expansion contributes to greater cardiac output during healthy pregnancy. RBC, red blood cell. Adapted from Heidemann and McClure (2003).

Pregnancy is also associated with reduced afterload, which may also contribute to greater SV. During gestation, arterial blood pressure (BP) is maintained at or just below non-pregnant levels (Melchiorre et al., 2012a), however systemic vascular resistance (SVR) is significantly lowered across gestation. The small reductions in BP and significant reduction in SVR are caused by peripheral vasodilation and increased arterial compliance (Macedo et al., 2008, Khalil et al., 2009, Robb et al., 2009, Iacobaeus et al., 2017) as well as the development of the low resistance fetoplacental circulation (Huppertz and Peeters, 2005, Wang and Zhao, 2010).

Vascular tone therefore plays a large role in the reduction in afterload during pregnancy. In healthy adults, the control of vascular tone is achieved through local and neurohormonal control (Levick, 2010), however these mechanisms are influenced by gestation. Elevated sympathetic activity typically causes vasoconstrictive responses (Levick, 2010), however this is not observed during pregnancy. The slope of the relationship between arterial pressure and MSNA, known as baroreflex gain, is reduced during pregnancy compared to non-pregnant females (Usselman et al., 2015a). Neurovascular transduction, or the translation of sympathetic activity into a vascular outcome, is also reduced during late pregnancy at rest and in response to a cold pressor test (Usselman et al., 2015b, Jarvis et al., 2012). The refractoriness of the vasculature to sympathetic innervation may also be result of increased local control. Nitric oxide (NO) is increased during pregnancy (Fu and Levine, 2009) via greater shear stress caused by higher blood flow (Levick, 2010) as well as oestrogen mediated upregulation of NO production. Pregnancy induces greater expression and activity of oestrogen receptors within the vasculature (Mata et al., 2015, Salerni et al., 2015) that lead to greater activation of endothelial NO-synthase and synthesis of NO (Charkoudian and Stachenfeld, 2016). Additionally, despite the vasoconstrictive actions of isolated progesterone, when combined with high levels of oestrogen, progesterone inhibits vasoconstriction via modification of angiotensin II receptors (Tkachenko et al., 2014). High concentrations of progesterone and oestrogen also have a thermogenic effect, increasing basal body temperature which also contributes to the systemic vasodilation (Charkoudian and Stachenfeld, 2016). Relaxin is also implicated in increased endogenous NO production and impairment of vasoconstrictive pathways (Conrad, 2011). Therefore, despite increased vasoconstrictive stimuli, several local mechanisms enable the maintenance of systemic dilation in the vasculature. These mechanisms facilitate

the maintenance or reduction in afterload during gestation, and in turn may contribute to greater SV and cardiac output.

In addition to the greater preload and reduced afterload, SV may be increased by greater myocardial contractility (Levick, 2010). As previously noted, pregnant females experience increased sympathetic activity (Usselman et al., 2015b, Jarvis et al., 2012) and upregulation of the RAAS, resulting in increased circulating catecholamines and angiotensin II (Fu and Levine, 2009). In healthy adults, these inotropic agents cause a cascade of cellular changes increasing the availability, uptake and transient of calcium ions within myocytes that result in shorter, more forceful myocardial contraction and greater SV (Klabunde, 2005). The increased sympathetic activity of gestation has been suggested to occur as compensatory response to both the activation of the RAAS and systemic vasodilation (Jarvis et al., 2012), However, whilst sympathetic activity has been measured during pregnancy, the effect on cardiac contractility specifically has not yet been assessed in isolation from the effects of haemodynamic load. Therefore it is unclear as to whether greater contractility contributes to elevated cardiac output during pregnancy.

2. 1. i. The influence of the fetoplacental unit on haemodynamic load across pregnancy

The fetoplacental unit mediates many of the gestational adaptations in the maternal cardiovascular system. The placenta is a temporary organ that allows direct contact between the maternal blood and the chorionic (foetal) villi for nutrient and oxygen transport to the foetus. It is responsible for regulating the maternal adaptation to pregnancy via hormone production and optimising maternal-foetal exchange (Pijnenborg et al., 2011). Placental development occurs after the trophoblast cells of the embryo adhere and invade into the maternal decidua

(Sharma et al., 2016) with further growth stimulated by increased placental growth hormone (Lacroix et al., 2002). Placental vascularisation occurs via local *de-novo* formation of small vessels (Wang and Zhao, 2010) and the remodelling of maternal uterine spiral arteries from low-flow, high-resistance to high-flow, low-resistance vessels to enable maternal-foetal exchange (Cartwright et al., 2010). In the first trimester, the dilated spiral arteries are 'plugged' and only allow plasma filtrate to enter the intervillous space, supporting the conceptus through histotrophic nutrition (Burton et al., 2010). Between 10 and 12 weeks gestation, the plugs degenerate and maternal blood perfuses the placenta. Intervillous blood flow is detected after 12 weeks gestation (Coppens et al., 1996), at which point, the maternal-placental (uteroplacental) and foetal-placental (fetoplacental) circulations are established (Pijnenborg et al., 2011). Haemotrophic exchange, facilitated by continuous radial flow and low pressure (approx. 10 mmHg) within the placental unit, meet the fetoplacental demands for oxygen and nutrients for the remainder of gestation (Huppertz and Peeters, 2005, Wang and Zhao, 2010). Maternal blood flow increases in response to foetal demand, and at term, reaches approximately 600 to 700 ml per minute, requiring a significant percentage of maternal cardiac output (Wang and Zhao, 2010). The fetoplacental unit therefore mediates the hormonal responses that enable the increase in preload (via blood volume expansion), reduction in afterload (via systemic vasodilation) and consequent increase in sympathetic activity, as well as drive the rise in metabolic demand.

Changes in haemodynamic load and metabolic demand are not stable across gestation (Figure 3), and therefore may contribute to fluctuations in the generation of cardiac output. Females during early gestation experience reductions in afterload through hormonally mediated systemic vasodilation, whereas the second trimester is associated with enhanced preload due to blood volume expansion

from the up-regulated RAAS. As such, cardiac output should be increased above non-pregnant levels in both the first and second trimester. However, the final trimester represents the phase of pregnancy with the greatest cardiovascular challenge, with maternal and foetal metabolic demands peaking (Bobrowski, 2010, Lof et al., 2005, Acharya et al., 2016), increasing afterload due to hormonal preparation for labour (Smith et al., 2002, Kamel, 2010), and potentially lower preload caused by compression of the inferior vena cava by the gravid uterus (Ryo et al., 1996, Armstrong et al., 2011, Higuchi et al., 2015). As a result, the changes in haemodynamic load and increased metabolic demand may explain the reducing cardiac output and SV previously reported in the third trimester.

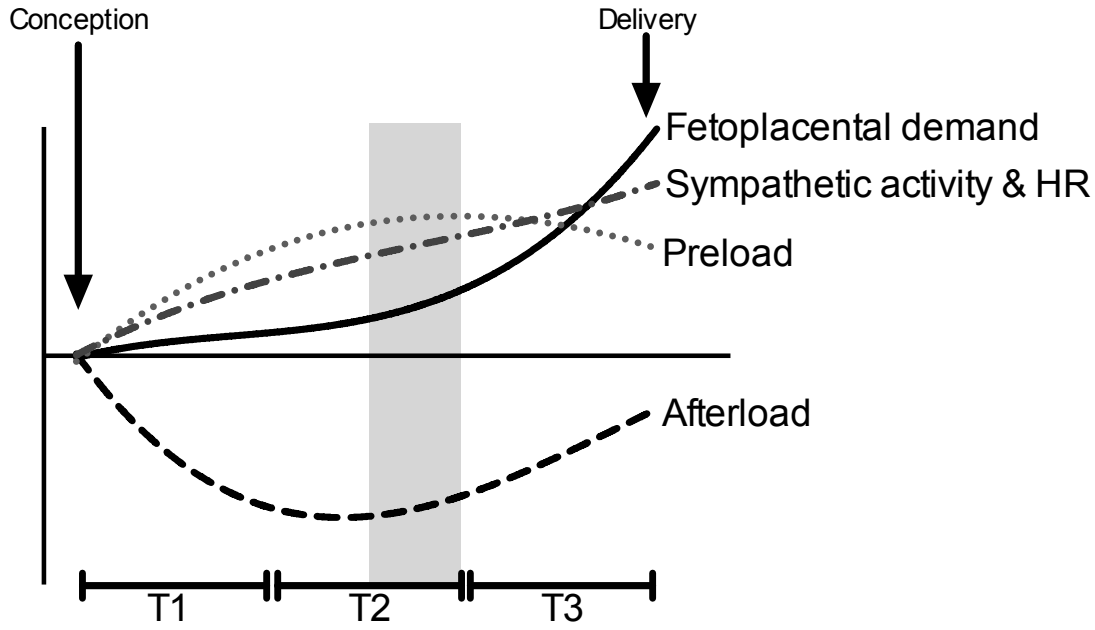


Figure 3. Changes in fetoplacental demand and haemodynamic load alter cardiac function across pregnancy. Trimester one (T1) is associated with low metabolic demand, reduced afterload, increasing preload and elevated sympathetic activity. Trimester two (T2) is associated with stabilisation of afterload, and preload, whilst sympathetic activity and metabolic demand increase. The third trimester (T3) is associated with peak fetoplacental demand and sympathetic activity and heart rate (HR), afterload is returning to non-pregnant levels and preload is potentially reduced, resulting in increased cardiac work. Preload, afterload and HR curves were interpolated from data presented in Chapter 3. The fetoplacental demand curve was interpolated from previously published maternal basal metabolic rate Lof et al. (2005).

Summary of global haemodynamics during healthy pregnancy

Healthy gestation results in increased cardiac output, however there is a lack of consensus in published literature regarding the magnitude and timing of adaptation. It is unclear if cardiac output rises progressively over gestation, peaks and then plateaus, or peaks and then declines until term. This lack of clarity has hindered the development of normative values for cardiac output and related haemodynamics in healthy gestation. Although the progressive rise in HR is established, changes in SV across pregnancy are not well understood. Favourable changes in haemodynamic load during gestation (including increased preload via blood volume expansion, reduced afterload mediated via systemic vasodilation and increased contractility via sympathetic innervation) would suggest SV should be enhanced however this has not been consistently reported. These inconsistent reports in global cardiac function are likely result of fluctuations in haemodynamic load and metabolic demand across gestation that are mediated by the developing fetoplacental unit. Additionally, the changes in haemodynamic load; specifically the volume overload, and increased demand provide stimuli for cardiac remodelling to facilitate the generation of greater cardiac output.

2.2. Cardiac remodelling during healthy pregnancy

Prolonged exposure to increased haemodynamic load causes changes in cardiac function that stimulate myocardial remodelling. Hypertrophy is categorised as eccentric or concentric and can be either pathological or physiological, as shown in Figure 4 (Lang et al., 2015). Healthy pregnancy results in physiological eccentric hypertrophy (Chung and Leinwand, 2014) and occurs in response to volume overload. The increased preload of gestation stimulates an increase in myocyte length that enables the accommodation of greater volume (Russell et al., 2000). It is well documented that healthy pregnancy results in increases in LV cavity size

with proportional increases in wall thicknesses (Savu et al., 2012, Geva et al., 1997, Mone et al., 1996, Desai et al., 2004, Poppas et al., 1997).

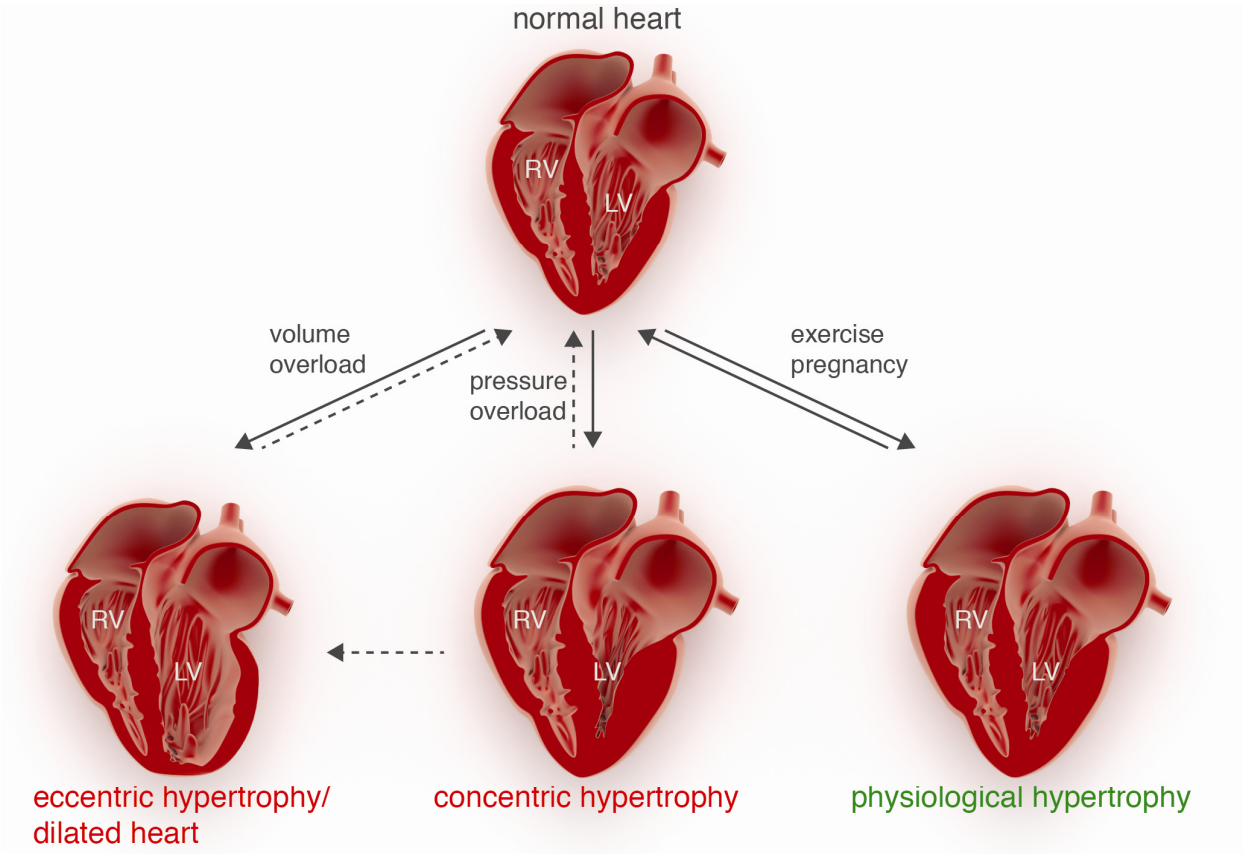


Figure 4. Physiological and pathological morphometric alterations of the myocardium in response to pressure or volume overload. Changes in cardiac loading through pressure and volume result in cardiac remodelling, as shown by solid arrows. Physiological hypertrophy occurs in response to chronic exercise and pregnancy and is reversible upon withdrawal of said stimuli. Physiological hypertrophy is considered favourable with proportional increases in chamber dimensions and wall thicknesses with normal or enhanced function. Pathological hypertrophy occurs in response to chronic increases in pressure or volume and is typically irreversible, indicated by dashed arrows. Adapted from Chung and Leinwand (2014).

Aerobic exercise training also results in physiological eccentric hypertrophy, consequently both pregnancy-associated and aerobic exercise-induced cardiac remodelling are often said to be similar (Chung and Leinwand, 2014, Mone et al., 1996). However, aerobic exercise training is an intermittent stimulus resulting in volume overload, whereas pregnant females are chronically exposed to greater HR and metabolic demand, as well as an increased volume load over a 40-week period (Chung and Leinwand, 2014). Additionally, the gene expression associated

with the remodelling processes is different in pregnant *versus* exercising mice despite achieving similar levels of cardiac hypertrophy (Chung et al., 2012). These regulatory differences have been attributed to female sex steroid hormones (Nio et al., 2015, Leinwand, 2003, Chung and Leinwand, 2014), with higher circulating oestrogen increasing hypertrophic processes through increased gene signalling and protein synthesis (Dworatzek et al., 2014). The molecular mechanisms that control structural adaptation in response to pregnancy and exercise are therefore different.

In addition to LV size, cardiac geometry may also be affected during healthy gestation. The sphericity index of the LV is decreased from the second trimester of healthy pregnancy onwards, recovering to non-pregnant values after delivery (Savu et al., 2012, Cong et al., 2015). A lower sphericity index (meaning a more spherical heart) has been linked to increased regional wall stress, documented in the late stages of health pregnancy previously (Estensen et al., 2013), and is a known stimulus for cardiac hypertrophy (Grossman et al., 1975, Mone et al., 1996). Additionally, decreased sphericity index can reduce contractility (Ghista, 2016, van Dalen et al., 2010). However, while decrements in systolic function have been reported in the third trimester (Zentner et al., 2009, Kametas et al., 2001, Estensen et al., 2013, Cong et al., 2015) it is unlikely that a more spherical LV is the isolated cause. Geometric indices and functional parameters only have a weak, non-significant correlation (Cong et al., 2015); therefore, factors other than changes in cardiac structure and geometry are likely important mediators of maternal cardiac function during healthy pregnancy.

2.3. Traditional and novel measures of cardiac function in pregnancy

Traditional measures of cardiac function and their limitations

Despite greater cardiac output, favourable changes in haemodynamic load, and LV remodelling during gestation, changes in cardiac function are not well understood. Previous literature has shown either unchanged (Melchiorre et al., 2016, Clapp and Capeless, 1997, Valensise et al., 2000, Savu et al., 2012, Geva et al., 1997, Mesa et al., 1999, Yoon et al., 2011, Papadopoulou et al., 2013, Sengupta et al., 2017) or reduced (Zentner et al., 2009, Kametas et al., 2001, Estensen et al., 2013, Cong et al., 2015) ejection fraction (EF) during the final trimester. Reductions in EF are used clinically to diagnose systolic dysfunction, and many authors interpret the lower EF in healthy pregnancy as 'impaired systolic function.' However, the reported reductions in EF during healthy pregnancy are modest and not clinically relevant (Poppas et al., 1997). Additionally, the interpretation of EF as an indicator of cardiac function during pregnancy is limited: firstly, EF does not distinguish between the contributions of contraction and relaxation to overall function, secondly, EF is not sensitive to changes in haemodynamic load (known to be altered in healthy gestation), and finally, EF is unresponsive to subtle changes in function (suggested to be the case in healthy pregnant females) (Konstam and Abboud, 2017). As shown in Figure 5, a similar EF may be result of markedly different haemodynamic loads, and without further cardiac evaluation, it is impossible to unpick what factors may be influencing the development of cardiac output. Therefore, additional assessments of systolic and diastolic function are required to fully understand the influence of healthy pregnancy on cardiac function.

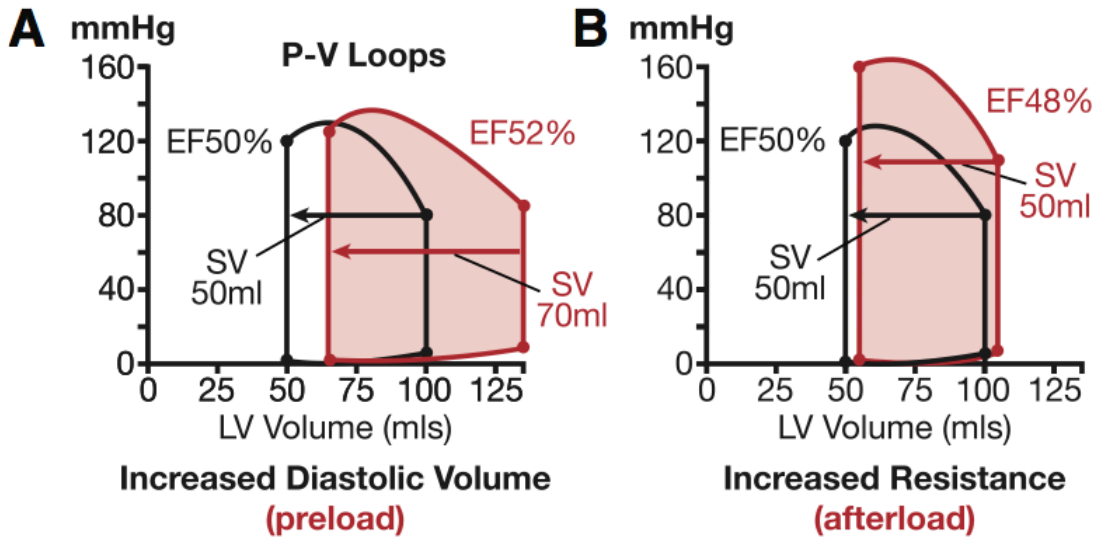


Figure 5. Ejection fraction is not a sensitive measure of cardiac function during alterations of preload and afterload. Changes in pressure volume (P-V) loops and ejection fraction (EF) and stroke volume (SV) of the healthy left ventricle to increased preload (Panel A) and increased afterload (Panel B). Increased end-diastolic volume causes an increase in SV and contractile force via the Frank-Starling mechanism, however EF is barely changed. Increased vascular resistance increases arterial pressure and contractile force, maintaining SV, however EF is again, barely changed. Adapted from Konstam and Abboud (2017).

To add to the confusion surrounding cardiac function in healthy pregnancy, long-axis displacement of the LV during systolic contraction (S') has been shown to increase early in pregnancy, before returning to non-pregnant levels late in pregnancy (Zentner et al., 2009, Savu et al., 2012, Bamfo et al., 2007). Previous authors have interpreted this decline from early to late gestation as an 'impaired' systolic function (Bamfo et al., 2007), however such findings may also reflect an enhanced function early in gestation that returns to normal levels. The lower systolic function may be linked to the higher HR in the final stages of gestation. A shorter diastolic period may reduce cardiac preload, lowering the contractile force through the Frank-Starling mechanism and/or the time for coronary artery perfusion, negatively impacting upon myocardial oxygen delivery and myocyte contraction (Kametas et al., 2001).

Similar to systolic function, previous authors have also suggested that diastolic function may also be reduced in the late stages of gestation (Kametas et al.,

2001). Passive and active ventricular filling is reflected in a biphasic pattern of early (E' and E) and late (A' and A) diastolic tissue and trans-mitral blood flow velocities, respectively. In the latter stages of pregnancy, E' and E are decreased, suggestive of reduced diastolic relaxation, whilst A' and A are increased, evidencing a greater reliance on atrial contraction for ventricular filling (Moran et al., 2002, Yosefy et al., 2012, Valensise et al., 2000, Song et al., 2015, Kametas et al., 2001, Sengupta et al., 2017, Estensen et al., 2013, Bamfo et al., 2007). Early diastolic filling during pregnancy is reduced due to reduced time for passive LV filling and a decreased trans-mitral pressure gradient causing resistance to passive diastolic flow (Yosefy et al., 2012, Moran et al., 2002, Song et al., 2015) (both reducing E and E'), whereas late diastolic filling is enhanced via greater blood volume causing a larger atrial stretch and therefore atrial contractile force (Song et al., 2015) (increasing A and A'). Overall, previous work has shown that the late stages of gestation are associated with reductions in both systolic and diastolic function that are inextricably linked and could contribute to the potential reduction in cardiac output in the third trimester. It is also possible that changes in haemodynamic load in late gestation, such as lower preload and greater afterload, discussed previously, may also contribute to reductions in filling and ejection.

Traditional methods of assessing cardiac function do not provide insight into the complexities of cardiac function (Mottram and Marwick, 2005). As a result, the understanding of functional adaptations to pregnancy based on these measures is compromised. In addition to the limitations of EF described previously, tissue velocity measures are also limited by a lack of consideration for regional differences in myocardial contractile velocity (Chahal et al., 2010) and deformation in other planes of motion (Mottram and Marwick, 2005). As a result, more advanced techniques are required to interpret functional changes comprehensively. Therefore, methods allowing the quantification of global and

multi-directional myocardial deformation across the cardiac cycle, known as LV mechanics, could be utilised to provide a comprehensive assessment of cardiac function. Using these methods, it may be possible to determine whether cardiac function is enhanced, unchanged or reduced during healthy pregnancy.

The role of ventricular mechanics in understanding cardiac function

Deformation of the myocardium describes the multi-directional multi–speed movement of heart segments during contraction and relaxation. LV mechanics, in the form of strain and rotation, can provide greater detail of systolic and diastolic function across the cardiac cycle (Armstrong and Ryan, 2009). LV mechanics are measured using speckle-tracking echocardiography (STE), described in more detail in Chapter 4. The following sections will provide an overview of LV mechanics, the impact of changes in haemodynamic loading on LV mechanics in healthy adults, and finally, changes in LV mechanics during healthy gestation.

Strain

Strain is a measure of tissue deformation and is defined as the change in length of a segment of myocardium relative to its resting length (Marwick, 2006). Strain is expressed as a percentage change from the original position and strain rate is the speed of this deformation (D'Hooge et al., 2000). Lagrangian strain can be measured in three planes: longitudinal, circumferential and radial strain (Figure 6) using speckle-tracking echocardiography (STE) (Collier et al., 2017). Negative strain implies shortening of a segment, whereas positive strain indicates lengthening of a segment. Normal myocardial contraction is defined by negative longitudinal and circumferential strain and positive radial strain (wall thickening) (Marwick, 2006, Armstrong and Ryan, 2009).

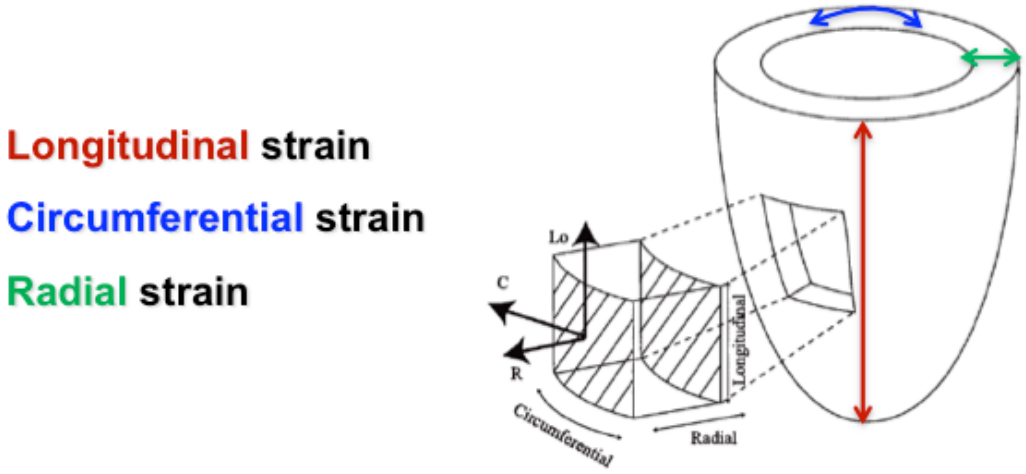


Figure 6. Left ventricular strain is measured in three myocardial axes: long, short and transmural, reflected by longitudinal, circumferential and radial strain, respectively. Longitudinal strain refers to the long-axis deformation towards the base and away from the apex of the heart. Circumferential strain refers to the deformation of the short-axis of the heart. Radial strain is defined as the deformation of the transmural myocardial axis, and reflects wall thickening. Adapted from D'Hooge et al. (2000).

Measurements of strain overcome some of the limitations of traditional functional parameters, discussed previously, by enabling assessment of segmental contractile properties in different planes of motion during both systole and diastole (Feigenbaum et al., 2012, Marwick, 2006). Therefore, strain is considered a more sensitive measure of cardiac function (Feigenbaum et al., 2012) and may be used to identify the subtle changes in function assumed to occur during healthy pregnancy (Poppas et al., 1997). In support of this, previous work has shown that reductions in strain occur prior to reductions in EF and the development of overt disease (Smiseth et al., 2016). Additionally, there is increasing support for the use of strain in the prediction of morbidity and mortality. Strain has been shown to be an independent predictor of all-cause mortality in heart failure (Sengelov et al., 2015), chronic kidney disease (Krishnasamy et al., 2015) and as an early predictor of cardiotoxicity in cancer therapy (Guerra et al., 2016). Strain is therefore considered a superior measure of cardiac function in comparison to EF and tissue velocities (Feigenbaum et al., 2012). The assessment of longitudinal and circumferential strain during healthy pregnancy may provide insight into whether

cardiac function is reduced prior to the overt changes in traditional measures observed in the late third trimester.

Twist and untwist

During ventricular contraction, obliquely orientated myofibres of the epicardium and endocardium cause the apex and base to rotate in opposite directions, resulting in a wringing motion of the LV (Sengupta et al., 2008, Stöhr et al., 2016). Clockwise basal rotation occurs in conjunction with anticlockwise apical rotation during systole, with net rotation termed twist (Figure 7a. and b.) (Stöhr et al., 2016, Sengupta et al., 2007). In healthy adults, apical rotation is dominant, as the larger radius of the epicardial layer results in greater torque through an increased lever arm (Figure 7c.). The basal endocardial fibres act as a brake to the momentum of twist and act as an opposing force to the epicardial contraction (Sengupta et al., 2008).

During diastole, the LV recoils to its unstressed length and shape, with this particular motion termed ‘untwist’. The rate of untwist is driven by the preceding twist (Thompson et al., 2010). During contraction, actin and myosin filaments generate active force within the sarcomeres (Linke and Hamdani, 2014). As a result, potential energy is generated and stored in cardiac proteins, such as titin (Anderson and Granzier, 2012) and sheer stress builds up between endo- and epicardial layers (Esch and Warburton, 2009). During early myocardial relaxation, this passive tension is released resulting in the rapid untwist of the endo- and epicardial helical fibres (Buckberg et al., 2011).

Assessment of LV twist mechanics can provide additional insight into ventricular contraction and relaxation. While LV twist facilitates the development of SV and hence cardiac output; increasing evidence supports an important role in equalising the distribution of transmural stress across the LV during systole (Young and Cowan, 2012, Stöhr et al., 2016, Vendelin et al., 2002). Additionally, untwisting

velocity (Figure 7d.) is an important aid to passive diastolic filling (Notomi et al., 2008, Rademakers et al., 1992). As discussed previously, diastolic filling (represented by E) is dependent on the trans-mitral pressure gradient (Levick, 2010). Untwisting generates a steep base-to-apex pressure gradient that causes a suction action in the LV that facilitates early diastolic inflow (Esch and Warburton, 2009, Notomi et al., 2008, Rademakers et al., 1992).

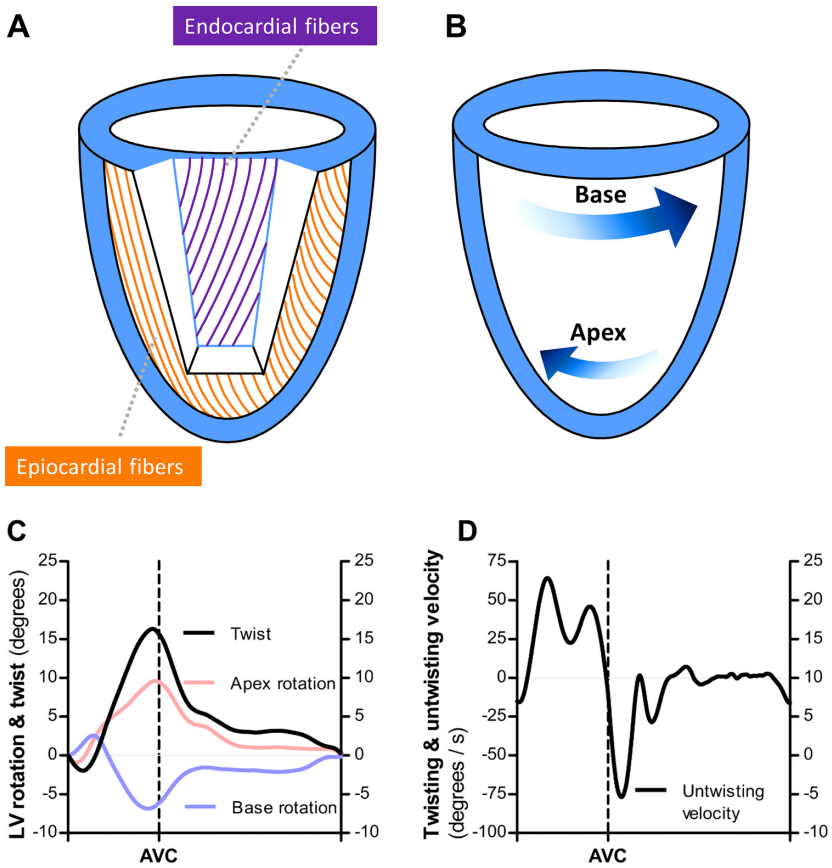


Figure 7. Left ventricular rotation occurs as a result of a. double helical myofibre orientation that causes b. anticlockwise and clockwise movement during systole of the apex and base, respectively. The net rotation is termed twist and the speed of relaxation from the preceding twist is the untwisting velocity, with typical curves for a healthy adult shown in c. and d., respectively. The left ventricle is separated into endocardial left-handed helical fibres and epicardial right-handed helical fibres. During contraction, the contraction of fibres in both helical arrangements results in opposite rotations of the base (clockwise) and apex (anticlockwise). As the heart contracts, potential energy develops and is transiently stored until release during early diastole. The rapid uncoiling of the obliquely orientated fibres, or untwist, produces a suction effect to aid passive ventricular filling. Adapted from Stöhr et al. (2016).

Due to the additional insight into cardiac function that LV twist mechanics provide, assessment of twist and untwist in healthy and clinical populations is increasing. However, the application of these parameters into clinical practice is currently limited by an understanding of what constitutes subclinical changes in LV twist mechanics and what impact changes in twist/untwist have on prognostic outcomes (Stöhr et al., 2016). Broadly, twist can be increased, unchanged or decreased in individuals with diagnosed cardiovascular disease and reduced or delayed

untwisting is suggested to cause impaired diastolic function (Stöhr et al., 2016). Therefore, population specific investigations are required to identify how physiology/pathology affects twist mechanics in order to derive normative values, that may in time be useful in the identification of abnormal function. Most importantly, the interpretation of cardiac function with either LV twist mechanics and/or LV strain must be considered in the context of haemodynamic load and cardiac structure, as these factors are known to impact upon LV mechanics. As discussed previously, healthy pregnancy results in cardiac remodelling as well as increased preload and contractility, with reduced afterload. Therefore, LV mechanics in healthy pregnancy are likely to be altered.

Effects of structure, preload, afterload and contractility on LV mechanics

Ventricular mechanics are influenced by cardiac structure and geometry (Oxborough et al., 2016, van Dalen et al., 2010, Young and Cowan, 2012). The measurement of strain removes the influence of size in measurement (% change from original position), however previous work in an athletic population has shown a relationship between longitudinal strain and wall thickness (Oxborough et al., 2016). Increased LV cavity size was suggested to increase the contribution of longitudinal deformation to ejection (Oxborough et al., 2016). Additionally, previous studies have shown that a larger heart experiences greater apical rotation, and therefore twist, due a greater lever arm of epicardial fibres (Young and Cowan, 2012). However, unpublished work from the Physiology and Health laboratory at Cardiff Metropolitan University has shown similar twist in untrained and endurance-trained males (the latter with significantly greater LV mass and LV length) (Cooke et al., 2017). It is likely that endocardial fibre contraction strength increases proportionally to the increased epicardial torque of larger hearts, leading to a normalisation of LV twist (Cooke et al., 2017). These findings have been verified by a longitudinal aerobic exercise training study in young males (Weiner et

al., 2010a, Weiner et al., 2015). During the initial response to training (90-day), twist and untwist were transiently increased (Weiner et al., 2010a), but not different from baseline after 3 years of continued training despite significantly greater LV size (Weiner et al., 2015). Therefore, chronic physiological adaptation appears to result in normalisation of LV twist, subsequent to gross LV remodelling. As mentioned previously, changes in cardiac geometry may impact cardiac contractility, and in this instance, have been shown to also influence LV twist (van Dalen et al., 2010). A more spherical LV causes suboptimal myofibre angles for contraction and results in reduced mechanical efficiency of the heart (van Dalen et al., 2010), causing decreased LV twist.

Haemodynamic load and sympathetic activity also influence LV mechanics (Sengupta et al., 2008, Marwick, 2006, Stöhr et al., 2016). In general, acute reductions in afterload or increases in preload and sympathetic activity cause increased LV twist, untwist, longitudinal and circumferential strain, as shown in Figure 8 (for LV twist only) (Burns et al., 2010b, Burns et al., 2010a).

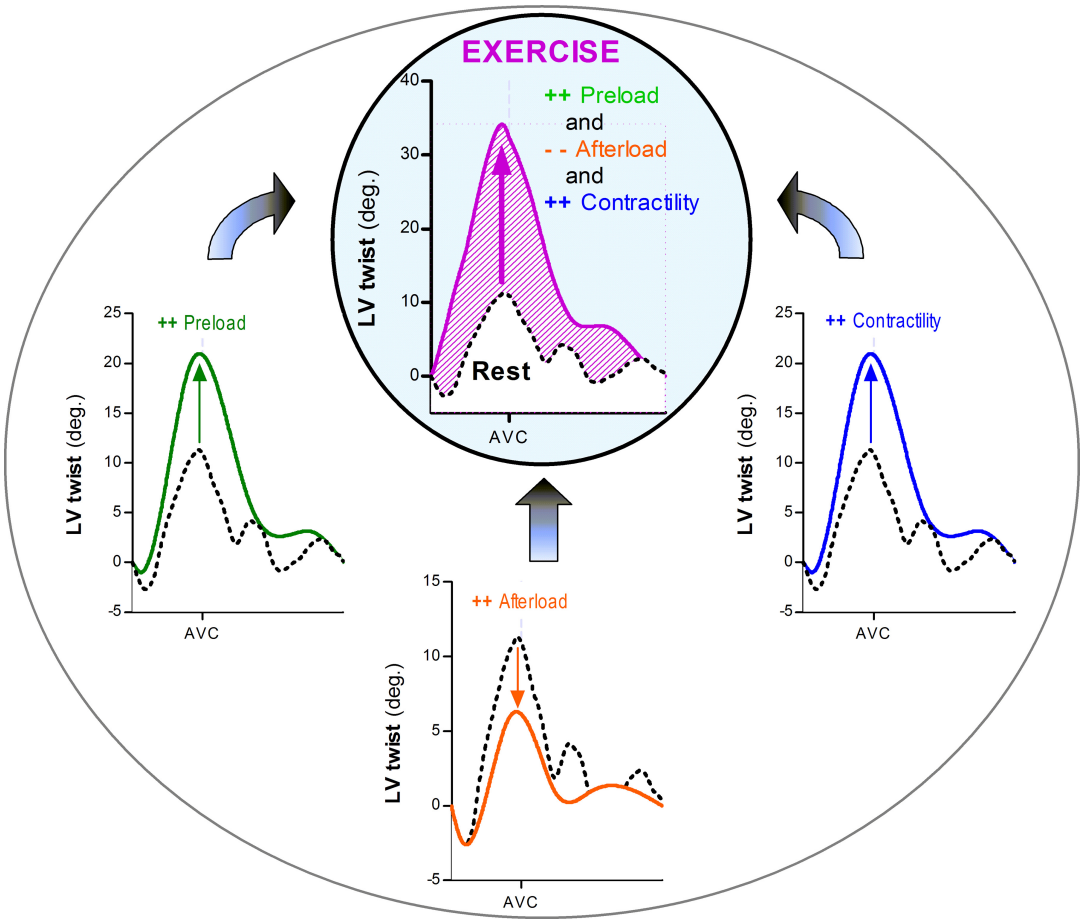


Figure 8. Left ventricular (LV) mechanics are affected by isolated and global alterations to cardiac loading. Specifically, in response to isolated increases in preload (left panel) and contractility (right panel), twist is increased, whereas in response to an isolated increase in afterload (bottom panel), twist is decreased. During physiological challenge, such as aerobic exercise, twist is increased in line with increased preload and contractility and altered afterload (top panel). Adapted from Stöhr et al. (2016).
N.B. *AVC,* aortic valve closure.

Changes in preload can influence cardiac function by altering LV volume loading (Figure 8, left panel). In response to acutely increased preload, mediated by saline infusion, EDV, longitudinal and circumferential strain and LV twist were increased (Weiner et al., 2010b). In contrast, acute reductions in preload, achieved through the use of lower body negative pressure, caused significant reductions in EDV, longitudinal and circumferential strain but increased LV twist (Williams et al., 2016). Greater EDV leads to significantly increased strain and twist suggestive of a greater myocardial contraction via the Frank-Starling mechanism.

Altered afterload can also impact LV mechanics (Figure 8, bottom panel). During isometric handgrip, in which systolic BP and HR were significantly increased, LV twist (Weiner et al., 2012, Balmain et al., 2016) and longitudinal strain (Weiner et al., 2012) were significantly decreased. The increased afterload reduces myocyte shortening and subsequently affects ejection and contractile function.

Global changes in haemodynamic load collectively influence LV mechanics. For example, during aerobic exercise (Figure 8, top panel), in which preload and contractility are increased and afterload reduced, LV twist and longitudinal strain are significantly increased (Stöhr et al., 2011b, Donal et al., 2011). However, alterations in LV mechanics may be influenced more greatly by one aspect of haemodynamic load. For example, administration of glycerol trinitrate, reducing preload and afterload, resulted in significantly increased circumferential strain and LV twist (Burns et al., 2010b, Burns et al., 2010a). In this instance, the decrease in afterload appears to have greater influence on LV mechanics, whereas the influence of reduced preload (associated with a reduction in LV mechanics) is negated. Collectively, these findings reinforce that any changes in LV mechanics must be interpreted alongside changes in the prevailing haemodynamics.

The majority of experimental work investigating the influence of haemodynamic load on LV mechanics has been completed using predominantly male cohorts (Weiner et al., 2012, Donal et al., 2011, Balmain et al., 2016, Stöhr et al., 2011b, Burns et al., 2010b, Burns et al., 2010a). Young healthy males and females are known to have significant differences in cardiac structure (Nio et al., 2015, Lang et al., 2015, Kuznetsova et al., 2008, Kaku et al., 2011) and function (Kaku et al., 2011, Williams et al., 2016, Celentano et al., 2003, Zhong et al., 2014, Kishi et al., 2015, Kuznetsova et al., 2008, Chung et al., 2006, Giraldeau et al., 2015). Healthy young females have significantly greater longitudinal and circumferential strain at rest when compared to males (Kishi et al., 2015, Hurlburt et al., 2007, Giraldeau et

al., 2015, Reckefuss et al., 2011, Williams et al., 2017, Lawton et al., 2011), although null findings have also been reported (Williams et al., 2016). Twist, torsion, apical and basal rotations are not different between the sexes at rest (Williams et al., 2016, Sun et al., 2013, Williams et al., 2017). However, in response to physiological challenges, such as reduced preload, females exhibit greater LV twist in comparison to males (Williams et al., 2016). Myocardial regulation is suggested to be different between the sexes. Specifically, the male heart appears to be more sympathetically mediated, whereas the female heart is volume sensitive with greater vagal control (Fu et al., 2004, Williams et al., 2017). At present there is only limited research addressing sex dimorphism in cardiac structure and function. The sex differences noted to date are linked to female sex steroid hormones (Fu et al., 2004, Williams et al., 2017, Nio et al., 2015), and therefore, these differences may be exacerbated by pregnancy, in which the hormonal profile of the female is significantly enhanced. As a result, hormonal influences or their downstream effects on haemodynamic load may explain changes in cardiac structure and function, specifically LV mechanics.

Left ventricular mechanics in healthy pregnancy

As discussed throughout this literature review, healthy pregnancy is associated with significant cardiac remodelling, as well as changes in haemodynamic load and sympathetic activity; therefore LV mechanics are likely to also be affected. However, LV mechanics during healthy pregnancy have only been assessed in nine previous studies: a summary of the research design and findings from each are presented in Table 1. Reduced longitudinal and circumferential strain have been observed in the first (Sengupta et al., 2017), second (Papadopoulou et al., 2013) and third trimester of pregnancy (Cong et al., 2015, Estensen et al., 2013, Savu et al., 2012), although no significant differences have also been noted in two

studies (Ando et al., 2015, Tzemos et al., 2008). The reductions in strain were interpreted as an impaired systolic function and support the findings of reduced EF and S', described earlier.

Contrasting the decline observed in traditional measures of cardiac function, LV twist and untwist have been reported to be greater in the final trimester of pregnancy (Hristova et al., 2016, Papadopoulou et al., 2013, Tzemos et al., 2008, Yoon et al., 2011) and previous authors have interpreted this as enhanced function (Hristova et al., 2016, Papadopoulou et al., 2013, Tzemos et al., 2008, Yoon et al., 2011). However, it must be noted that in comparison to healthy controls, different cardiovascular disease states are linked to both significantly greater and significantly lower LV twist (Stöhr et al., 2016). Therefore, a greater LV twist does not necessarily equate to enhanced function. As discussed above, the normative ranges for LV twist in specific populations are not yet understood, and this is certainly the case during healthy pregnancy. The increase in twist previously observed in the final trimester therefore requires further scrutiny.

The assessment of LV mechanics in addition to traditional echocardiographic measures will enable the understanding of whether cardiac function is enhanced, unchanged or reduced during healthy pregnancy. Furthermore, both strain and twist will help to unpick how the changes in haemodynamic load, such as increased preload and contractility with reduced afterload, contribute to the change in maternal cardiac function, and therefore the generation of cardiac output.

Table 1. Summary of observational studies investigating left ventricular mechanics in human pregnancy.

	Study description	Longitudinal strain	Circumferential strain	Twist
Ando et al. (2015)	Retrospective cross sectional study; 37 healthy pregnancies at T2 and at T3 with NP controls.	No change.	No change.	
Cong et al. (2015)	Longitudinal study; 68 healthy pregnancies; T1, T2, T3 and 8 months PP with NP controls.	↓T3 (38 wks).	↓T3 (38 wks).	
Estensen et al. (2013)	Longitudinal study; 65 healthy pregnancies; T1, T2, T3 and 6 months PP.	↓T3 (36 wks).		
Hristova et al. (2016)	Longitudinal study; 10 pregnancies; T1, T2, and T3.			↑T3.
Papadopoulou et al. (2013)	Longitudinal study; 27 healthy pregnancies; T1, T2, T3 and 8 months PP with NP controls.	↓T2 (21-28 wks) until term.	↓T2 (21-28 wks) until term (apical).	↑T3 (33-36 wks).
Savu et al. (2012)	Longitudinal study; 51 healthy pregnancies; T1, T2, T3 and 3-6 months PP with NP controls.	↓T3 (32-33 wks).	No change.	
Sengupta et al. (2017)	Longitudinal study; 30 healthy pregnancies; T1, T2, T3 and labour with NP controls.	↓T1 (10-12 wks) until term.	↓T1 (10-12 wks) until term.	
Tzemos et al. (2008)	Cross sectional study; 10 healthy pregnancies; T2; with 10 NP controls; 10 pregnant females with valve disease	No change.		↑T2.
Yoon et al. (2011)	Longitudinal study; 32 pregnant females; T2 and T3 with NP control; recruited in murmur evaluation.			↑T2 and T3.

Summary of cardiac functional adaptation to healthy pregnancy

In summary, gestational changes in haemodynamic load, sympathetic activity and cardiac structure cause alterations in systolic and diastolic function of the maternal heart. Healthy pregnancy is associated with increased preload (via blood volume expansion) and contractility (via increased sympathetic activity), as well as reduced afterload (via systemic vasodilation). Additionally, the maternal heart undergoes significant remodelling. These changes in haemodynamic load, sympathetic activity and structure collectively influence cardiac function, but presently there is a lack of clarity as to whether this reflects a reduction or enhancement during healthy pregnancy. As changes in cardiac function during healthy pregnancy are suggested to be subclinical (Poppas et al., 1997), traditional echocardiographic parameters may not be sensitive enough to determine these modest alterations, especially at time points prior to the third trimester. Therefore, the understanding of maternal cardiac function during pregnancy may be improved through the additional assessment of LV mechanics whilst bearing in mind the influence of changes in cardiac structure, haemodynamic load and sympathetic activity.

2.5. Cardiovascular responses to functional haemodynamic testing

So far within this review, maternal cardiac function has only been considered at rest, however, the resting state does not reflect the capacity of the heart to respond to additional demand or changes in haemodynamic load.

The functional capacity of the cardiovascular system, also known as cardiovascular reserve, reflects the ability of the heart to adapt to altered haemodynamic load and increased cardiovascular demand (Koelwyn et al., 2012). Impaired responses to physiological challenges may indicate a reduced cardiovascular reserve, and therefore help identify subtle changes in cardiac function that may not be evident at rest (Gibbons et al., 1997). At rest, during pregnancy, global function (cardiac output, HR, SV) are elevated above non-pregnant levels, which may result in a reduced functional reserve. Higher resting values of systolic function (specifically strain and twist) may lower the capacity for mechanics to adapt and meet the demands of additional physiological challenge (Sengupta et al., 2008). Therefore, challenging and assessing the maternal cardiovascular reserve during pregnancy may help to elucidate if cardiac function is reduced, maintained or enhanced during pregnancy (Ohuchi et al., 2013).

Acute physiological challenges, such as an increased afterload or submaximal aerobic exercise result in coordinated changes in LV function, vascular compliance and neurohormonal control in order to match cardiac work to demand (Fournier et al., 2014). The postulated reductions in cardiac function in the final trimester of pregnancy may be explained by the increasing afterload (Melchiorre et al., 2012a), reducing preload and peak metabolic demand (Lof et al., 2005) observed at this time point. It is therefore possible, that the third trimester of pregnancy experiences reduced resting systolic and diastolic function (Kametas et al., 2001, Bamfo et al., 2007) as a result of a reduced cardiovascular reserve. Undertaking physiological challenges that alter haemodynamic load and increase metabolic

demand in the first or second trimester may therefore acutely mimic the overall cardiovascular function of late pregnancy (Meah et al., 2013). It is possible that dynamic assessment of cardiovascular function in healthy pregnancy may provide some clarity on the changes in systolic and diastolic function in the maternal heart. Additionally, use of different physiological challenges may identify whether preload, afterload, contractility or increased metabolic demand drive the altered cardiac function during pregnancy.

Cardiovascular responses to increased afterload

Haemodynamic load can be altered through the use of an afterload challenge, such as isometric handgrip (IHG). During this stimulus, the mechano- and metabo-reflex are activated causing increased arterial BP (approximately 15 mmHg rise in DBP), HR, cardiac output (Balmain et al., 2016, Weiner et al., 2012) and sympathetic activity (Zygmunt and Stanczyk, 2010, Rowland and Fernhall, 2007, Lalande et al., 2014). The physiologically induced transient increase in afterload is suggested to be similar to those experienced in response to resistance exercise and in disease states such as hypertension (Weiner et al., 2012).

During IHG, increased afterload, HR and sympathetic activity influence cardiac function. Previous research has shown significant reductions in EF, S', longitudinal strain and LV twist in a healthy population during IHG (Weiner et al., 2012). Additionally, circulatory occlusion post-IHG led to more pronounced reductions in LV twist and untwist, despite a return of HR to resting values (Balmain et al., 2016). Overall, acutely increased afterload results in reduced systolic function in healthy hearts.

As discussed earlier, the majority of research investigating functional responses (particularly LV mechanics) to afterload challenges has been conducted in predominantly male cohorts (Weiner et al., 2012, Balmain et al., 2016). However,

young healthy females have shown attenuated HR and MAP (Wong et al., 2007, Melrose, 2005, Momen et al., 2006) and sympathetic activity (Jarvis et al., 2011) responses to increased afterload in comparison to young healthy males. It is therefore possible that the functional response in young healthy males and females to the same afterload stimulus may be different. However, a recent study found no significant differences in global haemodynamics and cardiac function between the sexes during IHG (Williams et al., 2017). Despite this finding, there is potential that young healthy males and females may respond differently to increased afterload, which may be further exacerbated by pregnancy.

Previous research has shown that isometric exercise elicits similar increases in cardiac output, HR and BP in healthy pregnant *versus* non-pregnant females (Avery et al., 1999) and healthy pregnant *versus* postpartum females (Lotgering et al., 1992b). However, specific measures of cardiac systolic or diastolic function were not assessed within these studies. It is not known if contraction and relaxation of the healthy maternal heart is reduced during increased afterload.

Cardiovascular responses to acute submaximal aerobic exercise

Submaximal aerobic exercise is a global challenge that requires an integrated and coordinated cardiovascular response to match blood supply with increased metabolic demand and altered haemodynamic load (Levick, 2010). At the onset of exercise, the sympathetic system is stimulated and vagal tone dampened, increasing HR and contractility to increase output. Despite an increase in arterial BP, metabolic-induced systemic vasodilation reduces SVR (Klabunde, 2005). Preload is increased up to moderate intensity activity via a greater venous return through the muscle and respiratory pumps (Klabunde, 2005). Therefore, submaximal exercise causes increases in preload, HR, sympathetic activity and decreases in afterload, which contribute to enhanced global cardiac function (Esch

and Warburton, 2009). This is evidenced by an increase in cardiac output, SV, EF, S' as well as LV strain and twist from rest to submaximal exercise in healthy adults (Levick, 2010, Donal et al., 2011, Stöhr et al., 2011b).

Cardiovascular responses to submaximal aerobic exercise are influenced by sex. During the same relative intensity of aerobic exercise, young healthy females have greater HR, lower cardiac output, SV (Carter et al., 2001) and BP (Deschenes et al., 2006), greater EF and longitudinal strain (Williams, 2016) compared to males. In contrast, no differences in LV twist mechanics have been observed between young healthy males and females during exercise (Nio et al., 2013, Williams, 2016). Whilst this area requires further investigation, current data suggest that the greater cardiac output required for aerobic activity is achieved via greater systolic contraction in females.

Healthy pregnant women respond to exercise in a similar manner as their non-pregnant counterparts. Non-pregnant and pregnant females achieve similar HRs in response to relative submaximal exercise, however pregnant females have a smaller increase from rest due to the increased resting HR (Khodiguian et al., 1996, Finkelstein et al., 2011). Some studies have observed an increased exercise HR in pregnant females, however this is likely the result of the use of absolute workloads to prescribe intensity as opposed to individualised loads (Wolfe and Weissgerber, 2003). Similar to previous work examining afterload challenges during pregnancy, cardiac output, SV and BP responses to submaximal aerobic exercise were unaffected by gestation (May, 2015, Finkelstein et al., 2011, Sady et al., 1989, Heenan et al., 2001). The systolic and diastolic responses of the maternal heart to exercise have been investigated previously in two studies. Veille et al. (1992) completed echocardiography in sixteen healthy pregnant women during incremental exercise. At maximal exercise, end-diastolic diameter (reflecting volume) and fractional shortening (an index of systolic function) were

increased, whilst end-systolic diameter was decreased, as observed during exercise in any healthy adult. The findings from this study suggest that the maternal LV can adequately respond to the additional physiological demand of exercise during pregnancy and that cardiovascular reserve was not reduced. A later longitudinal study by the same group assessed diastolic function in ten healthy women in each trimester and at 6 weeks postpartum (Veille et al., 2001). Similar to the observations of resting diastolic function described earlier, passive filling (E) was significantly decreased and the atrial contribution to filling significantly increased (A) during exercise, suggesting again that the altered dynamics of diastole observed at rest continue under additional challenge. Whilst these studies were novel, and remain so, the echocardiographic methods used are now out-dated. As discussed previously, the newer and more novel assessment of LV mechanics may improve the identification of subtle changes in maternal cardiac function prior to overt reductions in traditional measures late in pregnancy. The sensitivity of LV mechanics may be further strengthened during physiological perturbation, which allows further insight into maternal cardiovascular reserve and also enables an understanding of how increased demand and altered haemodynamic load affect function during gestation.

2.7. General summary

In conclusion, the cardiovascular system is modified by sex itself, and by sex specific changes in physiology, such as those observed during pregnancy. Using non-pregnant females as the reference point, the known differences in cardiovascular structure and function in healthy pregnant females are presented in Table 2. These differences appear to be related to an increased preload caused by a blood volume expansion, reduced afterload subsequent to systemic vasodilation and increased sympathetic activity. Although increases in cardiac output, HR and SV are well established in the literature, the timing and magnitude of these changes across gestation are less clear and likely fluctuate as a result of changes in haemodynamic load and increasing metabolic demands. Such changes also influence function of the maternal heart, and although a lack of consensus exists, previous reports generally suggest a modest reduction in systolic and diastolic contraction and relaxation in the final stages of pregnancy.

Table 2. Summary of differences in pregnant female cardiovascular structure and function in comparison to healthy non-pregnant females.

	Rest	***Afterload challenge***	***Acute exercise***
Heart rate	↑	=	=
Blood pressure	↓ or =	=	=
Cardiac output	↑		=
Stroke volume	↑		=
End-diastolic volume	↑		
LV mass	↑		
Sphericity index	↓ more spherical		
Ejection fraction	↓ or =		
S’	=		
E’ and E	↓		↓
A’ and A	↑		↑
Longitudinal strain	↓		
Circumferential strain	↓		
Twist	↑		
Untwist	↑		

N.B. LV, left ventricular; S’, systolic tissue velocity; E’ early diastolic tissue velocity; E, early mitral inflow velocity; A’ late diastolic tissue velocity; A, late mitral inflow velocity.

Functional haemodynamic testing during healthy pregnancy may help clarify whether cardiac function during gestation is enhanced, unchanged or reduced. Using acute physiological challenges to test cardiovascular responses will identify if the maternal heart is able to increase its capacity to meet increased demand. Specifically, increased afterload may mimic the altered haemodynamic load of late gestation and submaximal aerobic exercise may mimic the global cardiovascular function and increased metabolic demand of the third trimester. Further clarity in the changes to systolic and diastolic functions in healthy pregnancy would aid future research examining abnormal responses.

2.8. Aims and hypotheses

This literature review has provided an overview of cardiovascular physiology during healthy pregnancy. A lack of consensus related to the adaptations in cardiac structure and function across pregnancy warrants further research. Additionally, the interpretation of whether cardiac function is reduced or enhanced in healthy pregnancy is clouded by insensitive methods, a lack of consideration for haemodynamic loading and a lack of assessment of the functional capacity of the maternal cardiovascular system. Therefore, this thesis will aim to further the understanding of cardiac adaptation and functional cardiovascular responses in healthy pregnancy. This aim will be achieved by meeting certain objectives. Firstly, global cardiac function across healthy pregnancy will be characterised through use of meta-analyses, secondly, a comprehensive assessment of maternal cardiac function, with consideration of haemodynamic load, at rest will be completed, and thirdly, functional cardiovascular responses to physiological challenges will be investigated. The specific objectives of each chapter are presented below:

Chapter 3: ***Cardiac output and related haemodynamics during pregnancy: A series of meta-analyses***

Objective: To comprehensively describe the pattern and magnitude of change in cardiac output and related haemodynamics during healthy pregnancy at rest using previously published literature.

Chapter 4: ***General methodology for experimental studies***

Chapter 5: ***Cardiac structure and function at rest in non-pregnant, pregnant and postpartum females***

Objective: To investigate cardiac structure and function in the late second trimester of healthy pregnancy and in the postpartum period, with a specific focus on potential changes in underpinning mechanics and haemodynamic load.

Chapter 6: ***Functional cardiovascular responses to acute physiological challenges in non-pregnant, pregnant and postpartum females***

Objective: To examine functional responses to increased cardiovascular demand and altered haemodynamic load during the late second trimester of healthy pregnancy and in the postpartum period.

Chapter 7: ***General discussion***

Chapter 3. Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses

Foreword

Data from the present chapter has already been published in the following article:

Meah VL, Cockcroft JR, Backx K, Shave R, Stöhr EJ. (2016). Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. *Heart*. doi:10.1136/heartjnl-2015-308476.

Editorial:

Boardman H, Ormerod O, Leeson P. (2016). Haemodynamic changes in pregnancy: what can we learn from combined datasets? *Heart*. doi:10.1136/heartjnl-2015-309166.

3.1. Introduction

Cardiac output reflects the total demand placed on the maternal cardiovascular system at any specific point in time. As outlined previously, during pregnancy cardiovascular demand is increased due to the development of the fetoplacental circulation as well as increased blood flow to the maternal kidneys, breasts, skin and the heart itself (Desai et al., 2004, van Oppen et al., 1996, Wallenburg, 1990b). Despite a wealth of literature describing cardiac output during healthy gestation, there is a lack of consensus in published literature regarding the time course of adaptation (Desai et al., 2004, Clapp and Capeless, 1997, Savu et al., 2012, Mone et al., 1996, Easterling et al., 1990, Robson et al., 1989, Ogueh et al., 2009, Estensen et al., 2013, van Oppen et al., 1996, Melchiorre et al., 2012a). Previous reviews agree that cardiac output increases across pregnancy, however, there are discrepancies regarding the magnitude and pattern of change (van Oppen et al., 1996, Melchiorre et al., 2012a, May, 2015, Sanghavi and Rutherford, 2014). Specifically, cardiac output has been reported to follow three different patterns of change throughout pregnancy: (i) a continued increase until term (Desai et al., 2004, Clapp and Capeless, 1997, Savu et al., 2012); (ii) a continued increase to peak in the latter half of pregnancy, after which cardiac output decreases towards term (Mone et al., 1996, Easterling et al., 1990); (iii) a continued increase to peak in the latter half of pregnancy, after which cardiac output plateaus until term (Estensen et al., 2013, Robson et al., 1989, Ogueh et al., 2009).

The relative changes in the determinants of cardiac output also remain unclear across gestation (Melchiorre et al., 2012a). Increased preload (greater blood volume) and heart rate (HR), elevated sympathetic activity and reduced afterload (blood pressure and systemic vascular resistance; BP and SVR) may influence cardiac structural and functional adaptation in pregnancy (Clapp and Capeless,

1997, Savu et al., 2012, Melchiorre et al., 2012a, Robson et al., 1989, Ogueh et al., 2009, Mone et al., 1996, Mesa et al., 1999, Fok et al., 2006, Geva et al., 1997, Estensen et al., 2013, Easterling et al., 1990, Desai et al., 2004). The relative contribution of haemodynamic load to elevated cardiac output therefore requires further examination.

To improve the current understanding of normal cardiac adaption to pregnancy, insight from larger cohorts with greater statistical power than typically possible within pregnancy research is required (Dennis et al., 2012). Despite a wealth of literature in this area, there are many sources of heterogeneity that impact the interpretation of data. These include study designs, choice of control groups, patient characteristics, methodology, sample sizes, and gestational age at assessment. Therefore, meta-analyses were used within this chapter to combine previously published datasets to better inform our understanding of cardiac adaptation to healthy pregnancy. The overall objective of this study was to comprehensively describe the pattern and magnitude of change in cardiac output and related haemodynamics during healthy pregnancy at rest using previously published literature. It was hypothesised that:

(i) Cardiac output would be progressively greater across gestation, peaking in the latter half of pregnancy and declining towards term;
(ii) HR would be progressively greater across gestation, peaking at term;
(iii) SV and end-diastolic volume (EDV) would exponentially rise in the second trimester, alongside blood volume expansion, and be maintained until term;
(iv) Mean arterial pressure (MAP) would be lower in the second trimester, remaining at non-pregnant levels in the first and third trimester;
(v) SVR would be progressively lower across gestation, peaking in the latter half of pregnancy and returning to non-pregnant levels towards term;

(vi) Left ventricular (LV) mass would be progressively greater across pregnancy, peaking at term.

3.2. Methods

Ethical approval and search strategy

This study received ethical approval from the Cardiff Metropolitan University ethics board (12/06002R), (Appendix I.a.). A comprehensive literature search of the PubMed and Scopus databases for peer-reviewed publications examining the maternal cardiovascular responses to pregnancy was conducted. The pre-set search engine criteria, both on PubMed and Scopus, were restricted to studies using humans, females and publications written in the English language. Reviews, editorials, case reports and unpublished data were excluded. The keywords and phrases used in the online search included combinations of the words *cardiac output, maternal, cardiovascular, pregnancy, haemodynamic/hemodynamic, normotensive, and healthy,* referring to uncomplicated gestation. As the last review on cardiac output during pregnancy (van Oppen et al., 1996) was published in 1996, the search was limited to studies published between 1st January 1996 and 31st December 2014. There was no overlap of included studies between the last review and the current analyses.

Study selection criteria

Studies were eligible for inclusion in the meta-analyses if they met the following criteria;

(i) Examined uncomplicated, healthy, singleton pregnancies who conceived naturally;

(ii) Mean age of included participants was 19-35 years;

(iii) Participants were assessed during one or more of the following gestational ages, as shown in Figure 9: first trimester (6-13 weeks);

early second trimester (14-20 weeks); late second trimester (21-27 weeks); early third trimester (28-34 weeks), late third trimester (34 weeks-term); during the early (4-12 weeks) or late (13-52 weeks) postpartum period;

(iv) Provided the mean ($L \cdot min^{-1}$) and standard deviation of cardiac output;

(v) Assessed cardiac output using one of the following methods: magnetic resonance imaging (MRI), echocardiography, impedance cardiography or inert gas re-breathing.

Studies of longitudinal and cross sectional design were eligible to be included within the meta-analyses.

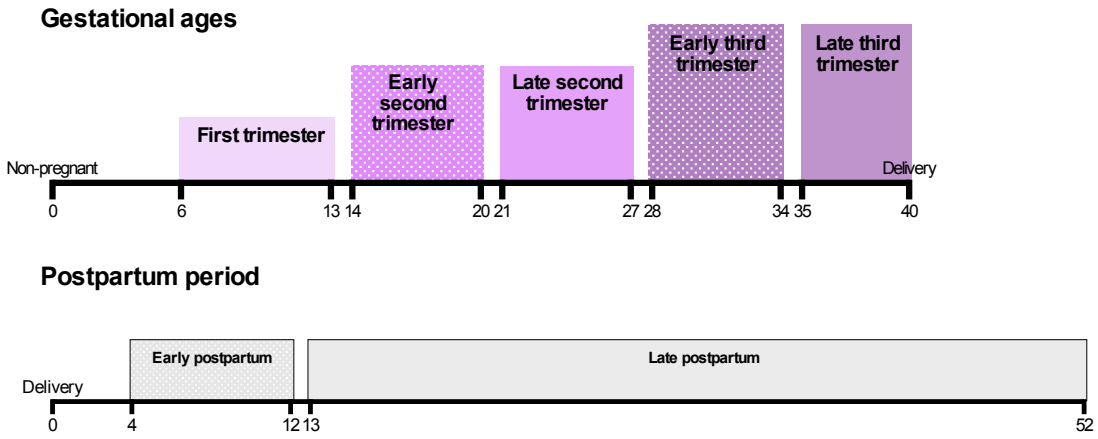


Figure 9. Schematic demonstrating the gestational ages and postpartum periods used for inclusion within the meta-analyses.

Outcome variables

The primary variable cardiac output was assessed across healthy pregnancy. Secondary variables were determinants of cardiac output, namely HR, SV, MAP, SVR, EDV and LV mass. MAP and SVR were used as indicators of cardiac afterload, EDV as a measure of cardiac preload, and LV mass as a measure of cardiac structure.

Study process

The titles and abstracts of all identified publications were independently screened and reviewed. Full text articles were retrieved for each study that was considered relevant from the initial evaluation. Full text articles were independently assessed by two reviewers (VM and EJS). Inclusion into the final dataset was based on the *a priori* selection criteria described. Consensus was sought on the final set of articles to be included and disagreements were resolved through discussion. Some issues could not be resolved according to the inclusion criteria set *a priori*. In studies where conception was not explicitly described, it was assumed that participants conceived naturally and not through use of reproductive therapies. Where cardiac output was only included in a graphical format or not reported in $L \cdot min^{-1}$ as a mean and SD, the corresponding authors of the original publications were contacted by email and asked to provide the required data. Suitable data provided by authors of original publications were included in the analyses. When data was not provided, the publications were excluded.

Data extraction

The lead author (VM) extracted all relevant data from the full-text articles to be included in the meta-analyses. The mean ± SD for cardiac output for each study was transferred into a predesigned form along with the sample sizes (Excel 2010, Microsoft Corp). Where reported, HR, SV, MAP, SVR, EDV and LV mass (mean ± SD, and sample size) were also extracted from the same studies.

Statistical analyses

As all parameters were continuous variables, sample size and mean ± SD were input into the analysis software (Comprehensive Meta-analysis software version 2.0, Biostat, Englewood, NJ, USA). Separate random-effects meta-analyses were applied to all primary and secondary outcome variables for each gestational stage (non-pregnant, first trimester, early and late second trimester, early and late third trimester, early and late postpartum). As the meta-analyses were based upon observational data obtained from populations that would unlikely have a common variance, a random-effects model was used. For each analysis, a weighted mean, standard error, variance, upper and lower limits were computed through the DerSimonian and Laird method (DerSimonian and Laird, 1986). The homogeneity of the reported data for each parameter was assessed, with no indication of skewness. Publication bias was evaluated through a funnel plot and, if present, was corrected through use of Duval and Tweedie's trim and fill method. Forest plots were created for each individual meta-analysis (Neyeloff et al., 2012) and presented as a compiled figure for each variable at each time point of analyses (Excel 2010, Microsoft Corp).

Use of a random effects model

True effects would inherently vary from study to study due to different effect sizes, and therefore a random-effects model was used to allow for variation between studies. Use of a random-effects model also allows for generalisation to similar studies that may be conducted in the future, allowing this dataset to serve as a potential reference point. A fixed-effects model was not appropriate for use in these analyses due to the assumption that there is only one true effect underlying all studies within the analyses. The methodological diversity within these analyses alongside the heterogeneity of the cohorts included created variability and hence bias, variably affecting the results of different studies. There is also an assumption

that the ‘intervention’ in this case, pregnancy, would have the same effect in all studies in both magnitude and direction, however as alluded to within the introduction of this chapter, previous studies have reported different trends in cardiac output, especially within the late third trimester. Through use of a random-effects model, inferences are also not limited to studies represented within this sample as when using a fixed-effects model (Hedges and Vevea, 1998). This allows generalisation of the reported data to similar, but non-identical studies, which may be conducted in the future i.e. provided the potential for the dataset to serve as a potential reference point. For the above rationale, the DerSimonian and Laird method was used to calculate the weighted means within the analyses software. This is a variation on the inverse-variance method and accounts for the assumption that different studies are estimating different - yet related - intervention effects as per the rationale for using a random effects model.

3.3. Results

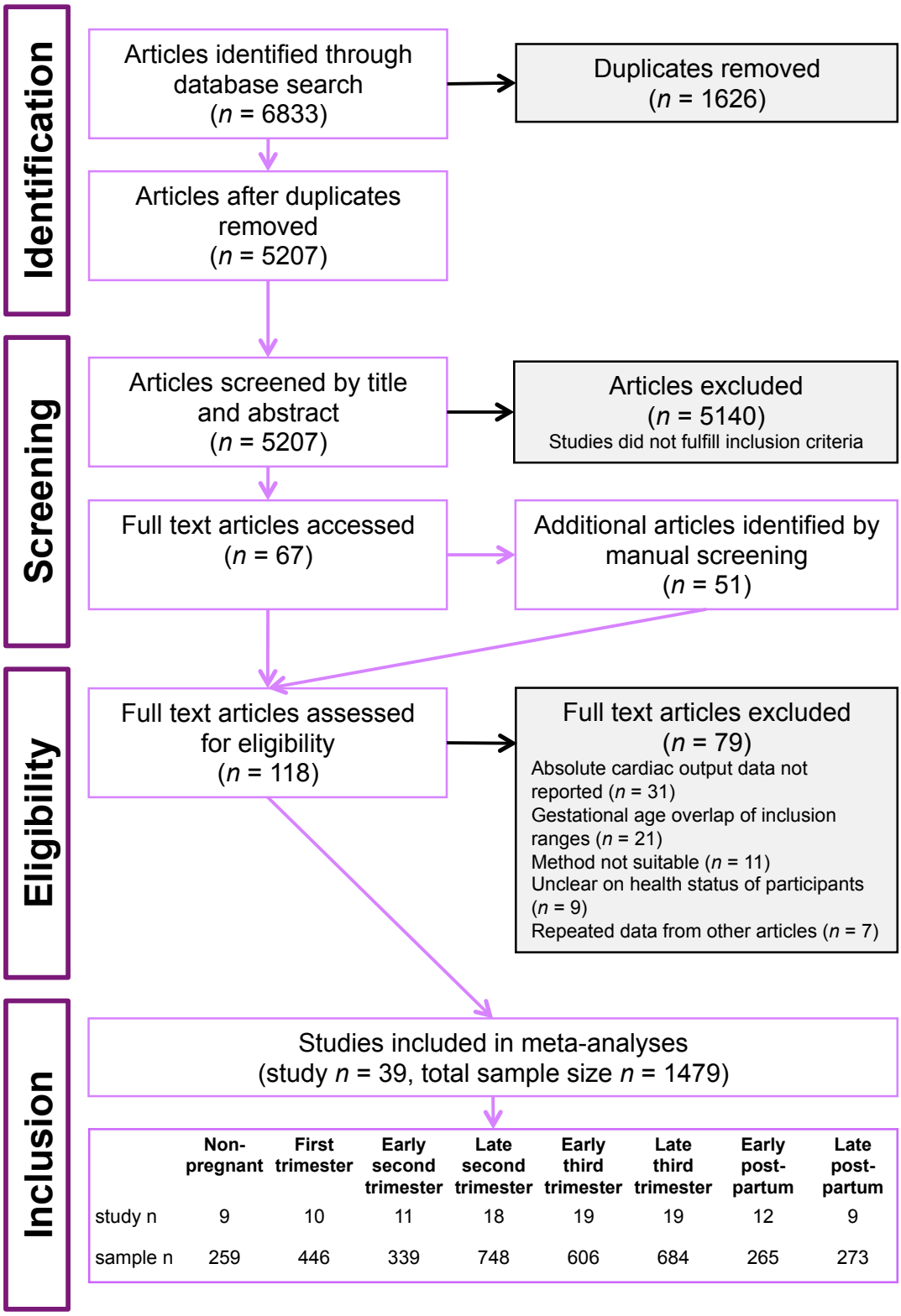
Search results

The search process, as illustrated in Figure 10, resulted in the inclusion of observational data from a total of 39 articles sourced from both the original database and reference list searches. Originally, only 32 articles were eligible as the numerical data were not reported as per requirement for inclusion, however, following email contact data was obtained for seven studies (Savu et al., 2012, Tamas et al., 2007, Armstrong et al., 2011, Cornette et al., 2011, Gyselaers et al., 2014, D'Silva et al., 2014, Vartun et al., 2014).

Four studies reported multiple data sets within one of the predetermined gestational age ranges *e.g.* data at week eight and week ten, both eligible to be included for first trimester (6 to 13 weeks) analyses (Ogueh et al., 2009, Mone et al., 1996, Moertl et al., 2012, Clapp and Capeless, 1997). In all cases, the data

was not included within the meta-analysis for that predetermined gestational age in order to avoid statistical bias that would arise from inclusion of multiple data sets from an individual study.

Following the review process, observational data from 39 studies were included within the final analyses however the number of studies included in the individual meta-analyses conducted for each of the eight time points ranged from 9 to 19, as shown in Figure 10. The non-pregnant data was collected from eligible studies that reported data for a non-pregnant control or preconception group (Clapp and Capeless, 1997, Mahendru et al., 2014, Ogueh et al., 2009, Savu et al., 2012, Vartun et al., 2014, Borghi et al., 2000, Lof et al., 2005, Schannwell et al., 2002, Yosefy et al., 2012). Details of the 39 included studies are reported in Table 3.



	Non-pregnant	First trimester	Early second trimester	Late second trimester	Early third trimester	Late third trimester	Early post-partum	Late post-partum
study n	9	10	11	18	19	19	12	9
sample n	259	446	339	748	606	684	265	273

Figure 10. Flow diagram of inclusion and exclusion process for studies identified within the meta-analyses.
N.B. SD, standard deviation.

Table 3. Details of studies included in meta-analyses.

Study	Design	*n*	Parity	Method	Position
Armstrong *et al.,* 2011	Cross-sectional	25	NP & MP	Suprasternal Doppler; Supra Q®	Left lateral
Bamfo *et al.,* 2007	Cross-sectional	17	NP & MP	Echo; 2D; LVOT	Left lateral
Borghi *et al*., 2000	Cross-sectional	10-35	NP & MP	Echo; 2D; LVOT	Left lateral
Clapp and Capeless, 1997	Longitudinal	30	NP & MP	Echo; 2D, Teichholtz	Left lateral
Cornette *et al.,* 2011	Cross-sectional	15	DNR	Echo; 2D; LVOT	Left lateral
Desai *et al.,* 2004	Longitudinal	6-33	NP & MP	Echo; 2D; LVOT	Left lateral
D'Silva *et al*., 2014	Longitudinal	28	DNR	ICG; Task Force©	Supine
Estensen *et al.,* 2012	Longitudinal	61-65	NP & MP	Echo; 2D; LVOT	Left lateral
Geva *et al.,* 1997	Longitudinal	34	NP & MP	Echo; 2D; LVOT	Left lateral
Gilson *et al.,* 1997	Longitudinal	76	NP	Echo; 2D; Simpson's	Left lateral
Gyselaers *et al.,* 2014	Cross-sectional	13	NP & MP	ICG; NICCOMO©	Supine
Hennessy *et al*., 1996	Longitudinal	26	DNR	Echo; 2D; LVOT	Left lateral
Jia *et al.,* 2010	Cross-sectional	103	DNR	ICG; BioZ DX©	Left lateral
Kuleva *et al*., 2011	Longitudinal	10	NP & MP	Echo; 2D; LVOT	Left lateral
Lof *et al*., 2005	Longitudinal	22	DNR	Echo; 2D; LVOT	Left lateral
Mahendru *et al.,* 2014	Longitudinal	54	NP & MP	Inert gas re-breathing; Innocor©	Left lateral
Mesa *et al*., 1999	Longitudinal	8-35	DNR	Echo; 2D; LVOT	Left lateral
Moertl *et al*., 2012	Longitudinal	48	DNR	ICG; Task Force©	Left lateral
Mone *et al*., 2006	Longitudinal	33	NP & MP	Echo; 2D; LVOT	Left lateral
Novelli *et al.,* 2012	Longitudinal	54	NP	Echo; 2D; LVOT	Supine
Ogueh *et al.,* 2009	Longitudinal	10-13	NP & MP	Echo; 2D; LVOT	Left lateral
Pandey *et al.,* 2010	Longitudinal	22	DNR	Echo; 2D, Teichholtz	DNR
Poppas *et al*., 2007	Longitudinal	14	NP & MP	Echo; 2D; LVOT	Left lateral
Rang *et al*., 2007	Longitudinal	16	NP	Echo; 2D; LVOT	Left lateral
San-Frutos *et al.,* 2005	Longitudinal	18	DNR	ICG; NCCOM3©	Left lateral
Savu *et al.,* 2012	Longitudinal	10-50	DNR	Echo; 2D; LVOT	Left lateral
Schannwell *et al*., 2002	Longitudinal	46	NP	Echo; 2D, Quinones	DNR
Tamas *et al.,* 2007	Cross-sectional	100	NP & MP	ICG; ASKIT 400©	Left lateral
Tyldum *et al.,* 2012	Longitudinal	19	NP & MP	Echo; 2D; LVOT/ Simpsons	Left lateral
Valensise *et al*., 2000	Longitudinal	43	NP	Echo; 2D; Teichholz	Left lateral
Valensise *et al*., 2001	Cross-sectional	21	DNR	Echo; 2D; Teichholz	Left lateral
Valensise *et al.,* 2006	Longitudinal	41	NP & MP	Echo; 2D; LVOT	Supine
Van der Graaf *et al*., 2013	Cross-sectional	116	NP & MP	Suprasternal Doppler; USCOM©	DNR
Vartun *et al*., 2014	Cross-sectional	54-108	NP & MP	ICG; Phillips©	Supine
Vasapollo *et al*., 2002	Cross-sectional	21	NP	Echo; 2D; Teichholz	DNR
Vlahovic-Spipac *et al.,* 2010	Longitudinal	12	DNR	Echo; 2D; Simpson's	DNR
Wolfe *et al*., 1999	Longitudinal	19	NP & MP	Echo; 2D	Left lateral
Yosefy *et al*., 2012	Cross-sectional	20	DNR	Echo; 3D	Left lateral
Yuan *et al.,* 2006	Cross-sectional	24	DNR	Echo; 2D; LVOT	Left lateral

N.B. NP, nulliparous; MP, multiparous; DNR, data not reported; Echo, echocardiography; ICG, impedance cardiography; 2D, two-dimensional; LVOT, left ventricular outflow tract; 3D, three-dimensional.

Search outcomes

For the 39 included studies, the total sample size included within the analyses of cardiac output was 1479, with numbers ranging from 259 – 748 in the individual analyses (Figure 10). Within the analyses of the additional haemodynamic variables, the required data were not consistently reported; therefore, total sample sizes were reduced in the analyses of additional parameters, as shown in Table 4.

Table 4. Total sample size of all meta-analyses.

	NP	T1	Early T2	Late T2	Early T3	Late T3	Immediate PP	Late PP
Cardiac output	259	446	339	748	606	684	265	273
HR	259	294	339	686	563	684	265	273
SV	216	446	327	736	551	635	236	258
MAP	185	446	320	609	586	615	216	220
SVR	239	365	222	602	489	479	246	175
LV mass	79	205	259	384	406	260	200	157
EDV	50	107	159	229	254	204	79	102

N.B. HR, heart rate; SV, stroke volume; MAP, mean arterial pressure; SVR, systemic vascular resistance; LV, left ventricular; EDV, end-diastolic volume; NP, non-pregnant; T1, T2, and T3, trimester one, two and three, respectively; PP, postpartum.

Publication bias

Examination of funnel plots indicated publication bias in two of the 8 time points within the meta-analyses for cardiac output. Original outputs were adjusted to reflect the presence of bias and these values were reported as the final results. Within the forest plot of cardiac output (Figure 11), the original outputs prior to any adjustment for bias are shown. Similarly, publication bias was also identified within some of the meta-analyses of HR, SV, MAP, SVR, LV mass and EDV and all analyses were corrected accordingly. The original outputs prior to any adjustment for bias are also shown within the forest plots for each parameter (Appendix II Figures A1-A6).

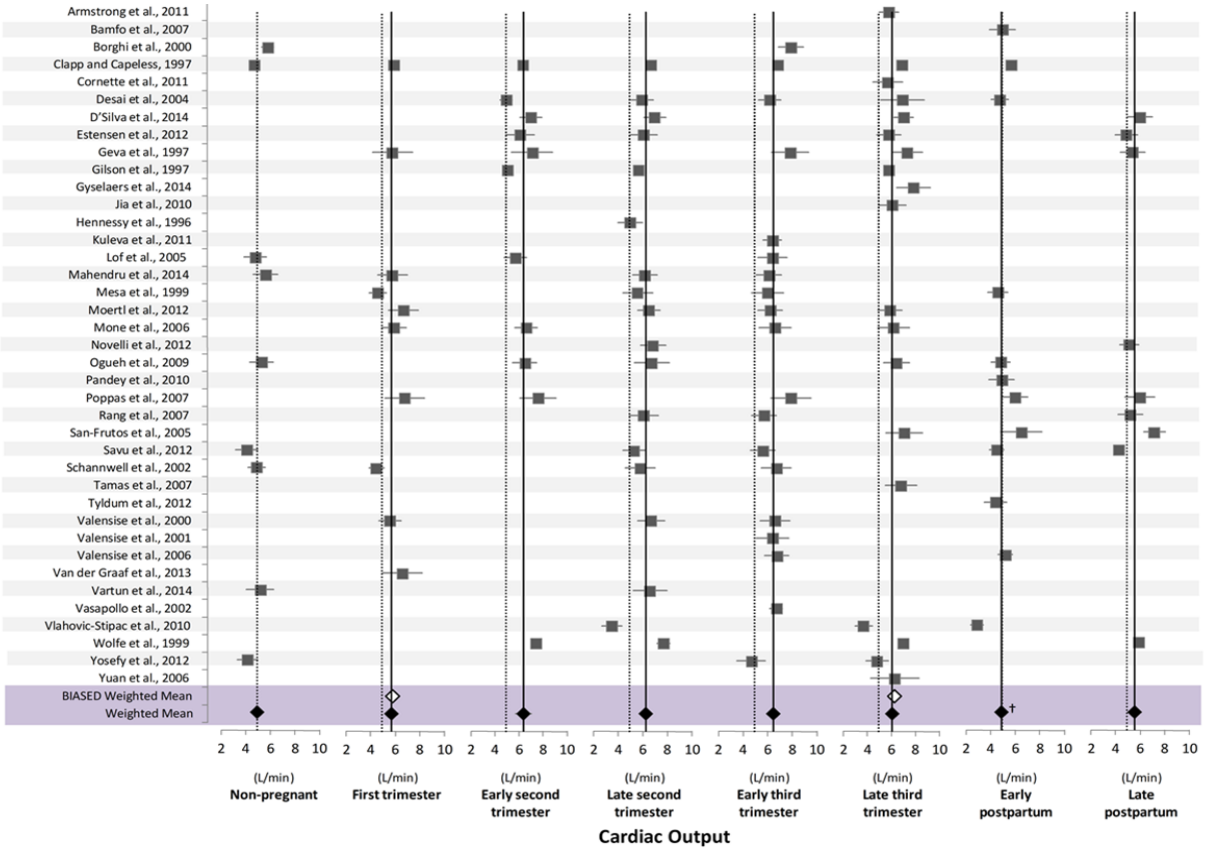


Figure 11. Forest plots for all meta-analyses of cardiac output across gestation and into the postpartum period.
Filled grey squares represent study outputs. Filled black diamonds represent the weighted mean as a result of the analyses. Unfilled diamonds represent outputs from biased analyses that were corrected for using Duval and Tweedie's trim and fill method. Dotted line represents non-pregnant weighted mean on all figures. Black solid line represents weighted mean for that individual gestational age. † Non-pregnant weighted mean at same value as weighted mean for early postpartum (4.96 vs. 4.91 $L \cdot min^{-1}$).

Findings from meta-analyses

A composite figure of forest plots for cardiac output at each time point of analyses is shown in Figure 11. Forest plots for all other variables are presented in Appendix II – Figures A1-A5. The summary effect, or weighted mean, and 95% confidence intervals for cardiac output and related haemodynamics at each gestational age are provided in Table 5 and presented in Figure 12. Observations of the results are discussed below; no statistical tests have been performed to infer differences between gestational ages, this is discussed in more detail in the limitations section of this chapter.

During the late first trimester, cardiac output was 0·74 $L \cdot min^{-1}$ (15%) higher than non-pregnant values. The peak value of 6·48 $L \cdot min^{-1}$ for cardiac output was observed in the early third trimester and was 1·5 $L \cdot min^{-1}$ (31%) greater than non-pregnant values. However, cardiac output did not increase linearly. Between the early second and early third trimester, a reduction of 0.11 $L \cdot min^{-1}$ (2%) was observed. After the observed peak in the early third trimester, cardiac output was lower by 0·41 $L \cdot min^{-1}$ (6%) in the late third trimester. In the early postpartum period, cardiac output was similar to that of non-pregnant values, after which, there was a modest increase in the late postpartum period by 0·63 $L \cdot min^{-1}$ (12%).

HR rose progressively over the course of gestation, reaching its highest value in the late third trimester 16 $beats \cdot min^{-1}$ (24%) above non-pregnant values. Following birth, HR was similar to non-pregnant values in both the early and late postpartum period. SV was greater by 6 ml (8%) in the first trimester above non-pregnant values, primarily as a result of a greater EDV (14 ml, 13%). The peak adaptation for both SV and EDV occurred in the early second trimester (greater than non-pregnant values by 10 and 20 ml; 13 and 20%, respectively). In the late second trimester, both SV and EDV dropped modestly, and then plateaued until delivery.

MAP remained relatively stable throughout pregnancy and did not exceed non-pregnant values at any gestational age. The lowest value observed in pregnant females occurred during the second trimester in which MAP was 8 mmHg (9%) below non-pregnant values. SVR was progressively lower than non-pregnant values over the course of pregnancy, with the peak value (396 $dyne \cdot s \cdot cm^{-6,}$ 30% below non-pregnant values) occurring during the early third trimester. As expected with the limited changes in MAP, SVR followed a similar pattern to that observed in cardiac output. Following birth, SVR was similar to that of non-pregnant values.

During the early third trimester, LV mass peaked and was 40 g (34%) greater than non-pregnant values. Despite returning to non-pregnant levels in the early

postpartum period, LV mass was greater by 9 g (8%) in the late postpartum period.

Table 5. Cardiac output and related haemodynamics across healthy pregnancy. Data presented as weighted mean and 95% confidence intervals.

	NP	T1	Early T2	Late T2	Early T3	Late T3	Immediate PP	Late PP
Cardiac output ($L \cdot min^{-1}$)	4·96 4·64-5·28	5·70 5·23-6·18	6·38 5·71-7·04	6·27 5·93-6·61	6·48 6·21–6·76	6·07 5·75–6·40	4·91 4·43–5·40	5·54 4·99–6·10
HR ($beats \cdot min^{-1}$)	66 62–71	72 67–77	73 70–76	79 75–83	81 77–85	83 80–85	69 64–73	69 66–72
SV (ml)	74 68–81	80 71–90	84 73–95	72 65–79	80 75–86	77 70–85	71 59–83	75 69–80
MAP (mmHg)	82 80–84	80 77–82	75 67–82	74 69–80	78 77–80	79 73–86	80 77–82	81 77–86
SVR ($dyne \cdot s \cdot cm^{-6}$)	1331 1226-1435	1170 1069-1270	974 912–1036	1027 985–1070	934 880–989	981 935-1027	1325 1228-1423	1156 912–1401
LV mass (g)	117 105-130	125 114–136	129 124–133	137 128–149	157 146–169	138 132–143	117 113–122	126 118–135
EDV (ml)	98 59–138	112 77–146	118 91–145	105 84–126	110 95–128	110 92–129	107 64-150	99 69–129

N.B. HR, heart rate; SV, stroke volume; MAP, mean arterial pressure; SVR, systemic vascular resistance; LV, left ventricular; EDV, end-diastolic volume; NP, non-pregnant; T1, T2, and T3, trimester one, two and three, respectively; PP, postpartum.

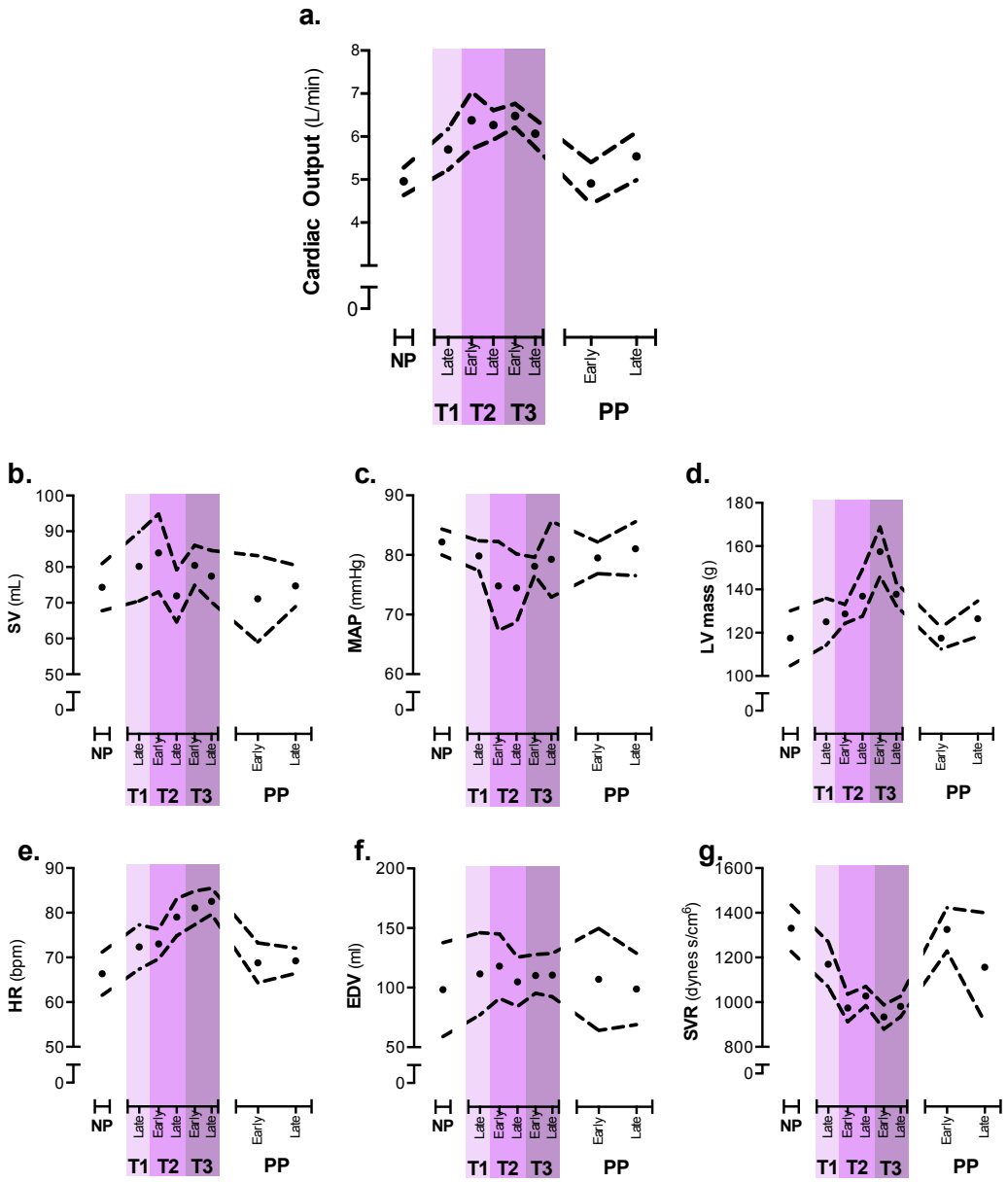


Figure 12. Compiled weighted mean and 95% confidence intervals derived from meta-analyses for a. cardiac output, b. stroke volume (SV), c. mean arterial pressure (MAP), d. left ventricular (LV) mass, e. heart rate (HR), f. end-diastolic volume (EDV), and g. systemic vascular resistance (SVR) across gestation and into the postpartum period. Coloured bars represent the first, second and third trimester of gestation. NP, non-pregnant; T1, trimester one; T2, trimester two; T3, trimester three; PP, postpartum.

3.5. Discussion

The objective of the present study was to comprehensively describe the pattern and magnitude of change in cardiac output and related haemodynamics during healthy pregnancy at rest using previously published literature.

The key findings from this study were:

(i) Cardiac output was progressively greater in a non-linear fashion during healthy pregnancy, peaking in the early third trimester, with a modest reduction towards term;

(ii) HR was progressively greater across gestation and peaked at term;

(iii) SV and EDV were greater than non-pregnant values in the late first and early second trimester, with a small reduction in the late second trimester and plateau until term;

(iv) MAP was lower in the second trimester, but similar to non-pregnant levels in the first and third trimester;

(v) SVR was progressively lower across gestation, peaking in the early third and returning towards non-pregnant levels towards term;

(vi) Left ventricular (LV) mass was greater across pregnancy, peaking in the early third trimester and reducing towards term.

These findings may have important implications in identifying healthy *vs.* abnormal adaptation of the maternal cardiovascular system during gestation.

Cardiac output changes in the first trimester

During healthy pregnancy, cardiac output is known to increase above non-pregnant levels; however the magnitude and time course of change remains unclear within the published literature. Discrepancies in the reported adaptation of cardiac output early in pregnancy exist and it is not well understood if the changes occur as a result of increases in SV, HR or a combination of both contributing

factors (Melchiorre et al., 2012a, van Oppen et al., 1996). Increased SV, as a result of an increased blood volume, was previously believed to be the main determinant of the increase in cardiac output in the first trimester (Clapp and Capeless, 1997, Flo et al., 2010, Robson et al., 1989). The present results show that both SV, through increased EDV, and HR contribute to the early increases in cardiac output (Mone et al., 1996, Ogueh et al., 2009). Supporting previous longitudinal data (Mahendru et al., 2014), the present study shows a reduction in SVR from pre-pregnancy to early first trimester indicating a reduction in afterload, which in turn, stimulates sympathetic activity (Usselman et al., 2015a, Jarvis et al., 2012), and consequently increases HR. The results from the present analyses indicate that increased cardiac output early in pregnancy is a result of reduced afterload, increased preload and elevated sympathetic activity.

Peak cardiac output in the early third trimester

As discussed in previous reviews (van Oppen et al., 1996, Melchiorre et al., 2012a), the third trimester has been associated with significant discrepancies in the pattern of cardiac output adaptation; with either a continual increase, decrease, or plateau within the final weeks of gestation. Supporting some of the previous data (Mone et al., 1996, Easterling et al., 1990), this study shows peak cardiac output is achieved in the early third trimester, followed by a decrease towards term. One explanation for this pattern could be that compression of the inferior vena cava as a result of considerable and progressive foetal growth occurring during the third trimester affects venous return (van Oppen et al., 1996). In addition, blood flow to the uteroplacental circulation is at its peak (approximately 12% of total cardiac output) during the late third trimester in order to meet peak foetal metabolic demands (Dowell and Kauer, 1997, Thaler et al., 1990). Both factors could contribute to a reduced cardiac preload and therefore cardiac output

during the late third trimester. The plateau in EDV observed in the late stages of pregnancy within this study indicates that volume may indeed be limited towards term. Additionally, as SV is further reduced in the late third trimester despite similar EDV, systolic function may be reduced via other mechanisms such as a progressively increasing afterload (MAP and SVR). Reduced systolic and diastolic function has also previously been observed in the late third trimester (Melchiorre et al., 2012a). The progressive increase in HR throughout pregnancy peaking in the late third trimester, identified previously (Mahendru et al., 2014, Savu et al., 2012, Sanghavi and Rutherford, 2014) and confirmed here, maintains cardiac output at a functional level until delivery.

The alterations in HR, SVR and LV mass reflect increased sympathetic activation, decreased vascular tone, and structural remodelling of the maternal heart, all of which may be secondary to hormonal surges and the increased physiological demand of gestation. In line with the decrease from peak cardiac output in the late third trimester, the results from the present analyses also show that LV mass declines prior to delivery. Whilst this finding has not been observed previously within the literature, the consistent confidence intervals in these meta-analyses suggest that this is a physiological phenomenon. Speculatively, this decline may be as a result of changes in LV wall stress (Opie et al., 2006) and/or reductions in hormonal concentrations in late third trimester, such as placental growth factor (Aasa et al., 2015), but future investigations are warranted.

Non-linear increase of cardiac output during pregnancy

The results of this study demonstrate the increase in cardiac output until peak during pregnancy may not be linear. From the non-pregnant state to term, a steady and progressive rise in cardiac output is interrupted by small reductions in the late second trimester and late third trimester, with the peak value achieved

between these points in the early third trimester. As shown in Figure 13a, this finding may not have been observed in previous literature due to the simple collation of data by trimester. As discussed previously, the reduction in cardiac output in the late third trimester is supported by previous literature and may be attributed to increasing afterload, stable preload, and a possible decline in systolic and diastolic function. However, the small reduction in cardiac output during the late second trimester has not previously been observed and may be attributed to changes in cardiac preload, as shown by the drop in EDV, leading to a reduction in SV. Maternal-foetal circulation within the placenta is only achieved after 14 weeks gestation yet uteroplacental blood flow remains stable until 20 weeks gestation (Coppens et al., 1996), after which it increases rapidly as a result of foetal growth and metabolic demand. Blood volume remains relatively unchanged during the second trimester which, when combined with a progressively increasing uteroplacental blood flow, may cause the drop in venous return and hence SV during the late second trimester (Chapman et al., 1998). It is possible that cardiac afterload and/or contractile function may be different at this time point in order to compensate for a reduced preload. Cardiac function may vary across gestation in order to balance fetoplacental demands and haemodynamic load. Appropriately powered longitudinal studies with assessments at regular intervals across gestation should be used to statistically confirm these findings.

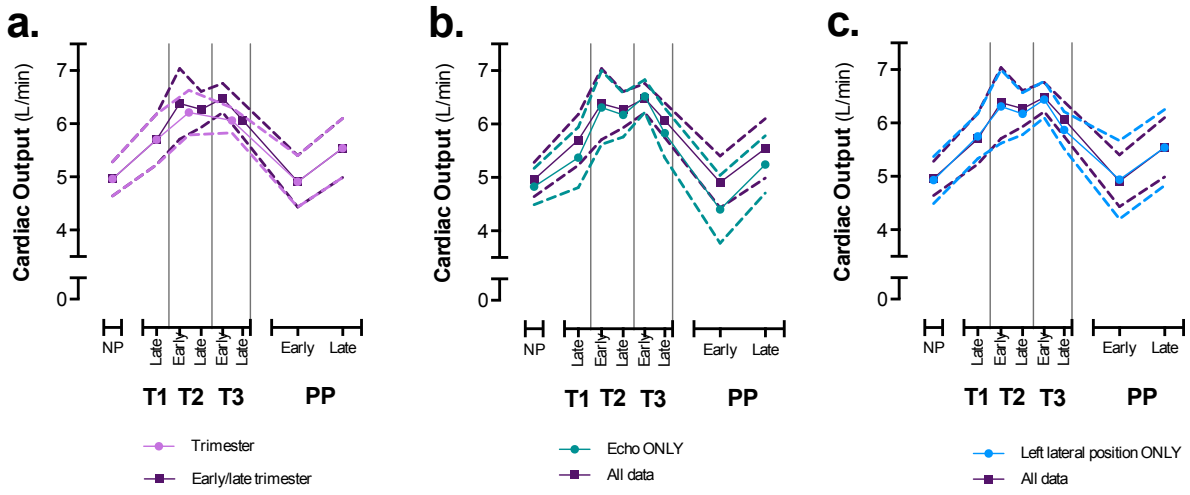


Figure 13. Additional analysis of meta-analyses data a. separated by trimester only, b. using only echocardiographic data, and c. using data collected in the left lateral position only.
NP, non-pregnant; *T1,* trimester one; *T2,* trimester two; *T3,* trimester three; *PP,* postpartum.

Postpartum cardiovascular function

As per the form follows function principle (Russell et al., 2000), cardiac output is considerably reduced after delivery in the early postpartum period returning to non-pregnant values (van Oppen et al., 1996). The rapid decline in cardiac output following birth is likely a consequence of reduced maternal cardiovascular demand and hormonal drive following delivery (Soldin et al., 2005). Within the extended postpartum period, cardiac output increases modestly above non-pregnant values (Melchiorre et al., 2012a). Previous studies have reported prolonged effects on cardiovascular function following gestation, including increased arterial compliance (Morris et al., 2015). In the late postpartum, SVR is reduced below non-pregnant and early postpartum levels. Favourable peripheral adaptations post-pregnancy may contribute to the increased cardiac output at this time point. In addition, many factors likely influence maternal cardiac output in the postpartum period. Breastfeeding and/or a return to physical activity may also explain the variability in cardiac structure and function observed in the postpartum state.

Clinical implications

An understanding of pregnancy-related cardiovascular complications, such as preeclampsia and gestational hypertension, is limited by the incomplete understanding of healthy cardiovascular adaptation to pregnancy. Whilst it is generally accepted that cardiac output increases during healthy pregnancy, the course of adaptation provides new insight into the expected timing and magnitude of responses. These meta-analyses have suitable power from a pooled observational dataset to provide a representative 'norm' of adaptations to cardiac output and related haemodynamics during uncomplicated gestation. The findings represent the healthy cardiac adaptation to pregnancy.

Limitations and future directions

Whilst the present meta-analyses offer new insight into the course of cardiovascular adaptation to healthy pregnancy, limitations of this study must be acknowledged. Despite the pooled sample sizes being greater than most pregnancy research studies, it must be highlighted that within each of the meta-analyses for cardiac output, the sample size ranged between 258 and 748 (data presented in Figure 10 and Table 4). The reductions in the sample size for additional haemodynamic parameters must also be considered. Careful interpretation of results from analyses with a low sample size is required. Inclusion in the meta-analyses was based on the mean gestational age at assessment fitting within a predefined time frame, and took no consideration of the range or SD of this mean, thus, overlap between gestational ages may be present within the analyses. Statistical significance was not analysed between the meta-analyses of each gestational age for each parameter so all reported results are observations of trends and must be interpreted carefully. Determining the differences between the time points was not possible on the included data because (i) original studies did

not supply the *P* – values of comparisons and (ii) authors of original studies were reluctant to provide the raw data of their investigations, therefore the reported changes in this study are not statistically verified.

There are limitations to each methodology included within these analyses that should be considered. The determination of cardiac output by inert gas rebreathing relies upon correct alveolar gas mixing and constant oxygen saturation during measurement (Farina et al., 2014), which cannot be confirmed without invasive procedures. The calculation of cardiac output from impedance cardiography is based upon assumptions that may not be appropriate during pregnancy as a result of the developing foetal unit (McIntyre et al., 2015). In echocardiography, the upward shift of the diaphragm may interfere with the image acquisition (Rang et al., 2007) which may alter the reliability of measurement. However, echocardiography is the preferred method for cardiac imaging during pregnancy (Waksmonski, 2014). To identify if the results of these analyses were altered due to the inclusion of varying methodologies, the analyses were re-run with studies using echocardiography only (n = 29). Only minor differences between the two outputs were noted (see Figure 13b). The values derived from incorporation of the differing techniques in these analyses may allow a wider application within clinical practice and research. It must be noted that these analyses did not include parameters of cardiac function that may provide further insight into changes in cardiac output. In particular, the decline in SV observed in the late third trimester occurred despite no decline in EDV, an indicator of LV preload. As such, the decline in SV may be result of increased afterload, observed in this study, and/or reduced contractility. Assessments of systolic and diastolic function may enable greater understanding of the factors that may be influencing this reduction in SV.

The left lateral position has been shown to be a preferable position for cardiac output measurement in pregnant women in order to avoid inferior vena cava

compression (Bamber and Dresner, 2003). In the interest of ensuring that different positioning was not a driver behind the reduction in cardiac output in the late second and third trimester, the meta-analyses were re-run on studies that collected data in the left lateral position only (n = 29). As presented in Figure 13c, there was minimal change in the absolute values that did not impact on the general trend when compared to the original analyses. This is supported by previously published data showing there is no significant effect of maternal position on cardiac output when measured using impedance cardiography (Bamber and Dresner, 2003). Comparison of these analyses to the total dataset suggested limited impact of maternal position.

Maternal characteristics may also influence cardiac adaptation to pregnancy. An influence of parity, ethnicity, and BMI on gestational cardiovascular changes has previously been observed (van Oppen et al., 1996, Melchiorre et al., 2012a), however it was not possible to control for these parameters within this study due to a lack of clear reporting within included studies. Additionally, the impact of breast-feeding on postpartum cardiovascular function has also not been addressed, as lactation status was also not consistently reported within the included studies. Therefore, these analyses were limited in control of these factors due to the inherent use of previously published data. Future studies should be conducted with consideration for maternal factors and should investigate their impact on the course of cardiovascular adaptation during and after healthy pregnancy.

3.6. Conclusions

Through use of meta-analyses based on observational data, this study shows that cardiac output is greater than non-pregnant levels as early as the first trimester, reaching its peak in the early third trimester. The greater cardiac output is achieved via a combination of favourable changes in haemodynamic load:

increased HR, preload (suggested from greater EDV) with reduced afterload (BP and SVR). The novel finding of a non-linear increase in cardiac output appears to be associated with a reduced SV and EDV in the late second trimester. The lower cardiac output in the late third trimester coincided with both MAP and SVR returning to non-pregnant levels. Therefore, the fluctuations in haemodynamic load and increased demand across healthy gestation may impact upon maternal cardiac structure and function.

Chapter 4. General methodology for experimental chapters

This chapter provides a description of the methods of data collection and analysis used within Chapters 5 and 6. The second and third objectives of this thesis were to provide a comprehensive assessment of maternal cardiac function at rest and in response to physiological challenge. In order to fulfil these objectives, a single cross-sectional repeated measures study of non-pregnant females, pregnant females and postpartum females was completed. The results of the experimental study will be presented within this thesis as follows:

Chapter 5: Cardiac structure and function at rest in non-pregnant, pregnant and postpartum females

Chapter 6: Functional cardiovascular responses to acute physiological challenge in non-pregnant, pregnant and postpartum females

As noted previously in the literature review, the majority of studies investigating LV mechanics during acute physiological challenges have been completed in male participants. Accordingly to validate the findings of this study, a male cohort was also included.

4.1. Overview of study design

Volunteers participated within the study as either: a non-pregnant female or male (no time control of inclusion), a pregnant female between 22-26 weeks gestation or a postpartum female between 12 – 16 weeks after delivery. During the time frames associated with each group, volunteers were asked to attend the laboratory on two separate occasions. A schematic representation of the visits and their relation to the respective experimental chapters is presented in Figure 14. The initial visit involved the measurement of anthropometrical characteristics (height, body mass and BMI), maximal voluntary handgrip and exercise capacity in preparation for cardiovascular assessments. During the second visit to the laboratory, a comprehensive cardiac ultrasound exam (“echocardiography”) and

blood pressure measurement were completed at rest, during a sustained isometric handhold and during aerobic cycling. The detailed methods of data collection are described later within this chapter. The specific research design for each individual study will be presented within the respective experimental chapter.

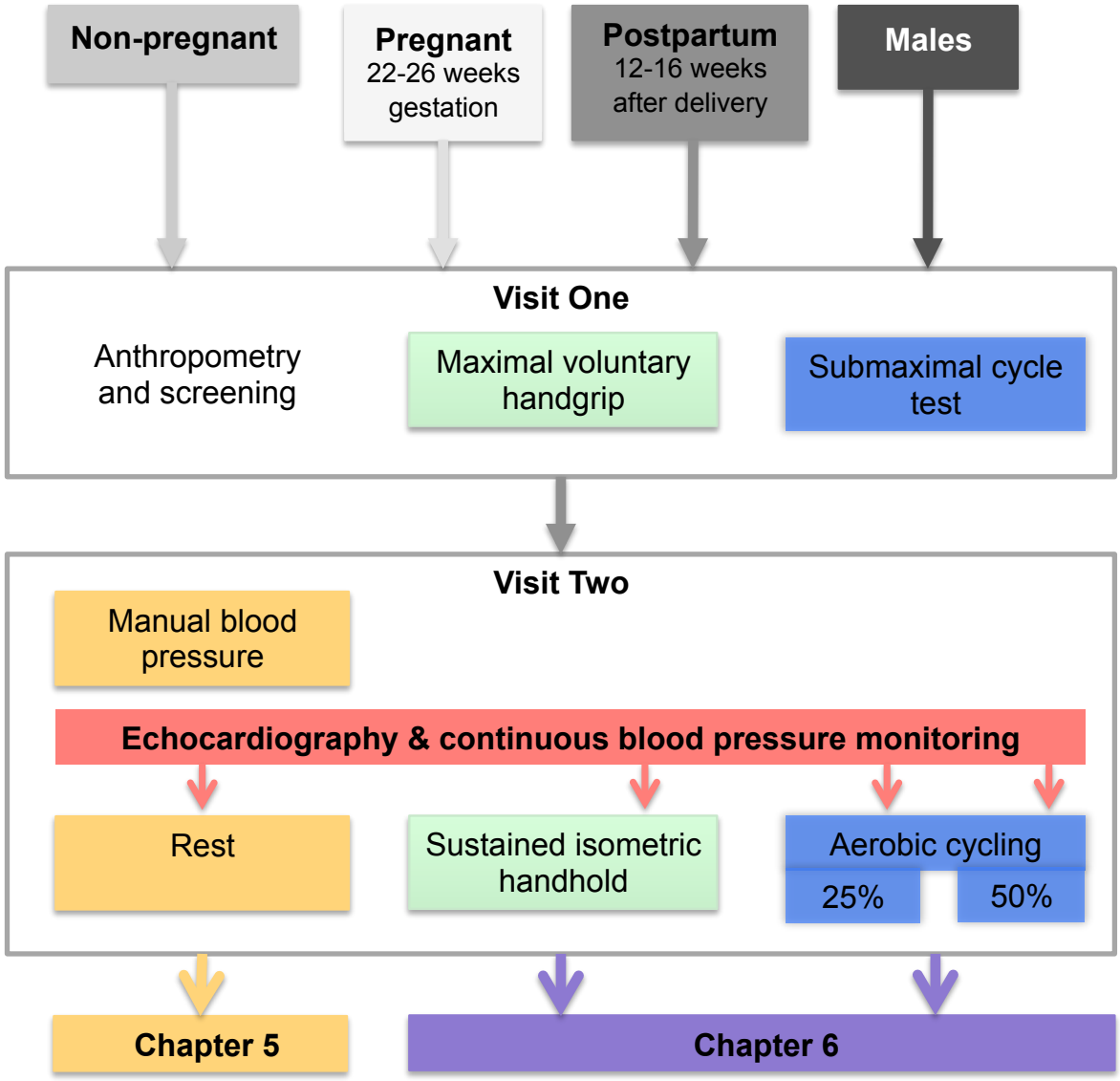


Figure 14. Protocol for experimental study presented in Chapters 5 and 6. Volunteers were recruited for a cross sectional study of non-pregnant, pregnant and postpartum females and males. All volunteers were asked to abstain from caffeine and physical activity for 24 hours prior to visiting the laboratory on both occasions. All visits took place at the same time of day to control for circadian rhythm.

4.2. General procedures

Ethical approval

Ethical approval (Appendix I.b.) was gained from Cardiff Metropolitan University (ethics code: 16/3/01R). A detailed study information sheet (Appendix III) was provided to all potential volunteers prior to enrolment in the study. At the point of consent, the principal investigator thoroughly discussed the study information sheet with the volunteer and invited any questions. The principal investigator ensured adequate understanding of the testing protocol and the requirements of the study. Informed consent was gained from all volunteers using a research consent form (Appendix IV). Volunteers were reminded of their right to withdraw from the study at any time. The study complied with the Declaration of Helsinki.

Power analyses

An *a priori* power analysis was used to decide upon an appropriate sample size. Previously published resting cardiac output data from a cross sectional study of non-pregnant and pregnant females (Yoon et al., 2011) was used as the basis of the power calculation. Firstly, the previously published group means and standard deviations (SD) were to estimate an effect size using software (G*Power, Version 3.1.7.). Effect size was estimated using the following equation:

$$Cohen's\ d = \frac{M_1 - M_2}{SD\ pooled}$$

Where M_1 and M_2 are the means for the 1st and 2nd samples, and SD_{pooled} is the pooled standard deviation for the samples. The effect size was then input into the software to determine an initial sample size with a statistical power greater than 0.8 and an alpha level less than 0.05. According to the analysis performed (G*Power, Version 3.1.7.), 27 volunteers per group were required to identify differences in cardiac output at rest between non-pregnant controls and pregnant females in the second trimester. As four groups were to be included in this study,

this increased the total cohort size (*n* = (4 x 27) = 108. Within developed countries, 10% of pregnancies are expected to suffer from complications (Roberts et al., 2011). Subsequently, the sample size was increased by 10% to accommodate for this potential confounding factor, resulting in an estimated total of 30 volunteers per group.

This initial power analyses determination was imprecise due to the estimation of the effect size from previously published data at rest only. Therefore, one year into the project (December 2015), additional power analyses were completed on preliminary data (non-pregnant female controls (*n* = 18) and pregnant females between 22 and 26 weeks (*n* = 9)) at rest and during exercise to determine an expected effect size in relation to the additional physiological challenge. As the original power analyses were based upon resting data only, the secondary analyses enabled the confirmation that sample sizes were appropriate to detect differences within this repeated measures research design. Cardiac output data for non-pregnant female controls and pregnant females at rest, during 25% and 50% aerobic cycling were analysed with a repeated measures analysis of variance (RMANOVA) using GraphPad Prism 5 (GraphPad software, San Diego, CA). Results from the RMANOVA analyses were used to calculate partial eta squared (η_p^2). Where *SS* is equal to the sum of standard deviations, η_p^2 was calculated using SS between treatments/(SS total – SS between subjects). The η_p^2 was entered into the G*Power software to compute an effect size, which allowed the determination of the required sample size. As previously noted, a statistical power of 0.8 and an alpha level of less than 0.05 were set. As a result of these secondary analyses, a sample size of 10 was required per group to detect differences in cardiac output at rest and during supine cycling. Again this sample size was applied for four groups (non-pregnant females, pregnant females, postpartum females, and males; total *n* = 40) and increased by 10% to account for the

potential development of pregnancy complications, resulting in a requirement of 11 volunteers per group (total *n* = 44). The adjustment to sample size was revised on the ethical approval.

Qualifications of investigator

A single investigator, Miss Victoria Meah, conducted the research. All experimental procedures were completed at the Physiology and Health laboratory at Cardiff Metropolitan University, Cyncoed Campus. The investigator was first aid qualified and trained in immediate life support. The investigator holds vocational qualifications in ante- and post-natal personal training and is accredited by the British Association of Sport and Exercise Science (BASES) as a Certified Exercise Practitioner.

Participants

Inclusion criteria

Healthy individuals between the ages of 20 and 39 years were recruited from the local community. The study had four distinct cohorts:

1) Non-pregnant females;
2) Pregnant females;
3) Postpartum females;
4) Males.

Non-pregnant females were nulliparous. All pregnant and postpartum females were primiparous. Pregnant females with uncomplicated, healthy, singleton pregnancies in the late second trimester (between 22 and 26 weeks) were included. This time point fell within the defined periods used in the meta-analyses of Chapter 3. Specifically, the late second trimester was chosen for practical reasons. Firstly, the included pregnant females would have recently had their second trimester foetal ultrasound and check up, confirming their health of

gestation to enable inclusion into the study. Secondly, the anatomical changes of gestation (increasing size of gravid uterus and breast enlargement) did not compromise the quality of echocardiographic data collection. Postpartum females in the late postpartum (between 12 and 16 weeks after delivery) were included. This time point was chosen to ensure that females were cleared to exercise by their physicians after caesarean section births (typically 8-10 weeks recovery).

Exclusion criteria

Individuals were excluded from participating in the study if they were current smokers, hypertensive (prior to or during pregnancy), had previous or existing cardiovascular disease or diabetes mellitus. Pregnant and postpartum females had to have conceived naturally, as pregnancies achieved using conceptual aids (such as in-vitro fertilisation, donor/intrauterine insemination) have been shown to have higher BP early in gestation, which may influence cardiac function (Ogueh et al., 2009).

Females who had experienced a miscarriage after 12 weeks of gestation in previous pregnancies were excluded from all cohort groups, however those who had experienced a miscarriage at or before 12 weeks of gestation were included within the study (pregnant n = 2; postpartum n = 2). It is challenging to completely eradicate the potential of early previous miscarriage within a reproductive female cohort. Approximately one third of embryos are lost before implantation (Wilcox et al., 1999) and a further third of early miscarriage occurs before the pregnancy is confirmed clinically (Macklon et al., 2002).

To confirm suitability to partake in the research, all volunteers were asked to complete a pre-participation screening questionnaire which allowed identification of the above listed exclusion criteria (Appendix V).

In order to determine an individual's suitability to exercise, all volunteers completed a physical activity readiness questionnaire (PAR-Q). Non-pregnant

females, postpartum females and males completed the American College of Sports Medicine (ACSM) PAR-Q, attached as Appendix VI (Balady et al., 1998). Pregnant females completed the PARMed-X for Pregnancy pre-participation screening questionnaire (Wolfe and Mottola, 2002), attached as Appendix VII). This questionnaire identified any contraindications to exercise during pregnancy as stated by American College of Obstetricians and Gynaecologists (ACOG). Pregnant volunteers were excluded from participating in the study if they suffered from any of the following contraindications in their current pregnancy: severe anaemia, cardiac arrhythmia, diabetes, chronic bronchitis, intrauterine growth restriction, lung disease, heart disease, incompetent cervix, second or third trimester bleeding, or ruptured membranes (Artal and O'Toole, 2003).

Pregnant females are at risk of developing anaemia due to a significant increase in plasma volume that is disproportionate to the increase in red blood cell mass. Reduced haemoglobin levels are therefore a common observation in pregnant females. Severe maternal anaemia is a contraindication of exercise during pregnancy and is diagnosed when the haemoglobin concentration falls below 110 g/L during gestation (WHO, 2001). Haemoglobin levels were measured in pregnant and postpartum volunteers through the collection of capillary blood samples from the ear lobe, analysed using a point of care testing device (HemoCue Hb 201+, HemoCue AB, Angelholm, Sweden) and averaged. Pregnant and postpartum females had average haemoglobin values of 132 ± 11 and 148 ± 13 g/L, respectively. No volunteer's haemoglobin levels were below 110 g/L, which represents the cut off point for contraindication to exercise and a subsequent referral to their physician.

The gestational health of postpartum volunteers was assessed through a questionnaire at the point of data collection (Appendix IX). The questionnaire requested basic descriptive information of pregnancy outcomes, such as

gestational age at delivery and foetal birth weight and also requested information regarding gestational, labour and/or infant complications. The information was recorded for descriptive purposes. Volunteers that participated in the study during their pregnancy were also asked to complete this questionnaire via telephone or email after their delivery. This was used to confirm their gestational health after study participation until term. Volunteers that developed pregnancy-related cardiovascular complications during their gestation were to be excluded from the study *post hoc*, however this issue did not arise.

Overview of general study population

A total of 62 volunteers were enrolled into the study. Two individuals did not complete the second study visit due to time constraints, resulting in a dropout rate of 3%. Therefore, 60 individuals across four cohort groups (non-pregnant females: n = 18, pregnant females: n = 14 and postpartum females: n = 13, males: n = 15) were included within the final analyses of one or more studies. The cohort sizes in each group met the power analyses requirements. Anthropometric characteristics are provided in the results section of Chapter 5.

The average gestational age at the time of assessment was 25.4 ± 0.6 weeks gestation in pregnant volunteers and 15.1 ± 1.3 weeks after delivery in postpartum volunteers. The average gestational age of delivery was 40.2 ± 2.1 and 39.7 ± 1.5 weeks for pregnant and postpartum volunteers, respectively (P = 0.449). The average birth weight and sex of infants of pregnant and postpartum volunteers was 3.44 ± 0.46 and 3.14 ± 0.50 kg (P = 0.113) and 50 and 54% males, respectively. Volunteers in the pregnant group delivered naturally (n = 10), by planned (n = 1) or emergency (n = 3) Caesarean section, with similar numbers in the postpartum volunteers (n = 10, 1 and 2, respectively). Six infants had infections at birth (n = 3 in each group). The majority of volunteers in the

postpartum group (93%, *n* = 12) reported breastfeeding and participation in physical activity at the time of inclusion in the study.

4.3. Data collection procedures

Echocardiography

Ultrasound is a non-invasive diagnostic technique that allows internal imaging of the human body for assessment of anatomy and function. It is a technique that is routinely used both in clinical and research settings for many varied purposes. In this thesis, ultrasound imaging of the heart, termed echocardiography, was used to assess myocardial structure, function and deformation. The echocardiographic images and techniques used to measure myocardial variables are fully discussed in this section. During pregnancy, echocardiography is the preferred screening method to assess maternal cardiac function due to its lack of radiation exposure, good availability, good mobility and relatively high temporal resolution (Regitz-Zagrosek et al., 2011). A commercially available ultrasound system (Vivid E9, GE Medical Systems, Horten, Norway) and 1.5 – 4.6 MHz phased array transducer (M55, GE Medical Systems, Horten, Norway) was used to collect data for this thesis, as shown in Figure 15. All echocardiographic data collection and analysis was completed by the author.

Figure 15. Vivid E9 ultrasound machine (left panel) and M5S ultrasound probe (right panel) used for echocardiographic data collection within this thesis.

Principles of echocardiography

Ultrasound is a form of acoustic energy that has a frequency much greater than that of human hearing. In medical ultrasound, a transducer probe emits ultrasound waves through the activation of piezoelectric crystals by an electric current (a transmitted wave). Transducer probes are also capable of detecting ultrasound waves that are reflected back at an acoustic interface, such as a boundary between human tissues (Armstrong and Ryan, 2009). The varying densities of human tissue, such as bone, soft tissue and fluid, affect the velocities and directions of transmitted ultrasound waves. At an acoustic interface, transmitted ultrasound waves may continue through the boundary (a refractory wave), or are reflected back to the transducer probe (a reflected wave) (Figure 16). Consequently, the differences in tissue density, also known as acoustic impedance, cause reflected waves to return to the transducer probe at differing intensities and transit durations (Armstrong and Ryan, 2009). At this point, the reflected waves cause the piezoelectric crystals to vibrate and produce an electrical impulse, which allows the generation of an ultrasound image.

Specifically, in echocardiography, the transducer probe is applied to the anterior surface of the chest, and reflected ultrasound waves allow the production of an image of myocardial tissues and blood.

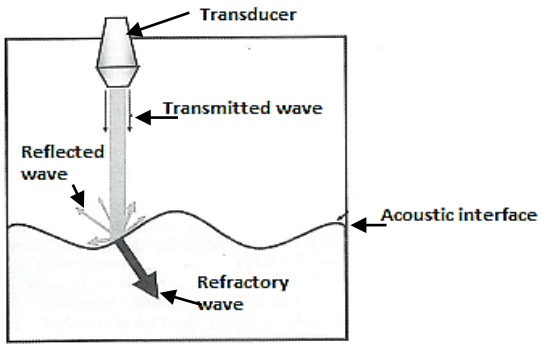


Figure 16. The principles of ultrasound wave transmission and reflection for echocardiographic image acquisition. A transducer probe emits ultrasound waves. When the transmitted wave meets an acoustic interface such as a boundary between two tissues, it may continue through the boundary, or be reflected back to the transducer. The time of transit and the intensity of the reflected wave allows the generation of an ultrasound image. Adapted from Feigenbaum et al. (2005).

Echocardiographic modalities

Ultrasound waves may be transmitted differently from the transducer, resulting in different echocardiographic imaging modalities. Motion-mode (M-Mode) echocardiography, the earliest form of echocardiography, uses a single interrogation beam whereas two-dimensional (2D) B-mode echocardiography uses multiple interrogation beams across a 90° arc (Armstrong and Ryan, 2009). The generated 2D ultrasound image is illustrated with the transducer position at the top of the image, with the superficial structures (those closest to the probe) in the upper part of the image, and the deeper structures (those furthest away from the probe) in the lower part of the image. Lateral structures are visualised on the right hand side of the screen, and medial structures on the left hand side of the screen (Fernández and Gómez de Diego, 2011).

Two dimensional B-mode echocardiography

Brightness mode (B-mode) 2D echocardiography is the standard acquisition mode within echocardiography and forms the basis of each collected image within this thesis. 2D echocardiography enables the collection of a plane of tissue across both width and depth via synthesis of multiple reflected waves to the transducer probe (Armstrong and Ryan, 2009). 2D echocardiography provides an improved view of LV structure than M-Mode, and allows other imaging modalities to be simultaneously performed. 2D echocardiography was used within this thesis to assess LV structure.

Doppler echocardiography

Doppler is a modality of echocardiography that can be used to determine blood flow velocity within myocardial chambers and arteries (Armstrong and Ryan, 2009), and was used within this thesis to determine blood velocity across the mitral valve as a marker of diastolic function. Doppler can be performed alongside 2D echocardiography in most modern ultrasound systems. In contrast to 2D echocardiography, Doppler interrogation is dependent on the change in frequency of transmitted ultrasound waves in relation to the moving heart, measured in KHz. The magnitude of change is converted using the Doppler equation to create a measurement of velocity (Armstrong and Ryan, 2009). Pulsed wave Doppler allows the determination of blood flow velocity at a precise location on cardiac anatomy through use of a focus beam, also referred to as a sample volume, and therefore, this thesis utilised the pulsed wave method throughout all Doppler acquisitions.

Tissue Doppler Imaging

Tissue Doppler imaging (TDI) relies on detection of the shift in frequency of ultrasound signals reflected from myocardial tissue motion (Ho and Solomon,

2006). In this method, the differences in frequency of transmitted and reflected waves are related to the velocity of the myocardial tissue. TDI of mobile sections of the myocardium can be used as an indicator of contraction and relaxation in the longitudinal plane (Armstrong and Ryan, 2009). Within this thesis, TDI was used to assess septal, LV lateral wall, and right ventricular tissue velocities as indicators of systolic and diastolic function.

Colour Doppler M-Mode imaging

Colour flow Doppler imaging uses pulsed wave interrogation to provide a colour flow map that allows the measurement of the direction and velocity of blood flow. Colour Doppler M-Mode imaging is primarily used in the assessment of valve integrity, however this was not within the scope of this thesis.

Echocardiographic image acquisition and standardisation

Participant position

The left lateral decubitus position allows the optimal acquisition of echocardiographic images. Through the gravitational effect of this position, the heart moves closer to the chest wall, reducing the depth required for penetration of the ultrasound wave. Raising the left arm allows the expansion of the rib cage, and therefore the intercostal space, improving access to the acoustic window (Fernández and Gómez de Diego, 2011). It is therefore the recommended position for all echocardiographic examinations. A specifically designed tilt bed may be used to place volunteers into the left lateral decubitus position, and can have a cycle ergometer attached for cardiac stress testing (Angio 2003, Lode, Groningen, Netherlands). The volunteer is asked to lie onto the bed in a supine position, and the bed is then electronically tilted up to 45° (Figure 17). This method of positioning is particularly useful in this population, as pregnant females are advised not to lie in the supine position for an extended period in the latter stages

of gestation, and are contraindicated from exercise in the supine position from 16 weeks' gestation. This is due to concerns that inferior vena cava compression from the gravid uterus may induce pre-syncopal symptoms. A left lateral tilt of greater than 15° has been shown to negate inferior vena cava compression (Lee et al., 2012). Consequently, in this study, the left lateral tilt used was between 30 and 45°, dependent on the comfort of the volunteer. This ensured the safety of pregnant volunteers at rest and during exercise, whilst allowing optimal echocardiographic image acquisition (Sicari et al., 2009).

Despite the advantages of its use, the specifically designed supine cycle ergometer is an unusual position for dynamic exercise. Familiarisation to the equipment is therefore essential and allows volunteers to become accustomed to the modality of exercise prior to data collection. Volunteers within this study were introduced to supine cycling exercise through the completion of two 4-minute exercise bouts at low intensities (15 and 30% supine peak power output). No data were collected during this familiarisation phase.

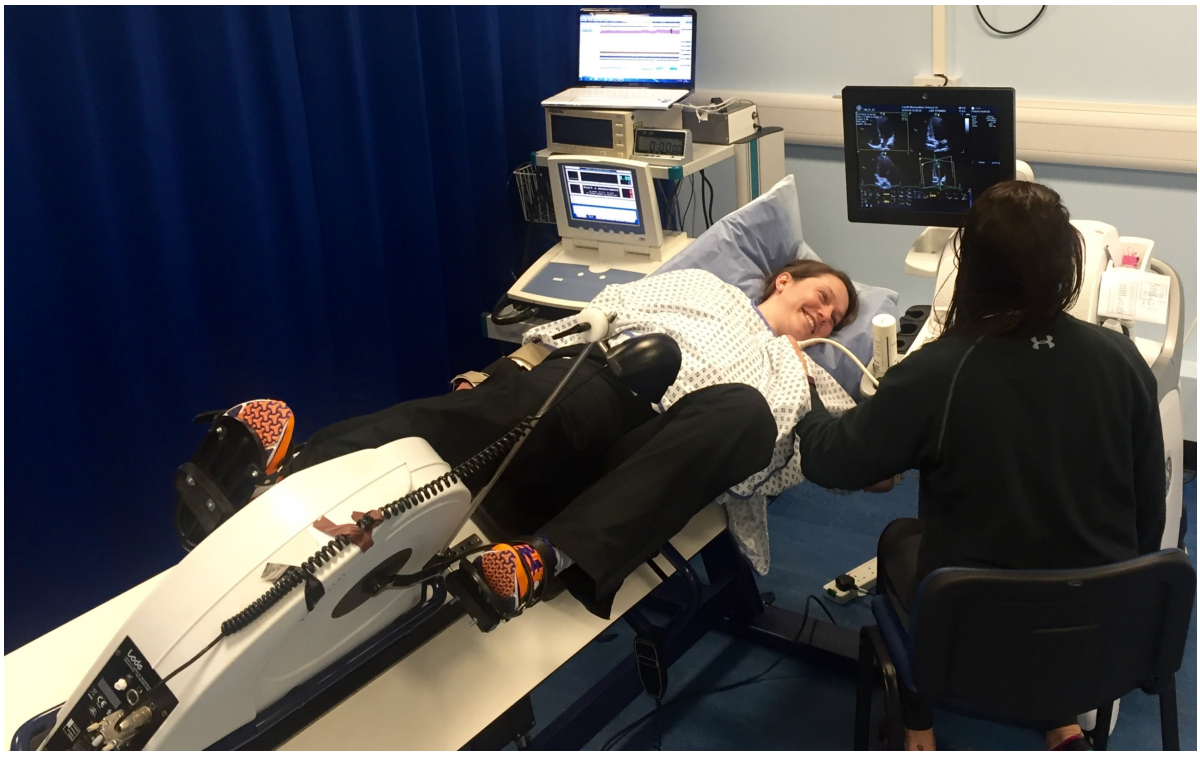


Figure 17. Exercise echocardiography completed on a supine cycle ergometer tilted in the left lateral position. The bed was tilted to between 30 and 45° for all volunteers.

Standardisation of image acquisition

The standardisation of image acquisition ensures the collection of high quality echocardiographic images. Acoustic windows describe locations on the anterior chest wall that allows the transmission of ultrasound waves to the cardiac structures. In adults, these sites are typically within the third and fifth intercostal space to the left of the sternum for the parasternal view, and in the 5th intercostal space on the left median axillary line for the apical view, as shown in Figure 18.

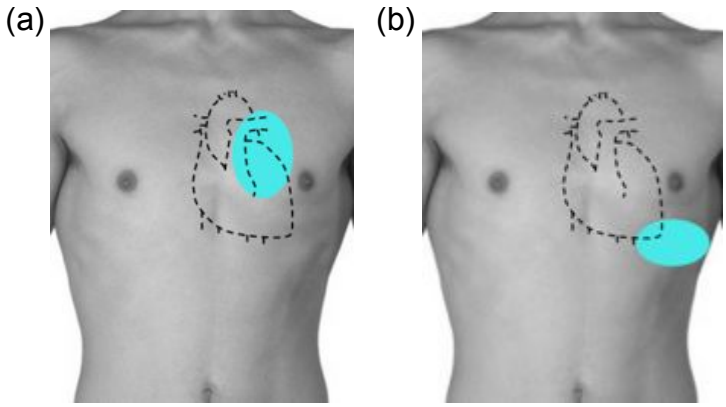


Figure 18. Acoustic windows at the a. parasternal and b. apical locations for imaging the adult heart. The parasternal acoustic window lies between the 3rd and 5th intercostal space left of the sternum. The apical acoustic window lies along the median axillary line in the 5th intercostal space.

The acoustic windows are highly variable, and exploration through movement of the transducer probe within these locations is required to identify the optimal image. As pregnancy progresses, the gravid uterus can cause alterations in the cardiac axis due to a general compression of the thoracic cavity. Sonographers involved in acquiring echocardiographic images must be aware of the potential adjustments that may need to be made in light of altered acoustic windows, and a longer duration of exploration may be required. The diaphragm may be elevated by up to 4 cm and the circumference of the chest wall expanded by 5-7 cm in some women (Hegewald and Crapo, 2011). Although an upward and leftward displacement of the maternal heart is hypothesised (Harvey, 1975), this positional change was not proven in a study using MRI when compared to the non-pregnant

state (Holmes et al., 2015). A suggested rotation of the cardiac axis and change in the anatomical relationship of the heart to the expanded chest wall, combined with the known cardiac hypertrophy may alter the optimal acoustic windows for echocardiography during pregnancy. It is therefore highly important that the sonographer use anatomical landmarks of the myocardium to dictate appropriate image acquisition in echocardiography in pregnant women. Anatomical landmarks used within the data collection for this thesis are presented later within this chapter in Table 6 and are in line with ASE recommendations for echocardiographic image acquisition (Lang et al., 2015).

The orientation of the transducer within an acoustic window allows the projection of an echocardiographic view. Images of the varying echocardiographic views are presented later in this chapter alongside descriptions for analyses. For example, orienting the transducer probe towards the volunteer's right shoulder will allow collection of the parasternal long-axis view (PLAX), and rotating the probe 90° towards the left shoulder will allow the collection of the parasternal short-axis view (PSAX). When in the PSAX view, holding the same orientation but leaning the probe towards the volunteer's feet will image the apex, whereas leaning the probe towards the volunteer's head will image the aortic valve. Adjustments through this plane allow the collection of images at mitral valve (MV) and papillary muscle (PM).

Echocardiographic image optimisation

Transducer frequency, size and focus all interact to affect image quality. Higher frequency probes result in the acquisition of images with greater spatial (as opposed to temporal) resolution and detail within the myocardium. Transducer size and frequency determine the length of the near field. A longer near field is optimal for image acquisition, and this is achieved by increasing the transducer size and frequency. However in practice, a larger transducer can be too large to image

within intercostal spaces and higher frequency results in lower penetration of the ultrasound beam. Focusing the beam is achieved through the use of a phased array transducer, in which a series of piezoelectric elements are interconnected. Adjusting the timing of excitation of these individual piezoelectric elements allows the beam to be steered and focused. A balanced approach to controlling transducer frequency, size and focus is required to maximise image acquisition. In this thesis, a 17 X 28 mm, 1.5–4.6 MHz phased array transducer with a depth of field up to 30 cm was used (M5S, GE Medical Systems).

Resolution is the ability to distinguish between two objects in close proximity and has two components: spatial and temporal. Spatial resolution refers to the distance that two targets must be separated by in order to be distinguishable, and has two dimensions: axial, differentiation of structures lying along the axis of the ultrasound beam, and lateral, differentiation of structures lying side by side relative to the ultrasound beam. Axial resolution is dependent on the frequency of the transmitted wave, with a higher frequency and shorter pulse creating a higher axial resolution. Lateral resolution is affected by the width of the ultrasound beam at a given depth. Wide beams are associated with distortion of objects, a loss of structures at the edge of the beam and artefacts. Gain, or the amplitude of the reflected wave, can be used to counteract such issues. Increasing the gain improves the acquisition of peripheral targets; however over-gaining an image may also cause image distortion, increased noise and an overall reduction in contrast resolution. Contrast resolution refers to the ability to distinguish between different shades of grey within an echocardiographic image. Contrast is a key determinant to the success of image analysis, as the differentiation between borders and structures within the myocardial tissue is imperative for measurements of all cardiac parameters. Finally, temporal resolution is the ability of the system to accurately track moving targets over time and is dependent on the frame rate of image acquisition, with

higher frame rates resulting in greater temporal resolution. It is important to consider that when acquiring images of structures with high velocity, narrowing the depth of field will optimise the temporal resolution.

During image acquisition, the sonographer considered the frequency of the transmitted wave, beam width, depth of image, gain, contrast and frame rate in order to optimise the image for subsequent analysis.

Echocardiographic image analysis

The echocardiography protocol used within this study abided by the Cardiff Metropolitan University cardiovascular ultrasound imaging approved protocol, attached as Appendix X. A three-lead electrocardiograph (ECG) was attached to the volunteer and connected to the ultrasound system. This allowed gating of the echocardiographic image to the electrical conduction cycle of the heart. For each image, five consecutive cardiac cycles were recorded at end expiration to limit displacement of the heart and changes in intrathoracic cavity pressure during respiration. Images were analysed offline (EchoPAC version 110.1.1, GE Medical, Horton, Norway). Three cardiac cycles were measured for each parameter and averaged. A single female sonographer performed all echocardiographic data acquisition and analysis (reliability presented later within this chapter).

The acquired echocardiographic images and measurement variables are presented later within this chapter in Table 6. The measurement of each parameter is described in more detail below.

Measurement of left ventricular structure and geometry

Internal linear dimensions of the LV were obtained from a parasternal long axis view (PLAX) using 2D echocardiography. Measurements were taken perpendicular to the LV long axis and at the level of the MV leaflet tips in line with recommendations (Lang et al., 2015). Using the caliper tool, the interventricular

septal wall thickness (IVST) was measured as the interface between the myocardial wall and the internal LV cavity. The LV internal diameter (LVID) was measured as the interface between the septal and posterior walls. The LV posterior wall thickness (PWT) was measured at the interface between the internal LV cavity and the pericardium. All measurements were taken at end-diastole and end-systole (d and s, respectively follow each acronym). LV length was measured from a 2D apical 4-chamber view at end-diastole, and is the distance between the base to the apex, specifically the middle of the mitral valve contour and the most distant part of the LV contour. Relative wall thickness (RWT) was calculated as (2*PWTd)/LVIDd. Sphericity index, a measure of LV geometry, was calculated as LV length/LVIDd. LV mass was calculated using the area-length method using LV length and mean wall thickness (Figure 19) (Lang et al., 2015) and allometrically scaled to height, as detailed later in this chapter. End-systolic wall stress (ESWS) was calculated as 1.33 * SBP * $\left(\frac{ESCA}{ESMA}\right)$, where SBP was a surrogate for LV end-systolic pressure, ESCA is end-systolic cavity area, and ESMA is end-systolic myocardial area, measured on a short axis view at the mid-papillary level (Haykowsky et al., 2001).

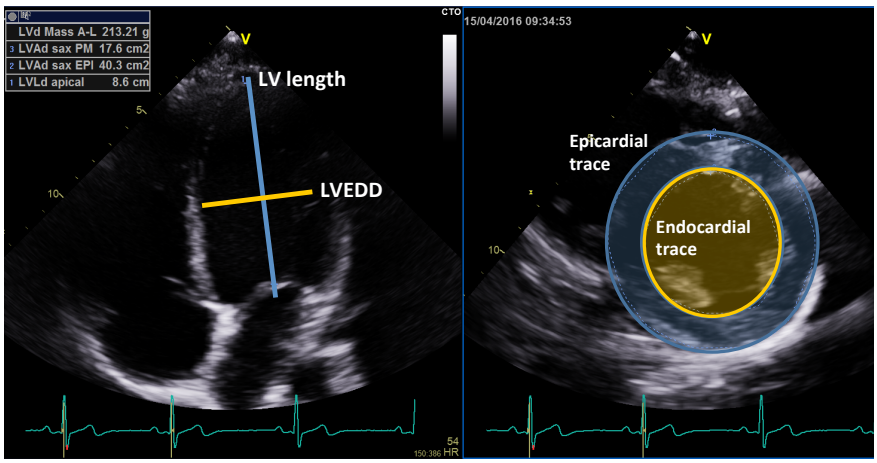


Figure 19. Left ventricular (LV) mass measurement using the area-length method. LV length from an apical 4-chamber (left panel) image at end-diastole and mean wall thickness from tracing the cross sectional area of the epicardial and endocardial border on a parasternal short axis view at the level of the papillary muscle at end-diastole (right panel). LV length and LV end-diastolic diameter (LVEDD) are used to calculate sphericity index.

Measurement of global left ventricular function

Stroke volume (SV) was measured using the Simpson's biplane method as recommended by the ASE (Lang et al., 2015). This method accounts for the shape of the LV along its entire length in two planes. The area change from end-diastolic to end-systolic is used to estimate LV volumes on both the 2D apical 4-Ch and apical 2-Ch view, as shown in Figure 20. Using the EchoPAC analysis software, a validated formula provides a composite end-diastolic volume (EDV) and end-systolic volume (ESV), and therefore SV (EDV-ESV). HR was averaged from the corresponding ECG trace on the analysed cardiac cycles, allowing the resultant calculation of cardiac output. Both SV and cardiac output were allometrically scaled to height to reduce the influence of body size on comparisons. Ejection fraction was also computed within this analysis. Using an average of MAP; measurement described later in this chapter), systemic vascular resistance (SVR) was calculated as MAP/cardiac output.

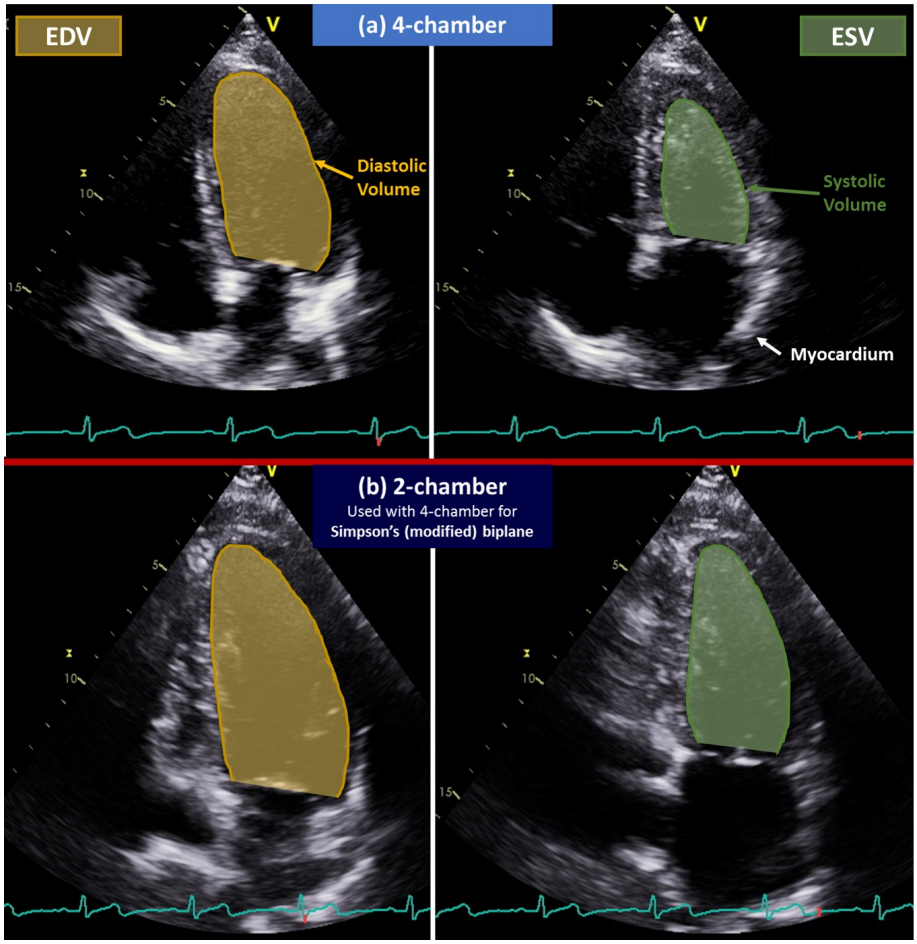


Figure 20. Echocardiographic single plane B-mode images of apical (a) 4-chamber and (b) 2-chamber views used primarily in the measurement of left ventricular volumes. Both views are used to calculate cardiac output via the Simpson's Biplane method. Stroke volume is estimated by measuring the area-change between end diastolic (EDV) and end systolic volumes (ESV) of the left ventricle, shown on the figure by yellow and green areas, respectively.

TDI was performed on an apical 4-Ch image to measure myocardial velocities during contraction and relaxation. The sample volume was placed at the level of the mitral valve annulus on the septum and left lateral wall and at the level of the tricuspid annulus on the right ventricle, as shown in Figure 21. The peak systolic (S'), early (E') and late (A') diastolic velocities of each wall were measured at each site. Septal and LV S', E' and A' were scaled to LV length (Batterham et al., 2008). All measures were made in triplicate over three cardiac cycles and averaged. TDI images were collected with frame rates >100 frames per second achieved by narrowing the sector width as much as possible.

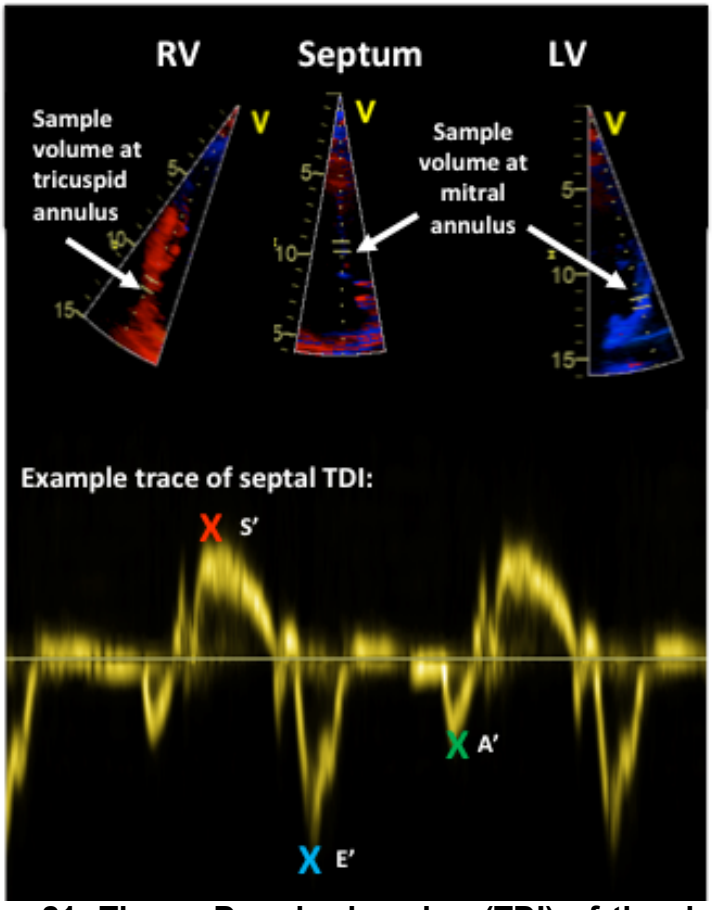


Figure 21. Tissue Doppler imaging (TDI) of the right ventricle (RV), septum and left ventricle (LV) to measure myocardial displacement velocities across the cardiac cycle. Peak systolic (S'), early (E') and late (A') diastolic velocities are measured at the valve annulus on each wall.

The E/A ratio was calculated to allow an assessment of diastolic function. E refers to the peak early diastolic filling velocity through active relaxation of the LV, whilst A refers to the peak active filling velocity as a result of atrial systole. Using pulsed wave Doppler, the mitral inflow velocity was measured on an apical 4-Ch view with a 1-2 mm sample volume between the tips of the mitral leaflets in the LV cavity. Using the caliper tool, peak E- and A-wave velocity were measured, as shown on Figure 22. Deceleration time, defined as the time interval from peak E to the cessation of the early filling phase, was determined as an indicator of LV compliance. E/E' ratio was calculated as a surrogate measure of LV filling pressure (Park and Marwick, 2011).

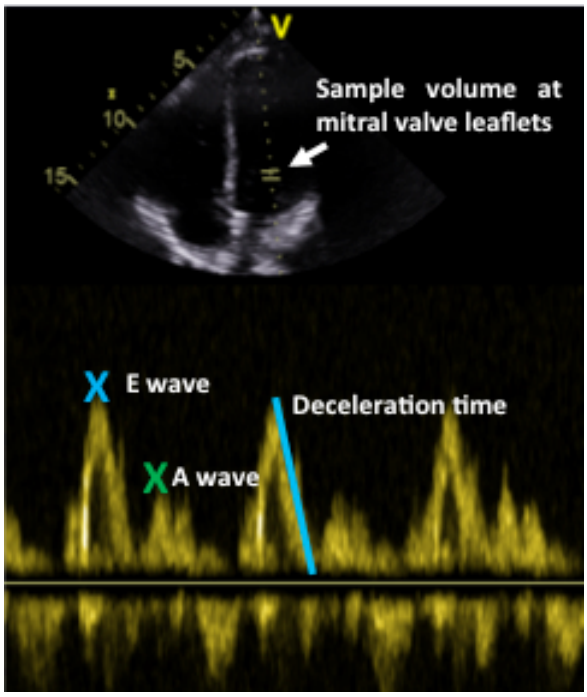


Figure 22. Measurement of trans-mitral filling velocities of the left ventricle, where the E and A wave represent passive and active filling. Deceleration time is the duration of the early filling phase, from peak to cessation.

Left ventricular mechanics

Measurement of LV rotation, longitudinal, circumferential and radial strain were completed using speckle tracking echocardiography. This is an offline analysis technique applied to 2D ultrasound images. Speckle tracking utilises the interference patterns or natural acoustic markers in grey scale 2D ultrasound images within myocardial tissue, known as speckles (Helle-Valle et al., 2005, Notomi et al., 2005, Modesto et al., 2006). These patterns are stable from frame-to-frame and blocks of speckles can be tracked using an automatic block matching process. The blocks of speckles then provide local displacement information in any direction within the imaging plane (Mor-Avi et al., 2011) as shown in Figure 23.

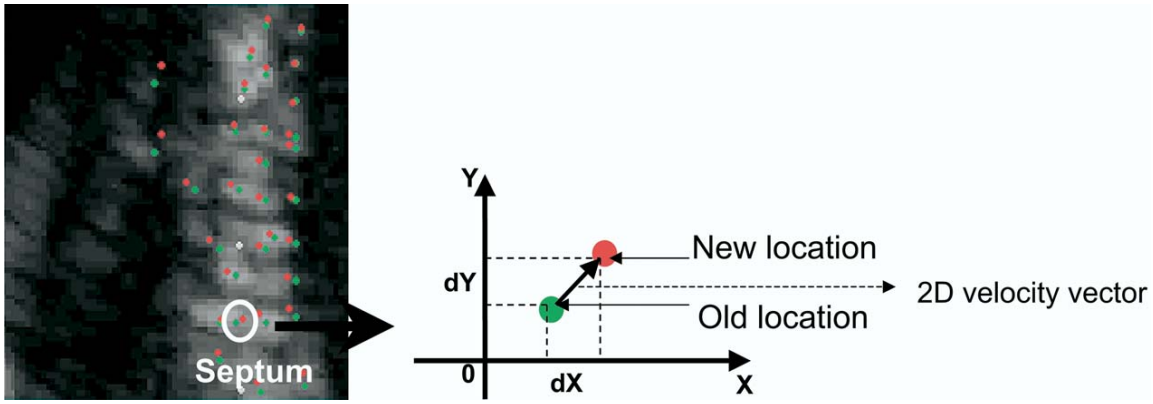


Figure 23. Left ventricular deformation is measured using speckle-tracking echocardiography, a method that utilises the displacement of natural acoustic markers, known as speckles, within the myocardium. The speckles are tracked from frame to frame across the cardiac cycle the pattern of this displacement allows assessment of strain in the axis of movement rather than in relation to the ultrasound beam, as with TDI. Adapted from Marwick (2006).

Speckle tracking analysis quality is dependent on the balance between spatial and temporal resolution of the 2D image. Low frame rates may result in a loss of speckles and under sampling, whereas high frame rates can reduce the spatial resolution (Mor-Avi et al., 2011). Therefore, images were acquired within a range of 70-90 frames per second (Stöhr et al., 2011a) and this was kept constant throughout all repeated measures. In addition, ultrasound artifact or image shadowing can reduce the quality of tracking, and therefore care was taken to avoid such issues during acquisition (Mor-Avi et al., 2011). Speckle tracking echocardiography has good correlation and agreement with MRI in humans for both strain (Amundsen et al., 2006) and LV twist (Helle-Valle et al., 2005), with r = 0.87 and 0.85, respectively.

Speckle tracking analysis is a semiautomatic method, which requires manual tracing of the endocardial border on a static image (either on the Ap-4, PSAX apex or mitral valve image) during offline analysis. The operator traces the region of interest and adjusts to cover the LV walls, avoiding the pericardium. The optimal tracking of the myocardial tissue was then assessed by the software and visually confirmed by the operator. Therefore, reproducibility of this method is dependent

on the operator's ability to collect 2D echocardiographic images with high temporal and spatial resolution and also, their ability to identify and trace the LV during analysis (reliability is reported in later within this chapter). After alteration and verification, cardiac cycles with insufficient tracking were excluded from the analysis. These exclusions will be noted within each affected dataset in each of the chapters.

Strain and/or rotation curves for three cardiac cycles were generated using the speckle tracking software. The software determines curves for six segments within the region of interest to provide measurement of regional function. In this thesis, the data presented is an average of the six segments and reflects global deformation. The raw frame-by-frame data was transformed using software (2D Strain Analysis Tool, Stuttgart, Germany) that applied a cubic spline interpolation. The total cardiac cycle was transformed to 1200 data points, with 600 data points in systole and 600 data points in diastole. The distinction between end-systole and diastole was made by the software using aortic valve closure. This allowed for data to be time-aligned, allowing for inter- and intra-individual variability in heart rate and individualised frame rate at image acquisition. Peak values represent the maximal value of strain/rotation observed across the cardiac cycle and were averaged for each group. Systolic peak values were taken during systole. Peak untwisting and diastolic values was taken during early diastole, and therefore greater values occurring at end-diastole were not included in the analyses. The time taken to achieve the peak for each parameter was expressed as a percentage of the cardiac cycle, and not in absolute values due to the anticipated differences in resting heart rate of pregnant females. Average curves for all strain and rotation parameters were also calculated for each independent group to allow presentation in a graphical format.

i. Strain

Strain is defined as the change in length of a segment of myocardium relative to its resting length and is expressed as a percentage change from the original position; strain rate is the rate of this deformation (D'Hooge et al., 2000). Strain is reported in three planes: longitudinal strain reflects the deformation of the long axis of the heart; circumferential strain reflects the deformation of the circumference; and finally, radial strain reflects the deformation of the transmural myocardial axis (previously shown in Chapter 2, Figure 6). Global longitudinal strain was determined using an apical 4-Ch image, whereas global circumferential and radial strain were derived from PSAX views at the apical and mitral valve levels. Speckle tracking of the myocardium on each respective image was completed to determine peak and time to peak (%) longitudinal, basal and apical circumferential strain. Strain rate peak and time to peak (%) were also reported.

ii. Left ventricular twist and torsion

Rotation is the circular movement of the LV about its long axis. The apex and base rotate in opposite directions, clockwise and anticlockwise, and result in a net movement, termed twist. Speckle tracking was used to determine basal and apical rotation using PSAX images at the mitral valve level and apex, respectively, shown in Figure 24. Twist was calculated by subtracting time-aligned basal rotation from apical rotation; whereby positive rotation occurs at the base and negative rotation at the apex in healthy individuals. Peak and time to peak twist (%), apical and basal rotation were measured and reported within this thesis. Twist was scaled to LV length to calculate torsion (°/cm) (Cameli et al., 2011). LV systolic twisting rate and untwisting rate were also reported.

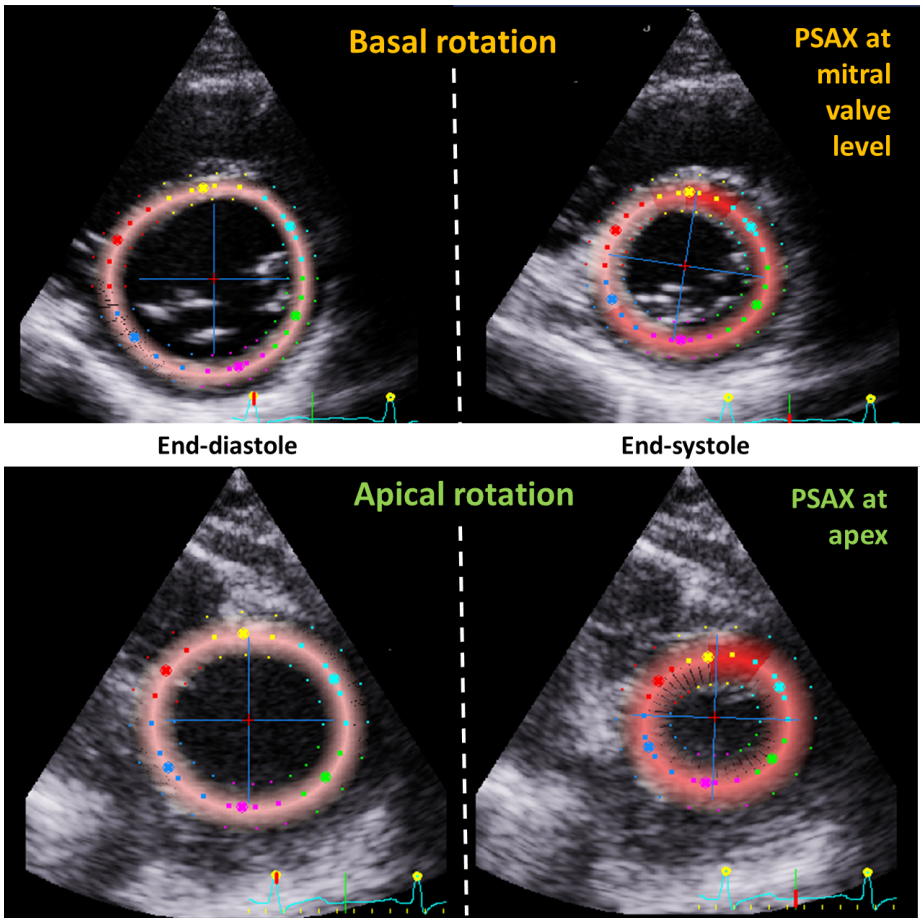


Figure 24. Apical and basal rotation at end-diastole and end-systole measured from a parasternal short axis (PSAX) images at the mitral valve level and apex. The net basal and apical rotation is termed twist.

In summary, echocardiographic images were collected for measures of cardiac structure and function. The images were collected according to recent recommendations and accepted best practice. The full acquisition protocol, anatomical landmarks and respective measurements are listed in Table 6.

Table 6. Acquired echocardiographic images, anatomical landmarks and respective measurement variables for experimental studies.

Image	Anatomical landmarks	Measurement of:
PLAX *	Between 3rd – 5th intercostal space; Left of sternum; Orientation of probe towards right shoulder; Visualisation of structures with posterior wall perpendicular to bottom of image; Visualisation of aortic and mitral valve leaflets; Unlikely to frame apex in image.	LV dimensions.
PSAX	Between 3rd – 5th intercostal space; Rotation of probe from PLAX 90° towards left shoulder.	
Mitral valve	Visualisation of the mitral valve leaflets; Anterior and posterior leaflet in upper and lower positions, respectively; Circular cross section.	LV basal rotation; Circumferential strain.
Papillary muscle *	Visualisation of the anterolateral and posteromedial papillary muscles inside of the LV cavity; Circular cross section.	Mean wall thickness from endo- and epi-cardial cross sectional area for LV mass.
Apex	Close to LV obliteration point; Move transducer to true apex in lower rib space if required.	LV apical rotation; Circumferential strain.
Apical 4-Ch	5th intercostal space on the left median axillary line; Orientation of probe towards volunteers left hand side; Visualisation of four chambers; Septum to be central to the image; Avoid chamber foreshortening.	LV end-diastolic and end-systolic volumes: Longitudinal strain; LV length; E/A ratio*; TDI of septum, left lateral* and right * wall.
Apical 2-Ch	Counter clockwise rotation from Apical 4-Ch view by approx. 45°; No visualisation of right chambers; Visualisation of coronary sinus.	LV end-diastolic and end-systolic volumes.

NB * acquired only at rest. *PLAX,* parasternal long axis; *PSAX,* parasternal short axis; *4-Ch*, four chamber; 2-*Ch*, two chamber; *2D*, two-dimensional; *LV,* left ventricular; *LVOT,* left ventricular outflow tract; *E/A*, early to late filling velocity; *TDI*, tissue Doppler imaging.

Scaling of cardiac parameters

The influence of body size on cardiovascular structure and function is well established; therefore, making comparisons between independent groups with known differences in body size becomes problematic. In general, males have a greater body mass and height when compared to females, whilst pregnant females have a greater body mass than non-pregnant and postpartum females. In general clinical practice (Dewey et al., 2008), and also specifically in research studies of pregnant populations (Melchiorre et al., 2011, Vartun et al., 2014, Moran et al., 2002), ratiometric scaling to body surface area (BSA), an indicator of metabolic mass, has been used. However, body mass, a key component of most BSA calculations, is known to alter across pregnancy as a result of extracellular fluid, foetal, placental and uterine growth, enlargement of breast tissue, and an increase in adipose tissue (Rasmussen and Yaktine, 2009). These unique alterations in maternal morphology do not construe the same internal physiology of the assumptions of BSA and may affect the accuracy of BSA estimation. The calculation of BSA is also based upon the assumption that the geometrical shape of the human body is a cylinder (Wang and Hihara, 2004). Some authors suggest that body shape can reduce the accuracy of BSA estimation (Lee et al., 2008), and this is evidenced through various reports of underestimation or overestimation, dependent on calculation, of BSA in obese individuals (Verbraecken et al., 2006). Body shape is altered significantly during pregnancy, with localised anterior mass as a result of foetal growth. The assumptions of a cylindrical body shape for BSA during pregnancy may therefore be compromised, and an alternative anthropometric variable should be used for this population. Height is not altered across the time course of pregnancy and is therefore a pregnancy-independent anthropometric variable. In the general population, the indexation of LV dimensions to height has been associated with greater error than BSA (Batterham

and George, 1998), however given the limitations of BSA in this population, this was deemed the most appropriate method.

Ratiometric scaling is dependent on an assumption that a linear relationship exists between the cardiovascular parameter and the anthropometric variable. However, variables scaled in this manner may still correlate with body size. As a result, allometric scaling is recommended as a more appropriate method to determine body size-independent scaled cardiovascular variables (Dewey et al., 2008). Allometric scaling involves division of a cardiovascular parameter by an anthropometric variable that is raised to a scalar exponent. In this thesis, cardiovascular parameters were allometrically scaled to height using previously published exponents (de Simone et al., 1992, de Simone et al., 1997) or predicted exponents determined by the theory of dimensionality (Batterham et al., 1999) where published exponents were not available, as shown in Table 7. Allometrically scaled data will be reported alongside absolute values in all cases.

Table 7. Allometric scaling exponent for height and cardiac parameter.

Cardiac parameter	Allometric exponent	Reference
LV mass (g)	2.70	de Simone et al. (1992)
Cardiac output ($L \cdot min^{-1}$)	1.83	de Simone et al. (1997)
Stroke volume (ml)	2.04	de Simone et al. (1997)
End diastolic and end systolic volumes (ml)	2.00	Batterham et al. (1999)
LV dimensions: IVS, LVPW, LVID, LV length (cm)	1.00	George et al. (1999)

Reliability of echocardiographic measurement

The quality and interpretation of echocardiographic measurement may be limited by a number of factors, including sonographer image acquisition and analysis. The reproducibility of measurements should be reported in all research, and sonographers should routinely assess the accuracy and reliability of their work to affirm standards are met (Popescu et al., 2009). For the purpose of this thesis, 21 volunteers were included in the analyses of intra-observer reliability (11 non-pregnant females; 1 pregnant female, 9 males). All volunteers provided voluntary informed consent for the collection and analysis of repeated, echocardiographic images for the purpose of reliability assessment. Characteristics for the group are presented in Table 8.

Table 8. Characteristics of population used within reliability assessment of echocardiographic image acquisition and analysis (*n* = 21). Data presented as mean ± SD.

Age (years)	28.4	±	10.8
Height (cm)	169.4	±	9.0
Mass (kg)	66.3	±	11.5
BMI ($kg \cdot m^2$)	23.1	±	2.1
Systolic blood pressure (mmHg)	113	±	11
Diastolic blood pressure (mmHg)	66	±	8

N.B. BMI, body mass index.

Echocardiographic images (PSAX: mitral valve, papillary muscle and apex; AP 4-Ch, TDI of the septum and AP 2-Ch) were collected for each individual at rest. The acquired images followed the same protocol as the repeated measures design used within the main study. An initial set of images was acquired within a 20-minute period, after which a second set of images was then collected.

A subgroup of volunteers (*n* = 10) completed an additional trial in which echocardiographic images were collected during low-intensity supine cycling. The power output prescribed was the average value for non-pregnant females and males at 25% of estimated maximum supine power output (40 and 60 W,

respectively, calculated from Visit 1 data). Ninety seconds after the initiation of exercise, an initial set of echocardiographic images was acquired as described above. A second set of images was collected immediately after the completion of the first set, whilst the volunteer remained cycling. The bout of exercise lasted no longer than 7 minutes in total. Blood pressure was measured continuously throughout each trial using non-invasive beat-by-beat finger plethysmography (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands). Cardiac images were analysed offline by the lead researcher (VM). As per the main study protocol, an average of three cardiac cycles was calculated for each parameter. The coefficient of variation was calculated and expressed as a percentage (Bland, 2015). The reliability measures for the most critical parameters of this thesis are included, as shown in Table 9.

Table 9. Coefficient of variation for echocardiographic parameters at rest and during low intensity supine cycling exercise.

Cardiac parameter	Units	Coefficient of Variation (%)	
		Rest (*n* = 21)	Exercise (n = 10)
Septal tissue velocities			
S'	$m \cdot s^{-1}$	5.8	5.1
E'	$m \cdot s^{-1}$	7.1	5.7
A'	$m \cdot s^{-1}$	5.5	6.0
Left ventricular volumes and global function			
End diastolic volume	ml	3.0	2.7
End systolic volume	ml	7.0	9.7
Ejection fraction	%	3.6	3.7
Stroke volume	ml	3.0	3.2
Heart rate	$beats \cdot min^{-1}$	2.6	3.0
Cardiac output	$L \cdot min^{-1}$	3.2	3.9
Left ventricular mechanics			
Twist	°	24.3	20.0
Basal rotation	°	29.5	27.4
Apical rotation	°	24.0	24.2
Basal circumferential strain	%	11.7	12.9
Apical circumferential strain	%	10.5	9.2
Longitudinal strain	%	7.7	8.5
Blood pressure (n = 10)			
Systolic	mmHg	0.9	1.5
Diastolic	mmHg	1.0	1.4
Mean arterial pressure	mmHg	0.8	1.7

N.B. S', systolic; E', early; A', late tissue velocity.

The intra-observer agreements calculated in this cohort are similar to that of published data of resting measurements in pregnant (Melchiorre et al., 2011) and non-pregnant (Armstrong et al., 2015) populations. Previously published data reporting intra-observer reliability during exercise is extremely limited. A previous study reported a coefficient of variation of 6% for the measurement of SV using Doppler at the suprasternal notch (Ihlen et al., 1987). The difference in echocardiographic methods prevents direct comparison to the current dataset; however, the coefficient of variation for global function measures during exercise is below this previously reported value.

Blood pressure measurement

Resting blood pressure

Resting systolic and diastolic blood pressure (SBP and SBP) were assessed via the auscultatory method using a stethoscope (Classic III, Littman, 3M Healthcare, Minnesota, USA) and sphygmomanometer (DuraShock DS54 Sphygmomanometer, Welch Allyn, New York, USA) placed on the bare upper left arm. Mean arterial pressure (MAP) was calculated as $\frac{1}{3}SBP + \frac{2}{3}DBP$. After 5 minutes of seated rest, two measurements were taken in line with the recommendations of the AHA (Pickering et al., 2005). Where a difference >5 mmHg was observed between the first and second reading, additional readings were taken until agreement between two consecutive values was found.

Continuous measurement of peripheral blood pressure

Continuous measurement of peripheral blood pressure is essential in determining the response to a functional haemodynamic challenge. In this thesis, peripheral blood pressure was measured continuously throughout the sustained isometric hold and aerobic cycling using a non-invasive beat-by-beat arterial blood pressure technique known as finger photoplethysmography (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands). This technique is based upon finger arterial pressure pulse contour analysis. The pulse pressure is assessed using photoelectric plethysmography in combination with a volume-clamp technique through an inflatable finger cuff, attached to the right middle finger. The volume-clamp technique maintains a constant diameter of the artery via external pressure from the cuff. Changes in arterial diameter are detected by an infrared photo-plethysmograph built into the finger cuff and are impeded by an inflatable air bladder to maintain the correct clamped diameter. Brachial blood pressure was used to calibrate the equipment using an integrated automated arm cuff prior to

data collection. Physical characteristics (age, height and weight) were input into the software to determine individual aortic pressure-area relationships. It is worth noting that the accuracy of these algorithms may be reduced during pregnancy due to changes in body habitus and remodelling of the aorta as a result of gestation. Despite these potential sources of inaccuracy in the absolute measurement during pregnancy, the system is able to effectively track change in blood pressure characteristics across time and in response to physiological challenges, such as an increase in afterload.

SBP, DBP and MAP (calculated as above) were recorded continuously (PowerLab, ADInstruments, Chalgrove, UK) and saved for later offline analysis. Average values were calculated from twenty continuous waveforms (cardiac cycles) selected from a clean trace within the final 2 minutes of each procedure (LabChart v7, ADInstruments, Chalgrove, UK). Cardiac output may be derived from this technique through analysis of the pulse contour using aortic characteristic impedance, compliance and estimated cardiac afterload (Dyson et al., 2010), however, the accuracy of photoplethysmography in estimating cardiac output has been contested in states of changing vascular resistance, such as that experienced during pregnancy (Elvan-Taspinar et al., 2003). Therefore, all cardiac output data presented in this thesis was determined using echocardiography, described previously in this chapter.

4.4. Statistical analyses

Descriptive data are presented as mean and standard deviation throughout this thesis. Statistical analysis was conducted using either SPSS (Version 22.0 IBM SPSS Statistics for Macintosh, IBM Corp., Armonk, NY) or GraphPad Prism software (GraphPad Prism for Mac, Version 7.0a, Dan Diego, California, USA). Alpha was set at 0.05. Statistical methods utilised will be detailed within the

methodology section of each chapter, however general protocols are described as follows.

ANOVA

One-way analyses of variance (ANOVA) were used to detect differences between groups. In all uses, data were checked to ensure assumptions of one-way ANOVA were met. Boxplots were used to identify outliers, Levene's test of equality of variances confirmed the homogeneity of variance (> 0.05) and normality was assessed using Shapiro-Wilks test (> 0.05). Outliers were included in the analyses without transformation, as data fell within normal physiological ranges. Tukey-Kramer *post hoc* test comparisons were completed if the main effects were statistically significant. In cases where variance was not equal and the assumption of homogeneity was violated, Welch's ANOVA was used and if significant, was followed by Games-Howell *post hoc* analyses. In cases where data were not normally distributed, the nonparametric Kruskall Willis H test was completed. Effect size, specifically partial eta squared (η_p^2), was estimated for all analyses using a full factorial univariate model within SPSS. Effect size was interpreted as 0.01 = small effect, 0.06 = medium effect and 0.14 = large effect (Cohen, 1988).

ANCOVA

Analyses of covariance (ANCOVA) were used to determine differences between groups after incorporation of one or more covariate parameters. Assumptions of ANCOVA were checked: linear relationships and homogeneity of regression slopes between parameters were assessed by visual inspection of scatterplots. Standardised residuals for groups and the overall model were assessed by Shapiro-Wilk's test ($P > .05$) for normal distribution. Homoscedasticity and homogeneity of variance were assessed by visual inspection of a scatterplot and Levene's test of homogeneity of variance, respectively. If statistical significance between groups was identified, *post hoc* analyses were performed with a

Bonferroni adjustment. Effect size, η_p^2, was estimated using a full factorial univariate general linear model (GLM).

Correlations

Pearson's correlation was used to identify relationships between continuous variables. Linearity and outliers were confirmed by visual assessment of scatter plots. Outliers were included in analyses without transformation if identified, as above. Normality was assessed using Shapiro-Wilks test (> 0.05). The correlation coefficient (r) was interpreted as $0.1 < r < 0.3$ small correlation; $0.3 < r < 0.5$ moderate correlation and $r > 0.5$ strong correlation, and + or – indicate positive and inverse relationships, respectively (Cohen, 1988). The coefficient of determination (r^2) was calculated to determine the variance between variables.

Chapter 5. Cardiac structure and function at rest in non-pregnant, pregnant and postpartum females

5.1. Introduction

Healthy pregnant females experience significant cardiac remodelling and functional adaptation to meet the additional metabolic demands of gestation (Melchiorre et al., 2012a, Meah et al., 2016). The maternal heart experiences increased preload (greater end-diastolic volume; EDV), increased heart rate (HR) and reduced afterload (decreased blood pressure and systemic vascular resistance; BP and SVR) contributing to greater cardiac output and stimulating eccentric hypertrophy, as shown in Chapter 3 and previous literature (Meah et al., 2016, Melchiorre et al., 2012a, Savu et al., 2012). After delivery, metabolic demand and changes in haemodynamic load result in the return of cardiovascular adaptation to the non-pregnant state (Savu et al., 2012, Meah et al., 2016).

Despite the structural adaptations noted above, impaired resting systolic and diastolic function in the third trimester of pregnancy has been reported (Zentner et al., 2009, Kametas et al., 2001, Estensen et al., 2013, Cong et al., 2015, Savu et al., 2012, Bamfo et al., 2007). The previously identified impairments in function were observed using traditional echocardiographic measures such as ejection fraction (EF) and tissue displacement velocity. These techniques oversimplify the complexities of myocardial deformation. Left ventricular (LV) mechanics, namely strain and twist, characterise this deformation across the cardiac cycle and provide greater insight into subtle changes in cardiac function than EF and tissue velocities alone. Therefore, assessment of these measures may identify how maternal cardiac function is affected during gestation.

Alterations in cardiac structure and haemodynamic load are known to influence LV mechanics. Acute increases in HR, preload and contractility, or decreases in afterload result in a greater LV strain and twist in the healthy human heart (Weiner et al., 2010b, Stöhr et al., 2016, Cameli et al., 2011, Burns et al., 2010a, Burns et al., 2010b). Therefore, it could be hypothesised that LV mechanics would be

enhanced during healthy pregnancy as a result of favourable changes in preload, contractility and afterload. However, previous research has shown conflicting results. During the late stages of healthy pregnancy, longitudinal and circumferential strain were unchanged (Ando et al., 2015, Tzemos et al., 2008) or decreased (Cong et al., 2015, Estensen et al., 2013, Savu et al., 2012, Sengupta et al., 2017, Papadopoulou et al., 2013), whereas twist was increased at rest (Hristova et al., 2016, Papadopoulou et al., 2013, Tzemos et al., 2008, Yoon et al., 2011). These findings have been interpreted as enhanced, maintained or impaired function adding confusion to the understanding of cardiac adaptation in healthy pregnancy. Additionally, the influence of haemodynamic load on LV mechanics during healthy pregnancy has not been considered, and it is not known if HR, preload, afterload or contractility underpins the changes observed in LV strain and twist.

The objective of this chapter is to investigate cardiac structure and function in the late second trimester of healthy pregnancy and in the postpartum period, with a specific focus on potential changes in the underpinning mechanics and haemodynamic load. It was hypothesised that, when compared to non-pregnant females at rest:

(i) Pregnant females would have a significantly greater preload (EDV), HR, and reduced afterload (SVR), resulting in significantly greater cardiac output and systolic function (*e.g.* increased LV twist, longitudinal and circumferential strain).

(ii) The greater LV mechanics in pregnant females would be significantly related to preload (EDV), afterload (MAP) and HR.

(iii) Postpartum females would present with a structural and functional phenotype similar to non-pregnant women.

The understanding of LV mechanics in healthy young females at rest is founded on a relatively small number of studies (Kishi et al., 2015, Hurlburt et al., 2007, Giraldeau et al., 2015, Reckefuss et al., 2011, Williams et al., 2017, Williams et al., 2016); therefore males were included in this study as a control group to validate the findings of the current dataset.

5.2. Methods

Ethical approval and volunteer inclusion

The experimental procedures were reviewed and approved by the Cardiff Metropolitan University Research Ethics Committee (Appendix I.b.). The study complied with the guidelines set out in the Declaration of Helsinki and written voluntary informed consent was gained from all volunteers.

Sixty-two individuals volunteered to participate in this study (non-pregnant females = 19, pregnant females = 15, postpartum females = 13, males = 16). Based on self-report, volunteers were healthy non-smokers, free from cardiovascular and/or metabolic diseases and were not taking any medication at the time of inclusion. All pregnant and postpartum females had uncomplicated, singleton pregnancies and were primiparous, although women who had suffered one previous miscarriage before 12 weeks gestation were included (pregnant n = 2; postpartum n = 2). The average time point of assessment for pregnant females was 25.4 ± 0.6 weeks gestation, and for postpartum females 15.1 ± 1.3 weeks after delivery. Non-pregnant women were nulliparous and had never tried to conceive.

General procedures

Volunteers attended the laboratory for resting cardiovascular assessments. Volunteers were asked to abstain from caffeine and/or alcohol for 18 hours and strenuous exercise for 24 hours prior to the visit. Volunteers' freestanding stature was measured (Holtain, Fixed Stadiometer, Pembs, UK), body mass was

determined using an electronic scale (SECA, Model 770, Vogel & Halke, Hamburg, Germany) and BMI was subsequently calculated through the division of body mass by the square of height.

Data collection and analysis

Resting blood pressure

Blood pressure was measured on the left arm after 5 minutes of seated rest using the auscultatory method with a stethoscope and sphygmomanometer. Two measurements of systolic and diastolic blood pressure (SBP and DBP) were taken and averaged. Mean arterial pressure (MAP) was calculated as described within Chapter 4.

Echocardiography

A trained sonographer acquired echocardiographic images after 15 minutes of quiet rest using a commercially available ultrasound system and 1.5 – 4.6 MHz phased array transducer (Vivid E9 and MS5, GE Medical Systems, Horten, Norway). The reliability of the sonographer in the acquisition and analysis of global haemodynamic and functional variables at rest is presented in Chapter 4, Table 9. A three-lead electrocardiograph was attached to the volunteer and connected to the ultrasound system for HR monitoring. Volunteers were scanned in the left lateral position tilted between 30 and 45° (Angio 2003, Lode, Groningen, Netherlands).

Echocardiographic images were collected to allow the assessment of LV structure and function in accordance with current guidelines (Lang et al., 2015, Nagueh et al., 2009). Two dimensional (2D) parasternal long axis and short axis views at the base (mitral valve), mid (papillary muscle) and apex levels and apical 4- and 2-chamber views were collected. Tissue Doppler imaging (TDI) of the septal mitral annulus and pulsed wave Doppler of mitral inflow velocities were measured from

the apical window. A full protocol was presented previously in Chapter 4, Table 6. 2D images were acquired within a range of 70-90 frames per second, whereas TDI images were collected with frame rates >100 frames per second. Five consecutive cardiac cycles were recorded at end expiration to limit displacement of the heart and changes in intrathoracic cavity pressure during respiration. Data were stored for later offline analysis (EchoPAC PC Version 112.1.0, GE Medical, Horton, Norway). Measurements were made in triplicate from different cardiac cycles and averaged.

a. Analysis of standard two-dimensional echocardiographic parameters

Linear dimensions including intraventricular septal wall thickness (IVS), LV internal diameter (LVID), posterior wall thickness (PWT) and LV length were measured at end-diastole (d) in line with recommendations (Lang et al., 2015). LV mass was calculated using the area-length method (Lang et al., 2015). Relative wall thickness (RWT), sphericity index and end-systolic wall stress were calculated as previously described (Chapter 4).

Cardiac volumes, including SV, EDV and end-systolic volume (ESV) were derived via the Simpson's biplane method. This allowed the subsequent calculation of cardiac output. Exponents for allometric scaling to height were used to remove the influence of body size on LV volumes (cardiac output [1.83], SV [2.04], EDV and ESV [2.00], detailed previously in Chapter 4, Table 7).

Traditional measures of systolic and diastolic function were also measured. Using the volume measurements above, EF was calculated. Peak systolic (S'), early (E') and late (A') diastolic septal velocities were measured to determine tissue displacement across the cardiac cycle and were scaled to LV length (Batterham et al., 2008). Trans-mitral blood flow velocities (E and A) were also measured and the E/A ratio calculated.

b. Analysis of left ventricular strain and twist

Speckle-tracking echocardiography (STE) was used to measure longitudinal, basal and apical circumferential strain, as well as basal and apical rotation to calculate LV twist, described in more detail in the Chapter 4. Strain and rotation curves for three cardiac cycles were generated and averaged. The raw frame-by-frame data was transformed using software (2D Strain Analysis Tool, Stuttgart, Germany) that applied a cubic spline interpolation to separate systole and diastole into 600 data points each. This enabled time alignment of data, allowing for inter- and intra-individual variability in HR and individualised frame rate at image acquisition. Cardiac cycles with insufficient tracking were excluded from the analysis. Exclusions will be noted within each affected dataset. Peak, time to peak (%), systolic and diastolic parameters of strain, rotation and twist were calculated.

Statistical analyses

Statistical methods are described in more detail within Chapter 4. Data are presented as mean ± SD. After confirmation that data met the assumptions of the method, differences between groups were identified using one-way ANOVA. Tukey-Kramer *post hoc* test comparisons were completed if the main effects were statistically significant. Alpha was set at 0.05. In cases where variance was not equal and the assumption of homogeneity was violated, Welch's ANOVA was used and if significant, was followed by Games-Howell *post hoc* analyses. In cases where data were not normally distributed, the nonparametric Kruskall Willis H test was completed. In all cases, this confirmed the statistical results of the one-way ANOVA, and therefore, the results of the latter are presented for consistency. Effect size, specifically partial eta squared (η_p^2), was estimated for all analyses.

In order to investigate the influence of haemodynamic load on LV mechanics, Pearson's correlation was used to identify the relationships to indices of haemodynamic load (HR, MAP, EDV). Additionally, ANCOVA analyses were

completed with parameters known to affect LV mechanics (HR, MAP, EDV, and ESWS) as covariates.

5.3. Results

Two individuals withdrew from the study; therefore 60 individuals across four cohort groups were included within the final analyses. Characteristics of the study population are presented in Table 10. There was inadequate tracking in STE for longitudinal strain for one postpartum female and for twist in one non-pregnant female.

Table 10. Characteristics of non-pregnant, pregnant, postpartum females and males included in experimental studies. Data presented as mean ± SD.

	Males	Non-pregnant	Pregnant	Postpartum	*P*	η_p^2
n	15	18	14	13		
Age (years)	27 ± 3 ‡	28 ± 4 ‡	32 ± 3 †	33 ± 2 †	**<0.0005**	0.410
Body mass (kg)	76 ± 11 ‡	64 ± 13 ‡	73 ± 8	67 ± 11	**0.019**	0.189
Height (cm)	176 ± 7 *	166 ± 7	167 ± 4	166 ± 4	**<0.0005**	0.370
BMI ($kg \cdot m^2$)	25 ± 3	23 ± 4	26 ± 4	23 ± 4	0.120	0.100

N.B. BMI, body mass index. *P*-value and effect size (η_p^2) from ANOVA. Multiple comparisons identified through *post hoc* Tukey-Kramer tests. * indicates significantly different from all other groups, † indicates significantly different to group(s) marked ‡.

Global haemodynamics and left ventricular structure

Pregnant females had significantly higher cardiac output than all other groups (mean difference: 0.5 $L \cdot min^{-1} \cdot m^{1.83}$, CI: 0.2 to 0.7 $L \cdot min^{-1} \cdot m^{1.83}$) (Figure 25). This was a result of statistically greater HR (mean difference: 12 $beats \cdot min^{-1}$, CI: 4 to 20 $beats \cdot min^{-1}$) and greater EDV and SV (mean difference: 6 $ml \cdot m^2$ and 5 $ml \cdot m^{2.04}$, CI: 1 to 10 $ml \cdot m^2$ and 1 to 8 $ml \cdot m^{2.04}$, respectively) compared to non-pregnant females (Figure 25). Pregnant females also had significantly lower SVR compared to non-pregnant and postpartum females (mean difference: 526 $dyne \cdot s \cdot cm^{-6}$, CI: 387 to 802 $dyne \cdot s \cdot cm^{-6}$, Figure 25). Postpartum females had signifcantly lower SBP, DBP and MAP (Figure 25) than non-pregnant females (mean difference: 10, 7 and 7 mmHg, CI: 2 to 17, 1 to 13, and 2 to 13 mmHg, respectively; Appendix XI Table A1).

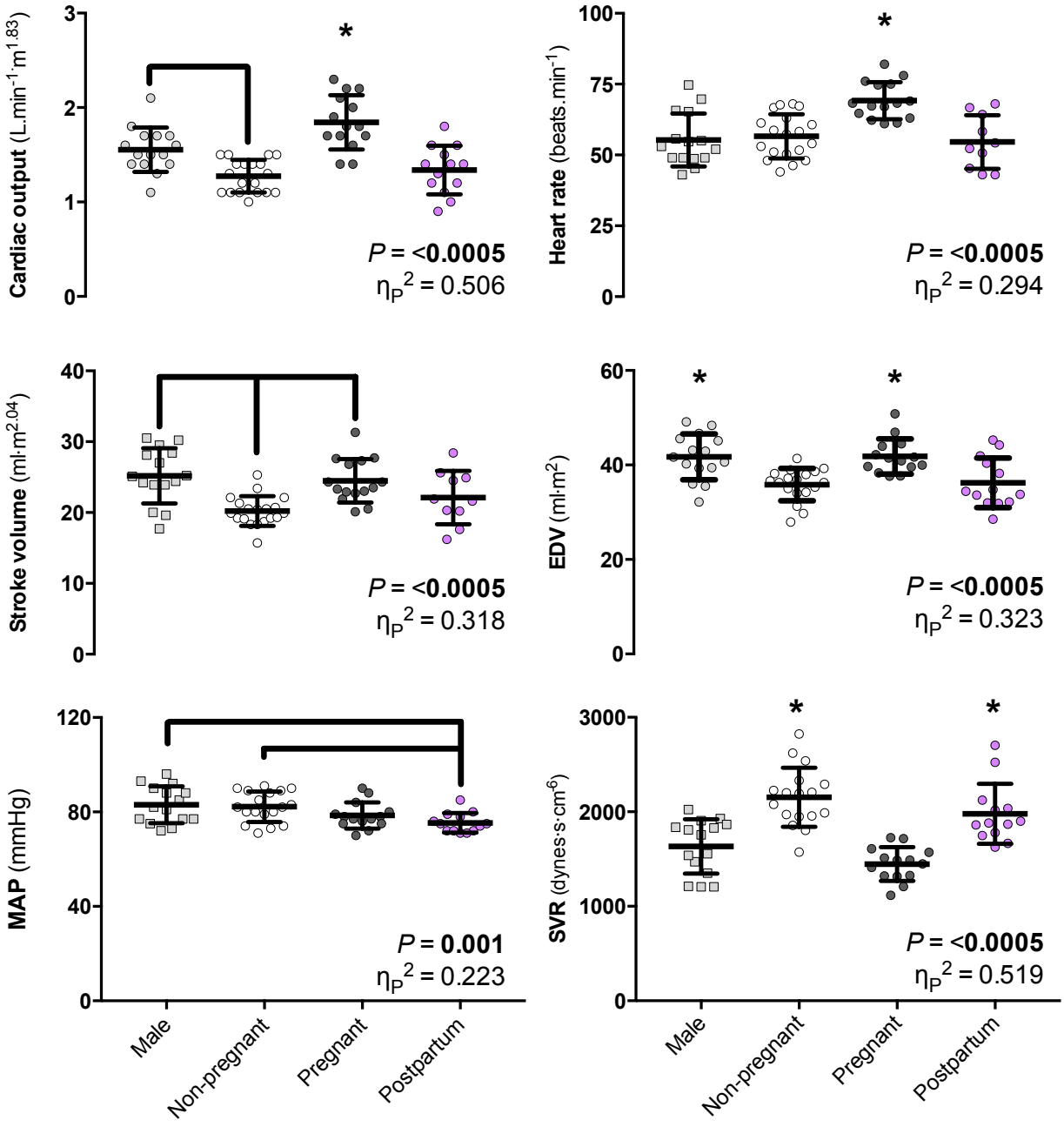


Figure 25. Healthy pregnant females in the late second trimester have greater cardiac output at rest compared to non-pregnant and postpartum females. This greater cardiac output was generated by significantly greater heart rate (top right panel) and stroke volume (middle left panel). Stroke volume in pregnant females was higher due to greater cardiac preload, evidenced by end-diastolic volume (EDV, middle left panel) and significantly lower afterload, evidenced by lower systemic vascular resistance (bottom right panel) without changes in mean arterial pressure (bottom left panel). Postpartum females had significantly lower mean arterial pressure (MAP) compared to non-pregnant females.

N.B. Data presented as mean ± SD. *P*-value and effect size (η_p^2) from ANOVA. Multiple comparisons identified through *post hoc* Tukey-Kramer tests. Capped lines indicate significant difference between respective groups ($P < 0.05$), * indicates significant difference to all groups ($P < 0.05$).

After allometric scaling, LV mass, posterior wall thicknesses and LV internal dimensions were similar between groups (Table 11). Additionally, there were no significant differences in RWT, sphericity index or ESWS. Absolute values for cardiac output, LV volumes, LV mass and dimensions and tissue velocities are presented in Appendix XI, Table A1.

Table 11. Left ventricular (LV) structure and geometry (scaled to height) in non-pregnant, pregnant and postpartum females and males. Data presented as mean ± SD.

	Males	Non-pregnant	Pregnant	Postpartum	*P*	η_p^2
IVSd (cm/m)	0.7 ± 0.1 †	0.6 ± 0.1 ‡	0.6 ± 0.1	0.6 ± 0.1	**0.002**	0.238
LVIDd (cm/m)	2.8 ± 0.2	2.7 ± 0.2	2.8 ± 0.2	2.8 ± 0.2	0.441	0.047
LVPWd (cm/m)	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.223	0.070
IVSs (cm/m)	0.8 ± 0.1 †	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1 ‡	**0.039**	0.138
LVIDs (cm/m)	2.0 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	0.197	0.080
LVPWs (cm/m)	0.6 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.1	0.399	0.051
Sphericity index	1.8 ± 0.2	1.8 ± 0.2	1.8 ± 0.3	1.8 ± 0.1	0.865	0.013
RWT	0.38 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.36 ± 0.04	0.243	0.071
LV length (cm/m)	4.6 ± 0.4	4.8 ± 0.5	4.8 ± 0.3	4.8 ± 0.3	0.502	0.041
LV mass ($g/(m^{2.7})$)	35 ± 6	33 ± 6	35 ± 5	33 ± 6	0.640	0.029
ESWS ($kilodyne \cdot cm^2$)	67 ± 12	75 ± 14	73 ± 14	67 ± 15	0.231	0.077

NB: IVS, intraventricular septum; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall; d, diastole; s, systole; RWT, relative wall thickness; ESWS, end-systolic wall stress. *P*-value and effect size (η_p^2) from ANOVA. Multiple comparisons identified through *post hoc* Tukey-Kramer tests. † indicates significantly different to group(s) marked ‡ ($P < 0.05$).

Systolic and diastolic function

There were no significant differences in EF, tissue velocities and trans-mitral blood flow velocities between non-pregnant, pregnant and postpartum females (Table 12). However, pregnant females had significantly greater peak longitudinal and basal circumferential strain and systolic strain rate compared to all other groups (peak strain mean difference: 4.5% and 6%, CI: 1.8 to 7.2% and 3 to 10%, and systolic strain rate mean difference: 0.3 and 0.5%/cm^{-1}, CI: 0.1 to 0.5%/cm^{-1} and 0.2 to 0.7%/cm^{-1}, respectively), (Figure 26 and Table 12). Additionally, pregnant females had significantly greater diastolic longitudinal strain rate compared to non-pregnant females (mean difference: 0.4%/sec^{-1}, CI: 0.1 to 0.7%/sec^{-1}).

There were no significant differences between groups in resting twist mechanics (Table 12) apart from a greater systolic twist velocity in pregnant females compared to non-pregnant females (mean difference: 30°/s, CI: 6 to 52°/s).

Although not of particular focus, compared to males, non-pregnant females had significantly faster mitral E and A wave velocities and significantly lower IVRT and deceleration time.

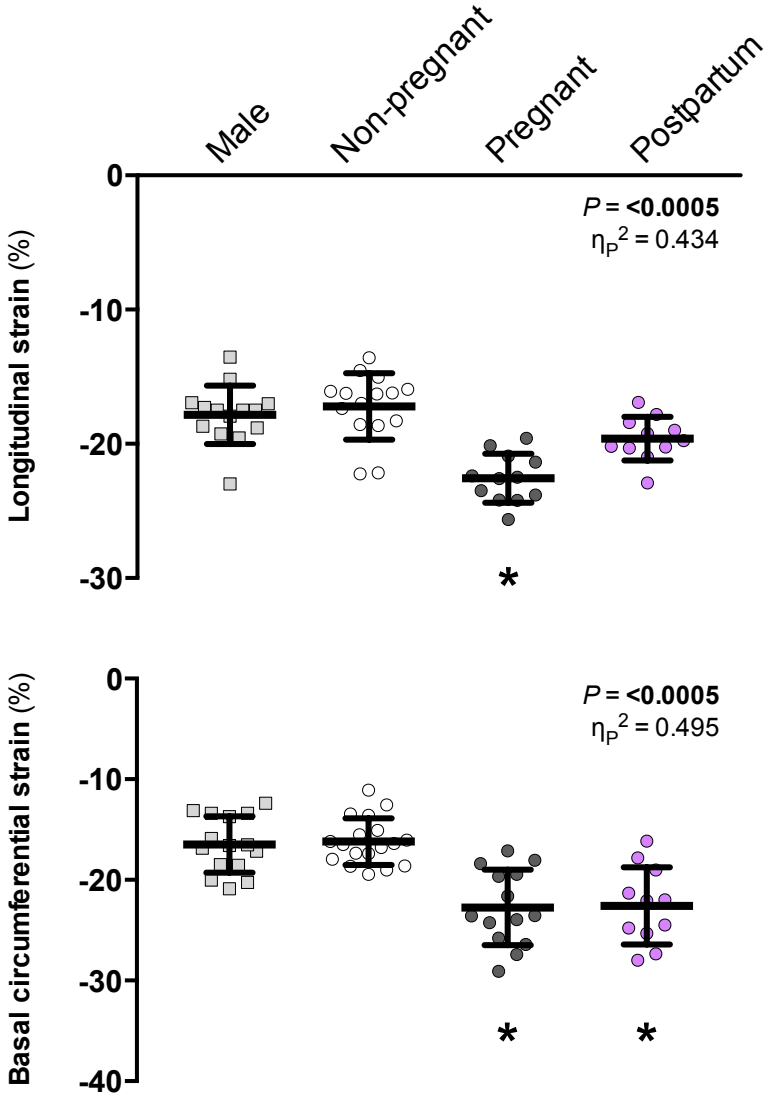


Figure 26. Healthy pregnant females in the late second trimester have greater peak longitudinal and basal circumferential strain at rest compared to non-pregnant females and males. Postpartum females also had a significantly greater basal circumferential strain compared to non-pregnant females and males. There were no differences in apical circumferential strain between groups were identified.

N.B. Data presented as mean ± SD. *P*-value and effect size (η_p^2) from ANOVA. Multiple comparisons identified through *post hoc* Tukey-Kramer tests. * indicates significantly greater than all other groups ($P < 0.05$).

Table 12. Left ventricular function at rest in non-pregnant, pregnant and postpartum females and males. Data presented as mean ± SD.

	Males	Non-pregnant	Pregnant	Postpartum	*P*	η_p^2
Systolic function						
Ejection fraction (%)	62 ± 6	57 ± 6	59 ± 5	60 ± 7	0.232	0.073
S' ($m \cdot s^{-1}$/cm)	0.009 ± 0.002	0.010 ± 0.002	0.010 ± 0.002	0.010 ± 0.001	0.545	0.038
Diastolic function						
Transmitral E ($m \cdot s^{-1}$)	0.8 ± 0.1 ‡	1.0 ± 0.1 †	0.9 ± 0.1	0.9 ± 0.1	**0.010**	0.184
Transmitral A ($m \cdot s^{-1}$)	0.4 ± 0.1 ‡	0.5 ± 0.1 †	0.4 ± 0.1	0.4 ± 0.1	**0.019**	0.164
E/A ratio	2.4 ± 0.7	2.2 ± 0.4	2.1 ± 0.3	2.3 ± 0.6	0.412	0.050
Deceleration time (ms)	251 ± 58	219 ± 101 *	248 ± 44	246 ± 31	**0.001**	0.247
E/E' ratio	5.8 ± 1.2	7.0 ± 1.7	7.0 ± 1.5	6.4 ± 1.4	0.068	0.120
LAP (mmHg)	9.2 ± 1.5	10.7 ± 2.2	10.7 ± 1.8	9.8 ± 1.8	0.070	0.119
E' ($m \cdot s^{-1} \cdot cm$)	0.016 ± 0.002	0.017 ± 0.003	0.015 ± 0.003	0.016 ± 0.003	0.292	0.065
A' ($m \cdot s^{-1} \cdot cm$)	0.009 ± 0.002	0.008 ± 0.002	0.009 ± 0.002	0.007 ± 0.002	0.122	0.099
IVRT (ms)	87 ± 14 *	64 ± 9	68 ± 14	60 ± 11	**<0.0005**	0.397
Longitudinal strain						
Peak strain (%)	-17 ± 3	-17 ± 3	-22 ± 2 *	-19 ± 3	**<0.0005**	0.434
Time to peak (%)	99 ± 6	100 ± 3	101 ± 4	101 ± 5	0.754	0.023
Systolic SR (%/sec^{-1})	-0.9 ± 0.2	-0.8 ± 0.2	-1.2 ± 0.2 *	-0.9 ± 0.2	**<0.0005**	0.387
Diastolic SR (%/sec^{-1})	1.2 ± 0.3 ‡	1.2 ± 0.3 ‡	1.6 ± 0.3 †	1.4 ± 2	**0.001**	0.272
Basal circumferential strain						
Peak strain (%)	-16 ± 3	-16 ± 2	-23 ± 4 *	-24 ± 6 *	**<0.0005**	0.495
Time to peak (%)	100 ± 1	101 ± 1	100 ± 8	97 ± 8	0.074	0.045
Systolic SR (%/sec^{-1})	-1.0 ± 0.2 ‡	-0.9 ± 0.2 ‡	-1.4 ± 0.2 †	-1.2 ± 0.4	**<0.0005**	0.376
Diastolic SR (%/sec^{-1})	1.4 ± 0.4	1.4 ± 0.4	1.7 ± 0.4	1.9 ± 0.8	0.063	0.158
Apical circumferential strain						
Peak strain (%)	-22 ± 4	-22 ± 5	-23 ± 3	-21 ± 4	0.720	0.024
Time to peak (%)	100 ± 1	100 ± 1	99 ± 1	100 ± 1	0.408	0.052
Systolic SR (%/sec^{-1})	-1.4 ± 0.4	-1.3 ± 0.3	-1.4 ± 0.3	-1.3 ± 0.4	0.759	0.021
Diastolic SR (%/sec^{-1})	1.9 ± 0.8	2.0 ± 0.7	2.1 ± 0.7	1.6 ± 0.4	0.395	0.053
Twist mechanics						
Peak apical rotation (°)	9.7 ± 5.6	7.9 ± 4.7	8.3 ± 5.3	7.7 ± 2.9	0.657	0.029
Peak basal rotation (°)	-6.0 ± 2.1	-5.8 ± 3.1	-8.8 ± 3.5	-7.6 ± 5.1	0.062	0.124
Peak twist (°)	14.5 ± 4.9	13.5 ± 4.0	16.5 ± 6.0	15.6 ± 4.6	0.335	0.059
Time to peak twist (%)	92 ± 8	92 ± 7	94 ± 7	94 ± 4	0.731	0.023
Torsion (°/cm)	1.7 ± 0.6	1.7 ± 0.5	1.9 ± 0.5	1.9 ± 0.6	0.613	0.032
Systolic twist velocity (°/s)	84 ± 26	75 ± 16 ‡	104 ± 25 †	91 ± 31	**0.015**	0.172
Untwisting velocity (°/s)	-85 ± 30	-97 ± 31	-93 ± 26	-110 ± 40	0.075	0.117

N.B. E, early filling; A, active filling; S', systolic tissue velocity; E', early-diastolic tissue velocity; A', late-diastolic tissue velocity; LAP, left atrial pressure; IVRT, isovolumetric relaxation time; SR, strain rate. *P*-value and effect size (η_p^2) from ANOVA. Multiple comparisons identified through *post hoc* Tukey-Kramer tests. * indicates significantly different to all groups ($P < 0.05$); † indicates significantly different to group(s) marked ‡ ($P < 0.05$).

Relationship of haemodynamic load indices to cardiac function

Longitudinal strain was significantly correlated to both HR and MAP, and basal circumferential strain was significantly correlated to MAP (Figure 27). There were no other significant correlations between LV mechanics and HR, EDV and MAP in either whole group data or individual group data (data presented in Appendix XI, Table A2).

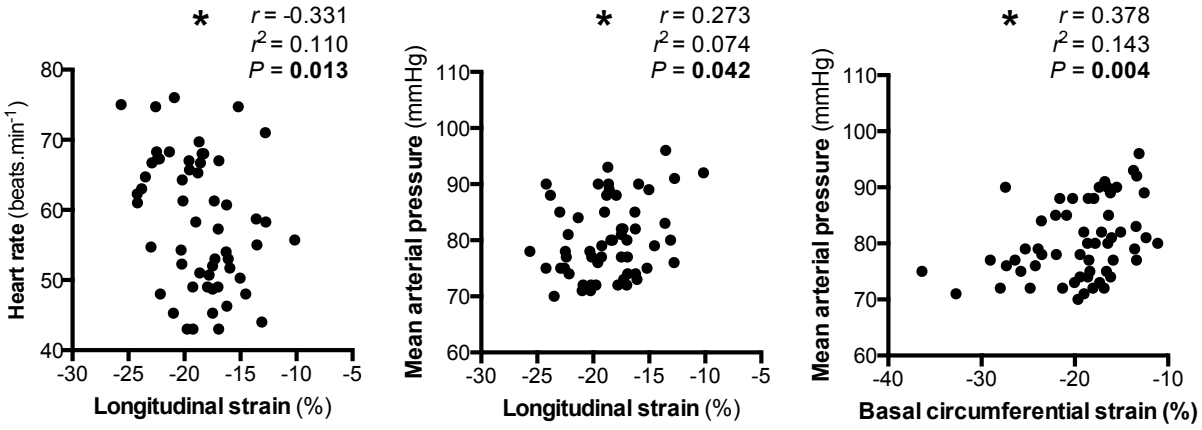


Figure 27. Significant weak/moderate correlations indicating that as MAP increases, longitudinal and basal circumferential strain decrease, and as HR increases, longitudinal strain increases.
N.B. * indicates significant correlation ($P < 0.05$).

Adjustment for factors known to influence LV mechanics (MAP, HR, ESWS and EDV) did not alter the statistical outcomes of the ANOVA analyses and longitudinal and basal circumferential strain remained significantly greater in pregnant females compared to non-pregnant females (data in Appendix XI, Table A3).

5.5. Discussion

Healthy pregnancy and the postpartum period result in altered cardiac loading and sympathetic activity in females. The objective of this study was to investigate the influence of healthy pregnancy and the postpartum period on cardiac structure and function at rest, specifically focusing on the underpinning mechanics. The main outcomes of this study were:

(i) In agreement with the initial hypotheses, healthy pregnant females in the late second trimester had significantly altered global haemodynamics (greater cardiac output, HR, SV, EDV, with reduced SVR) and significantly greater systolic function (greater longitudinal and basal circumferential strain and strain rate) when compared to non-pregnant females.

(ii) Despite significantly lower resting BP in postpartum females, there were no significant differences in cardiac structure or function between postpartum and non-pregnant females. These findings were in agreement with the initial hypotheses.

Overall, these findings suggest that healthy pregnant females in the late second trimester have an enhanced myocardial performance. The larger cardiac output at rest during pregnancy is generated by greater LV volumes, HR and elevated contractile function. After delivery, postpartum cardiac function and structure returns to non-pregnant values, although BP is lower than non-pregnant values.

Enhanced systolic function in healthy pregnancy in the late second trimester

In this study, there were no significant differences between groups in traditional measures of systolic function (EF or S'), however there were significant differences in LV mechanics, proving their additional insight into subtle alterations

in function. Specifically, longitudinal strain, basal circumferential strain and systolic strain rates were significantly greater in pregnant females in the late second trimester compared to non-pregnant females. The greater systolic function in pregnant females may be caused by (i) increased ventricular stretch via larger EDV and the Frank-Starling mechanism and/or (ii) greater contractility due to elevated sympathetic activity (Usselman et al., 2015a, Jarvis et al., 2012).

The elevated blood volume of pregnancy leads to higher EDV and may consequently contribute to greater activation of the Frank-Starling mechanism. During pregnancy, circulating blood volume is increased by approximately 45% compared to non-pregnant females in response to the hormonally mediated vasodilation of early gestation (Ouzounian and Elkayam, 2012, Hytten, 1985). The greater blood volume contributes to increased cardiac preload (May, 2015) evidenced in this study by the significantly greater scaled EDV in pregnant females in comparison to non-pregnant and postpartum females. As cardiac dimensions (LV mass, internal diameters, wall thicknesses) were not significantly different between non-pregnant and pregnant females, it can be speculated that the greater scaled EDV in pregnant females in the late second trimester may induce a more pronounced stretch of the LV consequently resulting in greater myofibre shortening and systolic function. However, within this study, strain parameters were not significantly correlated to EDV, suggesting other factors contribute to greater systolic function during healthy pregnancy.

The enhanced systolic function in pregnant females in the late second trimester is likely influenced by elevated sympathetic activity. Increased sympathetic activity has been evidenced previously in healthy pregnancy via measurement of muscle sympathetic nerve activity (Jarvis et al., 2012, Usselman et al., 2015a). Although not assessed within this study, the role of sympathetic drive in enhanced systolic function during pregnancy is suggested from a process of elimination. Firstly, a

significant moderate correlation was identified between longitudinal strain and HR, and between MAP and both longitudinal (weak correlation) and basal circumferential strain (moderate correlation), however these only accounted for 11, 7 and 14% of the variance in strain observed, respectively. These data support that differences in LV strain during pregnancy are not solely mediated by haemodynamic load and are not significantly related to EDV. Secondly, confounding factors known to affect LV mechanics (ESWS, HR, MAP and EDV) were included as covariates in additional ANCOVA analyses of longitudinal and basal circumferential strain (Sengupta et al., 2008, Marwick, 2006, Stöhr et al., 2016). After adjustment for the above haemodynamic parameters, longitudinal and basal circumferential strain remained significantly greater in pregnant females compared to non-pregnant females. Again, this supports that LV strain in healthy pregnancy must be influenced by additional factors over and above haemodynamic load and wall stress. Thirdly, the absolute increase in blood volume during pregnancy is similar to the average difference observed between young healthy males and non-pregnant females (Best et al., 2014). Despite similar scaled LV volumes and LV mass between males and pregnant females in this study, pregnant females had a significantly greater cardiac output, HR and LV strain compared to males. These parameters are mediated by changes in autonomic regulation, and in combination with the results above, lead to the suggestion that elevated sympathetic activity is a key contributor to greater LV strain in healthy pregnant females in the late second trimester.

Of interest, greater circumferential strain was evident at the base, but not the apex, of the maternal heart. This finding may be the result of the elevated circulating oestrogen associated with gestation. Previous work in animal models has shown that, in comparison to male hearts, female hearts have a higher density of calcium channels on the epicardium (Pham et al., 2001) and in the base of the

myocardium (Sims et al., 2008). Additionally, oestrogen upregulates the action of calcium ion channels in cardiomyocytes isolated from the base, but not the apex (Yang et al., 2012). Increased oestrogen during gestation may therefore allow greater calcium influx into the myocyte, increasing myocyte contraction in the basal epicardium, causing greater basal deformation, as shown within this study. However, further work is required to confirm this speculation. Additionally, a previous *in vivo* study of circumferential strain in healthy pregnant females reported reduced apical, but not basal, circumferential strain (Papadopoulou et al., 2013), contradicting the findings of the present study. Future research is therefore required to determine if elevated inotropy during healthy gestation (i) is linked to increased sex steroid hormones and (ii) exerts different effects on specific regions of the myocardium.

The enhanced systolic function in the late second trimester of pregnancy shown in this study is somewhat discordant to previously published literature. Both unchanged (Ando et al., 2015, Tzemos et al., 2008, Cong et al., 2015, Estensen et al., 2013, Sengupta et al., 2017) and decreased (Papadopoulou et al., 2013) longitudinal and circumferential strain have been reported at a similar stage of gestation previously. It is possible that broad time ranges of assessment and the inclusion of heterogeneous cohorts including twin pregnancies, multigravida females and women who developed complications such as gestational diabetes mellitus, may explain the differences between previous studies and the present study. The pregnant females included in this study were physically active prior to and during gestation. Although not yet fully understood, physical activity has been shown to influence pregnancy-related cardiac adaptation (May et al., 2016) and may explain the enhanced resting systolic function within this pregnant cohort.

Reduced strain and strain are associated with cardiac dysfunction and pathology (Smiseth et al., 2016) so in contrast; greater deformation of the myocardium likely

suggests an enhanced systolic function. In support, previous investigations have shown greater LV strain at rest in well-trained athletes, considered to have superior cardiovascular function compared to untrained healthy controls (Schattke et al., 2014). In light of the above, greater longitudinal and basal circumferential strain in pregnant females has been interpreted as enhanced cardiac function. It must also be considered that increased systolic function at rest may result in a lower capacity to adapt or respond to physiological challenge (Sengupta et al., 2008). It is possible that the greater LV strain observed at rest within this study does not actually reflect improved cardiac performance. Rather, the elevated resting systolic function may cause a reduction in cardiovascular reserve. Without the assessment of the dynamic responses to increased cardiovascular challenge, it remains to be fully understood if cardiac function is enhanced or impaired during the late second trimester of pregnancy.

Cardiac structure, geometry and wall stress do not contribute to differential LV mechanics in the late second trimester

Pregnancy is associated with increased cardiac size in comparison to non-pregnant females (Melchiorre et al., 2012a, Savu et al., 2012, Chung and Leinwand, 2014). In this study, however, there were no significant differences between groups in the scaled cardiac dimensions. These findings support previous longitudinal data in which scaled LV mass was not significantly different across pregnancy (Mone et al., 1996). Cardiac hypertrophy is stimulated by increased wall stress (Russell et al., 2000). In this study, ESWS was not significantly different between groups, supporting previous work in which wall stress at mid-gestation was not elevated in healthy pregnant females (Mone et al., 1996, Estensen et al., 2013). This may explain the lack of pregnancy-induced increases in cardiac size observed presently. Due to this lack of dimensional change, cardiac geometry was

also not altered in the late second trimester of healthy pregnancy. This finding is in contrast to previous work at a similar time point in which sphericity index was decreased (Savu et al., 2012). Overall, the findings from this study demonstrate that the structure of the maternal heart is not altered at this time point despite significantly greater LV volumes and HR.

Cardiac structure, geometry and wall stress are all known to influence LV mechanics in healthy adults (Oxborough et al., 2016, van Dalen et al., 2010, Young and Cowan, 2012, Vendelin et al., 2002). The similarities between groups in structure, geometry and wall stress suggest that morphology of the heart during pregnancy does not influence resting LV mechanics at this time point of gestation, at least in the present cohort of women. Elevated LV twist, a motion associated with the equalisation of wall stress, has previously been observed in the final trimester of healthy gestation (Tzemos et al., 2008, Yoon et al., 2011, Papadopoulou et al., 2013, Hristova et al., 2016), however the findings from this study indicate no differences in LV twist in non-pregnant and pregnant females in the late second trimester. Favourable changes in haemodynamic load during pregnancy (increased preload, contractility and reduced afterload) may be expected to increase LV twist, similar to the responses observed during acute challenges in healthy adults (Stöhr et al., 2016, Weiner et al., 2010b). However, LV twist was not altered in response to the chronic elevations in EDV, HR and reductions in SVR observed during gestation in this study. It is conceivable that despite changes in haemodynamic load, resting LV twist in the late second trimester of pregnancy was not altered because ESWS was not elevated. As pregnancy progresses, however, greater metabolic demand and altered haemodynamic load of the third trimester (shown previously in Chapter 2, Figure 3) may result in increased transmural stress and therefore greater LV twist. Physiological challenges in the late second trimester, that alter haemodynamic

load and increase metabolic demand, may mimic cardiac function at rest during late gestation. As discussed within Chapter 2, additional cardiovascular challenge in the late second trimester of healthy pregnancy may result in LV twist similar to that observed in the resting state of the third trimester, (Tzemos et al., 2008, Yoon et al., 2011, Papadopoulou et al., 2013, Hristova et al., 2016).

Cardiac structure and function is not significantly different between non-pregnant and postpartum females

Cardiac structural and functional adaptations of pregnancy are expected to return to a non-pregnant state after delivery in line with the 'form follows function' principle (Russell et al., 2000). The requirement for enhanced cardiac performance is removed during labour via the expulsion of the fetoplacental unit and a reduction in blood volume via blood loss (Macdonald et al., 2011). As a result, the altered metabolic demand, sympathetic activity and haemodynamic load of pregnancy is no longer a challenge on the female system leading to the reversal of adaptation. In the present study, there were no differences in cardiac structure or function between postpartum and non-pregnant females, supporting the 'form follows function' principle and previous research (Geva et al., 1997, Savu et al., 2012). It should be noted that postpartum females had significantly greater basal circumferential strain compared to non-pregnant females, however, there were no significant differences in any other systolic or diastolic parameter between non-pregnant and postpartum females. Therefore, the greater basal circumferential strain appears to be an anomalous result. The findings from the present study demonstrate that there is no carry over of pregnancy-related cardiac enhancements (greater cardiac output, HR and SV) early postpartum.

Breastfeeding is associated with reductions in resting BP in the postpartum period (Ebina and Kashiwakura, 2012, Groer et al., 2013). In this study, 12 out of 13

postpartum females were breastfeeding at the time of inclusion (all females breastfed for 12 weeks post delivery) and had significantly lower resting SBP, MAP and DBP compared to non-pregnant females. The reductions in BP as a result of breastfeeding have been attributed to a resetting of the arterial baroreflex, increased vagal tone (Altemus et al., 2001), increased oxytocin levels (Grewen et al., 2010), and/or hypervolemia via hydration changes from lactation (Murray and McKinney, 2013). Despite lower resting BP, cardiac function was not different between non-pregnant and postpartum females at rest. This is in contrast to previous observations of an increased LV strain and twist in response to acute reductions in afterload in healthy adults (Burns et al., 2010b, Burns et al., 2010a), but may be related to the chronic exposure of the lower BP in postpartum females.

The lower resting BP in postpartum females may cause differential BP responses during physiological challenge. A previous antenatal exercise intervention study found that the active group had significantly lower SBP and DBP compared to the non-active control group during the postpartum period, without differences in cardiac output, SV or HR between groups (Carpenter et al., 2015), similar to the findings of postpartum *versus* non-pregnant females in this study. Although no differences in resting global cardiac function were identified, the active group had significantly greater SV and significantly lower DBP and total peripheral resistance during an exercise challenge compared to the control group (Carpenter et al., 2015). Both lower DBP and vascular resistance during exercise led to a reduced afterload, which may have facilitated a greater ejection, and therefore increased SV. The results from the previous study show that the generation of cardiac output may be achieved differently in postpartum females with low resting BP due to the altered haemodynamic load. It is therefore possible that low resting BP in postpartum females may alter cardiac functional responses during physiological challenge.

Limitations

This study utilised a cross-sectional design to enable comparison of non-pregnant, pregnant and postpartum females and males and would have been more robust if a longitudinal design was used. Due to time constraints and feasibility, this was not possible. In addition, it was not possible to match the groups for age. Figures from the Office of National Statistics (2013) demonstrate that the maternal childbearing age has increased to an average of 30 years within the UK. In this study, non-pregnant females who had never been pregnant and had never attempted to conceive were recruited. This significantly lowered the age of volunteers in this cohort compared to the pregnant and postpartum groups. As aging is associated with a decline in cardiovascular function, additional ANCOVA analyses with age as a covariate were performed (method described in Chapter 4). Age did not have an influence on statistical outcomes and conclusions of this investigation.

Limitations of longitudinal strain must be considered when interpreting the measure as a marker of contractile function. Longitudinal strain does not account for differences in apical and basal shortening velocity or wall thicknesses (Stöhr et al., 2015, Samuel and Stohr, 2017) and is not directly reflective of myocyte contraction due to the double helical, and not long-axis, arrangement of LV myocytes (Sengupta et al., 2006). These limitations have been shown to underestimate the contribution of the whole LV to contraction (Stöhr et al., 2015). In this study, the greater longitudinal strain observed in pregnant females was supported by significantly higher basal circumferential strain, providing greater confidence that systolic performance was enhanced in pregnant females.

5.6. Conclusions

In conclusion, this study shows that healthy pregnant females in the late second trimester exhibited enhanced cardiac performance compared to non-pregnant females. Greater cardiac output was generated in pregnant females by higher SV, HR and systolic function (LV strain), without changes in ESWS, LV twist or cardiac structure. Elevated systolic function at rest in the late second trimester of healthy pregnancy is likely the result of higher sympathetic activity. However, it is not clear if elevated systolic function at rest impacts upon functional cardiovascular responses in healthy pregnant females. Further assessment of dynamic responses to physiological challenges would determine if systolic function is actually enhanced or reduced in the late second trimester of pregnancy.

After delivery, postpartum females had significantly lower afterload (BP) compared to non-pregnant females, however this did not alter resting LV structure or function. As shown previously (Carpenter et al., 2015), lower resting BP in postpartum females may cause a differential cardiovascular response to physiological challenge. Again, the assessment of dynamic cardiac function in healthy postpartum females may further the understanding of effects of pregnancy on the female cardiovascular system.

Chapter 6. Functional cardiovascular responses to acute physiological challenges in non-pregnant, pregnant and postpartum females

6.1. Introduction

Resting cardiac function during the late stages of healthy pregnancy has been reported as impaired, unchanged or enhanced. Previous literature has typically shown deterioration in both systolic and diastolic function in the third trimester (Sengupta et al., 2017, Papadopoulou et al., 2013, Yoon et al., 2011, Hristova et al., 2016, Bamfo et al., 2007), however the results in Chapter 5 are suggestive of an enhanced systolic performance at rest during the late second trimester of pregnancy. Greater left ventricular (LV) strain in pregnant females at this time point was attributed to greater inotropy as a result of elevated sympathetic activity and enhanced contractile force through favourable haemodynamic load (greater preload and reduced afterload) compared to non-pregnant females.

Increased systolic function at rest has been suggested to result in a reduced cardiovascular reserve (Sengupta et al., 2008). In the face of increasing cardiovascular demand, such as that observed at rest in the final trimester of pregnancy (Lof et al., 2005, Acharya et al., 2016), the maternal heart may be unable to increase function beyond that already achieved to meet maternal and foetal needs. Limitations in systolic and diastolic function in pregnancy may therefore only occur when cardiovascular demand exceeds cardiovascular reserve. Speculatively, additional cardiovascular demand, mediated through physiological challenges, during the late second trimester of pregnancy may unmask changes in maternal function as a result of reduced cardiovascular reserve.

In contrast to simply assessing the heart at rest, dynamic responses to physiological challenges, such as an increased afterload or aerobic exercise, provide greater insight into myocardial functional capacity (Gibbons et al., 1997, Ohuchi et al., 2013). Challenges that increase afterload, induced by isometric muscular contraction, have previously been shown to cause an acute increase in

blood pressure (BP), heart rate (HR) systemic vascular resistance (SVR) and sympathetic activity in healthy adults (Weiner et al., 2012, Balmain et al., 2016). These acute changes in haemodynamic load (increased afterload and HR) are similar to those experienced chronically in late gestation (Meah et al., 2016, Melchiorre et al., 2012a). Conversely, submaximal aerobic exercise results in increased global cardiovascular demand, similar to the increased metabolic demand of late pregnancy (Lof et al., 2005, Acharya et al., 2016), as well as alterations in sympathetic activity and haemodynamic load in healthy adults (increases stroke volume (SV), HR, BP, reduces SVR) (Donal et al., 2011, Stöhr et al., 2011b). Dynamic assessment of the maternal heart in the late second trimester (using increased afterload to mimic haemodynamic load and submaximal aerobic exercise to mimic increased global cardiovascular demand experienced in the third trimester) may help to elucidate if maternal cardiac function is enhanced, impaired or unchanged in healthy pregnancy.

Dynamic assessment of cardiac function in the postpartum period may also further the understanding of cardiovascular changes after delivery. Following birth, females, particularly breast-feeding mothers, have significantly lower BP at rest compared to non-pregnant females, without changes in cardiac structure (LV mass, dimensions and sphericity index) or function (cardiac output, HR, SV, EF and LV mechanics), as shown in Chapter 2, Chapter 4 and in previous research (Ebina and Kashiwakura, 2012, Groer et al., 2013, Savu et al., 2012). However, lower BP and greater SV during exercise in postpartum females with low resting BP has previously been demonstrated (Carpenter et al., 2015). These findings suggest the generation of cardiac output in postpartum females during additional challenge may be altered.

Accordingly, the objective of this study was to examine functional responses to increased cardiovascular demand and altered haemodynamic load during the late

second trimester of healthy pregnancy and in the postpartum period. Global haemodynamics and cardiac functional parameters, including LV strain and twist, will be used to provide a comprehensive assessment of dynamic function. It was hypothesised that, in comparison to non-pregnant females:

i. Pregnant females in the late second trimester would have a limited response to physiological challenges (evidenced by lower SV, EF, LV strain and twist) due a reduced cardiovascular reserve.
ii. Postpartum females would have lower BP and greater SV responses to physiological challenge as a consequence of lower resting BP.

As the majority of experimental work investigating the influence of haemodynamic load on LV mechanics has been completed using male cohorts (Weiner et al., 2012, Donal et al., 2011, Balmain et al., 2016, Stöhr et al., 2011b, Burns et al., 2010b, Burns et al., 2010a), a control group of males was included within this experimental study to verify the findings of LV mechanics during physiological challenges.

6.2. Methods

Ethical approval and volunteer inclusion criteria

The experimental procedures for both Chapter 5 and 6 were reviewed and approved by the Cardiff Metropolitan University Research Ethics Committee (16/3/01R Appendix I.b.). The study complied with the guidelines set out in the Declaration of Helsinki and written voluntary informed consent was gained from all volunteers.

As described previously in Chapter 5, sixty-two individuals volunteered to participate in this study (non-pregnant females = 19, pregnant = 15, postpartum = 13, males = 16). Volunteers were healthy non-smokers, free from known cardiovascular and/or metabolic diseases and were not taking any medication at

the time of inclusion based on self-report. Non-pregnant females were nulliparous and had never tried to conceive. Pregnant and postpartum females were primiparous, however females that had previously experienced a miscarriage before 12 weeks were included within the study (pregnant *n* = 2, postpartum *n* = 2). No females from either the pregnant or postpartum groups experienced any cardiovascular complications during their pregnancies.

Experimental protocol

Volunteers visited the laboratory on two occasions as outlined in Figure 28. Visits took place at least 24 hours apart and at the same time of day. Volunteers were asked to abstain from heavy exercise for 24 hours and caffeine for 12 hours prior to visiting the laboratory. Pre-exercise health screening was completed for all volunteers using standardised questionnaires (ACSM PAR-Q for non-pregnant females, postpartum females and males, and PARMed-X for pregnancy for pregnant females, attached as Appendix VI and VII, respectively). In the first visit to the laboratory, anthropometric characteristics (height, body mass and BMI) were determined, as described in Chapter 5. Maximal voluntary contraction force of the forearm was determined and maximal aerobic capacity was estimated. Finally, volunteers were familiarised to the experimental procedures. In the second visit, cardiovascular assessments were completed at rest, during a sustained isometric handhold and during two bouts of submaximal supine cycle exercise.

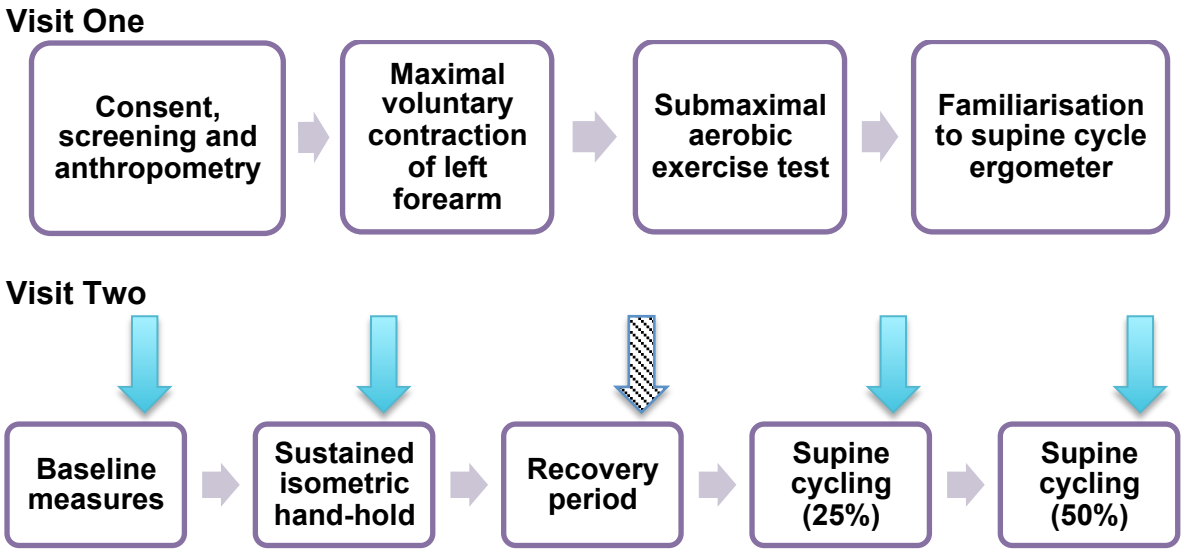


Figure 28. Schematic displaying when cardiovascular assessments were completed during the experimental study protocol. Echocardiographic data was collected at baseline and during each physiological challenge, indicated by light blue arrows. Baseline echocardiographic data was collected after 15 minutes of quiet rest and after 2 minutes into the sustained isometric handhold and each stage of submaximal aerobic exercise. **The experimental protocol was not randomised.** After the sustained isometric handhold, volunteers had 5 minutes recovery, followed by the collection of a second set of echocardiographic resting data to confirm cardiac function had returned to baseline, indicated by the striped arrow.

Sustained isometric handhold

To determine the individualised workload for the sustained isometric handhold in Visit 2, volunteers completed 1 s maximal voluntary contractions on a commercially available digital handgrip dynamometer (Grip-A, 5001, Takei Scientific Instruments Co Ltd. Shinagawa-ku, Tokyo, Japan). Whilst in the left lateral semi-recumbent position, volunteers performed two maximal contractions of the left arm with each effort separated by a minimum of 60 seconds. The average value (in kilograms) was calculated.

In the second visit to the laboratory, each volunteer performed a left-handed isometric hold at 30% of the individual's maximal voluntary contraction for a maximum of 5 minutes. This was completed using an adapted handgrip

dynamometer (Figure 29). The volunteer completed the task in the semi-recumbent left lateral position to facilitate the collection of echocardiographic images. No data were collected within the first 2 minutes of the hold. All volunteers were instructed to avoid the Valsalva manoeuvre and to breathe freely throughout the challenge.

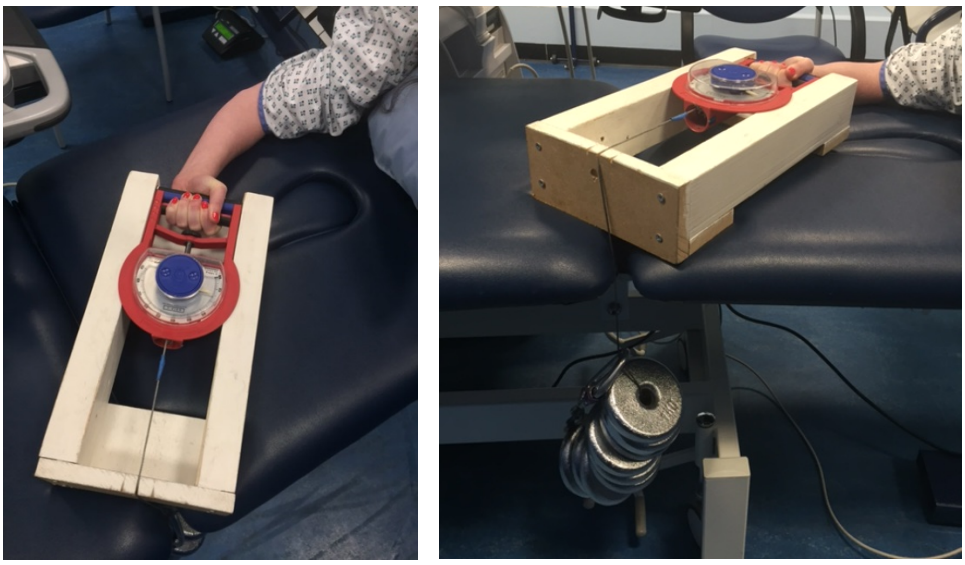


Figure 29. Handgrip dynamometer adapted to hold an external load for the sustained isometric handhold challenge.

Submaximal aerobic exercise

A submaximal incremental cycling test was used to estimate maximal aerobic capacity and to determine individualised workloads for the subsequent cardiovascular assessments during exercise. Prior to the start of the test, resting HR was recorded during 5 minutes of quiet rest (Polar Electro, RS400, Kemple, Finland) with the volunteer in a semi-recumbent position to calculate HR reserve. Volunteers then completed an incremental exercise protocol on an upright cycle ergometer (Lode, Corival, Lode B.V., Gronigen, The Netherlands). HR and oxygen consumption ($\dot{V}O_2$) were continuously measured (Jaeger, Oxycon Pro, Warwickshire, UK), after calibration as per the manufacturers guidelines. The test included a 2-minute warm up at a set cadence of 50 rpm at 0 Watts (W), followed by a ramped cycle protocol with a set cadence of 70 rpm and increments of 20 and

30 W·min^{-1} for females and males, respectively (Lode Ergometry Manager 9.2, Lode B.V., Gronigen, The Netherlands). The test was terminated at the target heart rate of 70% of the estimated heart rate reserve (HRR). This method allowed the prescription of exercise intensity whilst accounting for differences in resting heart rate and has been used previously in pregnant females (Davenport et al., 2008). The HR for test termination was therefore calculated as (Karvonen et al., 1957):

Target HR = [(maximum HR – resting HR) * 0.7] + resting HR

Maximum HR was estimated using the equation 208 - 0.7(age) (Tanaka et al., 2001), resting HR was measured during 5 minutes of quiet rest, as described previously, and 0.7 represents the target intensity of 70%.

Peak power output (PPO) and peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) were estimated via linear extrapolation to estimated maximal HR. Resting $\dot{V}O_2$ is known to increase during pregnancy, however resting HR is increased to a greater extent (Lotgering et al., 1992a) and therefore, the linear relationship of $\dot{V}O_2$ and HR that is assumed during exercise outside of pregnancy may be altered during in pregnant females. As a result, the accuracy of estimating maternal $\dot{V}O_{2peak}$ using a HR-$\dot{V}O_2$ extrapolation may be reduced (Lotgering et al., 1992a), therefore PPO was used to prescribe exercise intensities in the second experimental visit.

Estimated upright PPO from the sub-maximal aerobic exercise test completed in Visit One was adjusted (= multiplied by 0.7) for reduced cycling performance associated with the supine and left lateral position (Egana et al., 2013). The prescribed intensity for the aerobic cycling challenge was calculated from supine-adjusted PPO. Volunteers completed 5-minute bouts of supine cycling exercise at 25 and 50% PPO in the left lateral position (shown previously in Chapter 4, Figure 17). The position facilitates the collection of echocardiographic images and

enables the avoidance of inferior vena cava compression in pregnant females, described in more detail in Chapter 4.

Continuous blood pressure monitoring

Systolic, diastolic and mean arterial blood pressure (SBP, DBP and MAP, respectively) responses were measured continuously using non-invasive beat-by-beat finger photoplethysmography, described in detail in Chapter 4. Values were averaged over twenty continuous waveforms (cardiac cycles) at rest and during the final 2 minutes of each physiological challenge.

Echocardiography

Transthoracic echocardiography was performed using a commercially available ultrasound system and 1.5 – 4.6 MHz phased array transducer by one sonographer (VM), as described previously in Chapter 4 and Chapter 5. A three-lead electrocardiograph was used for HR monitoring. Echocardiographic images were collected after >15 minutes of quiet rest and after 2 minutes of the sustained isometric handhold and exercise challenges. The imaging protocol during physiological challenges is presented in Chapter 4, Table 6. Ultrasound settings, including image depth and frame rates (described previously), were kept the same during the assessments at rest and during each challenge. Five consecutive cardiac cycles were recorded at end expiration to limit displacement of the heart and changes in intrathoracic cavity pressure during respiration. Data were stored for later offline analysis and measurements were made in triplicate from different cardiac cycles and averaged.

Analysis of standard two-dimensional echocardiographic parameters

Cardiac output, SV, EDV and ESV were derived as described in Chapter 4 and 5 and allometrically scaled to height to remove the influence of body size on LV volumes (cardiac output [1.83] SV [2.04] (de Simone et al., 1997), EDV and ESV

[2.00] determined by the theory of dimensionality (Batterham et al., 1999)). Peak systolic (S'), early (E') and late (A') diastolic septal velocities were measured to determine tissue velocity across the cardiac cycle and were scaled to LV length (Batterham et al., 2008).

Left ventricular mechanics

Speckle-tracking echocardiography (STE) was used to measure longitudinal, basal and apical circumferential strain, basal and apical rotation and LV twist, as described previously. Strain and rotation curves for three cardiac cycles were generated. The raw data was transformed using software (2D Strain Analysis Tool, Stuttgart, Germany) that applied a cubic spline interpolation to separate systole and diastole into 600 data points each, which time aligned data to accommodate for inter- and intra-individual HR variability and individualised frame rate at acquisition. Peak and time to peak (%) strain, rotation and twist was calculated, defined as the maximal value across the cardiac cycle. Systolic and diastolic strain and twist and untwist velocities were also measured. The data presented are an average of all myocardial segments. The coefficients of variation for echocardiographic measurements during low intensity exercise (25% peak PO) are reported in Chapter 4, Table 8.

Statistical analyses

Measurements are presented as means ± SD. All statistical analyses were conducted using SPSS Statistics (Version 20.0, IBM Corporation, Chicago, IL). Graphical representations of data were produced using GraphPad Prism (Version 7 for Mac, GraphPad software, San Diego, CA). Statistical significance was accepted at $P < 0.05$. Effect size, specifically partial eta squared (η_p^2), was determined within each analyses.

To determine the effect of a sustained isometric handhold on cardiovascular

responses in non-pregnant, pregnant and postpartum females and males, ANCOVA analyses were completed with the resting value as a covariate. Assumptions of the ANCOVA were checked as described in Chapter 4. In some cases, within-group normality was skewed, however the ANCOVA was completed regardless as the test is considered robust to deviations from normality in groups of similar sample sizes (Olejnik and Algina, 1984).

Statistical differences between groups to both 25 and 50% aerobic cycling exercise were identified using a repeated measures general linear model (GLM), in which the resting value was used as a covariate. *P*-values were determined for between-subjects (group) and within-subjects (exercise intensity) differences, and the group*exercise (Grp*Ex) interaction. Assumptions of GLM were checked. Mauchly's test of sphericity was used to check the sphericity on the variance-covariance matrix of the dependent variables. No epsilon adjustments were required. Homogeneity of variance-covariance matrices was tested with the Box's M ($P > .05$).

In both ANCOVA and repeated measures GLM, some outliers were identified; however, they were included in the final analysis as the values were within physiological norms. *Post hoc* analyses of the ANCOVA and GLM were performed using a Bonferroni adjustment.

Previous literature has shown that blunted or exaggerated responses to physiologic challenge reflect a dysfunctional response (Lui et al., 2011, Schultz et al., 2015, Ohuchi et al., 2013). Although the differences between conditions were identified within the above statistical comparisons, the absolute changes from rest to during the sustained isometric handhold, from rest to 25% aerobic cycling and from 25 to 50% aerobic cycling were calculated and ANOVA analyses were completed. As described in Chapter 4, assumptions were checked and Tukey-Kramer *post hoc* tests were completed if statistical significance was detected.

6.3. Results

Of the 62 individuals enrolled into this study, two did not complete the second experimental visit due to time constraints; therefore 60 individuals across the four groups were eligible for inclusion in the final analyses. All pregnant and postpartum females attended the laboratory within the predefined time periods (during gestation range: 22 to 26 weeks, mean: 25.4 ± 0.6 weeks and after delivery range: 12 to 16 weeks, mean: 15.1 ± 1.3 weeks; respectively). Volunteer anthropometrics were presented in Chapter 5, Table 10, however characteristics relevant to this Chapter are outlined in Table 13.

Table 13. Volunteer maximal forearm contraction strength and aerobic exercise capacity estimation and individualised workloads used within experimental protocol.

	Males	Non-pregnant	Pregnant	Postpartum	*P*	η_p^2
n	15	18	14	13		
Forearm MVC (kg)	50 ± 9 *	30 ± 5	27 ± 5	27 ± 3	**<0.0005**	0.759
30% forearm MVC (kg)	15 ± 3 *	9 ± 1	8 ± 1	8 ± 1	**<0.0005**	0.761
$\dot{V}O_{2peak}$ (L.min^{-1})	3.4 ± 0.5 *	2.1 ± 0.5	2.1 ± 0.5	2.0 ± 0.5	**<0.0005**	0.592
Supine PPO (W)	245 ± 27 *	157 ± 28	149 ± 23	136 ± 22	**<0.0005**	0.744
25% PPO (W)	61 ± 7 *	39 ± 7	37 ± 7	34 ± 6	**<0.0005**	0.743
50% PPO (W)	123 ± 13 *	78 ± 14	75 ± 12	68 ± 11	**<0.0005**	0.746

N.B. MVC, maximal voluntary contraction; $\dot{V}O_{2peak}$, peak oxygen consumption; PPO, power output; * significantly different from all groups; † significantly different from group(s) marked ‡. *P*-value and effect size (η_p^2) from ANOVA. Multiple comparisons identified through *post hoc* Bonferroni adjustment.

All 60 volunteers successfully completed the sustained isometric handhold, however, 1 non-pregnant female (discomfort from unrelated injury) and 2 postpartum females (time constraints as result of breastfeeding) did not attempt the 50% aerobic exercise challenge. It was not possible to monitor blood pressure using finger photoplethysmography in 1 non-pregnant female, and a measurement error in 1 male caused non-physiological readings leading to the exclusion of that dataset from all blood pressure analyses. Some echocardiographic images were not analysable due to inadequate image quality. The *n* for each group is presented in each table in the interest of clarity.

All statistical outcomes presented in this chapter included the resting value as a covariate. This method was used to correct for the known differences in resting function between groups (shown in Chapter 5), which may affect the interpretation of cardiac responses during additional physiological challenge. Statistically adjusted mean values and absolute changes from rest to sustained isometric handhold and submaximal aerobic cycling are provided within the Appendix XII (Table A4 and A5, respectively).

Cardiovascular function at rest

Although not the focus of this chapter, the differences in resting cardiovascular function between groups (presented in Chapter 5) are briefly reviewed for the reader prior to discussion of the main findings associated with the specific objective of this study. Pregnant females had significantly greater HR, allometrically scaled cardiac output, SV, and EDV and significantly lower SVR compared to non-pregnant females. Pregnant females also had significantly greater longitudinal and basal circumferential strain and systolic strain rate when compared to non-pregnant females. Postpartum females had significantly lower BP compared to non-pregnant females, but no differences in cardiac structure or function were observed between groups.

Cardiovascular responses to acute physiological challenge

A control group of males was included within the study design to verify the findings of LV mechanics during physiological challenges. Males and young healthy females responded to both sustained isometric handhold and submaximal aerobic exercise as expected (Williams et al., 2016, Williams et al., 2017, Sanchez et al., 1980, Deschenes et al., 2006, Nio et al., 2013).

Effects of a sustained isometric handhold on global haemodynamics in non-pregnant, pregnant and postpartum females and males

During the sustained isometric handhold, there were no significant differences in BP, scaled cardiac output or LV volumes and SVR between non-pregnant, pregnant and postpartum females (Figure 30). Pregnant females had significantly higher HR (mean difference 10 beats·min^{-1}, CI: 1 to 19 beats·min^{-1}) compared to postpartum females (Figure 30).

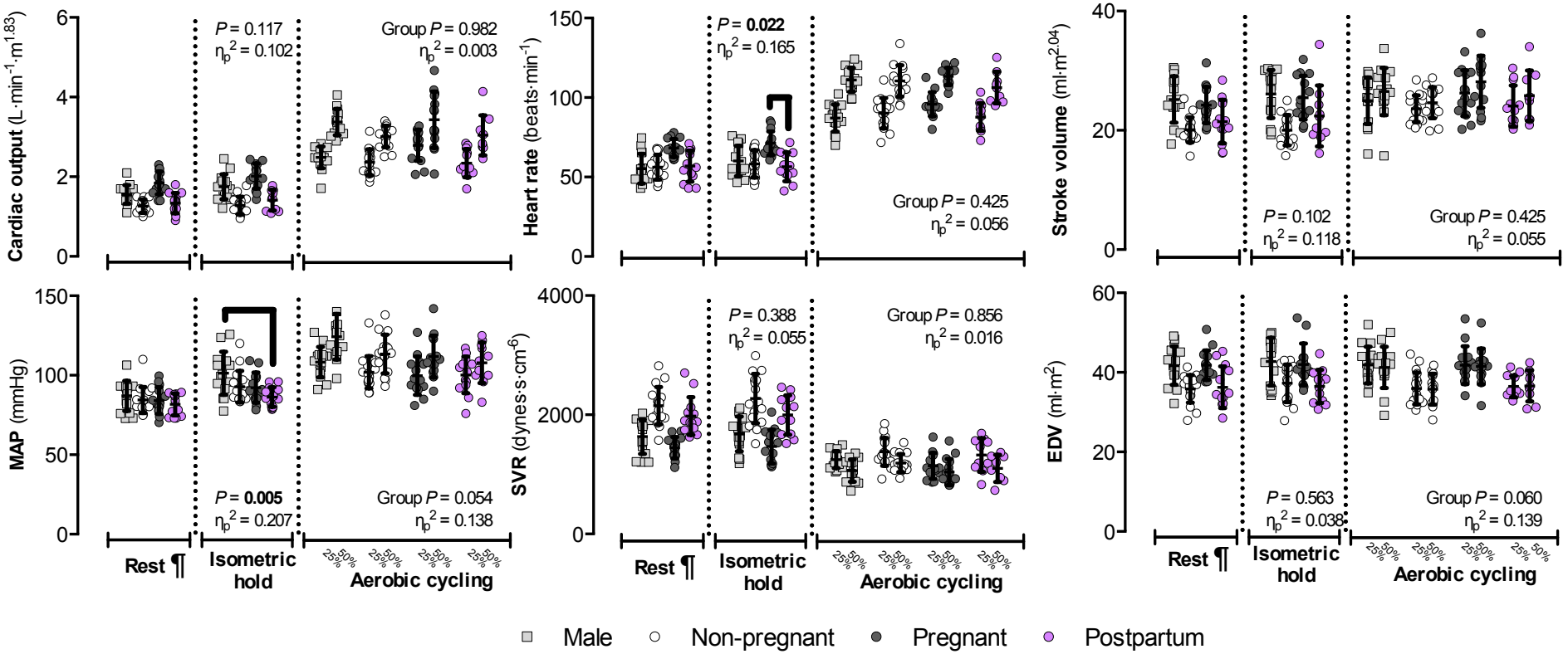


Figure 30. Sustained isometric handhold and submaximal aerobic exercise did not result in differential responses in cardiac output, stroke volume, end-diastolic volume (EDV) or systemic vascular resistance in non-pregnant, pregnant and postpartum females and males. Pregnant females had a significantly greater heart rate during the sustained isometric handhold when compared to postpartum females. Postpartum females had significantly lower mean arterial pressure (MAP) compared to males during the sustained isometric hold. Neither heart rate nor MAP were significantly different between groups during submaximal aerobic exercise.

N.B. ¶ No statistical differences are shown for resting data. Statistical analyses of isometric handhold and aerobic cycling data were performed with baseline value as covariate. *P*-value and effect size presented are for main group effect. Multiple comparisons identified through *post hoc* Bonferroni adjustment. Capped lines demonstrate significantly different groups from *post hoc* analyses ($P < 0.05$).

Effects of a sustained isometric handhold on cardiac function in non-pregnant, pregnant and postpartum females and males

There were no significant differences in traditional echocardiographic measures of systolic (EF and indexed S') or diastolic (indexed E' and A') parameters in response to sustained isometric handhold between all groups (Table 14). However, longitudinal and basal circumferential strain and systolic strain rates were significantly greater in pregnant females compared to non-pregnant females during sustained isometric handhold (peak strain: mean difference 4.3 and 8%, CI: 0.9 to 8% and 3 to 13% systolic strain rates: mean difference 0.18%/sec and 0.4%/sec, CI: 0.04 to 0.32 %/sec and 0.1 to 0.6%/sec, respectively) (Figure 31). There were no significant differences in apical circumferential strain, LV twist or any of the associated rotational parameters between groups during the isometric handhold (Table 15).

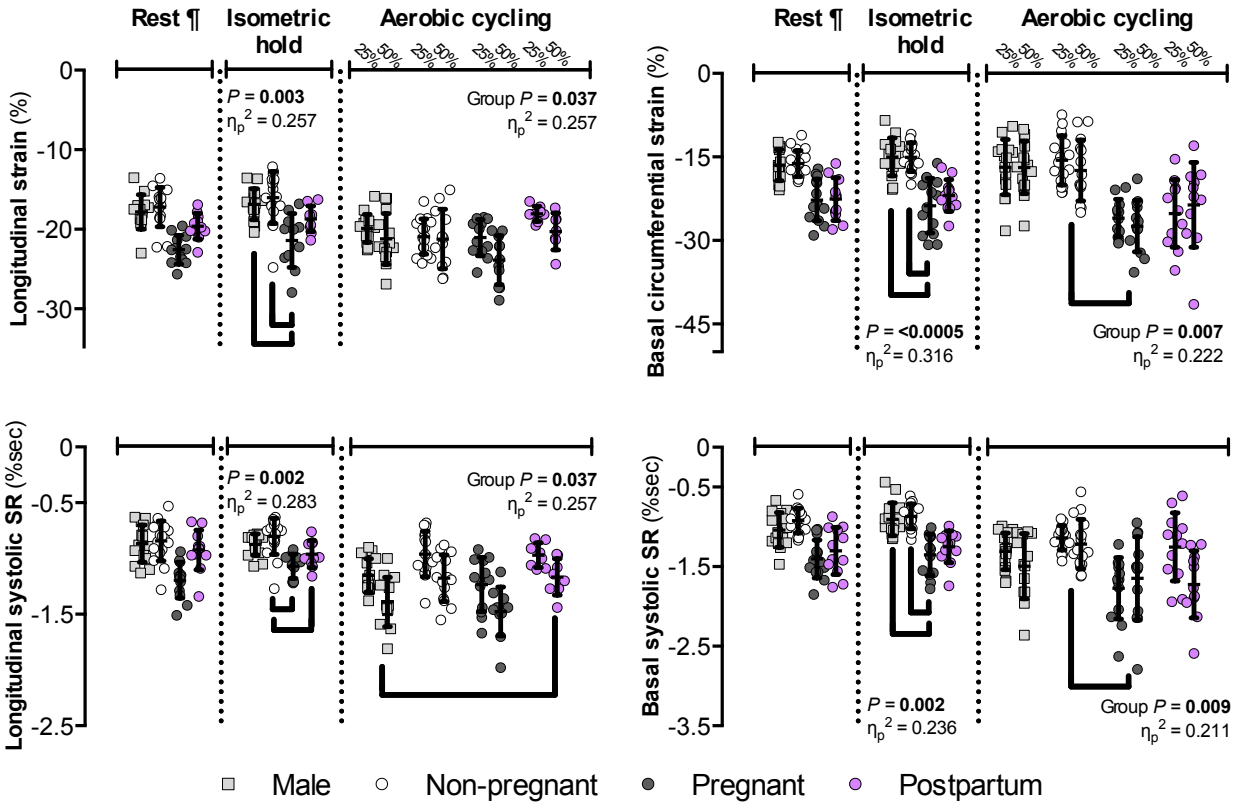


Figure 31. Healthy pregnant females in the late second trimester have greater left ventricular strain during physiological challenges. Pregnant females had significantly greater longitudinal and basal circumferential strain and strain rates during the sustained isometric handhold compared to non-pregnant females. During submaximal aerobic exercise, basal circumferential strain and strain rate were significantly greater in pregnant *versus* non-pregnant females.

N.B. ¶ No statistical differences are shown for resting data. Statistical analyses of isometric handhold and aerobic cycling data were performed with baseline value as covariate. *P*-value and effect size presented are for main group effect. Multiple comparisons identified through *post hoc* Bonferroni adjustment. Capped lines demonstrate significantly different groups from *post hoc* analyses ($P < 0.05$).

Table 14. Traditional measures of cardiac function in non-pregnant, pregnant and postpartum females and males during sustained isometric handhold and during submaximal aerobic cycling exercise. Data presented as mean ± SD.

		Males	Non-pregnant	Pregnant	Postpartum			
Sustained isometric handhold								
	n	14	16	14	12			
Systolic function						***P***	η_p^2	
Ejection fraction (%) **§**		62 ± 4	57 ± 6	61 ± 6	63 ± 8	0.088	0.113	
ESV ($ml \cdot m^2$)		16 ± 3	16 ± 3	16 ± 3	13 ± 3	0.138	0.098	
S' ($m \cdot s^{-1} \cdot cm$)		0.009 ± 0.001	0.010 ± 0.001	0.010 ± 0.002	0.010 ± 0.002	0.274	0.073	
Diastolic function								
E' ($m \cdot s^{-1} \cdot cm$)		0.015 ± 0.002	0.017 ± 0.003	0.016 ± 0.003	0.016 ± 0.003	0.569	0.038	
A' ($m \cdot s^{-1} \cdot cm$)		0.010 ± 0.001	0.008 ± 0.002	0.009 ± 0.002	0.008 ± 0.001	0.056	0.142	
Aerobic cycling								
	n	13	12	13	8			
	Intensity					**Group**	**Exercise**	**Grp * Ex**
Systolic function								
Ejection fraction (%) **§**	25%	63 ± 2 **†**	68 ± 2 **‡**	64 ± 2	67 ± 2	***P*** **0.029**	0.979	0.877
	50%	65 ± 2	72 ± 2	68 ± 2	71 ± 2	η_p^2 0.167	**<0.0005**	0.014
ESV ($ml \cdot m^2$)	25%	15 ± 3 **†**	12 ± 3 **‡**	15 ± 4	12 ± 2	***P*** **0.014**	0.665	0.760
	50%	14 ± 3 **†**	11 ± 3 **‡**	14 ± 4	10 ± 3	η_p^2 0.193	0.004	0.023
S' ($m \cdot s^{-1}/cm$)	25%	0.011 ± 0.002	0.011 ± 0.002	0.012 ± 0.002	0.011 ± 0.002	***P*** 0.056	0.180	0.276
	50%	0.012 ± 0.005	0.014 ± 0.001	0.015 ± 0.002	0.012 ± 0.001	η_p^2 0.160	0.041	0.085
Diastolic function								
E' ($m \cdot s^{-1}/cm$)	25%	0.017 ± 0.002	0.016 ± 0.005	0.017 ± 0.003	0.017 ± 0.004	***P*** 0.924	0.057	0.410
	50%	0.017 ± 0.008	0.019 ± 0.004	0.019 ± 0.002	0.019 ± 0.004	η_p^2 0.011	0.082	0.064
A' ($m \cdot s^{-1}/cm$)	25%	0.011 ± 0.002	0.011 ± 0.004	0.011 ± 0.002	0.010 ± 0.002	***P*** 0.209	0.059	0.595
	50%	0.010 ± 0.005	0.014 ± 0.002	0.010 ± 0.005	0.012 ± 0.003	η_p^2 0.101	0.082	0.044

N.B.: ESV, end-systolic volume (allometrically scaled to height); S', systolic tissue velocity; E', early systolic tissue velocity; A', late-diastolic tissue velocity (all tissue velocities indexed to left ventricular length). § indicates different cohort size: ejection fraction is reported for 15 males, 14 pregnant, 18 and 16 non-pregnant, and 12 and 9 postpartum females during sustained isometric handhold and aerobic cycling exercise, respectively. *P*-value and effect size (η_p^2) from ANOVA. Multiple comparisons identified through *post hoc* Bonferroni adjustment.**†** indicates significantly different to group(s) marked **‡**.

Table 15. Left ventricular mechanics in non-pregnant, pregnant and postpartum females and males during sustained isometric handhold and during submaximal aerobic cycling exercise.

		Males	Non-pregnant	Pregnant	Postpartum		
Sustained isometric handhold							
Longitudinal strain	*n*	15	15	11	10	***P***	η_p^2
Time to peak strain (%)		103 ± 6	99 ± 4	99 ± 4	99 ± 6	0.104	0.124
Diastolic SR (%/sec)		1.2 ± 0.2	1.1 ± 0.3	1.5 ± 0.4	1.3 ± 0.2	0.412	0.061
Basal circumferential strain	*n*	15	17	14	13		
Time to peak strain (%)		102 ± 6 ‡	101 ± 3 ‡	94 ± 10 †	100 ± 9	**0.015**	0.176
Diastolic SR (%/sec)		1.2 ± 0.3 ‡	1.3 ± 0.4	1.8 ± 0.7 †	1.5 ± 0.5	**0.021**	0.166
Apical circumferential strain	*n*	15	15	14	13		
Peak strain (%)		-20 ± 5	-20 ± 6	-24 ± 5	-22 ± 4	0.140	0.099
Time to peak (%)		100 ± 1	100 ± 1	101 ± 3	100 ± 1	0.666	0.030
Systolic SR (%/sec)		-1.2 ± 0.3	-1.1 ± 0.2	-1.4 ± 0.3	-1.4 ± 0.2	**0.012**	0.188
Diastolic SR (%/sec)		1.8 ± 0.6	1.9 ± 0.7	2.2 ± 0.8	1.9 ± 0.6	0.591	0.036
Rotation and twist	*n*	15	15	14	13		
Basal rotation (°)		-6 ± 2	-7 ± 4	-8 ± 3	-8 ± 5	0.427	0.050
Apical rotation (°)		9 ± 6	8 ± 5	9 ± 5	9 ± 3	0.630	0.033
Twist (°)		13 ± 7	14 ± 4	16 ± 6	17 ± 6	0.573	0.037
Torsion (°/cm)		1.5 ± 0.8	1.8 ± 0.5	1.8 ± 0.8	2.0 ± 0.8	0.442	0.051
Time to peak twist (%)		97 ± 14	91 ± 10	92 ± 8	94 ± 7	0.356	0.060
Systolic twist velocity (°/s)		77 ± 28	80 ± 22	98 ± 28	100 ± 32	0.559	0.039
Untwisting velocity (°/s)		-85 ± 28	-103 ± 34	-102 ± 35	-114 ± 42	0.666	0.030
Untwist/twist (°/s)/°		-7.3 ± 3.8	-7.5 ± 2.2	-7.0 ± 2	-7.3 ± 2.6	0.867	0.014

Table 15 cont.

		Males	Non-pregnant	Pregnant	Postpartum		Group	Exercise	Grp * Ex
Aerobic cycling	**Intensity**								
Longitudinal strain	*n*	13	13	11	7				
Time to peak (%)	25%	102 ± 8	103 ± 5	100 ± 3	103 ± 7	***P***	0.084	0.842	0.434
	50%	101 ± 6	107 ± 9	102 ± 5	103 ± 6	η_p^2	0.155	0.001	0.067
Diastolic SR (%/sec)	25%	1.6 ± 0.3	1.3 ± 0.3 ‡	1.6 ± 0.3 †	1.3 ± 0.1 ‡	***P***	**0.002**	0.097	0.055
	50%	1.9 ± 0.4	1.5 ± 0.4	2.2 ± 0.4	1.7 ± 0.4	η_p^2	0.322	0.069	0.175
Basal circumferential strain	*n*	14	15	13	11				
Time to peak (%)	25%	95 ± 9	101 ± 1	99 ± 7	97 ± 6	***P***	0.940	0.355	0.492
	50%	103 ± 9	100 ± 11	99 ± 7	101 ± 7	η_p^2	0.008	0.018	0.048
Diastolic SR (%/sec)	25%	1.7 ± 0.3	1.8 ± 0.5	2.5 ± 0.8	1.8 ± 0.7	***P***	0.149	0.500	**0.015**
	50%	2.1 ± 0.5	2.1 ± 0.9	2.4 ± 0.7	2.8 ± 0.6	η_p^2	0.104	0.010	0.195
Apical circumferential strain	*n*	14	14	13	11				
Peak strain (%)	25%	-25 ± 6	-24 ± 6	-26 ± 6	-23 ± 4	***P***	0.482	0.480	0.096
	50%	-30 ± 8	-30 ± 8	-23 ± 10	-26 ± 5	η_p^2	0.051	0.011	0.125
Time to peak (%)	25%	102 ± 6	100 ± 1	101 ± 4	100 ± 1	***P***	0.257	**0.019**	0.928
	50%	102 ± 4	100 ± 1	101 ± 4	100 ± 1	η_p^2	0.081	0.112	0.010
Systolic SR (%/sec)	25%	-1.6 ± 0.4	-1.6 ± 0.4	-1.7 ± 0.4	-1.5 ± 0.3	***P***	0.183	0.494	**0.024**
	50%	-2.4 ± 0.8	-2.4 ± 0.6	-2.1 ± 0.6	-1.9 ± 0.5	η_p^2	0.097	0.010	0.181
Diastolic SR (%/sec)	25%	2.7 ± 0.9	2.5 ± 1.0	2.8 ± 1.0	2.0 ± 0.6	***P***	0.163	0.227	0.058
	50%	3.4 ± 1.1	4.0 ± 1.7	3.1 ± 1.0	2.8 ± 0.9	η_p^2	0.102	0.031	0.146

Table 15 cont.

		Males	Non-pregnant	Pregnant	Postpartum				
Aerobic cycling	**Intensity**						**Group**	**Exercise**	**Grp * Ex**
Rotation and twist	*n*	13	13	13	11				
Basal rotation (°)	25%	-7 ± 3	-10 ± 5	-10 ± 4	-11 ± 6	***P***	0.341	0.722	0.374
	50%	-9 ± 4	-11 ± 5	-12 ± 5	-11 ± 6	η_p^2	0.068	0.003	0.064
Apical rotation (°)	25%	11 ± 4	12 ± 6	12 ± 6	9 ± 4	***P***	0.787	**0.018**	0.926
	50%	14 ± 7	15 ± 6	15 ± 9	13 ± 4	η_p^2	0.023	0.118	0.010
Twist (°)	25%	17 ± 4	20 ± 6	22 ± 7	19 ± 8	***P***	0.497	0.258	0.217
	50%	22 ± 6	24 ± 7	25 ± 12	24 ± 8	η_p^2	0.051	0.028	0.014
Torsion (°/cm)	25%	2.0 ± 0.5	2.5 ± 0.7	2.4 ± 0.7	2.3 ± 0.9	***P***	0.187	0.157	0.976
	50%	2.2 ± 0.9	2.9 ± 0.8	2.9 ± 1.2	2.9 ± 0.9	η_p^2	0.100	0.044	0.005
Time to peak (%)	25%	92 ± 5	96 ± 7	94 ± 3	90 ± 4	***P***	0.812	0.597	0.407
	50%	96 ± 10	94 ± 4	92 ± 9	94 ± 7	η_p^2	0.020	0.006	0.061
Systolic twist velocity (°/s)	25%	111 ± 31	119 ± 28	127 ± 17	139 ± 37	***P***	0.848	0.191	0.575
	50%	178 ± 63	175 ± 59	166 ± 76	174 ± 44	η_p^2	0.017	0.037	0.042
Untwisting velocity (°/s)	25%	-132 ± 42	-155 ± 51	-155 ± 49	-138 ± 48	***P***	0.056	**0.036**	0.122
	50%	-195 ± 50	-213 ± 57	-210 ± 68	-171 ± 63	η_p^2	0.150	0.092	0.117
Untwist/twist (°/s)/°	25%	-7.8 ± 2.7	-7.9 ± 2.0	-7.3 ± 1.6	-7.5 ± 1.6	***P***	0.075	0.124	0.159
	50%	-9.0 ± 2.0	-10.6 ± 3.5	-8.4 ± 1.2	-7.7 ± 2.5	η_p^2	0.141	0.052	0.108

N.B. SR, strain rate. *P*-value and effect size (η_p^2) from ANOVA. Multiple comparisons identified through *post hoc* Bonferroni adjustment. **†** indicates significantly different to group(s) marked **‡**.

Effects of submaximal aerobic exercise on global haemodynamics in non-pregnant, pregnant and postpartum females and males

Submaximal aerobic cycling exercise did not result in significant differences in BP, HR, cardiac output, SV, EDV, or SVR between non-pregnant, pregnant and postpartum females (Figure 30).

Although not of specific focus in this thesis, males had significantly greater SBP compared to all female groups (P = 0.003, mean difference: 17 mmHg, CI: 3 to 32 mmHg) and non-pregnant females had significantly lower ESV (mean difference: 3 $ml \cdot m^2$, CI: 0 to 6 $ml \cdot m^2$) resulting in a significantly higher EF during aerobic cycling exercise (mean difference: 6%, CI: 0 to 12%) compared to males (Table 14).

Effects of submaximal aerobic exercise on cardiac function in non-pregnant, pregnant and postpartum females and males

During aerobic cycling exercise, there were no significant differences in EF, systolic or diastolic indexed septal velocities between non-pregnant, pregnant and postpartum females. However, basal circumferential strain and systolic strain rate were significantly higher in pregnant *versus* non-pregnant females (mean difference: -7% and 0.5%/sec, CI: 1 to 13% and 0.1 to 0.8 %/sec, respectively (Figure 31). A significant group main effect was identified in peak longitudinal strain during aerobic cycling exercise, however *post hoc* comparisons did not detect differences between groups (Figure 31). Additionally, there were no significant differences between groups during aerobic cycling exercise in apical circumferential strain, LV twist or rotation parameters (Table 15).

6.5. Discussion

The objective of this study was to examine functional responses to increased cardiovascular demand and altered haemodynamic load during the late second trimester of healthy pregnancy and in the postpartum period. The results were considered alongside a male cohort in whom functional responses to physiological challenges are more established. The novel findings from the present study were:

(i) Haemodynamic responses to physiological challenges are similar between non-pregnant and pregnant females but the responses in pregnant females are underpinned by significantly greater LV strain.

(ii) Functional responses to physiological challenges are not significantly different between non-pregnant and postpartum females, despite lower resting BP in the latter group.

Overall, the findings provide support of enhanced cardiac function and adequate functional responses to physiological challenge during the late second trimester of healthy pregnancy.

Pregnant females have significantly altered cardiac function during sustained isometric handhold and submaximal aerobic exercise

Global haemodynamic responses (BP, HR, SV, cardiac output, ejection fraction) to both increased afterload and aerobic exercise challenges are not affected during the late second trimester of healthy pregnancy. In this study, there were no significant differences in cardiac output, BP, HR, LV volumes and SVR between non-pregnant and pregnant females during either physiological challenge. The findings from this study support previous investigations of global haemodynamics in response to isometric exercise (Avery et al., 1999, Lotgering et al., 1992b) and submaximal exercise (Veille, 1996, Petrov Fieril et al., 2016) in the latter half of

gestation. These previous studies, however, did not examine the factors that underpin global cardiac function.

In the late second trimester of healthy pregnancy, cardiac function is enhanced at rest and during physiological challenges. As shown in Chapter 5, both longitudinal and basal circumferential strain was significantly higher at rest in the late second trimester compared to non-pregnant females. Building on these resting data, the current study demonstrates that in response to either a sustained isometric handhold or sub-maximal aerobic exercise, LV strain is significantly greater in pregnant females in the late second trimester when compared to non-pregnant females. Specifically, healthy pregnant females in the late second trimester have greater longitudinal and basal circumferential strain during sustained isometric handhold, and significantly greater basal circumferential strain during submaximal aerobic exercise. The significant alterations in strain were identified without differences in traditional echocardiographic measures, reinforcing the greater sensitivity of LV mechanics to subtle changes in cardiac function.

The increased myocardial strain, both at rest and during physiologic stress, may reflect a greater degree of myocardial contractility in pregnant females. Previously, using mathematical cardiac modelling, greater longitudinal and circumferential shortening have been shown to contribute to greater ejection of blood (MacIver, 2012). Therefore, enhancement in systolic contraction facilitates the generation of greater cardiac output required during gestation. This is also reflected by greater absolute SV in pregnant females compared to non-pregnant females, shown in Appendix XII. During pregnancy, the contractile state is likely increased through a combination of elevated sympathetic activity and larger LV volumes. It is well established that pregnant females have greater sympathetic drive at rest and during physiological challenge (Usselman et al., 2015a, Usselman et al., 2015b,

Jarvis et al., 2012). The elevated sympathetic activity of pregnancy leads to greater chronotropy (Yang et al., 2000) and was suggested to influence inotropy, as discussed in the findings from Chapter 5. Additionally, larger EDV in pregnant females in the late second trimester may increase contractile force via the Frank-Starling mechanism. At rest, EDV was significantly higher in pregnant females at rest and remained larger during sustained isometric hold and aerobic cycling (Figure 29). Without changes in LV size, shown in Chapter 5, the LV of pregnant women experiences a greater stretch induced by the larger volume, which likely drives an increase in contractility via the Frank-Starling mechanism. Unfortunately, this study could not identify the true contributions of sympathetic activity and LV volumes to enhanced systolic function during pregnancy, as sympathetic activity was not directly assessed.

Despite greater LV strain observed within this study, LV twist was not significantly different between groups during physiological challenge. These findings further build upon the similarities in resting LV twist in non-pregnant and pregnant females in the late second trimester, shown in Chapter 5. LV twist is reflective of changes in the distribution of myocardial wall stress (Young and Cowan, 2012, Stöhr et al., 2016, Pokharel et al., 2014), which was not increased at rest in this population (Chapter 5). Due to a lack of differences in twist during physiological challenge, it may be suggested that transmural wall stress is similar in non-pregnant and pregnant females in the late second trimester at rest and during sustained isometric handhold and submaximal aerobic exercise. This time point may represent an optimal period in which increased cardiovascular demand and altered haemodynamic load do not result in increased myocardial stress. In support of this theory, a previous study has shown significantly lower ESWS at rest in the second trimester compared to both the first and third trimester of healthy pregnancy

(Estensen et al., 2013). Therefore, the 'twist reserve' or capacity to accommodate for increased transmural stress during physiological challenges is not impaired in the late second trimester. In contrast, physiological challenges in either early or late gestation, in which ESWS is greater at rest (Estensen et al., 2013), may exacerbate myocardial stress, and therefore result in increased LV twist.

The findings from this study support that healthy pregnant females in the late second trimester have the capacity to accommodate for both altered haemodynamic load and increased cardiovascular demand, at least up to the level assessed. Therefore, despite the increase in resting systolic function, the cardiovascular reserve of healthy pregnant females in the late second trimester is not reduced during submaximal challenges and is able to accommodate for increased cardiac work via different mechanisms, in comparison to non-pregnant females. Together, the elevated LV strain and similar LV twist compared to non-pregnant females suggests that the heart of a healthy pregnant female meets the additional output requirements without increased wall stress, and therefore is not compromised in response to increased cardiovascular demand and altered haemodynamic load during the late second trimester.

Global haemodynamic and cardiac functional responses to physiological challenges are not different between non-pregnant and postpartum females.

Postpartum breast-feeding females have a significantly lower resting BP, shown in Chapter 5 and in previous literature (Ebina and Kashiwakura, 2012, Groer et al., 2013), yet the implications of this in response to physiological challenge were unclear. In this study, haemodynamic and functional cardiac responses during sustained isometric handhold and aerobic cycling were not different between non-pregnant and postpartum females suggesting that the dynamic cardiovascular function is unaffected after delivery. The findings suggest that healthy females 12-

16 weeks after delivery have a normal cardiac response to a sustained isometric handhold and aerobic exercise, despite a significantly lower resting BP. Therefore, healthy pregnancy does not appear to exert any overt changes to the female cardiovascular system in the immediate postpartum period.

Generation of cardiac output is different between non-pregnant females and males during aerobic exercise

Although not the main focus of this study, it is pertinent to comment on the comparison between males and females. Young healthy males and non-pregnant females show differential responses in BP and systolic function during physiological challenges (Williams et al., 2016, Williams et al., 2017). In our study, physiological challenge resulted in similar increases in scaled cardiac output in males and non-pregnant females, however during aerobic cycling exercise, this was achieved differently between the sexes. Non-pregnant females had significantly lower ESV during aerobic cycling exercise, even when normalised for body size, resulting in a significantly greater EF compared to males, also shown previously (Williams, 2016).

Greater afterload in males may limit SV (Balmain et al., 2016, Weiner et al., 2012), hence the greater EF and lower ESV in females. Compared to males, females have a reduced sensitivity to, and potentially lower levels of, circulating catecholamines resulting in reduced vasoconstriction and therefore afterload during exercise (Joyner et al., 2016, Zouhal et al., 2008). This is supported by our findings of a significantly lower BP and significantly greater reductions in SVR from rest to exercise in non-pregnant females, indicative of greater systemic vasodilation compared to males (Joyner et al., 2016, Hulkkonen et al., 2014, Deschenes et al., 2006). The results from this study support the contention that the regulation of the heart is different between the sexes during physiological

challenge, which may be influenced by the different hormonal status of young females (Nio et al., 2017).

Limitations

The findings from the sustained isometric handhold challenge cannot be compared directly with previously published data from afterload challenges in healthy pregnancy. The sustained isometric handhold in this study caused only a modest increase in BP when compared to isometric handgrip used previously (Degani et al., 1985, Ekholm et al., 1994a, Eneroth-Grimfors et al., 1988, Nisell et al., 1987, Weiner et al., 2012, Balmain et al., 2016). This discrepancy between the expected *and* observed responses led to further investigation of the method utilised in this study. In short, the sustained isometric handhold was compared to an isometric handgrip at the same intensity using a force transducer (Appendix XIII). The isometric handgrip resulted in significantly larger increases in cardiac output, HR, and BP compared to the sustained isometric handhold. However, both afterload challenges significantly increased all of the above parameters from resting values. In this study, the significant differences in functional responses to sustained isometric handhold were identified; therefore the use of this challenge as a physiological stimulus was valid, although different to the methods previously reported. It is possible that the results may be different if a stronger stimulus had been used to increase afterload.

6.6. Conclusions

Non-pregnant, pregnant and postpartum females had similar global haemodynamic responses to the physiological challenges of sustained isometric handhold and submaximal aerobic exercise. However, pregnant females had enhanced dynamic systolic function compared to non-pregnant females,

evidenced by significantly greater LV strain. This increased contractile state is observed at rest in the late second trimester and maintained under altered haemodynamic load and increased cardiovascular demand. Therefore, cardiovascular reserve in the late second trimester of healthy pregnancy appears not to be reduced, despite elevated resting cardiac function.

Non-pregnant and postpartum females did not respond differently during physiological challenge, despite significantly lower resting BP in postpartum females. The similar dynamic function in both non-pregnant and postpartum females provides further support that the heart of a pregnant female returns to non-pregnant function after delivery.

The overall findings from this study showed that healthy pregnant females in the late second trimester and postpartum females do not have an impaired response to increased cardiovascular demand or altered haemodynamic loading, at least up to the level that was assessed. Therefore, it is possible that dysfunctional responses to these physiological challenges may indicate an elevated risk for cardiovascular complications of pregnancy and the postpartum period.

Chapter 7. General Discussion

7.1. Introduction

The aim of this thesis was to further the understanding of cardiac adaptation and functional cardiovascular responses in healthy pregnancy. Firstly, meta-analyses were completed to determine the adaptation of cardiac output and related haemodynamic parameters across healthy gestation (Chapter 3). Secondly, a comprehensive comparison of cardiac structure and function was completed in non-pregnant, pregnant and postpartum females at rest (Chapter 5). Thirdly, the cardiac responses to physiological challenges were measured in non-pregnant, pregnant and postpartum females to assess cardiovascular reserve (Chapter 6).

This chapter summarises the main findings, their importance, and the clinical relevance of this work, as well as making recommendations for future research and the acknowledgement of study limitations.

7.2. Summary of key findings

A schematic summarising the key findings of each chapter is presented in Figure 32. In agreement with previous literature (Mone et al., 1996, Easterling et al., 1990) and the initial hypothesis, the series of meta-analyses in Chapter 3 reported an increase in cardiac output across gestation, peaking in the early third trimester, declining towards term and recovering to non-pregnant values in the postpartum period. The decline in cardiac output at term occurred in conjunction with a higher afterload (higher MAP and SVR) that may negatively impact upon the generation of SV at this late stage of gestation. Additionally, a novel finding of a non-linear progression to peak cardiac output was identified, with a lower cardiac output in the late second trimester compared to values in the early second and early third trimester. The lower cardiac output value in the late second was associated with lower SV and EDV that may reflect a lower preload. The hypothesised reduction in

preload was suggested to be the result of large increases in fetoplacental blood flow, prior to the compensatory rise in blood volume, that may transiently reduce venous return. The findings from the meta-analyses highlighted the importance of the underlying haemodynamic load on the interpretation of global cardiac function across different gestational periods of healthy pregnancy.

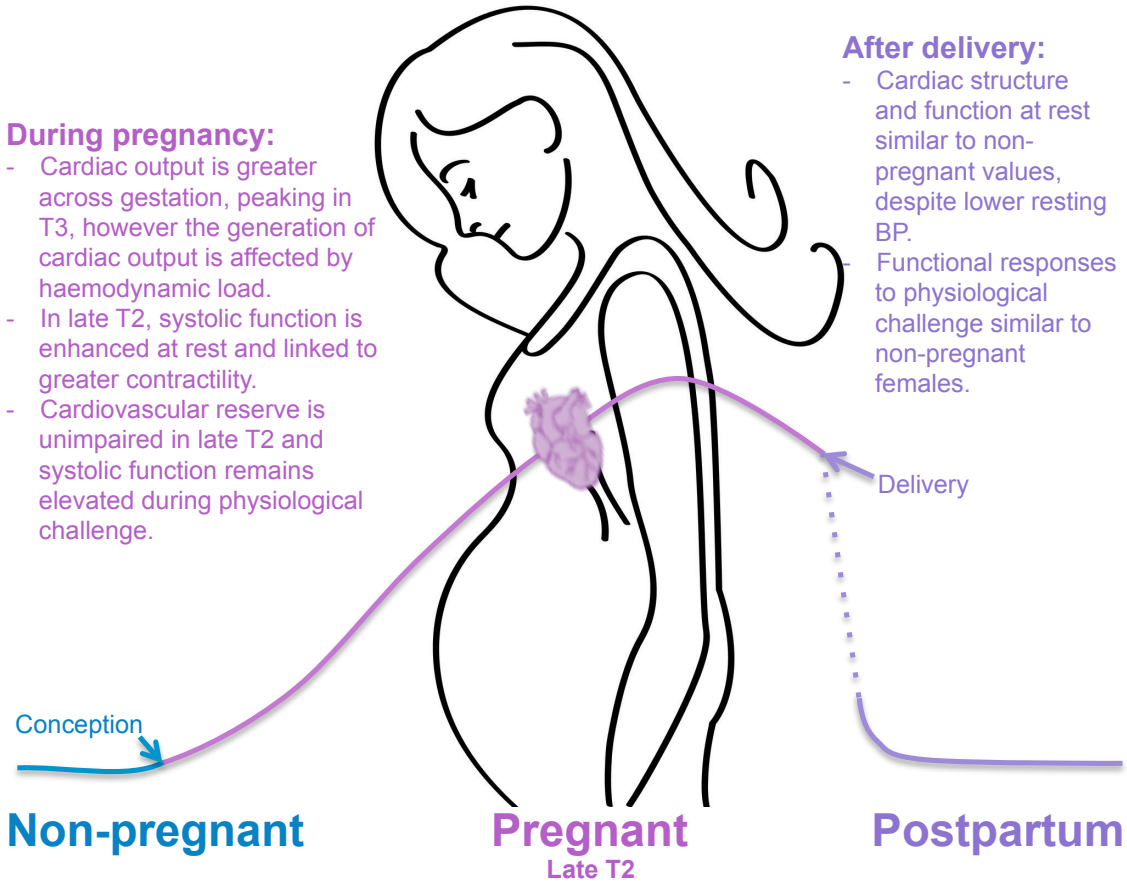


Figure 32. Schematic overview of main findings of this thesis. The series of meta-analyses completed in Chapter 3 identified the importance of gestational age and haemodynamic load in the assessment of cardiovascular function. Chapters 5 and 6 built on these observations through investigation of cardiac structure and function in non-pregnant, pregnant and postpartum females at rest and during physiological challenges. Pregnant females in the late second trimester had significantly greater cardiac output that was generated by greater HR and SV compared to non-pregnant females. The greater SV was suggested to be result of greater EDV and greater systolic function, which was observed in higher longitudinal and basal circumferential strain both at rest and during physiological challenge. Resting blood pressure (BP) was significantly lower in postpartum *versus* non-pregnant females, however there were no significant differences in cardiac structure or function between the groups at rest or during physiological challenges.
N.B. Interpolated linear curve (using data from Chapter 3) representing changes in cardiac output. Dotted line represents phase of unknown change post delivery. Curve is not to scale.

This thesis then investigated cardiac structure and function during the late second trimester of healthy pregnancy and in the postpartum period by completing a comprehensive cardiovascular assessment at rest (Chapter 5) and during physiological challenges (Chapter 6). The main outcomes of these experimental studies were:

(i) Pregnant females in the late second trimester at rest had significantly greater scaled cardiac output as a result of higher HR and larger SV. The larger SV was generated by a larger EDV, which likely reflects a greater preload, and enhanced systolic function, evidenced by greater LV strain in comparison to non-pregnant females. The novel finding of enhanced contractile function in pregnant females at rest remained evident even after controlling for changes in haemodynamic load (EDV, HR, and MAP) and was only moderately related to HR and MAP, but not related to EDV. These findings support that the underpinning contractility may be enhanced during pregnancy and could be the result of an increased sympathetic activity previously observed (Fu and Levine, 2009, Jarvis et al., 2012, Usselman et al., 2015a). Furthermore, the greater contractile function in pregnant females was also observed during physiological challenges of submaximal aerobic exercise and sustained isometric handhold. Collectively, these findings show that healthy pregnant females in the late second trimester have an enhanced systolic function and adequate cardiovascular reserve.

(ii) There were no significant differences in cardiac structure or function at rest or during physiological challenge between non-pregnant and postpartum females 12 – 16 weeks after delivery, despite significantly lower resting BP in the latter group (Ebina and Kashiwakura, 2012,

Groer et al., 2013). Accordingly, it appears that the heart of a postpartum female returns to non-pregnant values in line with the removal of increased metabolic demand, hormonal influence and the physical stress of gestation.

Collectively, the results of this thesis provide a comprehensive characterisation of maternal cardiac adaptation to healthy pregnancy, which is a fundamental step towards understanding the development of cardiovascular complications in gestation. Disorders such as preeclampsia, gestational hypertension, and gestational diabetes mellitus have substantial implications for both maternal and foetal mortality and morbidity and amalgamate with significant cardiac dysfunction (Simmons et al., 2002, Melchiorre et al., 2011, Cho et al., 2011, Appiah et al., 2016). Although the results of the meta-analyses provide normative healthy ranges for global cardiac parameters across gestation, clinicians may use these values to identify maladaptation prior to the manifestation of overt disease. However, the sensitivity of these global parameters to subtle changes in function is limited, yet this thesis demonstrates the benefit of additional novel measures of systolic and diastolic function, as well as physiological challenges to assess maternal cardiac function. Therefore, the assessment of LV mechanics during physiological challenge may allow even earlier identification of females at risk of cardiovascular complications (in contrast to comparing global function to normative data ranges alone), which could improve monitoring, treatment and outcomes for both the mother and infant.

7. 2. i. Haemodynamic load and influence on cardiac function across healthy pregnancy

It is well established that healthy pregnancy causes structural and functional cardiovascular adaptation, however, the pattern of change in fundamental cardiac parameters, such as cardiac output, has long been debated (van Oppen et al., 1996, Melchiorre et al., 2012a). Furthermore, the contribution of haemodynamic load (preload, afterload, and contractility) to the generation of SV and therefore, cardiac output across gestation remained inconclusive. The objective of Chapter 3 was to comprehensively describe the pattern and magnitude of change in cardiac output and related haemodynamics during healthy pregnancy at rest using previously published literature. The study built upon observational studies through the use of meta-analyses, which enabled the pooling of published data to create a larger cohort with greater statistical power than previously possible. The pooled observational dataset provides a representative normative range for global cardiac function at 5 time points during gestation and 2 time points in the postpartum period. These norm values have enabled new insight into the magnitude and timing of cardiac adaptation at rest in healthy pregnancy, which may allow earlier and more accurate identification of pregnancy related cardiovascular complications, such as gestational hypertension and preeclampsia (Boardman et al., 2016). For example, clinicians may use the normative ranges of global cardiac function across gestation to monitor adaptation, particularly in high-risk patients. A deviation outside of the normative ranges may indicate the progression towards complications prior to the overt manifestation, which would enable either increased monitoring or early medical intervention.

In healthy pregnancy, cardiac output is elevated above non-pregnant levels, but also fluctuates within trimesters according to haemodynamic load. Supporting

previous literature, a reduction in cardiac output at term was identified within the analyses (Mone et al., 1996, Mahendru et al., 2014, Easterling et al., 1990), which coincided with reduced SV. Additionally, the analyses identified a small reduction in cardiac output in the late second trimester, which was also associated with a reduced SV. However, the reductions in SV appeared to be mediated by differences in haemodynamic load. The drop observed in the late third trimester may be a consequence of a higher afterload (MAP and SVR) whereas the drop in the late second trimester occurred in line with a reduced EDV, likely reflective of reduced preload. The late third trimester may reflect a time point in which afterload limits the generation of SV. At this late stage, the production of oestrogen supersedes the production of progesterone, altering the balance between the hormones and acting as an antecedent to parturition (Smith et al., 2002). As both hormones play a role in systemic vasodilation throughout gestation (Charkoudian and Stachenfeld, 2016), it is possible that hormonal changes in the late third trimester contributes to the increasing SVR and MAP. The lower cardiac output in the late second trimester of healthy pregnancy was a novel finding of the analyses and can partially be explained by a lower SV and EDV. As discussed in Chapter 3, and noted above, it is possible that the drop in cardiac output is consequence of a reduced venous return. In the late second trimester, fetal growth and metabolic demand rise exponentially (Acharya et al., 2016) requiring a large increase in placental blood flow, however maternal blood volume remains relatively unchanged across this phase (Chapman et al., 1998). The stable maternal blood volume combined with rapidly increasing fetoplacental blood flow may result in maternal vascular underfill that causes a reduction in blood returning to the heart. These findings combined highlight that similar changes in cardiac output can occur in response to different changes in haemodynamic load (reduced afterload or

preload) across gestation. Therefore, the normative ranges for related haemodynamic variables (SV, HR, MAP, SVR, EDV and LV mass) are also useful for medical professionals. Determining the factor behind a pathologically reduced cardiac output during pregnancy may be improved by comparison to these data sets, which may facilitate quicker and more effective treatment.

7. 2. ii. Systolic function is enhanced late in the second trimester

Cardiac output and global haemodynamics (such as HR, SV, blood pressure) are useful measures in understanding the fundamental changes to the cardiovascular system during gestation. However, their analysis alone does not provide insight into how the underpinning haemodynamic load (preload, afterload and contractility) regulates the generation of cardiac output. Whilst cardiac output is viewed as a product of myocardial contraction, the relaxation of the heart facilitates passive ventricular filling, which in turn optimises the volume of blood ejected from the heart. Therefore both systolic and diastolic function of the maternal heart is important in the generation of cardiac output.

Previous investigations have reported reduced resting cardiac function in the latter half of gestation and consequently it was hypothesised in Chapter 5 that systolic function would be lower in pregnant females when compared to non-pregnant females. In this study, systolic function was assessed using traditional echocardiographic parameters such as EF and septal tissue velocity, as well as LV mechanics including strain and twist. Despite no significant differences between groups in EF and S', basal circumferential and longitudinal strain were significantly greater in pregnant females compared to non-pregnant females, supporting that LV mechanics are more sensitive measures to subtle changes in cardiac function than traditional echocardiographic measures (Smiseth et al.,

2016). The novel finding of greater LV strain is suggestive of an enhanced systolic performance during the late second trimester of healthy pregnancy. To determine the contributing factors to this elevated systolic function, additional statistical analyses were completed and showed that greater LV strain in pregnant females was still evident despite controlling for factors known to influence LV mechanics, and that LV strain was only moderately related to HR and MAP, but not EDV. Therefore, through a process of elimination, it was suggested that enhanced sympathetic activity ma be the predominant contributor to greater systolic function in healthy pregnant females in the late second trimester. The greater sympathetic tone in healthy pregnancy has previously been evidenced (Fu and Levine, 2009, Jarvis et al., 2012, Usselman et al., 2015a) and may result in increased myocardial contractility via catecholamine release (Levick, 2010). Additionally, high levels of circulating oestrogen, observed during pregnancy, have been shown to up regulate calcium ion channels to allow a greater influx of calcium into cardiomyocytes (Yang et al., 2012), that also enable a greater myocyte contraction. Together, increased circulating inotropic agents and female sex steroid hormones may increase the contractile function of the maternal heart. In contrast to these findings, both reduced (Papadopoulou et al., 2013) or unchanged (Ando et al., 2015, Tzemos et al., 2008, Cong et al., 2015, Estensen et al., 2013, Sengupta et al., 2017) longitudinal and circumferential strain at rest have been observed within the second trimester (12 to 28 weeks). The discordant results may be the result of the inclusion of heterogeneous cohorts, assessments over broad time ranges and a lack of consideration for the underlying haemodynamic load known to influence LV mechanics, all of which were considered within this study.

Although greater resting strain is typically interpreted as enhanced function, higher values at rest may be indicative of a reduced functional reserve (Sengupta et al.,

2008). Therefore, the present study used physiological challenge to determine the ability of the maternal heart to adapt to match cardiac output to increased demand and altered haemodynamic load (Fournier et al., 2014). A lack of response to such challenge may reflect a reduced capacity to adapt function and therefore, a reduced reserve. However, this was not the case in this study and pregnant females had significantly greater systolic function (evidenced by LV strain) during physiological challenges compared to non-pregnant females. This novel finding strengthened the results of enhanced systolic function at rest, but also confirmed that pregnant females in the late second trimester have adequate functional reserve during submaximal aerobic exercise and sustained isometric handhold. Although not directly comparable, this study supports the findings of an earlier longitudinal study investigating the effect of passive leg raising, a method to transiently increase venous return and hence preload, on haemodynamic function across pregnancy (Vartun et al., 2015). Functional haemodynamic responses to passive leg raising were positive (increased SV) between 20 and 31 weeks, but impaired (no change in SV) in healthy pregnancy after 32 weeks gestation. These results suggest that pregnant females in the second trimester have adequate functional capacity to adapt to altered haemodynamic load, but that this reserve may be reduced in the third trimester. Therefore, although the results of the present study suggest that cardiac reserve is not impaired late in the second trimester of healthy pregnancy during submaximal challenges, further investigation at different gestational ages and in different stimuli is required to fully understand the functional capacity of the maternal heart.

A reduced functional reserve or an inability of the maternal cardiovascular system to augment cardiac output in response to increased cardiac work may be indicative of complications later in gestation (Figure 33) (Meah et al., 2017). The

findings from Chapter 6 showed that cardiovascular function is enhanced during physiological challenges in the late second trimester of healthy pregnancy, however this may not be observed in females who later develop complicated pregnancies. Before this thesis, the functional reserve of healthy pregnant females in response to basic exercise tests was not known. Now, the quantification of this response in healthy pregnancy may enable the identification of abnormal responses. As such, this thesis has provided the foundation for developing the use of physiological challenges during pregnancy as screening tools for cardiovascular complications.

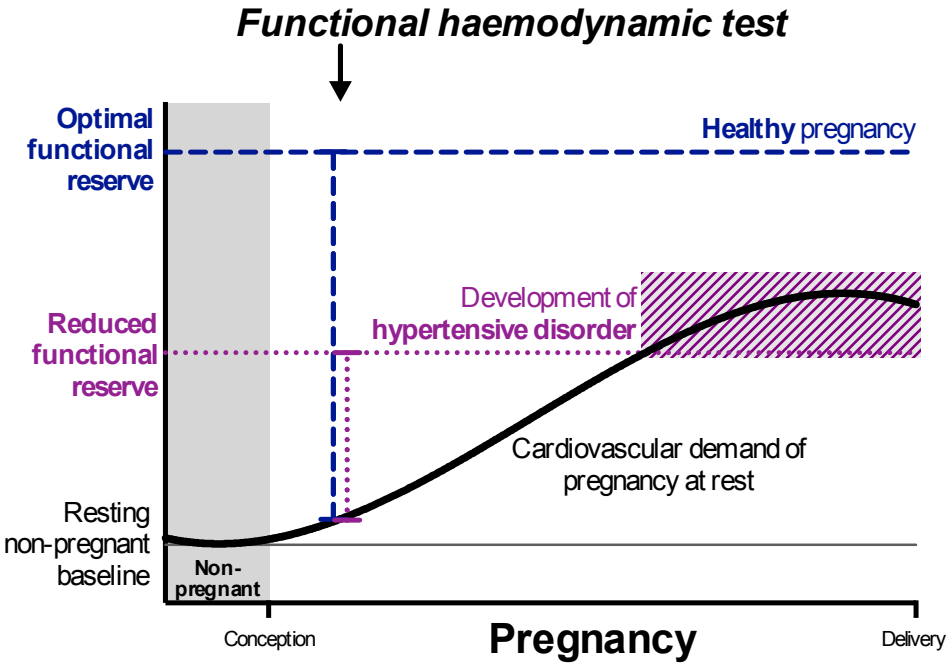


Figure 33. Functional haemodynamic testing early in pregnancy may predict the development of gestational cardiovascular complications. Black filled line indicates progressive cardiovascular demands of pregnancy. Horizontal dashed line indicates maternal cardiovascular reserve during healthy pregnancy; vertical dashed line indicates maternal cardiovascular response to functional haemodynamic test early in gestation. Horizontal dotted line indicates reduced maternal cardiovascular reserve during hypertensive pregnancy; vertical dotted line indicates reduced maternal cardiovascular response to functional haemodynamic test early in gestation. The filled striped section indicates the risk period for the clinical amalgamation of hypertensive pregnancy. If pregnant females have dysfunctional responses to functional haemodynamic testing early in pregnancy, this may indicate an inability to cope with the increased cardiovascular demands of later pregnancy, and therefore a reduced cardiovascular reserve. Adapted from Meah et al. (2017).

Physiologically-induced increases in afterload, although transient, are similar to those experienced in disease states, such as hypertension (Weiner et al., 2012). Maladaptive responses, such as an exaggerated BP response to a dynamic arterial load have been used as predictors of the development of hypertensive disorders in the general (Garg et al., 2013) and pregnant population (Degani et al., 1985, Ekholm et al., 1994a, Eneroth-Grimfors et al., 1988). Healthy pregnant and non-pregnant females exhibit similar cardiovascular responses to isometric contractions, shown in Chapter 6 and previous research (Ekholm et al., 1994b, Lotgering et al., 1992b). However hypersensitive responses to afterload challenges have been observed in high-risk pregnant females prior to the development of symptoms and diagnosis of disease. A previous study in pregnant women between 28 and 32 weeks found that afterload challenge at 50% maximal voluntary contraction demonstrated high sensitivity and specificity in identifying gestational hypertension through assessment of DBP alone (Degani et al., 1985). However, these findings have not been consistently reported (Ekholm et al., 1994a, Greenwood et al., 1998, Nisell et al., 1985, Nisell et al., 1987, Riskin-Mashiah and Belfort, 2004, Veille et al., 1984). The majority of this research has been completed in small cohort groups with out-dated and insensitive measures. It is possible that further investigations using an array of cardiac measures, such as those used in this thesis, would develop the understanding of the prognostic value of afterload challenges in identifying complications during gestation.

Physiological challenges that alter both haemodynamic load and metabolic demand, such as submaximal aerobic exercise, cause a greater stress on the system and may therefore unmask cardiovascular dysfunction that is not apparent at rest (Gibbons et al., 2002). Deviations from the normal integrative responses to,

and recovery from exercise may indicate a reduced cardiovascular reserve that can predict the development of disease (Shin et al., 2015, Balady et al., 2004, D'Amore and Mora, 2006). Submaximal aerobic exercise may therefore be useful in the identification of complicated pregnancies early in gestation (Meah et al., 2013, Meah et al., 2017). Previous, although limited, research in this area has shown a prognostic value of exaggerated DBP during submaximal exercise during otherwise healthy pregnancy (Watson et al., 1995), as well as a blunted heart rate during maximal exercise at preconception in females with congenital heart disease (Lui et al., 2011). Additionally, increased umbilical artery pulsatility index and absent end-diastolic flow during low-intensity exercise has been shown to be related to the development of intrauterine growth restriction later in gestation (Chaddha et al., 2005). Current research in this area is promising, and highlights a potential clinical value of exercise testing in pregnancy.

7. 2. iii. Factors influencing postpartum cardiovascular function

The postpartum period remains understudied and as a result, it is unclear how the female heart is affected after delivery. Evidence from a preconception longitudinal study suggests that the heart of a female in the postpartum period is functionally different to the heart of a female before pregnancy. Females in the postpartum period have been reported to have significantly greater cardiac output, SV, and EDV with lower SVR compared to pre-pregnancy values (Clapp and Capeless, 1997). However in contrast, no differences between non-pregnant and postpartum cardiac function have also been reported in previous literature (Savu et al., 2012, Mahendru et al., 2014). In support of the latter, the findings from Chapters 3, 5 and 6 show that in comparison to non-pregnant females, females in the postpartum period do not have significantly different cardiac structure (LV mass, RWT,

sphericity index) or function (cardiac output, SV, HR, EDV, EF, tissue velocities) 12-16 weeks after delivery.

The return of cardiovascular function to non-pregnant values after pregnancy fits with the 'form follows function' principle (Russell et al., 2000). This states that the myocardium remodels to accommodate for altered cardiac demand and haemodynamic load, such as that experienced during healthy gestation. At delivery, the expulsion of the fetoplacental unit leads to a withdrawal of pregnancy related hormones as well as reductions in metabolic demand and blood volume through blood loss (Macdonald et al., 2011). Therefore, the physiological challenge of pregnancy is removed, and the myocardium remodels to match the current (non-pregnant) haemodynamic load and demand. Although there are some indications that pregnancy has a long term affect on the female cardiovascular system, such as altered remodelling during subsequent pregnancy (earlier and greater magnitude of change in cardiac output, SV and EDV) (Clapp and Capeless, 1997) and reduced risk of cardiovascular disease in later life after one or two pregnancies (Lv et al., 2015), the results of this study suggest that there are no overt changes in cardiac structure or function in breastfeeding, physically active postpartum females.

It is possible that the return of cardiac function to non-pregnant values after pregnancy may be influenced by maternal characteristics or behaviour after delivery. The postpartum females in this study had significantly lower resting BP compared to non-pregnant females, and a number of factors have been linked to this post-delivery reduction. As discussed in Chapter 5, lactation has been shown to lower resting BP (Ebina and Kashiwakura, 2012, Groer et al., 2013), however physical activity may also impact upon cardiovascular function after gestation. Previous work has shown that females who were physically active during and after

pregnancy had lower resting BP compared to sedentary controls (Carpenter et al., 2015, Bisson et al., 2014). Additionally, active females had greater total blood volume at 12 weeks postpartum (Pivarnik et al., 1994) and elevated resting EDV, SV, and therefore cardiac output at 52 weeks postpartum (Clapp and Capeless, 1997). However, it is not clear if these changes are exercise-mediated or a carry over of gestation, or a combination of both factors. In this study, all postpartum females reported meeting physical activity guidelines during and after pregnancy and all females breastfed for at least 12 weeks after delivery. Therefore, it remains unclear if breastfeeding and/or postnatal physical activity caused the reduction in BP in this cohort. Previous research has suggested a protective effect of both breastfeeding and postpartum physical activity against the future development of cardiovascular disease (Aguilar Cordero et al., 2015, Groer et al., 2013), however how and if these behaviours positively affect the maternal cardiovascular system is yet to be elucidated.

7.3. Limitations

The methods utilised within this thesis are not without limitations. The limitations of the meta-analyses were fully discussed within Chapter 3. Briefly, the meta-analyses were limited by inclusion of heterogeneous cohorts (mixed parity, age, ethnicity, anthropometric measures) and different methodologies to calculate cardiac output (echocardiography, impedance cardiography, inert gas rebreathing). Statistical analyses between gestational ages were not possible without *P* values or the provision of raw data, resulting in descriptive findings only.

Some limitations of the experimental study have already been addressed within Chapters 5 and 6, however, the subsequent text will focus on the general limitations of the study. Firstly, Chapters 5 and 6 were part of a cross sectional

study, and not longitudinal in design as per recommendations (Melchiorre et al., 2012a, van Oppen et al., 1996). The successful completion of a study on longitudinal cohorts is possible, but often challenging and may be limited by sample size. Longitudinal studies suffer from high dropout rates over gestation (Melchiorre et al., 2012a), as shown in a recent preconception to postpartum longitudinal study. The study by Mahendru et al. (2014) had a successful completion rate of 51% of the 105 females originally recruited into the study. Preconception studies are also made more challenging by low conception rates and high unplanned pregnancies in the United Kingdom. Current statistics show a conception rate resulting in pregnancy of 0.078% (McLaren, 2014) and that only 55% of pregnancies are planned (Finer and Zolna, 2016). Previous projects have benefited from the provision of incentives for participation, such as ovulation and pregnancy test kits, and additional foetal ultrasound scans (Mahendru, 2012). Unfortunately, such incentives were not available and time constraints of the researcher limited the present study to a cross sectional design. Future research would benefit from longitudinal assessments during and after pregnancy where feasible.

Secondly, Chapters 5 and 6 assessed the structure and function of the heart but did not assess other systems that directly affect the cardiovascular system. In order to truly understand the generation of cardiac output during pregnancy, sympathetic activity, arterial and venous function must also be considered. As discussed throughout the literature review, each of these factors influence haemodynamic load and are primarily underpinned by changes in hormonal milieu during gestation. Understanding the function of the maternal heart, vessels, and autonomic control will assist in determining the regulating factors of cardiac adaptation to healthy pregnancy.

Lastly, the study cohort in Chapters 5 and 6 consisted of a physically active, Caucasian population, which is not reflective of a general population from either an ethnic or activity status. Only 15% of pregnant females meet physical activity guidelines according to objective physical activity assessment (Evenson and Wen, 2011). Within this study, 14 out of 15 pregnant females and all postpartum females reported meeting physical activity guidelines in a self-report questionnaire. It is therefore possible that exercise-induced changes in cardiac function may have influenced the findings in Chapters 5 and 6. The effects of chronic exercise on cardiac remodelling and function in a healthy adult population are well established (Chung and Leinwand, 2014) and have also been observed in pregnant and postpartum females. Previous research has shown that exercising mothers have significantly lower resting HR and higher heart rate variability compared to inactive mothers (May et al., 2016), supporting that exercise has a chronotropic effect irrespective of gestation. Additionally, after delivery, active females have been shown to have greater total blood volume, EDV, and cardiac output in comparison to inactive females, highlighting that exercise mediated haematological changes impact upon maternal cardiac function (Pivarnik et al., 1994, Clapp and Capeless, 1997). Ante and postnatal exercise may therefore alter cardiac adaptation during and after pregnancy.

7.4. Future directions

Summarised from the above sections, areas that require further research include:

- Cardiovascular responses to physiological challenge should be investigated in females with high risk for pregnancy related cardiovascular complications. This may provide prognostic value prior to clinical manifestation of the disease.

- Cardiac structure and function should be considered alongside sympathetic activity and vascular function to gain a comprehensive understanding of the regulation of cardiac output during healthy and complicated pregnancies.
- The relationship between maternal physical activity before, during and after pregnancy and its effect on cardiac adaptation to and return from gestation should be investigated to determine if this offers a protective effect against cardiovascular complications.

7.5. Conclusions

The results of this thesis highlight that healthy pregnancy causes significant structural and functional adaptation of the maternal heart that is reversed after delivery. Cardiac output is increased across healthy gestation, peaking in the third early third trimester with a modest reduction towards term. The generation of cardiac output during healthy pregnancy is highly influenced by haemodynamic load, which is altered across gestation. At rest, late in the second trimester of healthy pregnancy, EDV (likely reflective of preload) is significantly greater, SVR is significantly lower and contractile function is enhanced when compared to non-pregnant females, leading to a significantly greater cardiac output. The upregulation of the cardiovascular system at rest can lead to reductions in functional capacity, however systolic function in pregnant females in the late second trimester remained enhanced during physiological challenges that increased metabolic demand and altered haemodynamic load. These findings support that pregnant females in the late second trimester have an enhanced systolic function and adequate functional reserve. In contrast, cardiac structure and function in postpartum females was not significantly different from non-pregnant females, despite significantly lower BP, confirming that the cardiac adaptation of pregnancy returns to non-pregnant values after delivery.

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END OF THESIS VOLUME ONE

Appendices

Appendix I – Ethical Approval Documents

I.a. Approval for meta-analyses (Chapter 2)

CARDIFF METROPOLITAN UNIVERSITY
APPLICATION FOR ETHICS APPROVAL

When undertaking a research or enterprise project, Cardiff Met staff and students are obliged to complete this form in order that the ethics implications of that project may be considered.

If the project requires ethics approval from an external agency such as the NHS or MoD, you will not need to seek additional ethics approval from Cardiff Met. You should however complete Part One of this form and attach a copy of your NHS application in order that your School is aware of the project.

The document ***Guidelines for obtaining ethics approval*** will help you complete this form. It is available from the Cardiff Met website.

Once you have completed the form, sign the declaration and forward to your School Research Ethics Committee.

PLEASE NOTE:
Participant recruitment or data collection must not commence until ethics approval has been obtained.

PART ONE

Name of applicant:	Victoria Meah
Supervisor (if student project):	Dr Eric Stöhr
School:	Cardiff School of Sport
Student number (if applicable):	ST09001824 SM71724
Programme enrolled on (if applicable):	MPhil/PhD
Project Title:	Cardiovascular adaptation during and after pregnancy; a systematic review.
Expected Start Date:	17/06/2013
Approximate Duration:	6 months
Funding Body (if applicable):	None
Other researcher(s) working on the project:	Prof. Rob Shave Prof. John Cockcroft Jane Black
Will the study involve NHS patients or staff?	No
Will the study involve taking samples of human origin from participants?	No

In no more than 150 words, give a non technical summary of the project

Healthy pregnancy is a state of progressive physiological adaptation in the maternal cardiovascular (CV) system that allows optimal conditions for foetal development and growth. During healthy pregnancy, the CV system undergoes major structural and functional adaptation to the “stress” of pregnancy in order to supply an ever increasing demand for the developing foetus. Previous studies have reported an increase in cardiac output, caused by an increase in heart rate and stroke volume, with reduced vascular resistance in the second and third trimesters. However, there is some disagreement in the literature as to whether these CV parameters are maintained, continue to adapt, or regress towards delivery. The inconsistency of results is likely due to the varying gestational age at which measurements occur, as well as methods of measurement. The aim of this study is to characterise magnitude and timing of healthy CV adaptations throughout pregnancy and postpartum through a systematic review of previous literature.

Does your project fall entirely within one of the following categories:

Paper based, involving only documents in the public domain	Yes
Laboratory based, not involving human participants or human tissue samples	No
Practice based not involving human participants (eg curatorial, practice audit)	No
Compulsory projects in professional practice (eg Initial Teacher Education)	No

If you have answered YES to any of these questions, no further information regarding your project is required.

If you have answered NO to all of these questions, you must complete Part 2 of this form

DECLARATION:

I confirm that this project conforms with the Cardiff Met Research Governance Framework

Signature of the applicant: Date:

FOR STUDENT PROJECTS ONLY

Name of supervisor: Date:

Signature of supervisor:

Research Ethics Committee use only

Decision reached: Project approved ☑

Project approved in principle ☐

Decision deferred ☐

Project not approved ☐

Project rejected ☐

Project reference number: 12/06002R

Name: Peter O'Donoghue Date: 08/07/2013

Signature:

Details of any conditions upon which approval is dependant:

Click here to enter text.

CARDIFF METROPOLITAN UNIVERSITY APPLICATION FOR ETHICS APPROVAL

When undertaking a research or enterprise project, Cardiff Met staff and students are obliged to complete this form in order that the ethics implications of that project may be considered.

If the project requires ethics approval from an external agency such as the NHS or MoD, you will not need to seek additional ethics approval from Cardiff Met. You should however complete Part One of this form and attach a copy of your NHS application in order that your School is aware of the project.

The document ***Guidelines for obtaining ethics approval*** will help you complete this form. It is available from the Cardiff Met website.

Once you have completed the form, sign the declaration and forward to your School Research Ethics Committee.

PLEASE NOTE:
Participant recruitment or data collection must not commence until ethics approval has been obtained.

PART ONE

Name of applicant:	Victoria L Meah (VLM)
Supervisor (if student project):	Dr Karianne Backx
School:	School of Sport
Student number (if applicable):	ST09001824
Programme enrolled on (if applicable):	PhD
Project Title:	The assessment of cardiac mechanics and arterial haemodynamics during pregnancy and in the postpartum period at rest and during physical activity.
Expected Start Date:	01/12/2014
Approximate Duration:	32 months (end date 01/06/2017)
Funding Body (if applicable):	NA
Other researcher(s) working on the project:	Charlotte Leah Bitchell[1*], Aled Rees[1*], Prof. Rob Shave[1], Prof. John Cockcroft[2], Aimee Drane[1], Dr Eric J Stöhr[2] [1]Cardiff Metropolitan University, UK. [2]Colombia University, New York, USA.
Will the study involve NHS patients or staff?	No

Will the study involve taking samples of human origin from participants?	Yes

In no more than 150 words, give a non-technical summary of the project

Healthy pregnancy requires progressive adaptation of the maternal cardiovascular (CV) system. Current guidelines encourage expecting mothers to be physically active during pregnancy. However, it is not well understood how the mothers CV system copes with physical activity during pregnancy.

The aim of this study is to comprehensively characterise maternal CV function at rest and during physical activity across different stages of gestation and in the postpartum period. This will be achieved through describing the acute response of the maternal CV system to: (1) a dynamic CV challenge of low/moderate intensity, short duration cycling exercise; and (2) an increased cardiac afterload challenge, achieved through use of an isometric handgrip. In addition, the influence of maternal fitness on CV adaptation to pregnancy will be investigated.

Does your project fall entirely within one of the following categories:

Paper based, involving only documents in the public domain	No
Laboratory based, not involving human participants or human tissue samples	No
Practice based not involving human participants (eg curatorial, practice audit)	No
Compulsory projects in professional practice (eg Initial Teacher Education)	No

If you have answered YES to any of these questions, no further information regarding your project is required.

If you have answered NO to all of these questions, you must complete Part 2 of this form

DECLARATION:

I confirm that this project conforms with the Cardiff Met Research Governance Framework

Signature of the applicant: Date: 12.11.14

FOR STUDENT PROJECTS ONLY

Name of supervisor:	Date:
Dr Karianne Backx	12.11.14

Signature of supervisor:

Research Ethics Committee use only

Decision reached:	Project approved	☐
	Project approved in principle	☑
	Decision deferred	☐
	Project not approved	☐
	Project rejected	☐

Project reference number: 14/12/01R – Revised 15/9/01R – Revised 16/1/01R – Revised 16/3/01R

Name: Dr. Brendan Cropley Date: 25/01/2016

Signature: 

Details of any conditions upon which approval is dependant:

The participants must be given a longer 'cooling-off' period than being contacted by the researcher **48 hours** after initial contact. This is so that the participant has sufficient time to arrange to see their GP if necessary as well as giving their participation due consideration. The UEC have suggested that this follow-up should occur **no sooner than seven days** following the initial contact.

1. MSc students are not permitted to be involved directly in the sampling of human tissue. Only the PI and/or Supervisor who has been HTA Approved can be directly involved in the sampling of human tissue.

PART TWO

A RESEARCH DESIGN

A1 Will you be using an approved protocol in your project?	Yes

A2 If yes, please state the name and code of the approved protocol to be used[1]

Cardiovascular Ultrasound Imaging

A3 Describe the research design to be used in your project

Inclusion criteria and recruitment strategy. Healthy individuals between the ages of 20 and 39 years will be recruited from the local community. The study will have a cross sectional design with the aim to study five distinct cohorts:

1) a control group of non-pregnant women;
2) pregnant women with uncomplicated, healthy singleton pregnancies between 14 and 18 weeks,
3) pregnant women with uncomplicated, healthy singleton pregnancies between 22 and 26 weeks and
4) healthy women 12 – 16 weeks after giving birth;
5) an additional control group of males.

The cohort groups will be age-matched. All women must be primigravida (first pregnancy). The principal investigator (VLM) will recruit participants by approaching them at ante- and post-natal groups, mothers' meetings, word of mouth, and through use of advertisements (included as **Appendix A**). Social networking sites, media outlets - such as local newspapers, and posters in community areas will be utilised as a means of publicising the research project.

Exclusion Criteria. Individuals must not be current smokers, hypertensive (prior to or during pregnancy), have previous or existing cardiovascular disease or diabetes mellitus and all women must have conceived naturally without using conceptual aids (hormonal assistance for ovulation induction, in-vitro fertilisation, donor/intrauterine insemination). Women will be excluded from the non-pregnant control group if they have already experienced pregnancy and are mothers. Women who have had a miscarriage after 12 weeks of gestation will be excluded from all cohort groups, but those who have experienced a miscarriage before 12 weeks will be included.

Pregnant volunteers will be excluded from participation in the study if they suffer or develop any of the following contraindications for exercise during pregnancy (American College of Obstetricians and Gynaecologists (ACOG)) [1]: severe anaemia, cardiac arrhythmia, diabetes, chronic bronchitis, intrauterine growth restriction, lung disease, heart disease, incompetent cervix, second or third trimester bleeding, or ruptured membranes. These conditions will be identified through use of a PARMed-X for Pregnancy pre-participation screening questionnaire detailed below and attached as **Appendix B**.

Volunteers that participate in the study during their pregnancy or postpartum will be asked to complete a follow up questionnaire after delivery to determine the health of their

[1] An Approved Protocol is one which has been approved by Cardiff Met to be used under supervision of designated members of staff; a list of approved protocols can be found on the Cardiff Met website here

pregnancy. If volunteers did develop pregnancy-related cardiovascular complications during their gestation, they will be excluded from the study *post hoc.*

Sample Size. In previous versions of this application, an *a priori* power analysis was used to decide upon an appropriate sample size to detect differences in a relevant dependent variable, cardiac output, using previously published data at rest from study with a cross sectional research design [2]. According to this analysis (G*Power, Version 3.1.7.), 27 participants per group (total n = 108) were required in order to identify differences in cardiac output between non-pregnant controls and pregnant women in the second trimester with a statistical power greater than 0.8 and an alpha level less than 0.05.

As the principal investigator has now collected data at rest and during exercise in a number of volunteers, a power analysis was used to check appropriate sample size to detect differences in cardiac output at rest and DURING an aerobic exercise challenge between non-pregnant controls and pregnant women in the late second trimester. The alpha level was set at 0.05 with a statistical power above 0.8 as per the *a priori* analysis. Using outputs from repeated measures analysis of variance on the primary data, the power analysis on this primary data has indicated that 10 participants per group are required. Therefore the table below has been updated to reflect these changes.

To account for the possibility of the development of pregnancy complications, estimated to occur in 10% of all pregnancies within developed countries [3], the final sample size was increased by 10%, resulting in a total of 11 volunteers per group (total n = 55).

Group	**Sample size required**	**Sample collected**	**Sample to recruit**
Non-pregnant control women	11	19	0
Pregnant women between 14 and 18 weeks gestation	11	0	11
Pregnant women between 22 and 16 weeks gestation	11	9	2
Healthy women 12 to 16 weeks after giving birth	11	5	6
Control group of males	11	0	11
Total sample size	**55**	**33**	**30**

The first month of the 19 month study will be dedicated to pilot testing. The recruitment of on average 1-2 women per week needs to be achieved over the final 6 months of the 18 month study duration to meet the target sample size*. This equates to approximately 4-8 hours of testing time per week for the principal investigator (VLM), which is achievable alongside her part time work commitments. It is the intention to recruit non-pregnant and postpartum participants initially to ensure the research process runs as smoothly as possible for pregnant participants.

**6 months and 30 volunteers needed = minimum 5 volunteers per month need to be tested to meet required sample size.*

Average testing of 1-2 women per week (4-8 hours testing time total).

Informed consent. Potential volunteers will be provided with a participant information sheet (**Appendix C1 for female *unchanged*, Appendix C2 for males**). They will also be asked to provide a method of contact if they are willing to consider volunteering for the project. The individual will be encouraged to discuss participation in the project with their family, friends and midwife/GP. Seven days after the initial contact, the principal investigator (VLM) will contact the individual to determine if they are interested in participating in the research project and if positive, an appointment at the laboratory will be booked. In the event of an individual contacting the principal researcher regarding participation prior to the end of this seven day follow up period, an appointment at the laboratory will be booked. When arriving at the laboratory for the first Visit, the principal investigator or supervisor to the project (EJS) will discuss the participant information sheet with the volunteer and invite any questions. The principal investigator (VLM) or supervisor to the project (EJS) will ensure adequate understanding of the testing protocol and the requirements of the study. Informed consent will be sought from all volunteers using the appropriate consent form (**Appendix D**). Informed consent will be sought by individuals (VLM or EJS) trained in the informed consent procedure. Volunteers will be reminded of their right to withdraw from the study at any time.

Testing

All testing will take place at the Physiology and Health laboratory at Cardiff Metropolitan University, Cyncoed Campus. The study requires two Visits to the laboratory and a total time commitment of 3 to 4 hours over two visits. The procedures for each visit are listed below. Please refer to **Appendix E** for a detailed timeline of each Visit. Please refer to detailed descriptors of procedures within the Visit description section.

For males only: Additional researchers on the project, MSc Sport and Exercise Science students, Aled Rees and Charlotte Leah Bitchell will be completing the data collection for Visit One. Both students are working within this research study in order to complete their SSP7016 Independent Study module. In a physiology based Independent Study, the students are required to complete approximately 200 hours laboratory experience or a 5000 word essay, or weighted combination of the both laboratory experience hours and essay. The students are aiming to complete around 100 hours of laboratory experience individually through recruiting participants, setting up and completing data collection for Visit One, assisting VLM with data collection in Visit Two, and analysing data. The students will then write up individual projects (approximately 2500 – 3000 words) in a journal article style on a topic of their choice from the data collected. The students will be supervised for this project by Karianne Backx, also a member of this research team. The students have received training about lab procedures and data collection for each of the methods described in Visit One and their competence has been checked by VLM. The students will work independently in line with the learning outcomes of the module, but will receive guidance from VLM, EJS and KB when required. The students will not be able to take consent from volunteers as they have not received informed consent training. Gaining informed consent will be completed by VLM or EJS. The experience on this project and the data collected on this project will not form the basis of the student's dissertation projects.

Visit One

1. Pre-participation screening and anthropometric data collection;
2. Resting heart rate and oxygen consumption;
3. Haemoglobin measurement;
4. Maximal isometric hand grip test;
5. Aerobic exercise test to 70% heart rate reserve;
6. Pregnancy Physical Activity Questionnaire (PPAQ);
7. Chester Step Test;
8. Familiarisation exercise on the supine cycle ergometer;
9. Physical activity monitoring.

Visit Two

10. Blood pressure and pulse wave velocity;
11. Echocardiography scan and measurement of arterial haemodynamics at rest;
12. Echocardiography scan and measurement of arterial haemodynamics during a sub-maximal isometric hand grip test (30% of maximal isometric hand grip; lasting a maximum of 7 minutes).
13. Echocardiography scan and measurement of arterial haemodynamics during two bouts of low – moderate intensity exercise (25 and 50% estimated maximum power output; both bouts lasting a maximum of 7 minutes) on a supine cycle ergometer.

Throughout all testing visits, the laboratory will be kept at an ambient temperature of 20 - 23 degrees Celsius.

Visit One

Estimated time requirement for volunteer: 1.5 to 2 hours

1. ***Pre-participation assessment and anthropometry***.

Volunteers will be asked to complete a pre-participation health screening questionnaire. Male and female control and postpartum volunteers will be asked to answer the American College of Sports Medicine (ACSM) pre-participation screening questionnaire [4] whilst pregnant volunteers will be asked to complete the ACSM questionnaire and PARMed-X for Pregnancy [5] attached as **Appendices F** and **B** respectively. This will include questions that check for contraindications for exercise during pregnancy as set by ACOG [6]. Volunteers will be excluded from the study if they suffer from any contraindications as listed previously within the exclusion criteria. This is standard practice within the fitness industry prior to allowing ante-natal clients to partake in exercise [7, 8]. In order to confirm suitability to volunteer within the research, participants will also be asked to complete a pre-participation questionnaire (**Appendix G**).

Volunteers will then undergo measurement of stature (Holtain, Fixed Stadiometer, Pembs, UK) and body mass (SECA, Model 770, Vogel & Halke, Hamburg, Germany) from which BMI will be calculated. Anthropometric data will be collected through skinfold measurements (Baty International, Harpenden Skin Fold Calliper, Sussex, UK) at six sites on the right side of the body: triceps, biceps, subscapular, iliac crest, front thigh and mid-calf [9]. Limb circumferences will also be measured on the right side of the body using a measuring tape (SECA, 201, Vogel & Halke, Hamburg, Germany) at the wrist, upper arm, and thigh. The measurements will be taken twice to produce a mean average and will be

used to provide an indication of body composition during pregnancy. Estimations of body fat percentage are not valid during pregnancy as the assumptions and equations are unable to separate the mother and the foetus [10]. Therefore a sum of skinfold thicknesses and differences in limb circumferences will be used to identify changes in regional adipose deposition.

2. *Resting oxygen consumption and resting heart rate measurement.*

The volunteer will be asked to lie in a semi-recumbent position on a couch. Heart rate (HR) will be measured (Polar Electro, RS400, Kemple, Finland) which requires the volunteer to attach a strap around their chest to be placed at the bottom of the sternum. HR will be recorded and used to determine resting heart rate which will be used in the calculation of heart rate reserve for each individual. A mask to collect expired air will then be placed on the volunteer's face and resting oxygen consumption (VO_2) will be measured using a breath-by-breath analysis system (Jaeger, Oxycon Pro and Oxycon Mobile, Warwickshire, UK). Expired air will be collected for a period of 5 minutes in which the participant will be asked to relax and avoid conversation. Calibration of the ambient conditions, gas analyser and volume measurements of the breath-by-breath analysis system will be conducted prior to the commencement of each test. Resting VO_2 is known to increase during pregnancy, however resting heart rate (HR) is increased to a greater extent [11, 12] and therefore, the linear relationship of VO_2 and HR that is assumed during exercise is slightly altered during pregnancy. As a result, the validity of estimating maternal VO_{2max} using a HR-VO_2 extrapolation can be questioned [12]. With regards to the exercise testing employed within this study, a measurement of resting VO_2 will aid the interpretation and validity of the results.

3. *Haemoglobin measurement.*

Pregnant women are at risk of developing anaemia due to a significant increase in plasma volume that is disproportionate to the increase in red blood cell mass. Severe maternal anaemia is a contraindication of exercise during pregnancy and is diagnosed when the haemoglobin concentration falls below 11 g/dl during gestation [13]. Prior to the commencement of exercise, all volunteers will have their haemoglobin levels checked through collection of a capillary blood sample from the ear lobe. The sample will be analysed immediately (HemoCue Hb 201+, HemoCue AB, Angelholm, Sweden). If the result should fall below 11 g/dl, the volunteer will not be allowed to continue with the research project and will be encouraged to visit their GP or midwife for further assessment.

4. *Maximal isometric handgrip test.*

Figure 1. Hand grip dynamometer

Volunteers will be asked to complete maximal isometric contraction on a handgrip dynamometer (Grip-A, 5001, Takei Scientific Instruments Co Ltd. Shinagawa-ku, Tokyo, Japan) in preparation for a sustained endurance isometric handgrip during Visit two as described in detail hereafter. Volunteers will be asked to complete the maximal effort using their left hand in a tilted supine position which will be the required position during data collection in Visit two (discussed in full in '*familiarisation*'). To determine maximal handgrip force, volunteers will be asked to perform three maximal contractions on the handgrip dynamometer separated by a short period of rest (approx. 1

minute). The average value of the three efforts will be calculated. If one value deviates from the peak effort by over 25%, it will be excluded and the mean derived from the remaining two values. 30% of this maximal isometric handgrip test value will be used to set the intensity for the 4 minute endurance isometric handgrip test to be completed in Visit two.

5. ***Aerobic exercise test to 70% heart rate reserve****.*

A sub-maximal exercise test to 70% heart rate reserve (HRR) will be employed to provide an indication of individual aerobic fitness (Figure 2). Volunteers will be asked to complete an incremental exercise protocol on an upright cycle ergometer (Lode, Corival, Lode B.V., Gronigen, The Netherlands). Following the individual set up of the handlebar and saddle height and position, volunteers will be asked to warm up on the bike for 2 minutes at a set cadence of 50 rpm at 0 Watts. Upon completion of the 2 minute warm up, the workload will automatically increase in increments of 5 W every 15 seconds for all female volunteers (20 W per minute increase) and 7.5 W every 15 seconds for male volunteers (30 W per minute increase). Increments will be automatically controlled by the software (Lode Ergometry Manager 9.2, Lode B.V., Gronigen, The Netherlands) connected to the cycle ergometer. Volunteers will be asked to cycle at a constant cadence of 70 rpm throughout the duration of the test. Heart rate response will be recorded throughout the test (as above) and VO_2 measured using a breath-by-breath system (as above). Capillary blood samples will be collected from the ear to measure blood lactate prior to and at the end of the test. The sample will be analysed immediately after collection (Biosen, C-Line Sport, EKF-Diagnostic, Barleban, Magdeburg, Germany) and will provide data to indicate the individuals intensity of workload.

Figure 2. Completing the sub-maximal exercise test

The test will be stopped when the volunteer reaches 70% HRR. According to ACSM guidelines, maximal exercise testing in pregnant women is not recommended unless under supervision of a physician, however sub-maximal exercise tests can be completed up to 75% heart rate reserve (HRR) in women with no contraindications [8]. Heart rate reserve is calculated as:

HRR = heart rate maximum – resting heart rate

Maximum heart rate will be estimated using the equation:

Heart rate maximum = 208 – 0.7(age)

To calculate the target heart rate:

Target HRR = (HRR * desired intensity) + resting heart rate

For this study:

70% HRR = (HRR * 0.70) + resting heart rate (termination point of test)

75% HRR = (HRR *0.75) + resting heart rate (cut off point for testing according to ACSM

guidelines)

The value for resting heart rate taken from the heart rate measurement recorded previously in Visit one. As the test will be terminated at 70% HRR, the volunteer should not be in danger of reached the ACSM guideline threshold as described above. Heart rate, power output, and VO_2 will be extrapolated to estimate peak values according to the estimated heart rate (as above). The estimated maximal power output will be used to prescribe subsequent exercise intensities within the testing as outlined in detail in '*familiarisation*.'

Immediately following the end of the test, volunteers will be asked to cool down for 5 minutes at a power output of 25 watts. Volunteers will then have 10 minutes of rest in the sitting position and will be observed by the researcher for any adverse responses to exercise.

6. ***Physical activity assessment questionnaire.***

During the rest phase following the aerobic exercise test, volunteers will be asked to complete a physical activity questionnaire which should take no longer than 10 minutes. The Pregnancy Physical Activity Questionnaire (PPAQ) is a validated recall questionnaire to assess activity levels during gestation – attached as **Appendix H** [14]. Responders are asked to recall their activity within the previous 4 weeks and select a category that best approximates the amount of time spent in 33 activities ranging from household, occupational, exercise, transport and sedentary behaviour. Activities are classified by intensity: sedentary, light, moderate and vigorous. The duration of time spent in each activity is then multiplied by its intensity to arrive at a measure of average weekly energy expenditure ($MET\text{-}h^{-1}\text{-}week^{-1}$). Volunteers will be reminded not to include any of the physical exercise completed within the project. This will not be completed by the non-pregnant Control group.

7. ***Chester Step Test***.

Prior to commencing the Chester Step Test (CST), the researcher will ensure the volunteer's HR has returned to within 5 bpm of resting HR from the preceding aerobic challenge. The CST is a multistage, sub-maximal test that requires the volunteer to step on and off a low step (either 15, 20, 25 or 30 cms). The initial step rate is 15 steps per minute, set by a metronome, following which there is a 5 step per minute increase in cadence every 2 minutes. The test continues in this progressive manner until the volunteer reaches 70% HRR. At the end of each stage, HR will be recorded. Aerobic capacity will then be determined using the CST software (Chester Step Test calculator, ASSIST creative resources Ltd., Wrexham, UK). This test has not been validated for use during pregnancy however it has been chosen as it can be easily applied and replicated within clinical and applied practice. The estimate of VO_{2max} from this test will be compared to the estimate of VO_{2peak} from the submaximal exercise test completed previously.

8. ***Familiarisation to the supine cycle ergometer***.

The tilted supine cycle ergometer (Lode, Angio 2003, Groningen, Netherlands) is an unusual position for dynamic exercise due to the supine and left lateral tilt position of the body. The supine cycle ergometer is tilted laterally to 45° as this position optimises echocardiographic image collection (see Figure 3). Familiarisation to the equipment will allow the individual to become accustomed to the modality and duration of exercise required in Visit two. The supine cycle ergometer enables the collection of echocardiographic data during exercise within this special population. Pregnant women are advised not to exercise in the supine position from 16 weeks gestation due to inferior vena cava compression from the uterus that may induce pre-syncopal symptoms (please see section C for further discussion of potential risks and controls). These symptoms are also likely to be experienced if a pregnant woman lies in the supine position for an extended period of time and are particularly prevalent in the later stages of gestation. In everyday life, this compression is relieved in the pregnant woman by lying tilted on their left hand side supported by pillows or a foam wedge. Within this research study, the left lateral tilt position of the volunteer will be achieved through support from the tilted supine cycle ergometer and this will ensure safety of each individual and avoid compression of the vena cava compression during exercise. A tilt of greater than 15° has previously been shown to reduce inferior vena cava compression [15] and a recent study has also shown that pre-syncopal symptoms are unlikely to occur as a result of lying in the supine position during rest in the latter half of pregnancy [16] . In this study, left lateral tilt will be 30-45°.

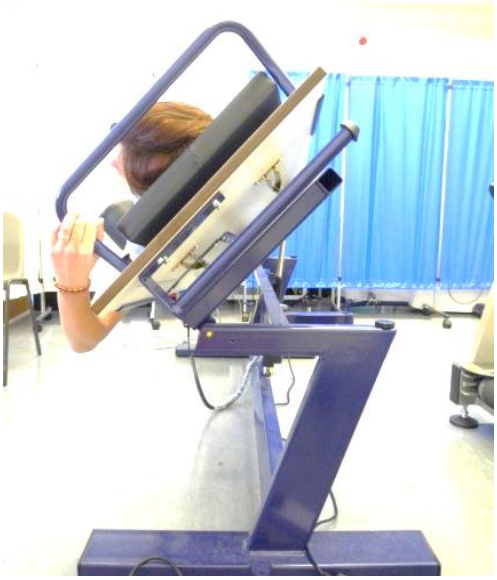

Figure 3. Supine cycle ergometer tilted to 45°

Familiarisation exercise on the supine cycle ergometer. Power output on a supine cycle ergometer is estimated to represent 70% of the power output achieved on an upright cycle ergometer; therefore, the estimated peak power output from the sub-maximal exercise test in Visit one will be adjusted accordingly (= multiplied by 0.7). Exercise intensities will be calculated from this supine-adjusted power output. The familiarisation exercise will be completed at 15 and 30% of the supine-adjusted power output. These exercise intensities are lower than the required intensities of Visit two (25 and 75%) to allow the volunteer to become comfortable with the duration and position. With the supine cycle ergometer at a 30-45° lateral tilt; volunteers will be asked to complete two 4 minute exercise bouts at 15 and 30% of the supine-adjusted power output followed by a period of 2 minutes rest in which the supine cycle ergometer will be returned from tilt to level ground (0°). No data will be collected during this familiarisation phase. Upon finishing the familiarisation exercise, the volunteer will be returned from tilt to level ground and will be encouraged to sit up before standing up to reduce the potential for dizziness and fainting. The volunteer will be monitored for a further 10 minutes of seated rest. They will also be invited to ask any questions or raise any concerns they may have at this point.

9. ***Physical activity monitoring.***

Volunteers will be asked to take home a physical activity monitor (Actigraph wGT3X-BT

Monitor, Actigraph LLC, Pensacola, FL, USA). These small monitors attach to a waist strap (Figure 4) and record 24 hour physical activity and sleep/wake measurements which can be analysed offline (Actilife 6 Data Analysis Software, Actigraph LLC, Pensacola, FL, USA). The use of the accelerometer will be fully explained to all willing volunteers prior to the individual leaving the laboratory. The physical activity monitor will be activated by the researcher (VLM) and the volunteer does not need to alter the monitor in any way for the duration of its use. The researcher (VLM) will deactivate the monitor on the designated day of completion arranged with the volunteer. The volunteer will be asked to wear the strap for a minimum of 5 days of which only the final 3 days data will be analysed. The volunteer will be asked to return the accelerometer to the laboratory when completed. Physical activity monitoring will not be completed by the non-pregnant control group.

Figure 4. Participant wearing an Actigraph waist strap.

Visit Two

Estimated time requirement for volunteer: 1.5 – 2 hours

10. ***Blood pressure and pulse wave velocity***.

The volunteer will be asked to lie in a semi-recumbent position on a couch. After 5 minutes of rest, blood pressure will be measured (Yamasu, 535, Yamasu, Tokyo, Japan). Arterial function, or pulse wave velocity, will be determined through the non-invasive method of radial tonometry (Sphygmocor, AtCor Medical, Sydney, Australia). Pulse wave velocity is the time it takes for the pressure wave generated from the heart to travel from the carotid artery to the brachial or femoral artery and is an indicator of arterial stiffness. The method involves the application of a tonometer, a pen-like instrument that measures pressure changes, to the carotid (neck), brachial (wrist) and femoral (inner thigh) arteries. Palpation will be used to determine the appropriate application point which will be marked using a washable marker pen. The distance between the supra-sternal notch (located at the top of the breast bone) to the carotid point, the supra-sternal notch to the brachial point and the supra-sternal notch to the femoral point will be measured using body callipers. Pulse wave velocity may alternatively be measured using a non-invasive method of brachial oscillometry (Mobil-O-Graph 24h PWA Monitor, I.E.M. GmbH, Stolberg, Germany). A common pressure cuff is placed upon the volunteer's upper left arm, and will inflate and then deflate under control of the equipment unit, similar to a blood pressure measurement. Generation of central aortic blood pressure curves based on brachial pulse waves is based on an algorithm which integrates arterial impedance and aortic haemodynamics into a mathematical model The pulse wave velocity method is routinely used in clinical practice and has been validated for use in research during pregnancy [17, 18] .***Echocardiography at rest***.

Echocardiography is a non-invasive method for imaging the heart. The collection of echo images will abide by the cardiovascular ultrasound imaging approved protocol. Images will be collected using a 1.5 – 4 MHz phased array transducer on a commercially available ultrasound system (Vivid E9, GE Vingmed Ultrasound, Horten, Norway). All images will be collected by a trained female sonographer in accordance with current guidelines [19, 20].

With the participant lying in the left lateral position on the supine cycle ergometer (45º), resting echocardiographic images will be collected (Figure 5a). Electrocardiographic (ECG) electrodes will be placed in 3 positions on the chest; on the right shoulder and above the right and left hip. From these pads, a heart rate trace will be created by the ultrasound system, thus allowing the recording of images for cardiac cycles. Imaging will require placement of the ultrasound transducer in two places on the volunteer's chest: left of their sternum (1) and on the left side of their rib cage (2) as shown in Figure 5b. A clear water-based gel will be applied between the transducer and the volunteer's skin. At rest, echocardiographic images that will allow the measurement of cardiac structure, function and mechanics will be collected. This resting scan will follow a standard image collection protocol, but prior to and during the exercise challenges, a shortened image collection protocol will be used. Images over 3-5 consecutive cardiac cycles will be recorded and analysed offline (EchoPAC, GE Medical, Horton, Norway). Blood pressure will be measured continuously though use of a non-invasive beat-by-beat arterial blood pressure monitoring system placed upon the right arm and hand and recorded continually for later off-line analysis of systolic and diastolic blood pressure, mean arterial pressure and pulse pressure (all equipment as above). The blood pressure monitoring system will remain attached to the volunteer for the duration of the testing Visit. Arterial function will be determined through the continual assessment of arterial wave reflection using a wristband placed on the right wrist (Colin CBM-7000, CMI, Komaki-City, Japan).

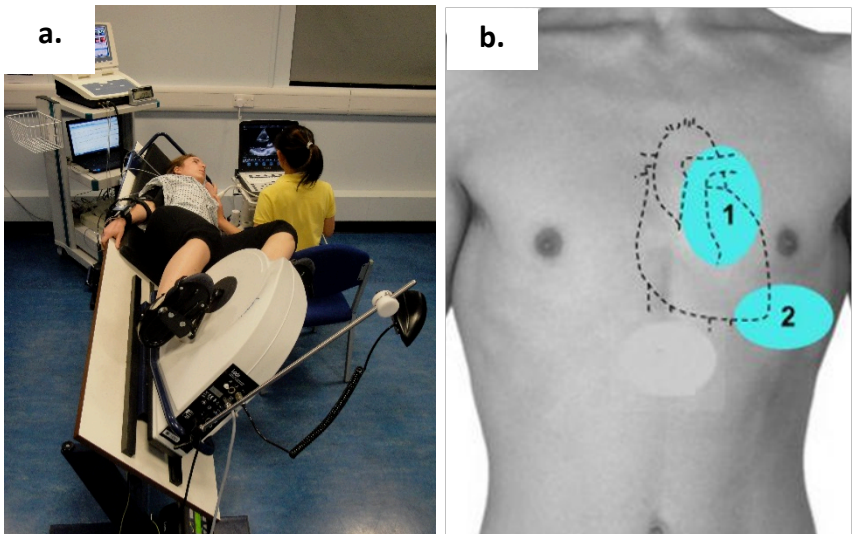

Figure 5. a. Collection of a resting echocardiography scan. **b.** Echocardiographic probe positions.

Upon completion of the resting scan, the participant will be given the option of a 5 minute break in which they can get up from the tilt bed and have a comfort break if required.

11. ***Echocardiography during a 30% maximum isometric handgrip****.*

Prior to starting the exercise challenge, the shortened image collection protocol will be used to collect resting images. Volunteers will be asked to perform a left handed isometric hold of a handgrip dynamometer adapted to hold a standard weight for a maximum of 7 minutes. The required sustained isometric hold will be 30% of the individuals achieved one repetition maximum. Echocardiographic images will be taken in the last two minutes of isometric handgrip in accordance with previous publications [21-23]. The number of echocardiographic images taken during isometric handgrip will be reduced from the number collected during the resting scan due to the time constraints, but will still allow the

measurement of cardiac structure, function and mechanics. The response of systolic and diastolic blood pressure, mean arterial pressure and arterial wave reflection to isometric handgrip hold will be recorded and analysed from the last two minutes of hold (as described above).

After completing the isometric handgrip protocol, the participant will be given the option of a 5 minute rest period in which they can get up from the tilt bed and have a comfort break if required.

12. ***Echocardiography during supine cycle ergometer exercise.***

Prior to starting the exercise challenge, the shortened image collection protocol will be used to collect resting images. Volunteers will be asked to perform two 4 minute bouts of supine cycling exercise at 25 and 50% the supine-adjusted power output. As discussed previously, the estimated peak power output on the upright cycle ergometer will be adjusted to reflect the different exercise position of supine cycle ergometer. The supine-adjusted power output will be used to determine the required exercise intensities of 25 and 50%. Volunteers will be asked to complete two exercise bouts lasting a maximum of 7 minutes at the supine-adjusted power outputs. Echocardiographic images will be taken in the last two minutes of exercise in accordance with previous publications [21-23]. The same echocardiographic images taken during the isometric handgrip hold will be taken during each bout of supine cycling exercise. The response of systolic and diastolic blood pressure, mean arterial pressure and arterial wave reflection to supine cycling exercise will be recorded and analysed during the last two minutes of each bout of exercise (as described above). Between the two bouts of exercise, a 2 minute rest will be given. HR response during the supine cycle exercise will be compared to observed HR at 25% and 50% on the upright submaximal cycle test to ensure achievement of the desired intensity. The key diagnostic criteria for ending the exercise is if the volunteer reaches 70% HRR; however it is highly unlikely that exercise at 25 and 50% supine power output will elicit such a response.

Upon finishing the exercise bouts, volunteers will be encouraged to complete a cool down at a reduced power output of 25 watts of 3 minutes. They will be asked to remain in the tilted position, as supine exercise is not appropriate after 16 weeks in pregnancy. When the volunteer is ready to do so, she will be returned from tilt to level ground and will be encouraged to sit up off the bed slowly to reduce the potential for dizziness and fainting. The volunteer will be monitored for a further 10 minutes of seated rest.

Follow Up Questionnaire

After giving birth, volunteers that took part in the project during their pregnancy will be asked to complete a general questionnaire that requests basic descriptive information of pregnancy outcomes such as gestational age at delivery and foetal birth weight. A copy of this questionnaire is attached with this application, as **Appendix I**. Volunteers will be given the opportunity to complete the questionnaire by email or over the telephone whenever suitable. Postpartum volunteers will be asked to complete the questionnaire upon attendance for Visit one at the laboratory. As detailed previously, if any volunteers identify they had experienced pregnancy related CV complications, they will be removed from the study *post hoc*. This will not be completed by the non-pregnant control group.

Statistical analyses. Descriptive data will be shown as means and standard deviations. Differences between groups will be identified using a one-way analysis of variance (ANOVA) with appropriate *post hoc* tests. A repeated measures ANOVA will be used to

identify differences within subjects between their resting and exercise (isometric hold and two stages of supine cycle exercise) function. Statistical significance will be set at $P < 0.05$.

A4 Will the project involve deceptive or covert research?	No

A5 If yes, give a rationale for the use of deceptive or covert research

NA

B PREVIOUS EXPERIENCE

B1 What previous experience of research involving human participants relevant to this project do you have?

For my undergraduate dissertation, I completed a dissertation entitled "*Cardiac structure and function at rest and during exercise across the menstrual cycle in healthy females*." I am experienced in conducting research and have assisted with other research studies within the physiology department at Cardiff Met. I am in the process of training as a sonographer, and I am now at the stage where I can comfortably acquire images in line with the current standards on a healthy population. I am also qualified as a pre and post natal personal trainer, cardiac rehabilitation instructor and am accredited by BASES as a Certified Exercise Practitioner. I am also working towards BASES accreditation as a sport and exercise physiologist. I have attended and presented at pregnancy-relevant conferences and have recently completed a meta-analysis assessing cardiac output and its determinants before, during and after pregnancy. I have the relevant qualifications and experience to supervise physical activity in pregnant women as well as to complete a research project in this area. I have also published a hypothesis article about the proposed research study:

Meah VL, Cockcroft J, Stöhr EJ. Maternal cardiac twist pre-pregnancy: potential as a novel marker of pre-eclampsia. *Fetal and Maternal Medicine Review.* 2013;24(4):289 - 295.

B2 **Student project only**

What previous experience of research involving human participants relevant to this project does your supervisor have?

Dr Eric Stohr is the Director of Studies for this project and has published extensively in the research area of exercise cardiovascular physiology. He has examined cardiovascular physiology in response to incremental exercise and is trained in the techniques of echocardiography central to the measurements in this project. Below are a list of relevant articles published using these techniques:

Meah VL, Cockcroft J, **Stöhr** EJ. Maternal cardiac twist pre-pregnancy: potential as a novel marker of pre-eclampsia. *Fetal and Maternal Medicine Review.* 2013;24(4):289 - 295.

Stöhr EJ, González-Alonso J, Pearson J, Low DA, Ali L, Barker H, Shave RE. Dehydration reduces left ventricular filling at rest and during exercise independent of twist mechanics. J Appl Physiol. 2011;111(3):897-897.

Stöhr EJ, González-Alonso J, Shave R. Left ventricular mechanical limitations to stroke volume in healthy humans during incremental exercise. Am J Physiol Heart Circ Physiol. 2011;301(2):H478-487.

Stöhr EJ, McDonnell B, Thompson J, Stone K, Bull T, Houston R, Cockcroft JR, Shave R. Left ventricular mechanics in humans with high aerobic fitness: adaptation independent of structural remodelling, arterial haemodynamics and heart rate. J Physiol. 2012;590(9):2107-2119.

Stöhr EJ, Shave R. Left ventricular apical mechanics during ectopy in an asymptomatic athlete. Heart. 2012;98(11):893-894.

Nio AQX, **Stöhr** EJ, Stembridge M, Shave R. Influence of the Menstrual Cycle on Resting and Exercise Left Ventricular Volumes and Twist Mechanics. Med Sci Sports Exerc. 2012;44(5S):724.

C POTENTIAL RISKS

C1 What potential risks do you foresee?

i) Blood sampling may result in risks of infection or cross contamination for both researcher and participant.

ii) The submaximal exercise test may be uncomfortable for individuals unaccustomed to exercise.

iii) Use of a non-disposable facemask for expired air analysis during the peak power test poses a risk of infection from poor sterilisation. Participants may also experience discomfort from unfamiliarity with wearing a facemask during exercise.

iv) Volunteers will be asked to complete a submaximal fitness test and a bout of submaximal supine cycle exercise during the study. Pregnant volunteers may be wary of the safety of physical activity during their pregnancy.

v) As echocardiography involves placement of the imaging probe on the skin surface above the location of the heart, female volunteers may feel uncomfortable with exposing this area of their body.

vi) Pregnant women should avoid lying flat on their back during the later stages of pregnancy due to potential compression of the inferior vena cava, resulting in reduced venous return to the heart. If the woman remains in the supine position for an extended period of time, blood flow may be reduced and result in foetal hypoxia as well as adverse maternal symptoms such as dizziness and syncope.

vii) The volunteer may experience discomfort from having the ultrasound probe pressed against the chest area in the later stages of pregnancy.

C2 How will you deal with the potential risks?

i) The researcher is trained and experienced in phlebotomy procedures and is hepatitis B immunised. The researcher will follow the Physiology and Health

laboratory health and safety policy and protocols when handling blood samples. A risk assessment of capillary blood sampling has been performed by physiology technicians (risk assessments attached as **Appendix J**). To protect both the volunteers and investigator(s), gloves will be worn when handling bloods in accordance with standard laboratory operating procedures.

ii) Equipment will be sterilised according to laboratory guidelines. Resting in a quiet environment whilst wearing the facemask will be enforced to familiarise the participant with breathing through the apparatus. A risk assessment for online gas analysis has been performed by physiology technicians (risk assessments attached as **Appendix J**).

iii) It is most likely that the submaximal exercise test will be stopped by the researcher prior to any discomfort as a result of exertion. Working to 75% of predicted heart rate max will be achievable for individuals unaccustomed to exercise. The submaximal exercise test will be stopped when heart rate reaches 75% of predicted heart rate max as described within the guidelines for sub-maximal testing during pregnancy as stated by ACSM. The participant will be reminded of their right to withdraw at any time.

iv) The sub-maximal exercise test will be conducted on a cycle ergometer as this is a safe and controlled activity providing physical support to any pregnant clients. The duration, intensity and type of exercise are well within the exercise guidelines as recommended by ACOG [1] and the design of the sub-maximal test is based upon guidelines as recommended by ACSM [8]. Physical activity is highly recommended throughout pregnancy in order to maintain health, and is also associated with reduced adverse symptoms of pregnancy. The principal researcher is an ante- and post-natal personal trainer and has relevant qualifications and experience to supervise, prescribe and instruct physical activity during pregnancy. The intensity of exercise used within this study represents activities of daily living, e.g. walking up and down stairs, carrying a shopping bag etc. However, the all tests will be stopped if one of the following events occurs: exhaustion, significant alterations in heart rate, difficulty breathing, general muscular discomfort or discomfort in the abdomen.

v) The purpose of echocardiography and the requirements for collection of echocardiographic images will be visually and verbally explained prior to the Visit two and included in the participant information sheet. A female researcher (VLM) will always complete all measurements throughout the study. Female volunteers will be provided with a gown to enable echocardiographic assessment. All echocardiographic examinations will be conducted in a closed room or behind screens, which will only be accessible to authorised personnel, to ensure the privacy of all volunteers. The risk assessment for echocardiography has been performed by the physiology technicians (risk assessments attached as **Appendix J**).

vi) In the left lateral position, the inferior vena cava is free from compression and provides no risk to the maternal or foetal blood supply [24]. Compression is dependent on the uterus volume [16]. Foetal growth and size, therefore uterus volume, exponentially rises in the third trimester and it is within this time point that compression is a high risk. The gestational age of assessment of volunteers within this study is early and late in the second trimester so the risk of compression is reduced due to a smaller uterus volume [16]. Inferior vena cava compression has been shown to be significantly reduced with a lateral tilt of above 15° [15] and in a recent study of pregnant women undergoing MRI in a

supine position, no individuals developed any adverse symptoms as a result of their position [16]. Volunteers will always be asked about their comfort and this is of upmost importance. Scanning will be stopped, the bed un-tilted, and volunteer asked to lie on their left hand side if they should develop any adverse symptoms or if they should wish to stop testing at any time. The volunteers blood pressure will be monitored continuously throughout the echocardiography assessments as well as the heart rate monitored through an ECG on the Vivid E9 ultrasound for any adverse reactions.

vii) The sonographer will ask the volunteer if they are experiencing discomfort as a result of the probe being pressed against the chest area. If volunteers experience any discomfort and would like to end the scan, the study will be stopped immediately.

When submitting your application you **MUST** attach a copy of the following:

- All information sheets
- Consent/assent form(s)

Refer to the document ***Guidelines for obtaining ethics approval*** for further details on what format these documents should take.

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Appendix II – Supplementary data for Chapter 3

On all figures: Filled grey squares represent study outputs. Filled black diamonds represent the weighted mean as a result of the analyses. Unfilled diamonds represent outputs from biased analyses that were corrected for using Duval and Tweedie's trim and fill method. Dotted line represents non-pregnant weighted mean on all figures. Black solid line represents weighted mean for that individual gestational age.

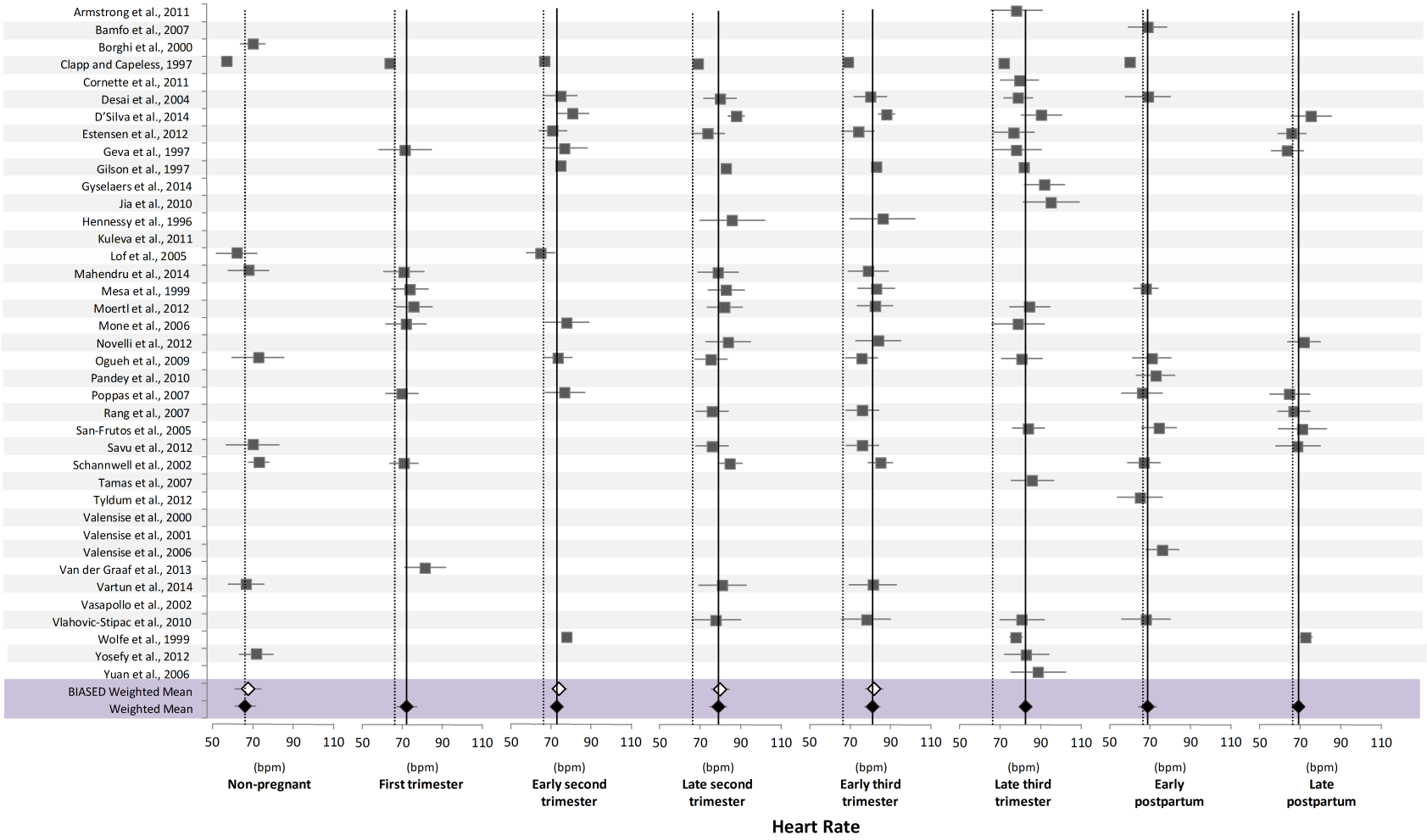


Figure A1. Individual forest plots for each meta-analyses of heart rate at different gestational ages.

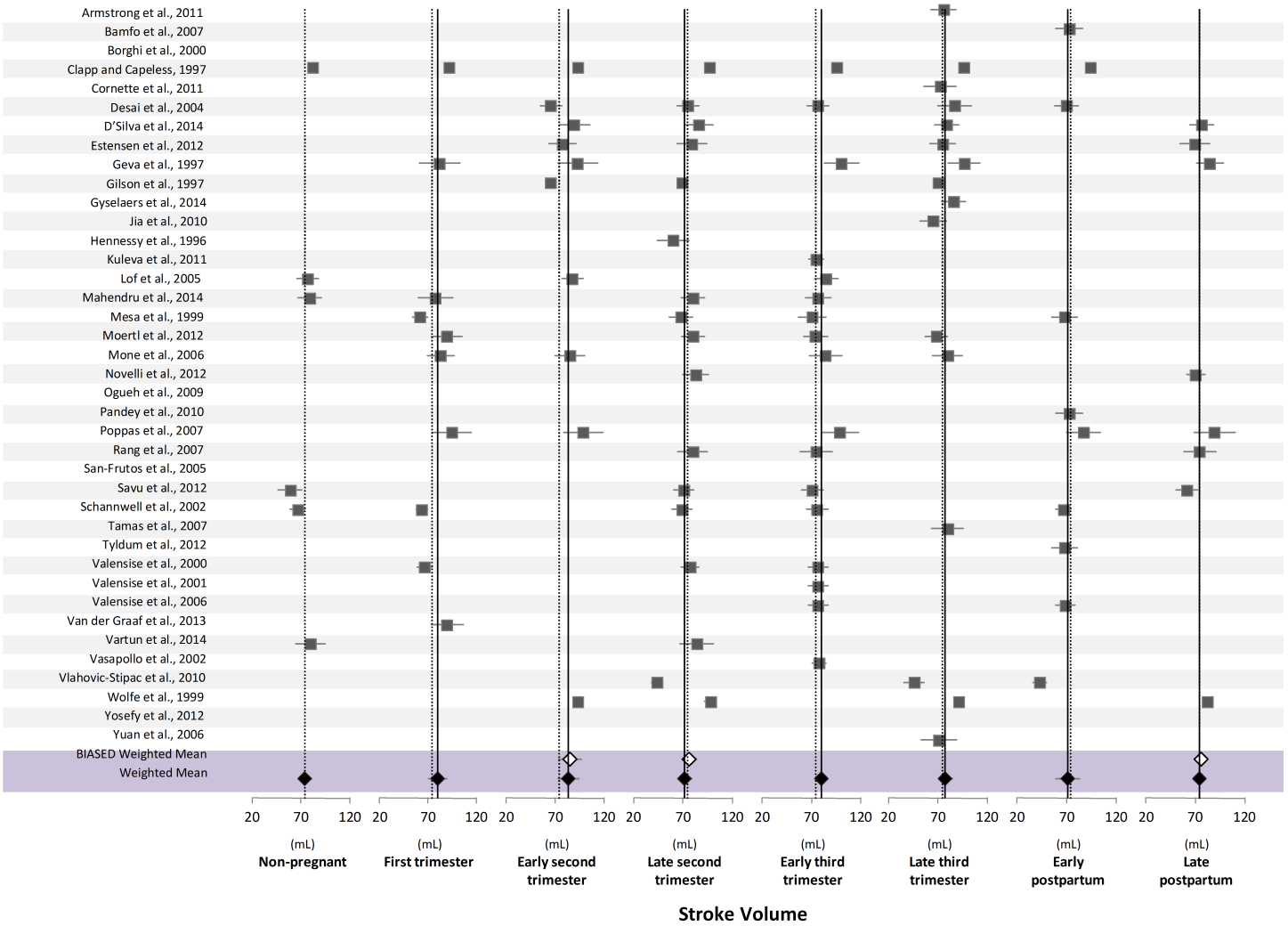


Figure A2. Individual forest plots for each meta-analyses of stroke volume at different gestational ages.

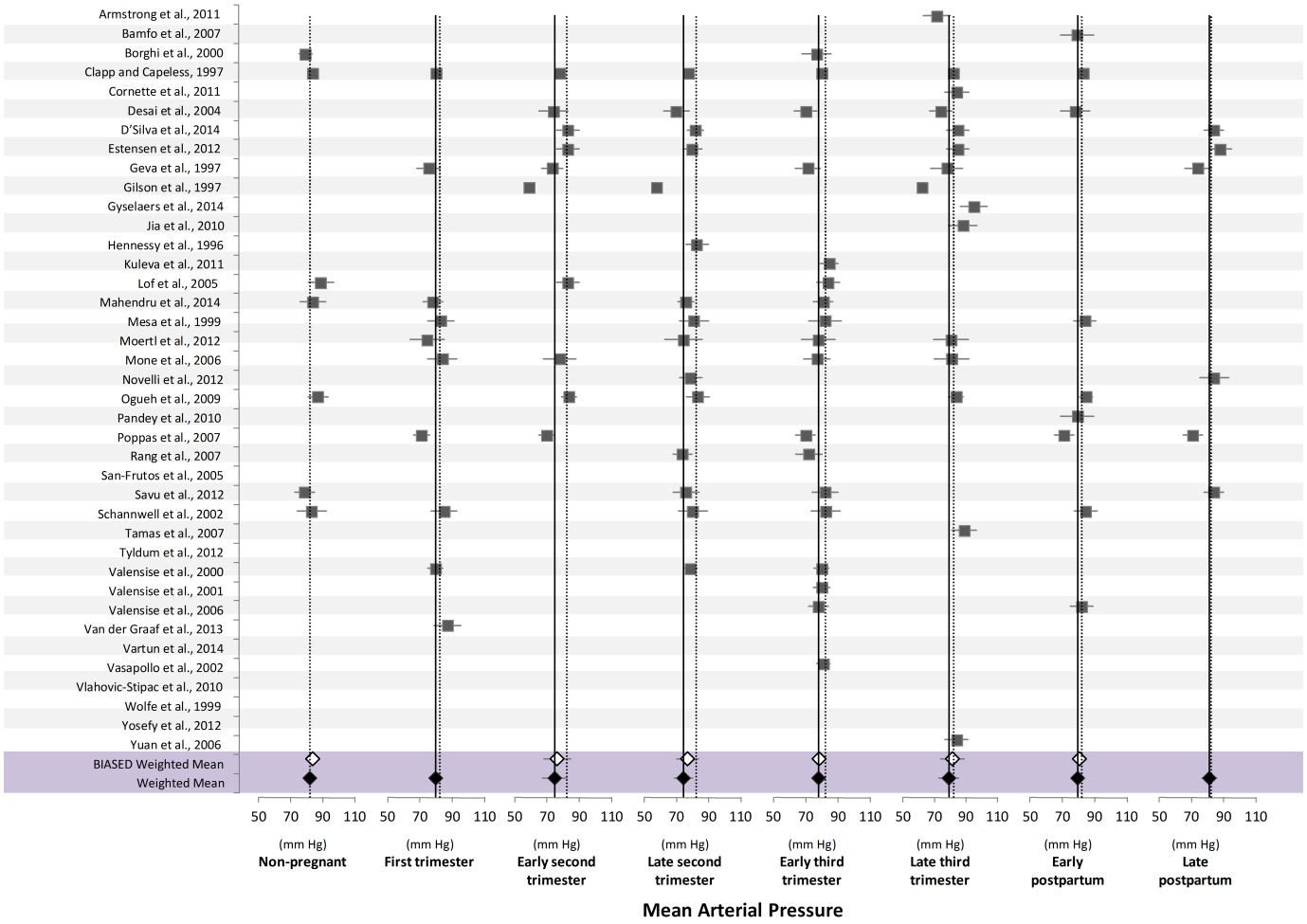


Figure A3. Individual forest plots for each meta-analyses of mean arterial pressure at different gestational ages.

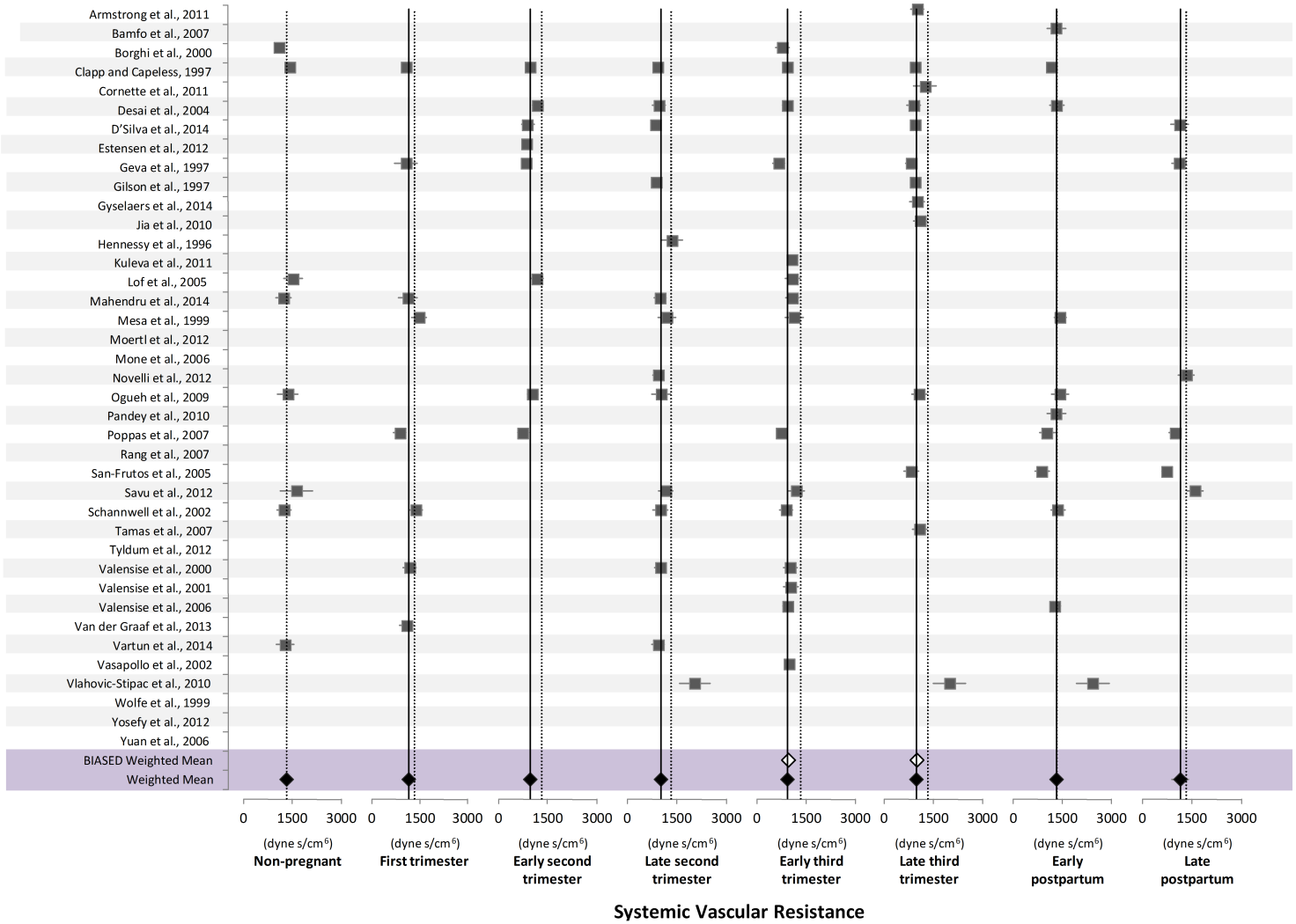


Figure A4. Individual forest plots for each meta-analyses of systemic vascular resistance at different gestational ages.

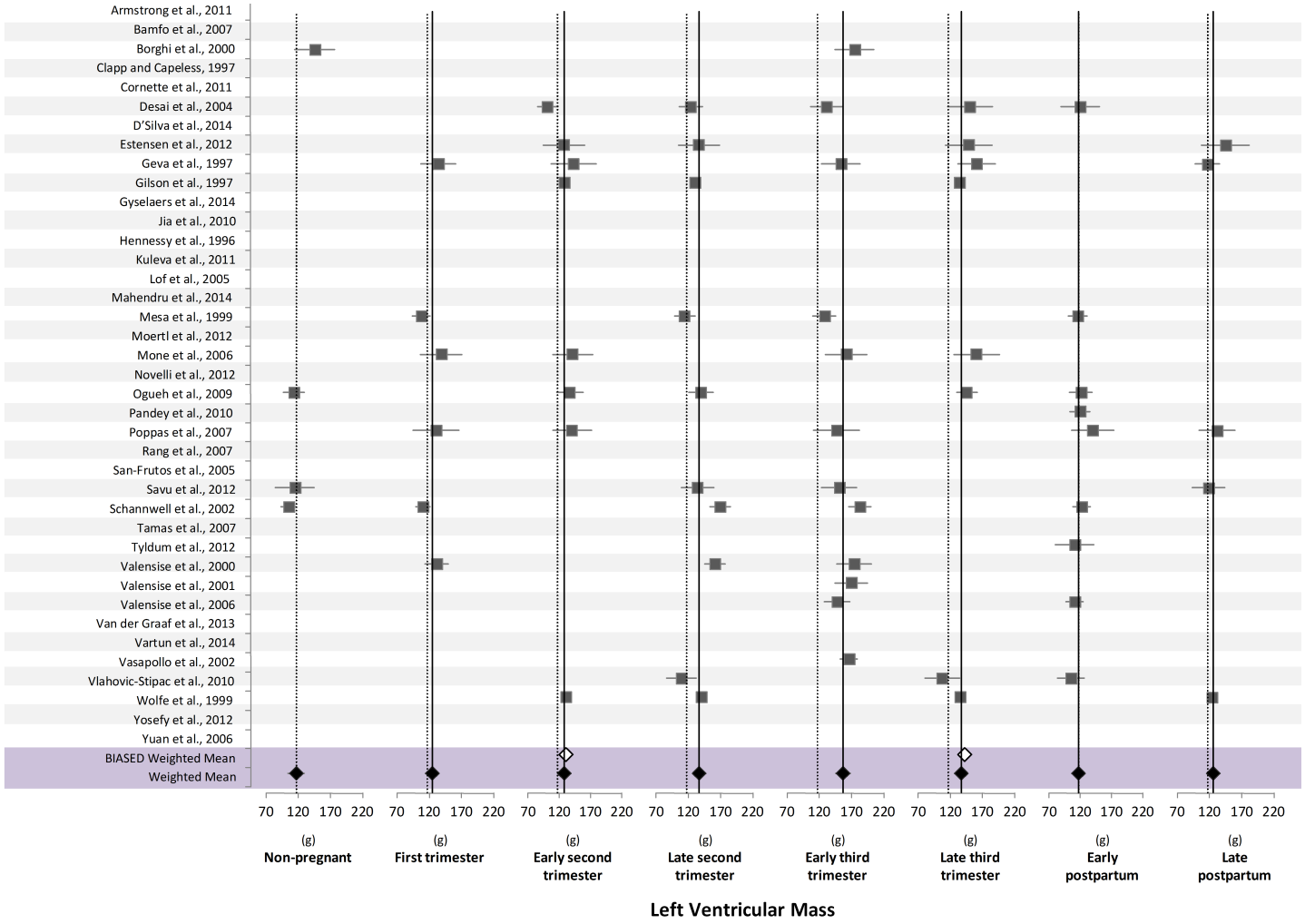


Figure A5. Individual forest plots for each meta-analyses of left ventricular mass at different gestational ages.

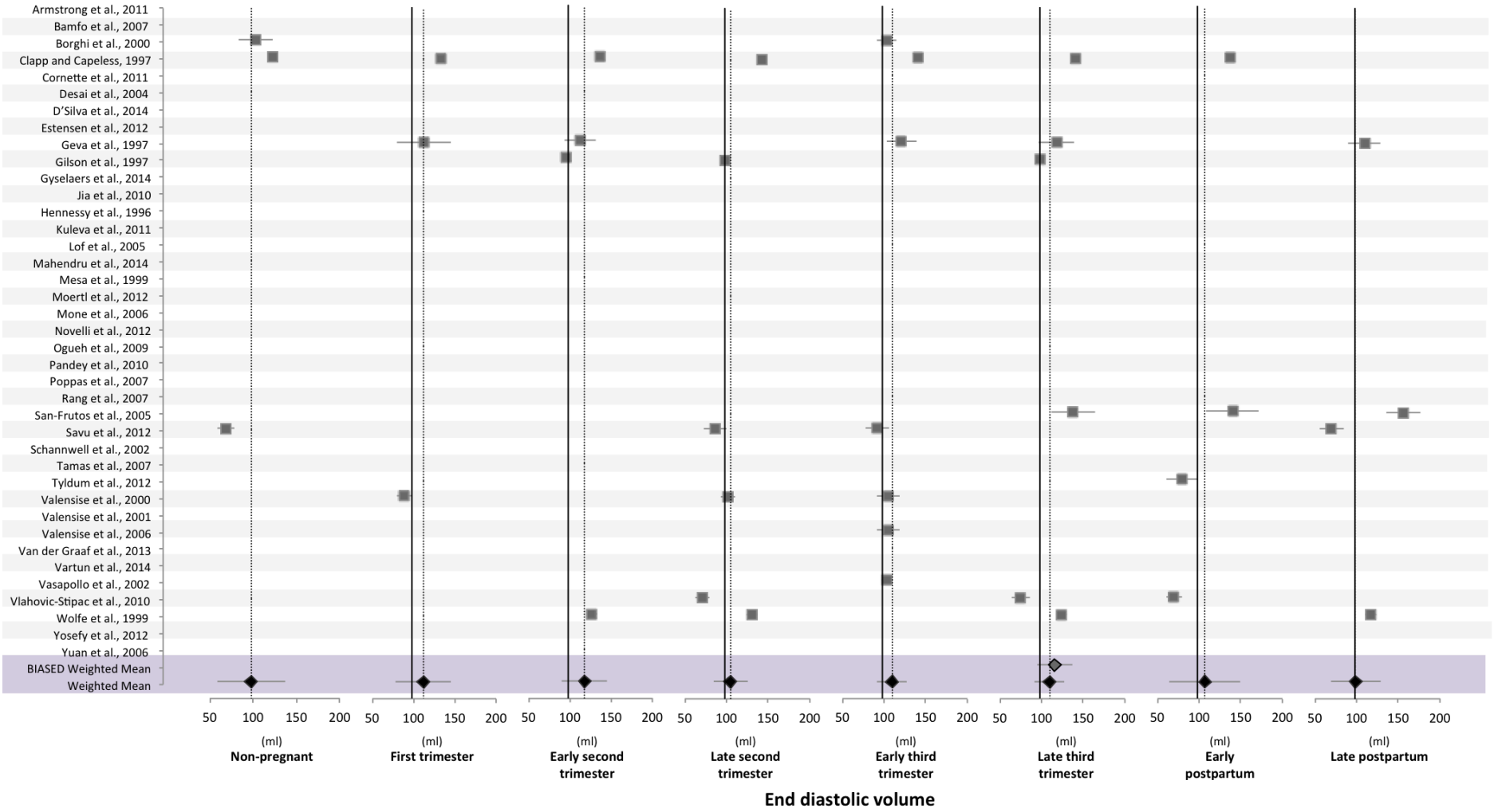


Figure A6. Individual forest plots for each meta-analyses of end-diastolic volume at different gestational ages.

Appendix III – Participant information for experimental study

Participant information sheet for female participants

A Mother's Heart:

A study of cardiac function during healthy pregnancy at rest and during physical activity

PARTICIPANT INFORMATION SHEET

Principal Investigator: Miss Victoria Meah (vimeah@cardiffmet.ac.uk)

We would like to invite you to take part in a research study run by Victoria Meah from the Health and Physiology laboratory at Cardiff Metropolitan University. The purpose of this study is to investigate the heart and the blood vessels in women before, during and after pregnancy at rest and during short physical activity. By participating in this research, you will contribute to our understanding of how the heart responds to pregnancy and physical activity.

Before you decide to take part, you must understand why the research is being carried out, and what is involved if you decide to take part. **Part One** provides an overview of this particular study, whereas **Part Two** provides general information about participating in research studies. Please take the time to read and carefully consider the following information. If you have any questions about the research or you would like further information, please do not hesitate to ask.

Note: Some of the information presented in here will not apply to non-pregnant women. This will be highlighted where applicable.

PART ONE

General overview of this project

What is the purpose of this study?

During pregnancy the mother's body changes extensively so that she can give birth to a healthy baby. In particular the heart and arteries, known as the cardiovascular system, must adapt in order to maintain a supply of blood to the growing baby. The heart will get bigger and becomes more efficient at pumping blood.

Physical activity can include day to day activities such as walking, carrying shopping, cleaning, and gardening as well as exercise activities such as running, swimming, cycling or playing sports. All individuals are encouraged to keep physically active for health benefits and that is no exception during pregnancy. At the moment, we have a good understanding of how a mother's cardiovascular system functions when she is at rest, but during physical activity our body must adapt so that we can perform physical tasks. In this research study, we want to find out how a pregnant woman's cardiovascular system copes during physical activity.

This study aims to examine the mothers' cardiovascular system at rest and during physical activity in **healthy** pregnancy and after delivery.

Why have I been invited to participate?

You have been invited to take part in the study because we are looking for healthy women between the ages of 20 and 39:

- Non-pregnant women who have not been pregnant before **OR**
- Between 14 – 18 weeks of your first pregnancy **OR**
- Between 22 – 26 weeks of your first pregnancy **OR**
- 12 – 16 weeks after you have given birth to your first child.

Do I have to take part?

The study is **voluntary** and it is up to you to decide whether or not you would like to take part. You have been given this information sheet to allow you to understand what is involved in the research, as well as how and why the research will be carried out. You should read this document carefully and discuss the research with family or friends and your midwife if you wish. A member of the research team will contact you by telephone within two days of you being given this information sheet. During the telephone call, the details of the study will be discussed and you can ask any questions you may have. If you decide to take part you will be asked to sign a consent form to show you have agreed to take part. **You are free to withdraw at any time without giving a reason**. This will not affect your relationship with Cardiff Metropolitan University.

What will happen to me if I decide to take part?

The study requires you to attend the laboratory on 2 different occasions. Each Visit will last around 1.5 to 2 hours. If you decide to take part, suitable dates and times will be arranged at your convenience.

Where will I need to go?

All testing Visits will take place in the Health and Physiology laboratory at Cardiff Metropolitan University, Cyncoed Campus. All of the research will be conducted by a female researcher. Other assistants may be present, you would be introduced to them prior to starting the tests.

Upon arrival at the laboratory you will be able to ask any questions or queries that you may have.

What am I required to do?

A brief description of each of the procedures is provided below but these will be fully explained to you in detail during your first visit.

Visit One

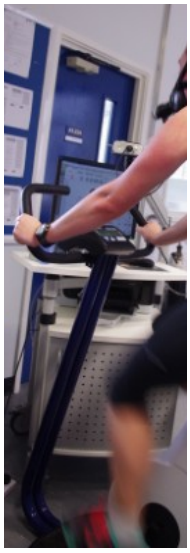
Time commitment: 1.5 to 2 hours
What is involved: Consent, participant inclusion assessment, body size measurements, pin-prick blood sample, hand grip test, sub-maximal exercise test, physical activity questionnaire, step test, and familiarisation exercise for Visit Two.

Description of procedures:

Consent: The researcher will fully explain the research project to you, and invite any questions. If you are willing to participate, you will be asked to complete a consent form

which shows you are happy to volunteer and fully understand what is required. *(approx. 10 min)*

Participant inclusion assessment and body size measurement: Prior to undertaking testing, you will be asked to complete a health questionnaire. Measurements of height, weight and body fat percentage will be taken. *(approx. 20 min)*



Resting heart rate and oxygen consumption: You will be asked to sit and rest for 5 minutes during which time your heart rate will be recorded. You will also be asked to wear a mask that collects the air you breathe out. *(approx. 5 min)*

Blood sample*:* Pregnant women are at risk of developing anaemia and you should not exercise without consent from your doctor. Therefore we will ask to take a very small blood sample from your ear lobe by pin-prick which will be analysed immediately. If we should measure a low value, we will stop the research and recommend you visit your GP for a check-up. *(approx. 5 min)*

Hand grip test*:* The hand grip test will require you to grip a dynamometer, a piece of equipment that measures force, as hard as you can for a maximum of 3 seconds. *(approx. 5 min)*

Sub-maximal exercise test*:* You will be asked to perform an exercise test on a stationary bike (see Figure 1). This will start at a low intensity and will get harder. The test will be stopped by the researcher when you hit a certain threshold which will reflect a moderate effort. You will be asked to wear a mask that collects the air you breathe out and a heart rate monitor. *(approx. 25 min)*

Physical activity questionnaire*:* Once you have cooled down, you will have 10 minutes of seated rest during which you will be asked to complete a short questionnaire about your physical activity levels. *(approx. 10 min)*

Step test*:* After you have completed the questionnaire, you will be asked to complete a low intensity exercise test that involves standing on and off an aerobics step to a set beat. *(approx. 5 min)*

Figure 1. Completing the sub-maximal exercise test

Preparation for Visit Two*:* The exercise in Visit Two requires you to exercise on a specially designed bed that tilts to the left between 30 and 45 degrees (see Figure 2a). This improves the quality of ultrasound images of your heart. Lying in this position and exercising in this position is not something you are likely to be familiar with. Therefore, a familiarisation of the equipment is required. You may be concerned that you should not lie flat on your back during pregnancy. As the bed tilts to the left, you will be in the recommended left lateral position which ensures you will have no adverse reactions. *(approx. 15 min)*

Physical activity measurement*:* You may be asked to take home a small monitor which attaches to a wrist or waist strap that will track your physical activity over a five day period. You should carry out your daily activities as normal and return the monitor at Visit two.

Non-pregnant volunteers will NOT be asked to complete the physical activity questionnaire or the physical activity measurement.

Visit Two

Time commitment: 1.5 to 2 hours

What is involved: Blood pressure, arterial stiffness assessment, heart scan at rest and during hand grip and cycle exercise.

Description of procedures:

Blood pressure and arterial stiffness assessment*:* Your blood pressure will be measured on your upper arm. An instrument, called a tonometer, will then be gently placed on specific points on your neck, wrist and leg to measure how 'stiff' your blood vessels are. *(approx. 15 min)*

Heart scan at rest: In order for the heart images to be taken, you will be asked to lie on the specially designed bed that you used in Visit one. Resting heart images will then be taken using an ultrasound (Figure 2a); this will be used to measure the structure and function of your heart. It is the same technology as is used when you have a scan of your baby during your pregnancy. A probe will be placed on two positions of your chest; just left of the breastbone and on the left hand side of the rib cage (Figure 2b). At the same time as the heart scan, your arterial stiffness will be measured from your right wrist and your blood pressure will be measured by a cuff on your right arm. *(approx. 30 min)*

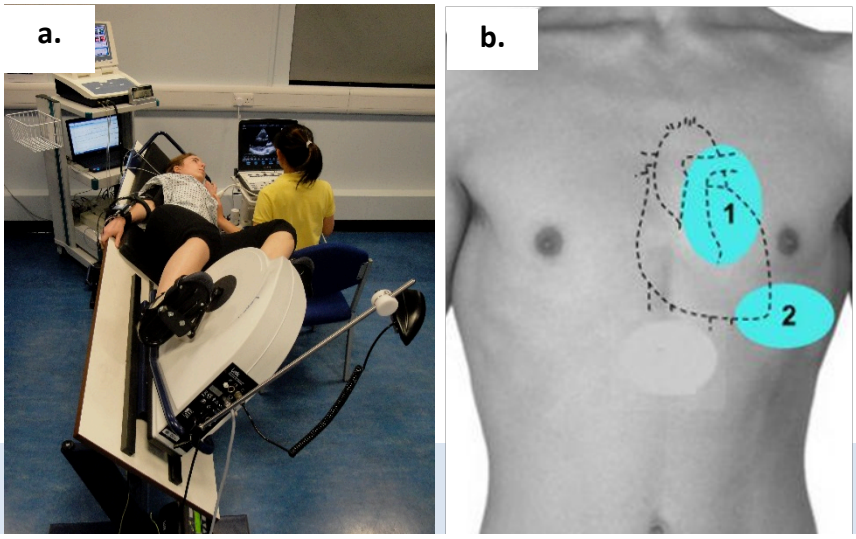


Figure 2. a. Collection of a resting heart scan. **b.** Heart scan probe positions.

Heart scan during continued handgrip: After a short break, you will be asked to hold the hand grip dynamometer (used in Visit one) at a low intensity for a maximum of 7 minutes. This should be the equivalent of holding a shopping bag in one hand. During this time, a heart scan will be taken, as well as measuring the arterial stiffness in your right wrist, and blood pressure from your right arm. *(approx. 15 min)*

Heart scan during cycle exercise: After a short break, you will be asked to exercise on the specially designed tilted cycle bed for two bouts of low and moderate intensity exercise lasting a maximum of 7 minutes each. During each stage, a heart scan will be taken, as well as measuring the arterial stiffness in your right wrist, and blood pressure from your right arm. *(approx. 20 min)*

What are the possible benefits of taking part?

Although this research will not directly benefit your own pregnancy, you will learn about the function of your heart and blood vessels when you rest and when you perform physical activity. You will also be provided with data about your fitness and physical activity levels. The data we will collect from you will contribute to our understanding of the changes to a mother's heart during pregnancy and will improve knowledge in the future for mothers, midwives, and doctors.

What are the possible disadvantages or risks of taking part in the study?

The procedures and exercise performed within this study are not associated with risks to a mother or her baby. However, a pregnant woman may have some concerns in relation to the procedures. More detailed information about the low levels of risk is provided below.

Concerns about exercise intensity during pregnancy. In the study, you will be asked to complete bouts of exercise. While this may be perceived as risky, you will be asked to complete a health questionnaire prior to any exercise, which will assess your safety to take part. In healthy mothers, there is no evidence that moderate exercise, like the exercise in this study, is associated with risk to your baby and completing moderate exercise is in fact encouraged throughout pregnancy. The recommendations for exercise during pregnancy currently state that women with healthy pregnancies may engage in exercise at a moderate intensity for up to 30 minutes per day on most, if not all, days of the week. The exercise within this study does not exceed moderate intensity which will result in a normal increase in heart rate and breathing rate and you will feel a moderate level of exertion. It is very unlikely that you will experience an adverse reaction to exercise but you will be monitored closely throughout all exercise bouts. If you want to stop exercising or you have any signs of an adverse reaction, the study will be stopped immediately. The exercise components within this study are routine procedures taking place in an accredited exercise laboratory. There will be a suitably trained exercise physiologist present for the duration of all exercise. For more information, please visit: http://www.nhs.uk/conditions/pregnancy-and-baby/pages/pregnancy-exercise.aspx#close.

Concerns about physical activity and miscarriage. Unfortunately, spontaneous miscarriage can occur in approximately 12% of pregnancies. As stated by the NHS website, there are a number of widely held assumptions about possible causes of miscarriage including participating in exercise; however there is no evidence to support such claims when exercise is completed in accordance with the recommended guidelines as applied in this study. The principal investigator is a registered and qualified ante- and post-natal personal trainer and has suitable experience in supervising and delivering exercise during pregnancy. All exercise required in this study is within the recommended guidelines and does not present an increased risk to you or your baby. For more information, please visit http://www.nhs.uk/Conditions/Miscarriage/Pages/Causes.aspx.

Concerns about procedures in this study. The study uses methods (blood pressure, arterial stiffness measurement, and a heart scan) that are regularly employed in clinical practice and provide no risks to mother or baby. The principal investigator is a trained phlebotomist and is experienced in taking blood samples from the ear. If any concerns are raised during the testing procedures that are within the expertise of the researchers,

with your permission, we would notify your midwife and encourage you to seek medical advice as soon as possible.

What if there is a problem?

Any complaint in the way you have been dealt with during the project or any possible harm you might suffer will be addressed. The detailed information on this report is given in Part Two.

What happens when the research project stops?

After you have participated in the study, you are free to contact the research team with any questions or queries you may have regarding the project. If you are interested in the data collected during your participation, we would be happy to send you a summary report. Results of this project will be published in scientific journals and presented at scientific meetings. Your identity will not be disclosed in any report, publication or presentation.

Will my participation in the project be kept confidential?

Yes. We will follow ethical and legal practice. All information about you will be handled in confidence. The details are included in Part Two.

Please read the additional information in Part Two about participating in research projects before making any decision.

PART TWO

General information about participating in research projects

What will happen if I change my mind about volunteering for the project?

You can withdraw from the project at any time, without giving a reason. If you do so, or decide not to take part, it will not affect your relationship with Cardiff Metropolitan University. If you do withdraw from the project, we will destroy all your identifiable samples.

What if there is a problem?

If you have a concern about any aspect of the study, you should ask to speak to a member of the research team (contact details on last page) who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by following the standard Cardiff Metropolitan University complaints procedure or you can contact the Community Health Council's free advocacy service (contact details on last page).

Harm

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for legal action for compensation against Cardiff Metropolitan University, but you may have to pay for your legal costs. The researchers in this project have suitable indemnity insurance if you are harmed due to taking part in this research. However, there are no special compensation arrangements for non-negligent harm.

Will my participation in the project be kept confidential?

Yes. We will follow ethical and legal practice and abide to the principles of scientific conduct. All information about you will be handled in confidence. Project data will be coded and stored at Cardiff Metropolitan University in locked cabinets and this will be kept separate from personal information (names and addresses).

No one, except the named researchers, will have access to your project data. No personally identifying details will appear in any published or presented work. It is possible that officials from regulatory authorities may need access to project data to check the quality of research. All members of the research team are aware of data protection issues and are bound by the Data Protection Act 1998. Once the project is complete, we will destroy any personally identifiable data after 7 years in line with the Cardiff Metropolitan University policy.

Involvement of the General Practitioner

If you decide to volunteer, you will be asked to complete a physical activity readiness questionnaire designed specifically for pregnancy prior to you taking part in exercise. This is standard practice in the fitness industry when pregnant or postnatal clients decide to exercise. If you highlight any medical conditions on the form, with your permission, we will request for further information from your midwife or GP on your suitability to exercise. You will be able to see all information exchanged. If any concerns are raised during your participation in the study, with your permission, we would notify your midwife

and encourage you to seek medical advice as soon as possible. This is in the best interests of you and your baby.

What will happen to any blood samples I give?

The blood samples collected from your ear will be analysed immediately and then disposed of.

Will any genetic tests be done?

No genetic testing will be carried out on any of your samples.

What will happen to the results of the research study?

Results of the study will be published in scientific journals and presented at scientific conferences. You will not be identified in any report, publication or presentation. If you are interested in the data collected during your participation we would be happy to provide you with a summary report.

Who is organising the research?

The project is being organised by the Cardiff School of Sport within Cardiff Metropolitan University, Cardiff, CF23 6XD, UK.

Who has reviewed the study?

All research is evaluated by an independent group called the Research Ethics Committee. Their aim is to protect participants' and researchers' safety, rights, well-being and dignity. This project has been reviewed and given favourable opinion, and thus been given the ethics code: 15/9/01R.

Further information and contact details

If you would like more information, then please contact any of the people listed below.

Miss Victoria Meah (Chief investigator for the project) Tel: 02920 416 503
Dr Eric Stöhr (Academic supervisor for the project) Tel: 02920 416 531
Community Health Councils Free Advocacy Service **Tel:** 01646 697 610

Thank you for taking the time to read this information sheet and for your consideration in taking part in this project.

MALE PARTICIPANT INFORMATION SHEET

Control Group for Study: A Mother's Heart: A study of cardiac function during healthy pregnancy at rest and during physical activity

Principal Investigator: Miss Victoria Meah (vimeah@cardiffmet.ac.uk)

We would like to invite you to take part in a research study run by Victoria Meah from the Health and Physiology laboratory at Cardiff Metropolitan University. The purpose of this study is to investigate the heart and the blood vessels at rest and during short physical activity. By participating in this research, you will contribute to our understanding of how the heart responds differently in **men** and women (before, during and after pregnancy) to physical activity.

Before you decide to take part, you must understand why the research is being carried out, and what is involved if you decide to take part. **Part One** provides an overview of this particular study, whereas **Part Two** provides general information about participating in research studies. Please take the time to read and carefully consider the following information. If you have any questions about the research or you would like further information, please do not hesitate to ask.

PART ONE

General overview of this project

What is the purpose of this study?

During pregnancy a woman's body changes extensively so that she can give birth to a healthy baby. In particular the heart and arteries, known as the cardiovascular system, must adapt in order to maintain a supply of blood to the growing baby. The heart will get bigger and becomes more efficient at pumping blood.

Physical activity can include day to day activities such as walking, carrying shopping, cleaning, and gardening as well as exercise activities such as running, swimming, cycling or playing sports. All individuals are encouraged to keep physically active for health benefits and that is no exception during pregnancy. At the moment, we have a good understanding of how a mother's cardiovascular system functions when she is at rest, but during physical activity our body must adapt so that we can perform physical tasks. In this research study, we want to find out how a pregnant woman's cardiovascular system copes during physical activity and how this compares to non-pregnant women, women after pregnancy and also **men**.

This study aims to examine cardiovascular system at rest and during physical activity in **men** and in women before, during and after healthy pregnancy.

Why have I been invited to participate?

You have been invited to take part in the study because we are looking for healthy men between the ages of 20 and 39 to act as a control group.

Do I have to take part?

The study is **voluntary** and it is up to you to decide whether or not you would like to take part. You have been given this information sheet to allow you to understand what is involved in the research, as well as how and why the research will be carried out. You should read this document carefully and discuss the research with family or friends if you wish. A member of the research team will contact you by telephone within two days of you being given this information sheet. During the telephone call, the details of the study will be discussed and you can ask any questions you may have. If you decide to take part you will be asked to sign a consent form to show you have agreed to take part. **You are free to withdraw at any time without giving a reason**. This will not affect your relationship with Cardiff Metropolitan University.

What will happen to me if I decide to take part?

The study requires you to attend the laboratory on 2 different occasions. Each Visit will last around 1.5 to 2 hours. If you decide to take part, suitable dates and times will be arranged at your convenience.

Where will I need to go?

All testing Visits will take place in the Health and Physiology laboratory at Cardiff Metropolitan University, Cyncoed Campus. Both male and female researchers will conduct the research. Other assistants may be present; you would be introduced to them prior to starting the tests.

Upon arrival at the laboratory you will be able to ask any questions or queries that you may have.

What am I required to do?

A brief description of each of the procedures is provided below but these will be fully explained to you in detail during your first visit.

Visit One

Time commitment: 1.5 to 2 hours
What is involved: Consent, participant inclusion assessment, body size measurements, pin-prick blood sample, hand grip test, sub-maximal exercise test, physical activity questionnaire, step test, and familiarisation exercise for Visit Two.

Description of procedures:

Consent: The researcher will fully explain the research project to you, and invite any questions. If you are willing to participate, you will be asked to complete a consent form which shows you are happy to volunteer and fully understand what is required. *(approx. 10 min)*

Participant inclusion assessment and body size measurement: Prior to undertaking testing, you will be asked to complete a health questionnaire. Measurements of height, weight and body fat percentage will be taken. *(approx. 20 min)*

Resting heart rate and oxygen consumption: You will be asked to sit and rest for 5 minutes during which time your heart rate will be recorded. You will also be asked to wear a mask that collects the air you breathe out. *(approx. 5 min)*

***Blood sample**:* We will ask to take a very small blood sample from your ear lobe by pin-prick which will be analysed immediately.

***Hand grip test**:* The hand grip test will require you to grip a dynamometer, a piece of equipment that measures force, as hard as you can for a maximum of 3 seconds. *(approx. 5 min)*

***Sub-maximal exercise test**:* You will be asked to perform an exercise test on a stationary bike (see Figure 1). This will start at a low intensity and will get harder. The test will be stopped by the researcher when you hit a certain threshold which will reflect a moderate effort. You will be asked to wear a mask that collects the air you breathe out and a heart rate monitor. *(approx. 25 min)*

Figure 1. Completing the sub-maximal exercise test

***Step test**:* After you have completed the questionnaire, you will be asked to complete a low intensity exercise test that involves standing on and off an aerobics step to a set beat. *(approx. 5 min)*

***Preparation for Visit Two**:* The exercise in Visit Two requires you to exercise on a specially designed bed that tilts to the left between 30 and 45 degrees (see Figure 2a). This improves the quality of ultrasound images of your heart. Lying in this position and exercising in this position is not something you are likely to be familiar with. Therefore, a familiarisation of the equipment is required. *(approx. 15 min)*

Visit Two

Time commitment: 1.5 to 2 hours
What is involved: Blood pressure, arterial stiffness assessment, heart scan at rest and during hand grip and cycle exercise.

Description of procedures:
***Blood pressure and arterial stiffness assessment**:* Your blood pressure will be measured on your upper arm. An instrument, called a tonometer, will then be gently placed on specific points on your neck, wrist and leg to measure how 'stiff' your blood vessels are. *(approx. 15 min)*

Heart scan at rest: In order for the heart images to be taken, you will be asked to lie on the specially designed bed that you used in Visit one. Resting heart images will then be taken using an ultrasound (Figure 2a); this will be used to measure the structure and function of your heart. It is the same technology as is used when you scan of a baby during your pregnancy. A probe will be placed on two positions of your chest; just left of the breastbone and on the left hand side of the rib cage (Figure 2b). At the same time as the heart scan, your arterial stiffness will be measured from your right wrist and your blood pressure will be measured by a cuff on your right arm. *(approx. 30 min)*

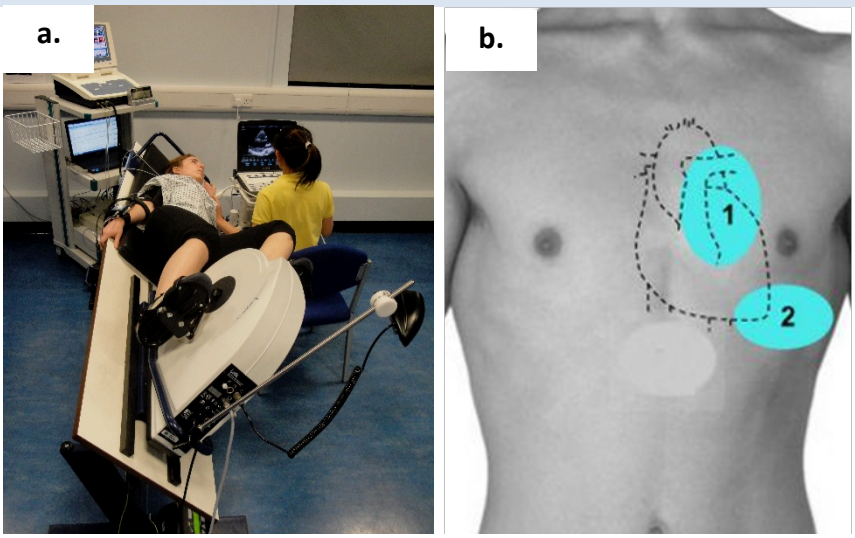


Figure 2. a. Collection of a resting heart scan. **b.** Heart scan probe positions.

Heart scan during continued handgrip: After a short break, you will be asked to hold the hand grip dynamometer (used in Visit one) at a low intensity for a maximum of 7 minutes. This should be the equivalent of holding a shopping bag in one hand. During this time, a heart scan will be taken, as well as measuring the arterial stiffness in your right wrist, and blood pressure from your right arm. *(approx. 15 min)*

Heart scan during cycle exercise: After a short break, you will be asked to exercise on the specially designed tilted cycle bed for two bouts of low and moderate intensity exercise lasting a maximum of 7 minutes each. During each stage, a heart scan will be taken, as well as measuring the arterial stiffness in your right wrist, and blood pressure from your right arm. *(approx. 20 min)*

What are the possible benefits of taking part?

Although this research will not directly benefit you, you will learn about the function of your heart and blood vessels when you rest and when you perform physical activity. If requested, you will be provided with data about your fitness and physical activity levels. The data we will collect from you will contribute to our understanding of the physiological changes in **men** and women's hearts during physical activity.

What are the possible disadvantages or risks of taking part in the study?

The procedures and exercise performed within this study are not associated with high risk to an individual. However, you may have some concerns in relation to the procedures. More detailed information about the low levels of risk is provided below.

Concerns about procedures in this study. The study uses methods (blood pressure, arterial stiffness measurement, and a heart scan) that are regularly employed in clinical practice and provide no risks an individual. The researchers are experienced and competent in taking blood samples from the ear. If any concerns are raised during the testing procedures that are within the expertise of the researchers, with your permission, we would notify your GP and encourage you to seek medical advice as soon as possible.

What if there is a problem?

Any complaint in the way you have been dealt with during the project or any possible harm you might suffer will be addressed. The detailed information on this report is given in Part Two.

What happens when the research project stops?

After you have participated in the study, you are free to contact the research team with any questions or queries you may have regarding the project. If you are interested in the data collected during your participation, we would be happy to send you a summary report. Results of this project will be published in scientific journals and presented at scientific meetings. Your identity will not be disclosed in any report, publication or presentation.

Will my participation in the project be kept confidential?

Yes. We will follow ethical and legal practice. All information about you will be handled in confidence. The details are included in Part Two.

Please read the additional information in Part Two about participating in research projects before making any decision.

PART TWO

General information about participating in research projects

What will happen if I change my mind about volunteering for the project?

You can withdraw from the project at any time, without giving a reason. If you do so, or decide not to take part, it will not affect your relationship with Cardiff Metropolitan University. If you do withdraw from the project, we will destroy all your identifiable samples.

What if there is a problem?

If you have a concern about any aspect of the study, you should ask to speak to a member of the research team (contact details on last page) who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by following the standard Cardiff Metropolitan University complaints procedure or you can contact the Community Health Council's free advocacy service (contact details on last page).

Harm

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for legal action for compensation against Cardiff Metropolitan University, but you may have to pay for your legal costs. The researchers in this project have suitable indemnity insurance if you are harmed due to taking part in this research. However, there are no special compensation arrangements for non-negligent harm.

Will my participation in the project be kept confidential?

Yes. We will follow ethical and legal practice and abide to the principles of scientific conduct. All information about you will be handled in confidence. Project data will be coded and stored at Cardiff Metropolitan University in locked cabinets and this will be kept separate from personal information (names and addresses).

No one, except the named researchers, will have access to your project data. No personally identifying details will appear in any published or presented work. It is possible that officials from regulatory authorities may need access to project data to check the quality of research. All members of the research team are aware of data protection issues and are bound by the Data Protection Act 1998. Once the project is complete, we will destroy any personally identifiable data after 7 years in line with the Cardiff Metropolitan University policy.

Involvement of the General Practitioner

If any concerns are raised during your participation in the study, with your permission, we would notify your GP and encourage you to seek medical advice as soon as possible.

What will happen to any blood samples I give?

The blood samples collected from your ear will be analysed immediately and then disposed of.

Will any genetic tests be done?

No genetic testing will be carried out on any of your samples.

What will happen to the results of the research study?

Results of the study will be published in scientific journals and presented at scientific conferences. You will not be identified in any report, publication or presentation. If you are interested in the data collected during your participation we would be happy to provide you with a summary report.

Who is organising the research?

The project is being organised by the Cardiff School of Sport within Cardiff Metropolitan University, Cardiff, CF23 6XD, UK.

Who has reviewed the study?

All research is evaluated by an independent group called the Research Ethics Committee. Their aim is to protect participants' and researchers' safety, rights, well-being and dignity. This project has been reviewed and given favourable opinion, and thus been given the ethics code: 16/1/01R.

Further information and contact details

If you would like more information, then please contact any of the people listed below.

Miss Victoria Meah (Principal investigator for the project) Tel: 02920 416 503
Dr Eric Stöhr (Academic supervisor for the project) Tel: 02920 416 531
Community Health Councils Free Advocacy Service **Tel:** 01646 697 610

Thank you for taking the time to read this information sheet and for your consideration in taking part in this project.

Appendix IV – Consent Form for experimental study

Study Number: 16/3/01R

Cardiff Metropolitan University | Prifysgol Metropolitan Caerdydd

CONSENT FORM

Title of Project:

The assessment of cardiac mechanics and arterial haemodynamics during pregnancy and in the postpartum period at rest and during physical activity.

Name of Researcher: **Miss Victoria L. Meah**

Please **initial** boxes

1. I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.

3. I understand that data collected will not be used for commercial purposes.

4. I understand that any images or data collected in this study are not for the basis of clinical diagnosis, and that the researchers are NOT able to provide such clinical information. If the researcher should however identify an area of concern which may constitute a referral to my GP or midwife, I **would/would not** (delete as appropriate) like to be informed.

5. I agree that the principal investigator and co-investigators of this study may use my data anonymously in other research projects with the purpose of answering new research questions.

6. I agree to take part in the above study.

Name of participant	Date	Signature
Name of person taking consent	Date	Signature

Name of Study/reason for blood taking:	The assessment of cardiac mechanics and arterial haemodynamics during pregnancy and in the postpartum period at rest and during physical activity. **Ethics code: 16/1/01R**

Blood Donation Declaration

1.	Do you agree to donate blood?	YES ☐	NO ☐
2.	Have you ever been advised not to give blood?	YES ☐	NO ☐
3.	Are you taking any medication which may affect the ability of your blood to clot?	YES ☐	NO ☐

Donor	Print Name _____	Signature _____	Date _____
Phlebotomist	Print Name _____	Signature _____	Date _____

Appendix V – Pre-participation screening questionnaire

Screening questionnaire for females

Patient Identification Number:

Date of Completion:

PRE-PARTICIPATION QUESTIONNAIRE

Title of Project:

The assessment of cardiac mechanics and arterial haemodynamics during pregnancy and in the postpartum period at rest and during physical activity.

Name of Researcher: Miss Victoria L. Meah

In order to describe the participants in this study, we ask that you complete this short questionnaire. The questions ask you about your general health and some basic information about you. If you are unsure or cannot remember, or do not want to answer a question; you are under no obligation to do so.

Health

Do you smoke or have you smoked on a regular basis within the last two years?

No ☐ Yes ☐

Have you ever been diagnosed with high blood pressure or cardiovascular problems?

No ☐ Yes ☐

Have you ever been diagnosed with diabetes?

No ☐ Yes ☐

Have you ever undergone fertility treatment? E.g. IVF, hormonal assistance.

No ☐ Yes ☐

Have you ever had a miscarriage or termination after 12 weeks of pregnancy?

No ☐ Yes ☐

Basic information

What is your post code?

_____
_

What is your highest level of education?

_____
_

Screening questionnaire for males

Patient Identification Number:

Date of Completion:

MALE PRE-PARTICIPATION QUESTIONNAIRE

Title of Project:
The assessment of cardiac mechanics and arterial haemodynamics during pregnancy and in the postpartum period at rest and during physical activity.

Name of Researcher: Miss Victoria L. Meah

In order to describe the participants in this study, we ask that you complete this short questionnaire. The questions ask you about your general health and some basic information about you. If you are unsure or cannot remember, or do not want to answer a question; you are under no obligation to do so.

Health
Do you smoke or have you smoked on a regular basis within the last two years?

No ☐ Yes ☐

Have you ever been diagnosed with high blood pressure or cardiovascular problems?

No ☐ Yes ☐

Have you ever been diagnosed with diabetes?

No ☐ Yes ☐

Basic information
What is your post code?

_____
_
What is your highest level of education?

_____
_

Appendix VI – American College of Sports Medicine Physical Activity Screening Questionnaire (ACSM PAR-Q)

Patient Identification Number:

Date of Completion:

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

Title of Project:

The assessment of cardiac mechanics and arterial haemodynamics during pregnancy and in the postpartum period at rest and during physical activity.

Name of Researcher: Miss Victoria L. Meah

Please answer the following questions so that we are able to determine your suitability to exercise.

1. Has a doctor ever said that you have a heart condition and recommended only medically supervised activity?

   No ☐ Yes ☐

2. Do you have chest pain brought on by physical activity?

   No ☐ Yes ☐

3. Have you developed chest pain in the past month?

   No ☐ Yes ☐

4. Have you on 1 or more occasions lost consciousness or fallen over as a result of dizziness?

   No ☐ Yes ☐

5. Do you have a bone or joint problem that could be aggravated by proposed physical activity?

   No ☐ Yes ☐

6. Has a doctor ever recommended medication for your blood pressure or heart condition?

   No ☐ Yes ☐

7. Are you aware, through your own experience or a doctor's advice, of any physical reason that would prohibit you from exercising without medical supervision?

   No ☐ Yes ☐

8. **FEMALES ONLY**: Are you pregnant, given birth within the last 6 months and/or breast feeding?

   No ☐ Yes ☐

Appendix VII - Canadian Society for Exercise Physiology (CSEP) PARMed-X for Pregnancy pre-participation screening questionnaire

Physical Activity Readiness
Medical Examination for
Pregnancy (2002)

PARmed-X for PREGNANCY PHYSICAL ACTIVITY READINESS MEDICAL EXAMINATION

PARmed-X for PREGNANCY is a guideline for health screening prior to participation in a prenatal fitness class or other exercise.

Healthy women with uncomplicated pregnancies can integrate physical activity into their daily living and can participate without significant risks either to themselves or to their unborn child. Postulated benefits of such programs include improved aerobic and muscular fitness, promotion of appropriate weight gain, and facilitation of labour. Regular exercise may also help to prevent gestational glucose intolerance and pregnancy-induced hypertension.

The safety of prenatal exercise programs depends on an adequate level of maternal-fetal physiological reserve. PARmed-X for PREGNANCY is a convenient checklist and prescription for use by health care providers to evaluate pregnant patients who want to enter a prenatal fitness program and for ongoing medical surveillance of exercising pregnant patients.

Instructions for use of the 4-page PARmed-X for PREGNANCY are the following:

1. The patient should fill out the section on PATIENT INFORMATION and the PRE-EXERCISE HEALTH CHECKLIST (PART 1, 2, 3, and 4 on p. 1) and give the form to the health care provider monitoring her pregnancy.
2. The health care provider should check the information provided by the patient for accuracy and fill out SECTION C on CONTRAINDICATIONS (p. 2) based on current medical information.
3. If no exercise contraindications exist, the HEALTH EVALUATION FORM (p. 3) should be completed, signed by the health care provider, and given by the patient to her prenatal fitness professional.

In addition to prudent medical care, participation in appropriate types, intensities and amounts of exercise is recommended to increase the likelihood of a beneficial pregnancy outcome. PARmed-X for PREGNANCY provides recommendations for individualized exercise prescription (p. 3) and program safety (p. 4).

NOTE: Sections A and B should be completed by the patient before the appointment with the health care provider.

A PATIENT INFORMATION

NAME _____

ADDRESS _____

TELEPHONE _____ BIRTHDATE _____ HEALTH INSURANCE No. _____

NAME OF PRENATAL FITNESS PROFESSIONAL _____ PRENATAL FITNESS PROFESSIONAL'S PHONE NUMBER _____

B PRE-EXERCISE HEALTH CHECKLIST

PART 1: GENERAL HEALTH STATUS

In the past, have you experienced (check YES or NO):

	YES	NO
1. Miscarriage in an earlier pregnacy?	❑	❑
2. Other pregnancy complications?	❑	❑
3. I have completed a PAR-Q within the last 30 days.	❑	❑

If you answered YES to question 1 or 2, please explain:

_____

Number of previous pregnancies? _____

PART 2: STATUS OF CURRENT PREGNANCY

Due Date: _____

During this pregnancy, have you experienced:

	YES	NO
1. Marked fatigue?	❑	❑
2. Bleeding from the vagina ("spotting")?	❑	❑
3. Unexplained faintness or dizziness?	❑	❑
4. Unexplained abdominal pain?	❑	❑
5. Sudden swelling of ankles, hands or face?	❑	❑
6. Persistent headaches or problems with headaches?	❑	❑
7. Swelling, pain or redness in the calf of one leg?	❑	❑
8. Absence of fetal movement after 6th month?	❑	❑
9. Failure to gain weight after 5th month?	❑	❑

If you answered YES to any of the above questions, please explain:

_____

PART 3: ACTIVITY HABITS DURING THE PAST MONTH

1. List only regular fitness/recreational activities:

_____

_____

INTENSITY	FREQUENCY (times/week) 1-2	2-4	4+	TIME (minutes/day) <20	20-40	40+
Heavy	___	___	___	___	___	___
Medium	___	___	___	___	___	___
Light	___	___	___	___	___	___

2. Does your regular occupation (job/home) activity involve:

	YES	NO
Heavy Lifting?	❑	❑
Frequent walking/stair climbing?	❑	❑
Occasional walking (>once/hr)?	❑	❑
Prolonged standing?	❑	❑
Mainly sitting?	❑	❑
Normal daily activity?	❑	❑
3. Do you currently smoke tobacco?*	❑	❑
4. Do you consume alcohol?*	❑	❑

PART 4: PHYSICAL ACTIVITY INTENTIONS

What physical activity do you intend to do?

_____

Is this a change from what you currently do? ❑ YES ❑ NO

*NOTE: PREGNANT WOMEN ARE STRONGLY ADVISED NOT TO SMOKE OR CONSUME ALCOHOL DURING PREGNANCY AND DURING LACTATION.

© Canadian Society for Exercise Physiology
Société canadienne de physiologie de l'exercice

Supported by: Health Canada Santé Canada

1

PARmed-X for PREGNANCY

PHYSICAL ACTIVITY READINESS MEDICAL EXAMINATION

C CONTRAINDICATIONS TO EXERCISE: to be completed by your health care provider

Absolute Contraindications

Does the patient have:

	YES	NO
1. Ruptured membranes, premature labour?	❑	❑
2. Persistent second or third trimester bleeding/placenta previa?	❑	❑
3. Pregnancy-induced hypertension or pre-eclampsia?	❑	❑
4. Incompetent cervix?	❑	❑
5. Evidence of intrauterine growth restriction?	❑	❑
6. High-order pregnancy (e.g., triplets)?	❑	❑
7. Uncontrolled Type I diabetes, hypertension or thyroid disease, other serious cardiovascular, respiratory or systemic disorder?	❑	❑

Relative Contraindications

Does the patient have:

	YES	NO
1. History of spontaneous abortion or premature labour in previous pregnancies?	❑	❑
2. Mild/moderate cardiovascular or respiratory disease (e.g., chronic hypertension, asthma)?	❑	❑
3. Anemia or iron deficiency? (Hb < 100 g/L)?	❑	❑
4. Malnutrition or eating disorder (anorexia, bulimia)?	❑	❑
5. Twin pregnancy after 28th week?	❑	❑
6. Other significant medical condition?	❑	❑

Please specify: _____

NOTE: Risk may exceed benefits of regular physical activity. The decision to be physically active or not should be made with qualified medical advice.

PHYSICAL ACTIVITY RECOMMENDATION: ❑ Recommended/Approved ❑ Contraindicated

Prescription for Aerobic Activity

RATE OF PROGRESSION: The best time to progress is during the second trimester since risks and discomforts of pregnancy are lowest at that time. Aerobic exercise should be increased gradually during the second trimester from a minimum of 15 minutes per session, 3 times per week (at the appropriate target heart rate or RPE to a maximum of approximately 30 minutes per session, 4 times per week (at the appropriate target heart rate or RPE).

WARM-UP/COOL-DOWN: Aerobic activity should be preceded by a brief (10-15 min.) warm-up and followed by a short (10-15 min.) cool-down. Low intensity calesthenics, stretching and relaxation exercises should be included in the warm-up/cool-down.

F	I	T	T
FREQUENCY	**INTENSITY**	**TIME**	**TYPE**
Begin at 3 times per week and progress to four times per week	Exercise within an appropriate RPE range and/or target heart rate zone	Attempt 15 minutes, even if it means reducing the intensity. Rest intervals may be helpful	Non weight-bearing or low-impact endurance exercise using large muscle groups (e.g., walking, stationary cycling, swimming, aquatic exercises, low impact aerobics)

"TALK TEST" - A final check to avoid overexertion is to use the "talk test". The exercise intensity is excessive if you cannot carry on a verbal conversation while exercising.

PRESCRIPTION/MONITORING OF INTENSITY: The best way to prescribe and monitor exercise is by combining the heart rate and rating of perceived exertion (RPE) methods.

TARGET HEART RATE ZONES

The heart rate zones shown below are appropriate for most pregnant women. Work during the lower end of the HR range at the start of a new exercise program and in late pregnancy.

Age	Heart Rate Range
< 20	140-155
20-29	135-150
30-39	130-145

RATING OF PERCEIVED EXERTION (RPE)

Check the accuracy of your heart rate target zone by comparing it to the scale below. A range of about 12-14 (somewhat hard) is appropriate for most pregnant women.

6	
7	Very, very light
8	
9	Somewhat light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

The original PARmed-X for PREGNANCY was developed by L.A. Wolfe, Ph.D., Queen's University. The muscular conditioning component was developed by M.F. Mottola, Ph.D., University of Western Ontario. The document has been revised based on advice from an Expert Advisory Committee of the Canadian Society for Exercise Physiology chaired by Dr. N. Gledhill, with additonal input from Drs. Wolfe and Mottola, and Gregory A.L. Davies, M.D.,FRCS(C) Department of Obstetrics and Gynaecology, Queen's University, 2002.

No changes permitted. Translation and reproduction in its entirety is encouraged.

Disponible en français sous le titre «Examination medicale sur l'aptitude à l'activité physique pour les femmes enceintes (X-AAP pour les femmes enceintes)»

Additional copies of the PARmed-X for PREGNANCY, the PARmed-X and/or the PAR-Q can be downloaded from: www.csep.ca/publications
For more information contact the:

Canadian Society for Exercise Physiology
18 Louisa Street, Suite 370, Ottawa, Ontario CANADA K1R 6Y6
tel.: 1-877-651-3755 www.csep.ca

PARmed-X for PREGNANCY

PHYSICAL ACTIVITY READINESS MEDICAL EXAMINATION

Prescription for Muscular Conditioning

It is important to condition all major muscle groups during both prenatal and postnatal periods.

WARM-UPS & COOL DOWN:
Range of Motion: neck, shoulder girdle, back, arms, hips, knees, ankles, etc.

Static Stretching: all major muscle groups

(DO NOT OVER STRETCH!)

EXAMPLES OF MUSCULAR STRENGTHENING EXERCISES

CATEGORY	PURPOSE	EXAMPLE
Upper back	Promotion of good posture	Shoulder shrugs, shoulder blade pinch
Lower back	Promotion of good posture	Modified standing opposite leg & arm lifts
Abdomen	Promotion of good posture, prevent low-back pain, prevent diastasis recti, strengthen muscles of labour	Abdominal tightening, abdominal curl-ups, head raises lying on side or standing position
Pelvic floor ("Kegels")	Promotion of good bladder control, prevention of urinary incontinence	"Wave", "elevator"
Upper body	Improve muscular support for breasts	Shoulder rotations, modified push-ups against a wall
Buttocks, lower limbs	Facilitation of weight-bearing, prevention of varicose veins	Buttocks squeeze,standing leg lifts, heel raises

PRECAUTIONS FOR MUSCULAR CONDITIONING DURING PREGNANCY

VARIABLE	EFFECTS OF PREGNANCY	EXERCISE MODIFICATIONS
Body Position	• in the supine position (lying on the back), the enlarged uterus may either decrease the flow of blood returning from the lower half of the body as it presses on a major vein (inferior vena cava) or it may decrease flow to a major artery (abdominal aorta)	• past 4 months of gestation, exercises normally done in the supine position should be altered • such exercises should be done side lying or standing
Joint Laxity	• ligaments become relaxed due to increasing hormone levels • joints may be prone to injury	• avoid rapid changes in direction and bouncing during exercises • stretching should be performed with controlled movements
Abdominal Muscles	• presence of a rippling (bulging) of connective tissue along the midline of the pregnant abdomen (diastasis recti) may be seen during abdominal exercise	• abdominal exercises are not recommended if diastasis recti develops
Posture	• increasing weight of enlarged breasts and uterus may cause a forward shift in the centre of gravity and may increase the arch in the lower back • this may also cause shoulders to slump forward	• emphasis on correct posture and neutral pelvic alignment. Neutral pelvic alignment is found by bending the knees, feet shoulder width apart, and aligning the pelvis between accentuated lordosis and the posterior pelvic tilt position.
Precautions for Resistance Exercise	• emphasis must be placed on continuous breathing throughout exercise • exhale on exertion, inhale on relaxation using high repetitions and low weights • Valsalva Manoevre (holding breath while working against a resistance) causes a change in blood pressure and therefore should be avoided • avoid exercise in supine position past 4 months gestation	

PARmed-X for Pregnancy - Health Evaluation Form

(to be completed by patient and given to the prenatal fitness professional after obtaining medical clearance to exercise)

I, _____ PLEASE PRINT (patient's name), have discussed my plans to participate in physical activity during my current pregnancy with my health care provider and I have obtained his/her approval to begin participation.

Signed: _____ Date: _____
(patient's signature)

HEALTH CARE PROVIDER'S COMMENTS:

Name of health care provider: _____

Address: _____

_____

Telephone: _____

_____
(health care provider's signature)

3

Appendix IX – Pregnancy health follow up questionnaire

Patient Identification Number:

Date of Completion:

FOLLOW UP QUESTIONNAIRE

Title of Project:

The assessment of cardiac mechanics and arterial haemodynamics during pregnancy and in the postpartum period at rest and during physical activity.

Name of Researcher: Miss Victoria L. Meah

In order to describe the pregnancy outcomes of the participants in this study, we ask that you complete this short questionnaire. The questions ask you about your health during and after your pregnancy, your method of delivery, and basic information about your baby. If you are unsure or cannot remember, or do not want to answer a question; you are under no obligation to do so.

Pregnancy Health

During your pregnancy were you diagnosed with any medical issues?

In particular, any medical issues that are specifically related to pregnancy i.e. preeclampsia, gestational diabetes, gestational hypertension?

No ☐ Yes ☐

If yes, please provide details:

_____

_____

__

During your pregnancy, were you physically active?

No ☐ Yes ☐

If yes, what activities did you do?

Walking	☐	Weight training	☐	Pilates or Yoga	
Running	☐	Swimming	☐	Antenatal exercise classes	☐
Cycling	☐	Sport	☐	Other: _____	☐

If yes, do you think you met with daily recommendations for physical activity?

The recommendation for physical activity during pregnancy is 30 minutes of moderate activity on most, if not all days of the week.

No ☐ Yes ☐ Sometimes ☐

Please provide details:

_____

_____

__

Delivery

What was your delivery date? _____

How many weeks were you at delivery? _____ weeks

How was your baby delivered?

Natural Caesarean – Emergency Other:_____

Caesarean - Planned

Were there any complications during your delivery?

No ☐ Yes ☐

If yes, please provide details:

_____
_____
__

How much did your baby weigh at birth? _____ pounds

What sex what your baby?

Boy ☐ Girl ☐

Were there any complications with your baby?

No ☐ Yes ☐

If yes, please provide details:

_____
_____
__

Postpartum

Have you breast-fed?

No ☐ Yes ☐

If yes, for how long do you plan to breastfeed? _____

Have you started any physical activity or exercise since giving birth?

The recommendation for physical activity during pregnancy is 30 minutes of moderate activity on most, if not all days of the week.

No ☐ Yes ☐

The research team sincerely thank you for volunteering your time to participate in this research project. We wish you and your family the best for the future.

If you should have any further questions, or you would like to know more about the outcomes of the research project, please do not hesitate to contact Victoria.

Appendix X – Cardiff Metropolitan University cardiovascular ultrasound imaging approved protocol

Proposer:	Prof. Robert Shave, Dr. Eric Stöhr
School:	School of Sport

BEFORE COMPLETING THIS FORM, PLEASE REFER TO THE NOTES ABOVE

A – DETAILS OF PROTOCOL

A1 Proposed title of protocol

Cardiovascular Ultrasound Imaging

A2 What is the purpose of this protocol?

To image the structure and function of the heart and vasculature for research purposes

A3 What procedure will the protocol encompass?

Introduction:

Participant's will come in to the laboratory and undergo cardiac (figure 1) and/or vascular (figure 2) ultrasound imaging. This will entail a transducer being placed on the participant's chest, neck, arm or thigh. A series of images will be produced and analysed later. The procedure is non invasive and will be used for research purposes and not as a clinical diagnostic tool.

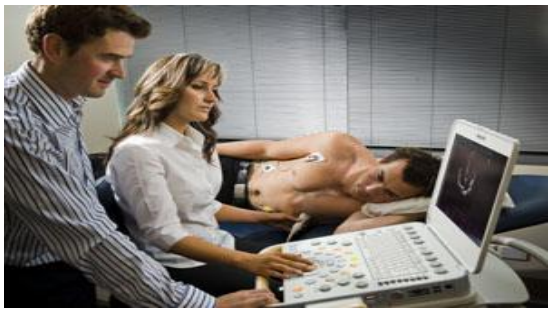


Figure 1: Participant undergoing cardiac ultrasound

Figure 2: Participant undergoing vascular ultrasound

Preparation:

If male, the participant will be asked to remove all items of clothing on their upper body. Prior to the examination females will have been informed to wear a loose fitting sports bra/bikini top and asked to remove all clothing on upper body except sports bra/bikini top. To respect privacy, the location for this imaging will only be accessible by the researcher, the participant and a chaperone, if requested.

Procedure:

1. 3 ECG electrodes will be placed on the participant's left and right clavicle and at the bottom of the right side of their rib cage.

2. For cardiac imaging, participants will be asked to lie on a medical plinth in the left lateral decubitus position with their left arm positioned behind their head (see figure 1). A transducer will be placed on two locations on the participant's chest, (1) just left of sternum (2) on lateral side of the upper body.

3. For vascular imaging, participants will be asked to lie supine on a medical plinth (see figure 2). A transducer will be used to image up to four locations on the participant's body (1) upper arm (2) neck (3) upper inner thigh (4) mid inner thigh.

4. For both cardiac and vascular scans a clear water based gel will be applied between the transducer and the participant's skin.

5. Ultrasound imaging is a non-invasive procedure.

6. Following the procedure (~30 minutes), the water based gel and ECG electrodes will be removed.

Within this setting all of the outlined procedures are non-clinical and non-diagnostic. Whilst ultrasound is a commonly used diagnostic tool in the clinical environment, imaging of the heart and vasculature in the physiology laboratory at UWIC will be performed solely for research purposes.

A4 Are these procedures well established in the participant community?	No
A5 If YES, please provide evidence	
Click here to enter text.	
A6 If NO, please provide an explanation of why this is the case	
Intended participants are male and female healthy individuals within the university and the general population. Generally, cardiac and vascular ultrasound imaging is not a routine procedure unless the participant presents in a clinical or research setting. However, within such settings, cardiac and vascul ultrasound imaging is very well established and is one of the most commonly used non-invasive tool looking at cardiac and vascular structure and function.	

A7 How many projects carried out in your School in the last three years have used these procedures?	3 ongoing and approx. 10 in planning for 2011
A8 Does the proposed protocol contain identical procedures to those used in these projects?	YES

A9 If NO, what are the differences?

B – POTENTIAL RISKS

B1 What are the risks associated with the procedures (for both the participant and the researcher) and how will these risks be managed?

There are no physiological or psychological risks known for humans.

Whilst the researchers are not clinicians they do possess an understanding of cardiac and vascular pathophysiology and it is possible that an abnormality in cardiac/vascular structure or function may be identified. In the unlikely event of this happening the participant will be advised to make an appointment with their G.P. to follow up on the findings.

C – DETAILS OF INDIVIDUALS INVOLVED

C1 Please provide details of academic staff qualified to supervise use of the protocol. You should include details of each individual's experience in using the procedures and any relevant qualifications.

Prof. Robert Shave has 15 years experience in the use of cardiac and vascular ultrasound for research purposes. Dr. Eric Stohr has 4 years experience in the use of cardiac and vascular ultrasound for research purposes. Both Prof Shave and Dr. Stohr have used this technique to examine cardiac and vascular structure and function in both athletic and general populations with a wide age range (18-65).

C2 Which groups of students do you envisage using this protocol eg what areas of study will they be involved in, will they require any particular qualifications or experience in order to use the protocol?

Post Graduate students whose projects require the examination of cardiac or vascular structure and function will use this protocol. They will only be able to use the techniques outlined within this protocol once they have received appropriate training and have demonstrated proficiency in the use of this technique. To ensure proficiency a weekly training programme run by Dr. Eric Stohr has been instigated for all intended users of this equipment.

C3 Will there be any requirements placed on participants on whom this protocol will be used? NB If participant characteristics would require ethics approval then protocol approval will not be granted

Participants will be required to attend the physiology laboratory for ~ 30 minutes, following the scans there will be no further requirements of the participant. Full ethical approval for physiological interventions performed for research purposes will be sought separately.

Appendix XI – Supplementary data for Chapter 5

Table A1. Absolute left ventricular volumes, dimensions and tissue velocities at rest non-pregnant, pregnant and postpartum females and males. Data presented as mean ± SD.

	Males	Non-pregnant	Pregnant	Postpartum	*P*	η_p^2
Left ventricular volumes						
Cardiac output ($L \cdot min^{-1}$)	4.4 ± 0.7 *	3.2 ± 0.5	4.8 ± 0.8 *	3.4 ± 0.5	**0.000**	0.557
SV (ml)	80 ± 13 †	57 ± 6 ‡ §	69 ± 8 §	60 ± 10 ‡	**0.000**	0.502
EDV (ml)	129 ± 16 *	100 ± 11 ‡	117 ± 10 †	99 ± 12 ‡	**0.000**	0.539
ESV (ml)	49 ± 10 ‡	43 ± 9	48 ± 8	39 ± 8 †	**0.009**	0.184
Blood pressure						
SBP (mmHg)	117 ± 9 ‡	112 ± 7 ‡	109 ± 8	105 ± 6 †	**0.001**	0.252
DBP (mmHg)	66 ± 8	67 ± 6 ‡	63 ± 5	61 ± 4 †	**0.005**	0.174
MAP (mmHg)	83 ± 8 ‡	82 ± 6 ‡	78 ± 5	75 ± 4 †	**0.001**	0.223
Dimensions						
IVSd (cm)	1.2 ± 0.1 *	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	**0.000**	0.367
LVIDd (cm)	4.9 ± 0.4 †	4.4 ± 0.3 ‡	4.7 ± 0.3	4.6 ± 0.3 ‡	**0.001**	0.241
LVPWd (cm)	1.0 ± 0.0 †	0.9 ± 0.1 ‡	0.9 ± 0.1	0.8 ± 0.1 ‡	**0.001**	0.246
IVSs (cm)	1.4 ± 0.2 †	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.1 ‡	**0.006**	0.200
LVIDs (cm)	3.6 ± 0.3 *	3.2 ± 0.2	3.2 ± 0.3	3.2 ± 0.3	**0.001**	0.253
LVPWs (cm)	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.117	0.099
LV length (cm)	8.9 ± 0.6 ‡	8.2 ± 0.8 †	8.8 ± 0.6 ‡	8.2 ± 0.4 †	**0.002**	0.239
LV mass (g)	160 ± 28 †	130 ± 19 ‡	139 ± 22	128 ± 16 ‡	**0.004**	0.285
Septal tissue velocities						
S' ($m \cdot s^{-1}$)	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.132	0.095
E' ($m \cdot s^{-1}$)	0.14 ± 0.02	0.14 ± 0.01	0.13 ± 0.03	0.13 ± 0.02	0.580	0.034
A' ($m \cdot s^{-1}$)	0.08 ± 0.01	0.07 ± 0.01 ‡	0.08 ± 0.02 †	0.06 ± 0.01 ‡	**0.003**	0.222

N.B. SV, stroke volume; EDV, end-diastolic volume; ESV, end-systolic volume; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; IVS, intraventricular septum; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall; d, diastole; s, systole; RWT, relative wall thickness; LV, left ventricular; S', systolic tissue velocity; E', early-diastolic tissue velocity; A', late-diastolic tissue velocity * indicates significantly different to all groups; † indicates significantly different to group(s) marked ‡; § indicates significantly different groups.

Table A2. Correlation outcomes for LV strain parameters and haemodynamic load indices in all group data and in non-pregnant, pregnant and postpartum females and males group data.

		Longitudinal strain			Basal circumferential strain		
		HR	EDV	MAP	HR	EDV	MAP
ALL GROUPS	***r***	-0.331	0.032	0.273	-0.207	0.168	0.378
	r^2	0.110	0.001	0.074	0.043	0.028	0.143
	P	**0.013**	0.813	**0.042**	0.120	0.207	**0.004**
Non-pregnant	***r***	-0.347	0.452	0.232	-0.088	0.090	0.221
	r^2	0.120	0.204	0.054	0.008	0.008	0.049
	P	0.172	0.069	0.370	0.727	0.724	0.378
Male	***r***	0.003	0.238	0.167	-0.007	0.313	0.274
	r^2	0.000	0.057	0.028	0.000	0.098	0.075
	P	0.992	0.393	0.552	0.980	0.256	0.324
Pregnant	***r***	0.024	-0.076	-0.257	0.268	0.152	-0.378
	r^2	0.001	0.006	0.066	0.072	0.023	0.143
	P	0.940	0.813	0.421	0.377	0.620	0.203
Postpartum	***r***	0.376	0.124	0.148	0.018	0.079	0.219
	r^2	0.141	0.015	0.022	0.000	0.006	0.048
	P	1.642	0.156	0.223	0.954	0.797	0.472

N.B. HR, heart rate; EDV, end-diastolic volume; MAP, mean arterial pressure.

Table A3. Resting left ventricular strain parameters, adjusted for heart rate, mean arterial pressure, end-diastolic volume and end systolic wall stress, in non-pregnant, pregnant and postpartum females and males.

	Males	Non-pregnant	Pregnant	Postpartum	*P*	η_p^2
Peak longitudinal strain (%)	-18 ± 1 ‡	-16 ± 1 ‡	-22 ± 1 †	-19 ± 1	**0.000**	0.331
Peak basal circumferential strain (%)	-17 ± 1 ‡	-16 ± 1 ‡	-24 ± 1 †	-24 ± 1 †	**0.000**	0.450

Appendix XII – Supplementary data for Chapter 6

Table A4. Global haemodynamic parameters, adjusted for resting values, in non-pregnant, pregnant and postpartum females and males in response to a sustained isometric handhold at 30% maximum and during aerobic cycling exercise at 25 and 50% estimated maximum. Data presented as mean ± SD. Cardiac output, stroke volume, end-diastolic (EDV) and end-systolic volume (ESV) allometrically scaled to height.

		Males	Non-pregnant	Pregnant	Postpartum				
Sustained isometric handhold									
	n	15	18	14	12		***P***	η_p^2	
Cardiac output ($L \cdot min^{-1} \cdot m^{1.83}$)		1.7 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	1.5 ± 0.1		0.117	0.102	
Stroke volume ($ml \cdot m^{2.04}$)		25 ± 1	21 ± 1	24 ± 1	25 ± 1		0.102	0.118	
End diastolic volume ($ml \cdot m^2$)		41 ± 1	39 ± 1	39 ± 2	39 ± 1		0.563	0.038	
End systolic volume ($ml \cdot m^2$)		16 ± 1	16 ± 1	16 ± 1	13 ± 1		0.138	0.098	
SVR ($dyne \cdot s \cdot cm^{-6}$)		1809 ± 79	2003 ± 87	1795 ± 91	1835 ± 91		0.388	0.055	
Aerobic cycling									
	n	15	16	14	9				
	Intensity						**Group**	**Exercise**	**Interaction**
Cardiac output ($L \cdot min^{-1} \cdot m^{1.83}$)	25%	2.4 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	***P***	0.982	0.056	0.366
	50%	3.3 ± 0.1	3.2 ± 0.1	3.2 ± 0.2	3.2 ± 0.2	η_p^2	0.003	0.072	0.062
Stroke volume ($ml \cdot m^{2.04}$)	25%	24 ± 1	25 ± 1	25 ± 1	26 ± 1	***P***	0.425	0.649	0.980
	50%	25 ± 1	26 ± 1	26 ± 1	27 ± 1	η_p^2	0.055	0.004	0.004
End diastolic volume ($ml \cdot m^2$)	25%	40 ± 1	37 ± 1	41 ± 1	39 ± 2	***P***	0.060	0.472	0.787
	50%	41 ± 1	37 ± 1	41 ± 1	38 ± 2	η_p^2	0.139	0.011	0.021
End systolic volume ($ml \cdot m^2$)	25%	15 ± 1 †	12 ± 1 ‡	15 ± 1	13 ± 1	***P***	**0.014**	0.665	0.760
	50%	14 ± 1	11 ± 1	13 ± 1	11 ± 1	η_p^2	0.193	0.004	0.023
SVR ($dyne \cdot s \cdot cm^{-6}$)	25%	1309 ± 53	1260 ± 62	1277 ± 63	1231 ± 73	***P***	0.856	0.520	0.751
	50%	1098 ± 50	1116 ± 58	1134 ± 59	1048 ± 69	η_p^2	0.016	0.009	0.025

N.B. SVR, systemic vascular resistance. † indicates significantly different to group(s) marked ‡.

Table A5. Magnitude of change and absolute values of global haemodynamics and cardiac function during a sustained isometric handhold in non-pregnant, pregnant and postpartum females and males. Data presented as mean ± SD.

		Males	Non-pregnant	Pregnant	Postpartum	*P*-value	η_p^2
Systolic blood pressure (mmHg)	*Δ*	15 ± 11	10 ± 8	10 ± 9	7 ± 7	0.177	0.087
Diastolic blood pressure (mmHg)	*Δ*	11 ± 9 †	5 ± 6	6 ± 6	3 ± 4 ‡	**0.021**	0.164
Pulse pressure (mmHg)	*Δ*	4 ± 5	4 ± 6	3 ± 5	4 ± 4	0.877	0.012
Mean arterial pressure (mmHg)	*Δ*	14 ± 10 †	8 ± 5	8 ± 7	5 ± 5 ‡	**0.011**	0.186
Cardiac output ($L \cdot min^{-1}$)	*IH*	4.9 ± 1	3.4 ± 0.7	5.1 ± 0.8	3.5 ± 0.6		
	Δ	0.5 ± 0.7	0.2 ± 0.6	0.4 ± 0.5	0.1 ± 0.6	0.129	0.097
Cardiac output ($L \cdot min^{-1} \cdot m^{1.83}$)	*Δ*	0.2 ± 0.2	-0.1 ± 0.4	0.2 ± 0.2	-0.2 ± 0.7	0.130	0.104
Heart rate ($beats \cdot min^{-1}$)	*Δ*	5 ± 4 †	2 ± 4	3 ± 4	-1 ± 4 ‡	**0.013**	0.177
Stroke volume (ml)	*IH*	83 ± 14	58 ± 9	73 ± 10	64 ± 14		
	Δ	3 ± 7	1 ± 7	3 ± 7	3 ± 11	0.833	0.016
Stroke volume ($ml \cdot m^{2.04}$)	*Δ*	1 ± 2	-2 ± 7	1 ± 3	-3 ± 12	0.583	0.037
End diastolic volume (ml)	*IH*	133 ± 22	103 ± 15	117 ± 14	99 ± 11		
	Δ	3 ± 13	4 ± 12	0 ± 12	0 ± 14	0.792	0.019
End diastolic volume ($ml \cdot m^2$)	*Δ*	1 ± 4	1 ± 4	0 ± 4	0 ± 5	0.762	0.021
End systolic volume (ml)	*IH*	50 ± 11	44 ± 10	45 ± 9	36 ± 7		
	Δ	1 ± 11	2 ± 8	-3 ± 9	-3 ± 8	0.357	0.057
End systolic volume ($ml \cdot m^2$)	*IH*	16 ± 3	16 ± 3	16 ± 3	13 ± 3		
	Δ	0 ± 3	1 ± 3	-1 ± 3	-1 ± 3	0.419	0.050
Ejection fraction (%)	*Δ*	0 ± 5	-1 ± 5	2 ± 5	2 ± 5	0.343	0.058
SVR ($dyne \cdot s \cdot cm^{-6}$)	*Δ*	43 ± 224	106 ± 348	17 ± 138	62 ± 375	0.813	0.017
Longitudinal strain (%)	*Δ*	0 ± 2	-1 ± 6	-2 ± 7	3 ± 8	0.867	0.015
Basal circumferential strain (%)	*Δ*	1 ± 3	2 ± 4	-1 ± 3	1 ± 5	0.149	0.095
Apical circumferential strain (%)	*Δ*	2 ± 5	2 ± 5	-1 ± 5	-1 ± 6	0.218	0.081
Twist (°)	*Δ*	-1.4 ± 5.2	0.6 ± 5.6	-0.8 ± 6.7	1.0 ± 5.7	0.677	0.029

N.B.: *Δ*, change. † indicates significantly different to group(s) marked ‡.

Table A6. Magnitude of change and absolute values of global haemodynamics and cardiac function during aerobic cycling exercise at 25 and 50% maximum in non-pregnant, pregnant and postpartum females and males. Data presented as mean ± SD.

	Intensity	Males	Non-pregnant	Pregnant	Postpartum		Group	Exercise	Interaction
Systolic blood pressure	*Δrest to 25%*	35 ± 16 *	26 ± 8	26 ± 14	25 ± 9	*P*	**0.001**	**0.002**	0.872
(mmHg)	*Δ25% to 50%*	30 ± 11 *	18 ± 11	18 ± 11	16 ± 3	η_p^2	0.317	0.164	0.013
Diastolic blood pressure	*Δrest to 25%*	14 ± 6 †	12 ± 6	10 ± 6 ‡	12 ± 6	*P*	**0.024**	**0.000**	0.701
(mmHg)	*Δ25% to 50%*	10 ± 7 †	5 ± 5	6 ± 7 ‡	5 ± 3	η_p^2	0.168	0.229	0.027
Mean arterial pressure (mmHg)	*Δrest to 25%*	21 ± 7 †	17 ± 7	16 ± 9 ‡	18 ± 8 ‡	*P*	**0.015**	**0.000**	0.761
	Δ25% to 50%	16 ± 9 †	12 ± 7	12 ± 6 ‡	9 ± 3 ‡	η_p^2	0.180	0.229	0.022
Pulse pressure (mmHg)	*Δrest to 25%*	21 ± 14 *	13 ± 6	16 ± 8	14 ± 5	*P*	**0.002**	0.400	0.679
	Δ25% to 50%	20 ± 7 *	14 ± 8	12 ± 12	11 ± 3	η_p^2	0.243	0.014	0.029
Cardiac output ($L \cdot min^{-1}$)	*25%*	7 ± 0.7	6 ± 0.9	7.1 ± 1	5.9 ± 0.9				
	50%	9.5 ± 1.2	7.7 ± 0.6	8.7 ± 1.7	7.7 ± 1.2				
	Δrest to 25%	2.6 ± 0.2	2.9 ± 0.2	2.4 ± 0.2	2.7 ± 0.3	*P*	0.055	**0.001**	0.208
	Δ25% to 50%	2.5 ± 0.3	1.7 ± 0.3	1.6 ± 0.3	1.6 ± 0.4	η_p^2	0.140	0.194	0.086
Cardiac output ($L \cdot min^{-1} \cdot m^{1.83}$)	*Δrest to 25%*	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.8	1 ± 0.1	*P*	0.310	**0.001**	0.262
	Δ25% to 50%	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	η_p^2	0.069	0.198	0.076
Heart rate ($beats \cdot min^{-1}$)	*Δrest to 25%*	34 ± 2 ‡	34 ± 2 ‡	27 ± 2 †	33 ± 2	*P*	**0.001**	**0.001**	0.496
	Δ25% to 50%	24 ± 1 ‡	21 ± 1 ‡	17 ± 2 †	19 ± 2	η_p^2	0.400	0.582	0.046
Stroke volume (ml)	*25%*	79 ± 10	67 ± 8	75 ± 11	67 ± 11				
	50%	84 ± 13	70 ± 7	77 ± 15	73 ± 12				
	Δrest to 25%	-6 ± 3 †	11 ± 3 ‡	5 ± 3	7 ± 3	*P*	**0.011**	0.785	**0.041**
	Δ25% to 50%	5 ± 3 †	3 ± 3 ‡	3 ± 3	3 ± 4	η_p^2	0.198	0.002	0.151
Stroke volume ($ml \cdot m^{2.04}$)	*Δrest to 25%*	-1.6 ± 0.8	3.9 ± 0.8	1.7 ± 0.8	2.5 ± 1.1	*P*	**0.006**	0.653	0.050
	Δ25% to 50%	1.5 ± 1.0	1.3 ± 0.9	1.0 ± 1.0	1.2 ± 1.3	η_p^2	0.218	0.004	0.143
End diastolic volume (ml)	*25%*	128 ± 18	100 ± 15	116 ± 12	100 ± 10				
	50%	130 ± 17	100 ± 13	116 ± 11	101 ± 10				
	Δrest to 25%	-5 ± 3	2 ± 3	1 ± 4	2 ± 4	*P*	0.813	0.907	0.477
	Δ25% to 50%	2 ± 3	-1 ± 3	-1 ± 3	-2 ± 4	η_p^2	0.019	0.001	0.048
End diastolic volume ($ml \cdot m^2$)	*Δrest to 25%*	-1.8 ± 1.2	0.6 ± 1.2	0.2 ± 1.2	0.9 ± 1.5	*P*	0.819	0.906	0.529
	Δ25% to 50%	0.6 ± 1.1	-0.3 ± 1.1	-0.3 ± 1.2	-0.6 ± 1.4	η_p^2	0.018	0.000	0.043
End systolic volume (ml)	*25%*	47 ± 12	33 ± 11	42 ± 11	33 ± 6				
	50%	44 ± 10	30 ± 10	38 ± 10	28 ± 7				
	Δrest to 25%	-2 ± 3	-9 ± 3	-4 ± 3	-5 ± 4	*P*	0.167	0.677	0.650
	Δ25% to 50%	-3 ± 2	-4 ± 2	-4 ± 2	-5 ± 3	η_p^2	0.096	0.003	0.032
End systolic volume ($ml \cdot m^2$)	*Δrest to 25%*	-0.7 ± 0.9	-3.4 ± 0.9	-1.5 ± 1.0	-1.7 ± 1.2	*P*	0.120	0.610	0.651

	Δ25% to 50%	-1.0 ± 0.7	-1.5 ± 0.7	-1.5 ± 0.7	-1.7 ± 0.9	η_p^2	0.109	0.005	0.032
Ejection fraction (%)	*Δrest to 25%*	0 ± 2 †	10 ± 2 ‡	4 ± 2	6 ± 2	***P***	**0.001**	0.299	0.078
	Δ25% to 50%	3 ± 1 †	4 ± 1 ‡	4 ± 1	4 ± 2	η_p^2	0.289	0.022	0.126
Systemic vascular resistance	*Δrest to 25%*	-433 ± 56 †	-914 ± 56 ‡	-349 ± 58 †	-801 ± 77 ‡	***P***	**0.001**	**0.001**	**0.001**
(dyne·s·cm^{-6})	*Δ25% to 50%*	-185 ± 51 †	-201 ± 51 ‡	-88 ± 52 †	-220 ± 69 ‡	η_p^2	0.603	0.643	0.294
Longitudinal strain (%)	*Δrest to 25%*	-3 ± 2 ‡	-4 ± 2 ‡	1 ± 3	2 ± 2 †	***P***	**0.003**	0.367	**0.003**
	Δ25% to 50%	-2 ± 3 ‡	-1 ± 4 ‡	-3 ± 3	-2 ± 4 †	η_p^2	0.309	0.021	0.309
Basal circumferential strain (%)	*Δrest to 25%*	-1 ± 4	1 ± 4	-4 ± 5	2 ± 7	***P***	0.084	0.852	0.163
	Δ25% to 50%	0 ± 5	1 ± 5	-1 ± 4	-3 ± 6	η_p^2	0.126	0.001	0.098
Apical circumferential strain	*Δrest to 25%*	-3 ± 6	-2 ± 7	-4 ± 5	-2 ± 5	***P***	0.405	0.902	0.248
(%)	*Δ25% to 50%*	-4 ± 7	-5 ± 6	1 ± 7	-3 ± 6	η_p^2	0.058	0.000	0.082
Twist (°)	*Δrest to 25%*	3 ± 4	8 ± 6	5 ± 8	5 ± 9	***P***	0.699	0.446	0.472
	Δ25% to 50%	5 ± 5	4 ± 6	4 ± 7	3 ± 8	η_p^2	0.030	0.013	0.053

N.B.: *Δ*, change. † indicates significantly different to group(s) marked ‡, * indicates significantly different to all other groups.

Appendix XIII - Comparison of haemodynamic responses to different methods of inducing an afterload challenge.

Introduction

Isometric handgrip (IHG) is a functional haemodynamic test designed to specifically increase cardiac afterload through increasing arterial pressure. During IHG, activation of the mechano- and metabo-reflex cause an increase in sympathetic nervous system activity alongside an increased blood pressure (approximately 15 mmHg rise in diastolic blood pressure) and heart rate, as well as alterations in left ventricular function and vasomotor tone of the vasculature (Zygmunt and Stanczyk, 2010). Previous literature (Weiner et al., 2012, Balmain et al., 2016) has utilised IHG to induce an increase in cardiac afterload. In application, participants must maintain an active grip to a relative intensity (typically 30-40% maximal voluntary contraction) for approximately 2- 3 minutes, although some may reach volitional exhaustion prior to the desired duration. Participants are instructed to breathe freely, however may inadvertently perform a Valsalva manoeuvre, a potential source of bias (Zygmunt and Stanczyk, 2010) and importantly, a contraindication during pregnancy (Mottola, 2016). IHG has previously been used as an afterload challenge in pregnant populations (Degani et al., 1985, Ekholm et al., 1994, Eneroth-Grimfors et al., 1988, Nisell et al., 1987), however issues associated with Valsalva and the potential for bias suggest the avoidance of this method in this population.

Therefore the aim of this study was to determine the differences in global haemodynamic responses to a traditional afterload challenge, IHG, and a novel method of inducing afterload, a sustained isometric handhold.

Methods

Nine young healthy volunteers (6 males, 3 females; age: 24±4 years; height: 1.73±0.09 m; body mass: 73.2±14.7 kg and BMI: 24±2) underwent cardiovascular measurements at rest, during an isometric handgrip (IHG) and during a sustained isometric handhold. Participants were asked to abstain from heavy exercise for 24 hours and caffeine for 12 hours prior to visiting the laboratory.

Experimental models for inducing an increase in cardiac afterload

In all assessments, volunteers were supine and tilted 30 - 45° laterally to the left using a supine tilt bed (Angio 2003, Lode B.V., Groningen, The Netherlands). Volunteers completed both afterload challenges in this position to facilitate the collection of echocardiographic images. As result of the position, all volunteers completed the IHG and isometric handhold using their left hand. All volunteers were instructed to avoid the Valsalva manoeuvre and breathe freely throughout the challenge. A minimum of 10 minutes of rest was given in between each intervention and the subsequent investigation did not begin until blood pressure had returned to resting values.

Isometric handgrip

The IHG was instrumented using a commercially available grip force transducer (MLT003/D Grip Force Transducer, ADInstruments, Chalgrove, UK) at 30% of maximal voluntary contraction until volitional fatigue. Maximal voluntary contraction was determined through a maximal effort grip after the collection of resting data. The target force (as a percentage) and real-time measurement of force generation were displayed on a computer monitor to guide the effort.

Isometric handhold

Volunteers completed 1 s maximal voluntary contractions on a commercially available digital handgrip dynamometer (Grip-A, 5001, Takei Scientific Instruments Co Ltd. Shinagawa-ku, Tokyo, Japan). The individual intensity for the isometric handhold was 30% of the individual's maximal voluntary contraction strength. The isometric handhold was completed using an adapted handgrip dynamometer designed to hold an external load and held for up to 5 minutes. The challenge was not completed until fatigue: when cardiovascular assessments were completed, the dynamometer was removed from the volunteer.

Cardiovascular assessments

Systolic, diastolic and mean arterial pressure (SBP, DBP and MAP, respectively) were recorded continuously (PowerLab, ADInstruments, Chalgrove, UK) and saved for later offline analysis. Average values were calculated from twenty continuous waveforms (cardiac cycles) at rest and within the final minute of IHG and sustained isometric handhold (LabChart 7 Pro, ADInstruments, Chalgrove, UK).

Transthoracic echocardiography was performed using a commercially available ultrasound system (Vivid E9, GE Medical Systems, Horten, Norway) and 1.5 – 4.6 MHz phased array transducer (M55, GE Medical Systems, Horten, Norway). A three-lead electrocardiograph (ECG) was attached to the participant and connected to the ultrasound system for heart rate monitoring. Echocardiographic images were collected after >5 minutes of quiet rest and after 1 minute from the initiation of each challenge. Two-dimensional (2D) and colour tissue Doppler (TDI) imaging were performed at parasternal and apical windows. The imaging protocol included collection of 2D parasternal short axis (base and apex), 2D apical 4- and 2-chamber, and TDI of the septal mitral annulus using the apical 4-chamber image. Five consecutive cardiac cycles were recorded at end expiration to limit displacement of the heart and changes in intrathoracic cavity pressure during respiration. Data was stored for later offline analysis (EchoPAC PC Version 112.1.0, GE Medical, Horton, Norway). Measurements were made in triplicate from different cardiac cycles and averaged. Ultrasound settings, including image depth and frame rates, were kept the same during the assessments at rest and during the sustained isometric handhold.

Cardiac parameters were measured in accordance with ASE recommendations (Lang et al., 2015). Left ventricular (LV) end-diastolic volume and end-systolic volume were calculated through Simpson's biplane method, involving tracing of the endocardial border at end-diastole and end-systole in the apical 4- and 2-chamber views. Stroke volume was calculated as the difference between end-diastolic and end-systolic volume and ejection fraction calculated as stroke volume divided by end-diastolic volume x 100. Cardiac output was calculated as the product of stroke volume and heart rate, as averaged from the respective ECG trace of the biplane measurements. TDI was performed on an apical 4-chamber image with the sample volume placed on the septum at the level of the mitral valve annulus. The peak systolic (S'), early (E') and late (A') diastolic velocities were measured.

Statistical analyses

All data are presented as mean ± SD. All statistical analysis was conducted using SPSS Statistics (Version 20.0, IBM Corporation, Chicago, IL). Statistical significance was set at 0.05. Two-way repeated measures analysis of variance (ANOVA) was used to determine the effect of different afterload challenges over time on cardiovascular function. All

assumptions were checked: outliers were assessed through examination of studentised residuals for values greater than ±3; normal distribution was assessed by Shapiro-Wilk's test of normality on the studentised residuals ($p > .05$); sphericity for the two-way interactions was tested through Mauchly's test of sphericity. If a statistically significant interaction term was identified, simple main effects were completed. Where there was no significant interaction, the main effects of the within-subject factors of time and afterload challenge were analysed. *Post hoc* pairwise comparisons were made using the Bonferroni adjustment. Partial eta squared effect sizes were interpreted as 0.01 = small effect, 0.06 = medium effect and 0.14 = large effect (Cohen, 1988).

Results

During the IHG, volunteers maintained a grip force of approximately 28 ± 1% until fatigue at an average of 2 min 39 s ± 26 s. The mean maximal voluntary contraction handgrip force was 53 ± 14 kg, therefore the average external load for the isometric handhold was 16 ± 4 kg, held for a duration of 3 min 3 s ± 30 s before removal of the dynamometer.

Interaction effect of time and afterload challenge on global haemodynamics
There was a significant interaction for time and afterload challenge for cardiac output ($F(1, 8) = 16.429$, $p = 0.004$, partial $\eta^2 = 0.673$), heart rate ($F(1, 8) = 35.266$, $p = 0.0005$, partial $\eta^2 = 0.815$), systolic blood pressure ($F(1, 8) = 13.953$, $p = 0.007$, partial $\eta^2 = 0.666$), diastolic blood pressure ($F(1, 8) = 10.260$, $p = 0.015$, partial $\eta^2 = 0.594$), and mean arterial pressure ($F(1, 8) = 15.034$, $p = 0.006$, partial $\eta^2 = 0.682$).

There was no statistical significant two-way interaction between time and afterload challenge for stroke volume ($F(1, 8) = 0.255$, $p = 0.648$, partial $\eta^2 = 0.027$). The main effect of time ($p = 0.986$, partial $\eta^2 = 0.000$) and main effect of afterload challenge ($p = 0.505$, partial $\eta^2 = 0.057$) on stroke volume were not statistically significant.

Haemodynamic function at rest
There were no significant differences at rest in cardiac output ($p = 0.225$, partial $\eta^2 = 0.178$), heart rate ($p = 0.489$, partial $\eta^2 = 0.062$), stroke volume ($p = 0.282$, partial $\eta^2 = 0.143$), systolic blood pressure ($p = 1.000$, partial $\eta^2 = 0.000$), diastolic blood pressure ($p = 0.381$, partial $\eta^2 = 0.111$) and mean arterial pressure ($p = 0.785$, partial $\eta^2 = 0.011$).

Haemodynamic response from rest to during isometric handgrip
Cardiac output was significantly greater during IHG compared to rest ($p = 0.001$, partial $\eta^2 = 0.768$, CI: 0.9 to 2.2 $L \cdot min^{-1}$). Heart rate was significantly greater during IHG compared to rest ($p = 0.0005$, partial $\eta^2 = 0.868$, CI: 14 to 28 $beats \cdot min^{-1}$). Systolic blood pressure ($p = 0.001$, partial $\eta^2 = 0.790$, CI: 21 to 57 mmHg), diastolic blood pressure ($p = 0.006$, partial $\eta^2 = 0.686$, CI: 12 to 50 mmHg) and mean arterial pressure ($p = 0.001$, partial $\eta^2 = 0.828$, CI: 19 to 45 mmHg) were significantly increased during IHG. Mean differences are presented in Table 2.

Haemodynamic response from rest to during sustained isometric handhold
Cardiac output was not significantly different from rest to during sustained isometric handhold ($p = 0.455$, partial $\eta^2 = 0.071$, CI: -0.5 to 1.0 $L \cdot min^{-1}$). Heart rate was also significantly greater during the isometric handhold ($p = 0.046$, partial $\eta^2 = 0.409$, CI: 1 to 8 $beats \cdot min^{-1}$). Systolic blood pressure ($p = 0.010$, partial $\eta^2 = 0.635$, CI: 7 to 39 mmHg), diastolic blood pressure ($p = 0.015$, partial $\eta^2 = 0.595$, CI: 5 to 33 mmHg) and mean arterial pressure ($p = 0.006$, partial $\eta^2 = 0.677$, CI: 8 to 32 mmHg) were significantly increased during the sustained isometric handhold. Mean differences are presented in Table 2.

Differences in haemodynamic function during isometric handgrip and during sustained isometric handhold

Haemodynamics variables during both IHG and during sustained isometric handhold are presented in Table 2 alongside *p*-values and effect sizes. Cardiac output was significantly greater during IHG compared to isometric handhold (mean difference 1.322, CI: 0.602 to 2.042). Heart rate was significantly higher during IHG compared to isometric handhold (mean difference 17, CI: 11 to 22 beats·min^{-1}). Systolic blood pressure (mean difference 16 mmHg, CI: 5 to 27 mmHg), diastolic blood pressure (mean difference 12 mmHg, CI: 4 to 20 mmHg) and mean arterial pressure (mean difference 12 mmHg, CI: 5 to 20 mmHg) were significantly higher during IHG compared to isometric handhold.

Table 2. Haemodynamic responses to an isometric handgrip and to a sustained isometric handhold in healthy young volunteers (*n* = 9). Data presented as mean ± SD.

	Isometric handgrip	**Sustained isometric handhold**	***p*-value**	**η_p^2**
Haemodynamic				
Heart rate (beats·min^{-1})	78 ± 13	61 ± 9	**< 0.0005**	0.857
Δrest	21 ± 8	4 ± 4		
Stroke volume (ml)	75 ± 19	72 ± 20	0.568	0.042
Δrest	1 ± 11	-1 ± 12		
Cardiac output (L·min^{-1})	5.7 ± 1.2	4.3 ± 1.1	**0.003**	0.692
Δrest	1.5 ± 0.9	0.3 ± 1.0		
Blood pressure				
Systolic (mmHg)	164 ± 22	150 ± 19	**0.012**	0.614
Δrest	39 ± 22	23 ± 18		
Diastolic (mmHg)	101 ± 23	90 ± 17	**0.010**	0.634
Δrest	31 ± 22	19 ± 17		
Mean arterial pressure (mmHg)	121 ± 16	109 ± 13	**0.007**	0.668
Δrest	32 ± 16	20 ± 14		

N.B. Δrest, mean difference from rest to during afterload challenge. *p*-value and effect size presented from *post hoc* pairwise comparisons between afterload challenges.

Conclusions

A significant increase in cardiac afterload can be achieved through use of both isometric handgrip and sustained isometric handhold challenges. However, IHG and sustained isometric handhold result in significantly different haemodynamics responses and therefore, should be considered as separate stimuli. IHG causes a greater magnitude of response in cardiac output, heart rate, and systolic, diastolic and mean arterial pressure when compared to sustained isometric handhold. The intensity of the sustained isometric handhold stimulus is lower than that of IHG and may be useful in specific populations where transient but large increases in blood pressure should be avoided. Tolerance and adherence to the afterload challenges was greater in the sustained isometric handhold, and may also be useful in experiments requiring a longer window of opportunity for data collection.

IHG results in increased peripheral blood pressure, alongside increases in heart rate, an independent influence on cardiac function outside of the afterload challenge. Balmain et

al. (2016) sought to counteract the increase in heart rate through use of circulatory occlusion of the limb immediately post-IHG. The model demonstrated that increased heart rate attenuated the cardiac response to an afterload challenge. Although the sustained isometric handhold significantly increased heart rate, the mean difference was minimal (4 beats·min^{-1} compared to 21 beats·min^{-1} in IHG), and therefore may be a useful method in avoiding large changes in heart rate during an afterload challenge.

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Appendix XIV – Invited talks relating to this thesis

International Congress on Maternal Haemodynamics in Pregnancy 2016, Rome, Italy

Second International Congress on Maternal Hemodynamics
Rome, 12 – 14 May 2016
Residenza di Ripetta
Via di Ripetta, n° 231 – Rome

Rome, 08th October 2015

Dear Professor Victoria Meah,

On behalf of the Organising Committee of the forthcoming ***"International Congress Maternal Haemodynamics in Pregnancy"*** that will be held in Rome from the 12th to the 14th of May 2016, we would like to invite you to participate and present a lecture on the

14th May 2016
10.15 – 10.30 IP 21: Exercise and cardiovascular function in pregnancy

All invited speakers will receive complementary registration and hotel stay for three nights at the Congress Hotel. We regret that we cannot reimburse travel expenses however.
The Congress is organized with key notes lectures, invited presentations and guided discussion.

An important space is left for submitted peer reviewed presentations and we hope that the researchers of your group will be interested in presenting their last work. The idea is to foster an informal but educational atmosphere with senior and junior researchers in all fields able to rub shoulders and discuss topics in this rapidly evolving area.

The Congress Venue has been organized in the centre of Rome at walking distance from all the major cultural attractions and places of interest. We hope this might help in the combination of scientific endeavor and leisure interest.

Thank you for your response. You'll then be contacted from NICO Congressi for the organizational details early next year.

Best regards

Herbert Valensise **Christoph Lees** **Wilfried Gyselaers** **Enrico Ferrazzi**

On behalf of the IWGMH steering group

Organizing Secretariat:

NICO Congressi
Organizzazione Congressi ed Eventi
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Women and Children's Health Research Institute 2016, University of Alberta, Canada

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January 26, 2016

File: 1609

Dr. Margie Davenport
Faculty of Physical Education & Recreation
University of Alberta

Sent by email

Dear Dr. Davenport:

RE: WCHRI Scientific Knowledge Exchange Program Grant 2016 – Offer of Award

On behalf of the Women and Children's Health Research Institute, we would like to offer you support for the upcoming visit of Ms. Victoria Meah through the WCHRI Scientific Knowledge Exchange Program.

Details of this offer are below and in the attached documentation.

Event Title:	A mother's heart: a study of cardiac function during healthy pregnancy at rest and during physical activity, March 2, 2016
Value of Award:	$1000.00
Funding Source:	"This research has been funded by the generosity of the Stollery Children's Hospital Foundation and supporters of the Lois Hole Hospital for Women through the Women and Children's Health Research Institute."
Speedcode:	Please use 25WKS and 25WKR (50%/50%) equally when claiming against these funds.
Term of Award:	February 1, 2016 – April 30, 2016
Reporting:	The awardee must submit an award report. The purpose of the final report is to provide details of the impact of your research to our funders.

In order to request implementation of your Offer of Award, WCHRI will require completion of the enclosed Commencement & Compliance form.

A copy of the Terms and Conditions pertaining to this award are included in the Commencement & Compliance form for your reference. By accepting funding from WCHRI, you agree to abide by these Terms and Conditions.

Should you have any questions regarding your award, please contact WCHRI Grants Administration at wcgrants@ualberta.ca.

Again, congratulations on your successful application. We hope that your WCHRI Scientific Knowledge Exchange Program Grant is highly productive!

Sincerely,

Dr. Lorin Charlton
Research Officer
Women & Children's Health Research Institute

Appendix XV – Conference abstracts relating to this thesis

ARTERY 2017, Pisa Italy – Poster presentation

Cardiovascular responses to increased pressure during healthy pregnancy

A long-standing question is whether pregnant females, who bear an increased biological stress, experience exacerbated cardiovascular responses during physiological challenge. At rest, pregnant females have reduced blood pressure, increased cardiac output, heart rate and stroke volume (1), with reported reductions in cardiac contraction and relaxation (2). Increased cardiac work may potentially exasperate impairments in function observed at rest. The aim of this study was to investigate the cardiovascular responses to an isolated increase in pressure in healthy nulliparous non-pregnant, primiparous pregnant (22 - 26 weeks gestation; *n* = 14) and primiparous postpartum (12 - 16 weeks after delivery; *n* = 13) females.

The pressure challenge was elicited through a sustained isometric hold for approximately 5 minutes at 30% of maximum using an externally loaded handgrip dynamometer. Echocardiographic images were collected to measure cardiac volumes and mechanics. Blood pressure was monitored continuously using finger photoplethysmography. Analyses of covariance, with baseline measures as covariate, were completed to determine differences between groups (*P*=<0.05). *Post hoc* analyses were performed with a Bonferroni adjustment.

There were no significant differences between groups in cardiac volumes or blood pressure during the challenge however; pregnant females had a greater heart rate (68±2 *versus* 62±2 beats·min^{-1}) and longitudinal strain (-20.6±1.0% *versus* -17.1±0.7%) than non-pregnant females.

Increased longitudinal strain and heart rate are likely result of increased contractility mediated by greater myocardial sympathetic innervation (3). In healthy pregnant females, increased pressure does not result in impaired cardiovascular function, however dysfunctional responses may predict hypertensive disorders of pregnancy.

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The Physiological Society Annual General Meeting 2017, London, UK – Oral presentation and winner of affiliate competition

CARDIOVASCULAR RESPONSES TO INCREASED PRESSURE AND AEROBIC EXERCISE DURING HEALTHY PREGNANCY

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INTRODUCTION

A long standing question in physiology is whether pregnant females, who already bear an increased biological stress, experience an exacerbated cardiovascular demand during exercise. Healthy pregnancy is associated with adaptations to the maternal cardiovascular system in order to accommodate for the additional demands of the developing foetus as well as the physiological stress experienced by the mother. At rest, the maternal heart increases in size and pumping capacity, whilst the vessels dilate resulting in a reduced resistance to blood flow (Meah et al., 2016, Melchiorre et al., 2012). Despite an increased cardiac output at rest, previously published data has suggested an impairment in systolic contraction and diastolic relaxation of the maternal heart with advancing gestation (Melchiorre et al., 2012).

Both resistance and aerobic exercise during healthy pregnancy are highly recommended for maternal and foetal health. Previous research has shown that pregnant females respond to increased pressure, such as that experienced in resistance exercise, and aerobic exercise with similar heart rate, cardiac output and blood pressure increases as non-pregnant females (Avery et al., 1999, Veille et al., 1992). Although the global haemodynamic responses of pregnant women to exercise are established, systolic and diastolic function of the maternal heart under additional demand remains understudied. It is not known if the stress of exercise further exasperates the cardiovascular stress of pregnancy. Additional myocardial work required to meet the demands of exercise may transiently exasperate the reduced systolic function observed at rest during pregnancy.

The aim of this study was to investigate the cardiovascular responses to an isolated increase in pressure and during aerobic exercise in healthy non-pregnant, pregnant and postpartum females, with particular focus on left ventricular mechanics. Left ventricular mechanics (e.g. longitudinal strain and left ventricular twist) provide insight into cardiac deformation across the cardiac cycle. Longitudinal strain and left ventricular twist have been suggested as more sensitive measures of function than traditional measures such as ejection fraction. It was hypothesised that global haemodynamic response of pregnant females to a pressure and an exercise challenge would not be significantly different from non-pregnant and postpartum females. However, pregnant females would have lower systolic function at rest and during interventions when compared to non-pregnant and postpartum females, thereby representing a greater cardiovascular stress in this population.

METHODS

Healthy nulliparous non-pregnant (n = 18), primiparous pregnant (22 - 26 weeks gestation; n = 14) and primiparous postpartum (12 - 16 weeks after delivery; n = 13) females participated in the study. As determined through power analyses, the groups exceeded the required sample size (n = 10) to detect differences in cardiac output between groups at rest and during exercise. All volunteers completed an initial visit to the laboratory including maximal handgrip and submaximal exercise testing on a cycle ergometer. In the subsequent visit, cardiovascular data were collected at rest, during a pressure challenge and during aerobic cycling exercise. The pressure challenge was elicited through a sustained isometric hold at 30% of maximum using an externally loaded handgrip dynamometer. The dynamic exercise challenge consisted of aerobic cycling at 50% maximal peak power output on a supine cycle ergometer. Echocardiographic images were collected to measure left ventricular volumes and mechanics. Cardiac output and left ventricular volumes were allometrically scaled to height. To account for differences in

cardiac size, left ventricular twist was scaled to left ventricular length to calculate torsion. Blood pressure was monitored continuously using finger photoplethysmography. Analysis of variance (ANOVA) tests were used to determine differences in response to increased pressure and aerobic cycling and in resting function between groups. *Post hoc* analyses were performed using Tukey-Kramer. Subsequent analysis of covariance (ANCOVA), with baseline measures as the co-variate, were completed to determine differences in cardiovascular responses between groups to both increased pressure and aerobic cycling. *Post hoc* analyses were performed with a Bonferroni adjustment. Alpha was set at 0.05 for all analyses.

RESULTS

All results are presented as mean ± SD and are shown in Table One. Pregnant females had a significantly larger cardiac output at rest than non-pregnant and postpartum females. This was the result of a significantly greater heart rate and stroke volume. Peak longitudinal strain was also increased at rest in pregnant females, as shown in Figure One.

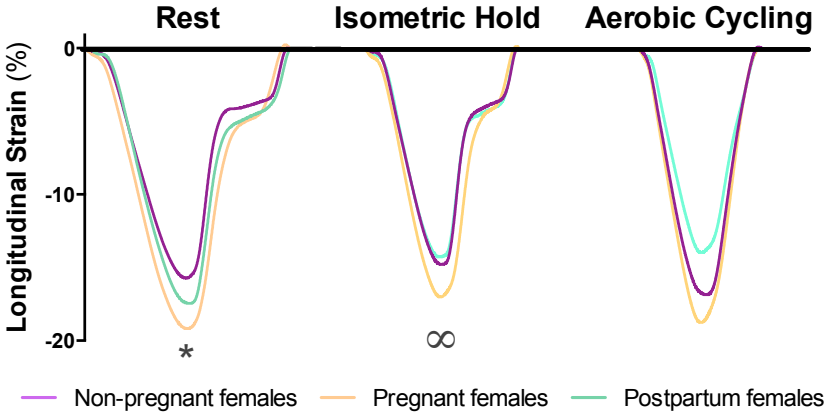

Figure One. Interpolated global longitudinal strain curves for non-pregnant, pregnant and postpartum females at rest and during isometric hold at 30% maximum and aerobic cycling at 50% maximum peak power output.
N.B. * indicates pregnant females significantly different ($p < 0.05$) to all groups; ∞ indicates pregnant females significantly different ($p < 0.05$) to non-pregnant females when adjusted for resting value.

In all groups, the sustained isometric hold significantly increased cardiac output (from 1.5±0.4 to 1.6±0.5 $L.min^{-1}/m^{1.83}$) and mean arterial pressure (from 83±8 to 91±9 mmHg) from resting values. Aerobic cycling exercise significantly increased cardiac output (from 1.5±0.4 to 3.2±0.6 $L.min^{-1}/m^{1.83}$), heart rate (from 61±10 to 110±9 $beats.min^{-1}$), and mean arterial pressure (from 83±8 to 111±12 mmHg) from rest.
When adjusted for the resting value, pregnant females had a greater heart rate and longitudinal strain during the pressure challenge compared to non-pregnant females (heart rate: 68±2 *versus* 62±2; longitudinal strain: -20.6±1.0% *versus* -17.1±0.7%, respectively). During aerobic cycling, there were no significant differences between groups.

Table One. Cardiovascular function at rest, during a sustained isometric hold, and during submaximal aerobic cycling activity in non-pregnant, pregnant and postpartum females.

		Non-pregnant (*n* = 18)		**Pregnant** (*n* = 14)		**Postpartum** (*n* = 13)
Cardiac output (L·min^{-1}/(m$^{1.83}$))	*Rest*	1.3 ± 0.2		1.8 ± 0.3	*	1.3 ± 0.3
	IH	1.3 ± 0.2		2.0 ± 0.3		1.4 ± 0.3
	CYC	3.0 ± 0.3		3.4 ± 0.7		3.0 ± 0.5
Heart rate (beats·min^{-1})	*Rest*	57 ± 8		69 ± 7	*	57 ± 10
	IH	59 ± 8		73 ± 9	∞	56 ± 9
	CYC	110 ± 10		113 ± 5		106 ± 10
Stroke volume (ml/(m$^{2.04}$))	*Rest*	20 ± 2		24 ± 3	†	22 ± 4
	IH	20 ± 3		26 ± 4		22 ± 5
	CYC	25 ± 3		27 ± 5		26 ± 4
End diastolic volume (ml/(m^2))	*Rest*	36 ± 4		42 ± 4	*	36 ± 5
	IH	37 ± 5		42 ± 5		37 ± 4
	CYC	56 ± 4		42 ± 5		37 ± 4
End systolic volume (ml/(m^2))	*Rest*	16 ± 3		17 ± 3		14 ± 3
	IH	16 ± 3		16 ± 3		13 ± 3
	CYC	11 ± 3		14 ± 4		10 ± 3
Peak longitudinal strain (%)	*Rest*	-17 ± 3		-22 ± 2	*	-19 ± 3
	IH	-16 ± 4		-21 ± 3	∞	-19 ± 2
	CYC	-20 ± 6		-22 ± 8		-19 ± 4
Torsion (°/cm)	*Rest*	1.7 ± 0.5		1.9 ± 0.5		1.9 ± 0.6
	IH	1.8 ± 0.5		1.8 ± 0.8		2.0 ± 0.8
	CYC	2.9 ± 0.8		2.9 ± 1.2		2.9 ± 0.9

N.B. All data presented is unadjusted for resting values. IH, sustained isometric hold at 30% maximum; CYC, aerobic cycling at 50% maximum peak power output; * indicates significantly different ($p < 0.05$) to all groups; † indicates significantly different ($p < 0.05$) to non-pregnant females; ∞ indicates significantly different ($p < 0.05$) to non-pregnant females when adjusted for resting value.

CONCLUSIONS

During the second trimester of healthy pregnancy, pregnant females have a greater resting cardiac output, heart rate and stroke volume compared to non-pregnant females, as shown within the literature (Sengupta et al., 2017). In contrast to previous findings (Sengupta et al., 2017), pregnant females appeared to have enhanced long-axis deformation as measured by longitudinal strain, and therefore had greater systolic function than non-pregnant females. In response to increased pressure, pregnant females had a greater heart rate and longitudinal strain compared to non-pregnant females. Global haemodynamics and left ventricular mechanics during aerobic exercise were similar in non-pregnant, pregnant and postpartum females.

The increased longitudinal strain is likely result of an increased heart rate during pregnancy. At rest and during increased pressure, pregnant females had a greater heart rate than non-pregnant females, leading to increased longitudinal strain. During aerobic exercise where heart rates were similar between groups, there were no differences in

longitudinal strain. Resting sympathetic activity is increased during gestation (Usselman et al., 2015), contributing to a higher resting heart rate. Aerobic exercise may attenuate the gestational rise in sympathetic innervation to result in similar cardiac function between non-pregnant, pregnant and postpartum females.

Functional responses to physiological challenges allow further insight into healthy adaptation to pregnancy. This dataset provides support that pressure and exercise challenges do not compromise the function of the maternal cardiovascular system during pregnancy. Inadequate responses to such challenges may be indicative of maladaptation to pregnancy and the later development of complications such as gestational hypertension and preeclampsia.

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Left ventricular mechanics in healthy females are not significantly altered in response to isometric handgrip

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Left ventricular (LV) mechanics characterize myocardial deformation across the cardiac cycle and are sensitive to changes in cardiac load. Previous research in a predominantly male cohort showed reduced LV mechanics during an afterload challenge mediated by isometric hand grip (IHG). There are known differences between male and female cardiac structure and function; it is possible that LV mechanics in females may respond differently to IHG.

PURPOSE: To quantify LV mechanics in healthy, young females during IHG.

METHODS: Healthy females (n=18, age 28±4 yrs) performed an IHG (30% maximal strength; 9±1 kg) for 5 min. Cardiac images were collected using echocardiography at i) REST, ii) DURING and iii) 5 min POST IHG and analyzed offline for longitudinal, circumferential and radial strain, rotation and twist using speckle tracking. Blood pressure was measured using photoplethysmography. Repeated measures ANOVA was used to identify significant differences with alpha set at 0.01.

RESULTS: Without significant change in heart rate or cardiac output ($P > 0.01$), systolic blood pressure was significantly increased DURING IHG compared to REST and POST measurements (SBP: 123±13 *vs.* 113±12 *vs.* 114±13 mmHg respectively, $P < 0.01$). Similarly, systemic vascular resistance was increased DURING IHG compared to REST measurements (2306±361 *vs.* 2125±312 dynes·sec·cm^{-5} $P < 0.01$, POST: 2246±274 dynes·sec·cm^{-6}) confirming that IHG augmented afterload. Except peak basal circumferential strain, there were no significant differences in LV mechanics from during to POST IHG (Table 1).

Table 1. Peak LV mechanics in response to isometric hand grip.

	REST	DURING	POST
Longitudinal strain (%)	-16.6 ± 3	-15.3 ± 4	-15.7 ± 3
Basal circumferential strain (%)	-15.8 ± 2	-13.9 ± 3 *	-16.4 ± 4
Apical circumferential strain (%)	-21.9 ± 5	-20.4 ± 5	-21.7 ± 5
Basal radial strain (%)	48.6 ± 15	45.9 ± 14	48.6 ± 14
Apical radial strain (%)	24.4 ± 10	25.0 ± 14	22.4 ± 9
Basal rotation (°)	-5.3 ± 3	-6.0 ± 4	-6.6 ± 3
Apical rotation (°)	8.3 ± 5	8.3 ± 4	8.9 ± 4
Twist (°)	14.9 ± 4	14.2 ± 5	14.2 ± 5

* $P < 0.01$ *vs*. POST.

CONCLUSION: In contrast to previous investigations in a predominantly male cohort, LV mechanics in healthy females do not appear to be markedly altered during acute IHG. These findings suggest that LV mechanics in response to an acute afterload challenge may be different between sexes.

CARDIOVASCULAR RESPONSES TO AEROBIC EXERCISE ARE SIMILAR IN NON-PREGNANT, PREGNANT AND POSTPARTUM FEMALES.

VL Meah, R Shave, K Backx, EJ Stöhr

Pregnancy presents a challenge on the female cardiovascular system. While the effects of pregnancy have been investigated extensively under resting conditions, this lacks insight into the functional responses of pregnant women to activities in daily life. Healthy non-pregnant (n=19), pregnant (n=9) and postpartum (n=7) women performed cycling exercise at 25 and 50% relative intensities. Global cardiovascular variables, septal tissues velocities (echocardiography) and blood pressure (finger photoplethysmography) were recorded at rest and during exercise. Mixed measures ANOVA was used to identify significant differences (P<0.05). No between-subjects differences in heart rate or blood pressure were observed, but increased with exercise. In comparison to non-pregnant and postpartum, pregnant women had significantly higher cardiac output (CO) at rest (3.1±0.4 and 2.9±0.5 *versus* 4.4±0.4 $L \cdot min^{-1}$, respectively) and during exercise bouts (25% exercise: 5.3±1.2 and 5.3±0.6 *versus* 6.9±1.1 $L \cdot min^{-1}$; 50% exercise: 6.6±1.1 and 6.8±0.6 *versus* 7.9±1.4 $L \cdot min^{-1}$, respectively). There were no significant differences between the magnitudes of change in CO from rest to exercise between groups. With no differences in heart rate, the higher CO was the product of a greater stroke volume during pregnancy. Septal systolic tissue velocity was significantly higher in pregnant women at rest and during exercise, but no between-group differences existed in diastolic tissue velocities. These data reveal that submaximal exercise during pregnancy presents similar cardiovascular responses to non-pregnant females and does not place undue challenge on the maternal cardiovascular system.

Appendix XVI – Journal articles relating to this thesis

MEAH, V. L., BACKX, K., DAVENPORT, M. H. & INTERNATIONAL WORKING GROUP ON MATERNAL, H. 2017. Functional Haemodynamic Testing in Pregnancy: Recommendations of The International Working Group on Maternal Haemodynamics. Ultrasound Obstet Gynecol.

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Guideline

Functional Haemodynamic Testing in Pregnancy: Recommendations of The International Working Group on Maternal Haemodynamics

Victoria L. Meah ✉, Karianne Backx, Margie H. Davenport,

On behalf of the International Working Group on Maternal Haemodynamics

Accepted manuscript online: 30 August 2017 Full publication history

DOI: 10.1002/uog.18890 View/save citation

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Accepted Articles

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Abstract

In the general population, functional haemodynamic testing, such as submaximal aerobic exercise, isometric handgrip, and the cold pressor test, has long been utilised to unmask abnormalities in cardiovascular function. During pregnancy, functional haemodynamic testing places additional demands on an already stressed maternal cardiovascular system. Dysfunctional responses to such tests in early pregnancy may predict the development of hypertensive disorders that develop later in gestation. These recommendations cover a description, an overview of the current understanding of clinical application, test protocol, equipment and considerations of each of the above functional haemodynamic tests during pregnancy.

MEAH, V. L., COCKCROFT, J. R., BACKX, K., SHAVE, R. & STÖHR, E. J. 2016. Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. Heart, 102, 518-26.

Downloaded from http://heart.bmj.com/ on January 22, 2016 - Published by group.bmj.com
Heart Online First, published on January 21, 2016 as 10.1136/heartjnl-2015-308476

ORIGINAL ARTICLE

Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/heartjnl-2015-308476).

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ABSTRACT

Objective Cardiac output, a fundamental parameter of cardiovascular function, has consistently been shown to increase across healthy pregnancy; however, the time course and magnitude of adaptation remains equivocal within published literature. The aim of the present meta-analyses was to comprehensively describe the pattern of change in cardiac output during healthy pregnancy.

Method A series of meta-analyses of previously published cardiac output data during healthy, singleton pregnancies was completed. PubMed and Scopus databases were searched for studies published between 1996 and 2014. Included studies reported absolute values during a predetermined gestational age (non-pregnant, late first trimester, early and late second trimester, early and late third trimester, early and late postpartum). Cardiac output was measured through echocardiography, impedance cardiography or inert gas rebreathing. Observational data were meta-analysed at each gestational age using a random-effects model. If reported, related haemodynamic variables were evaluated.

Results In total, 39 studies were eligible for inclusion, with pooled sample sizes ranging from 259 to 748. Cardiac output increased during pregnancy reaching its peak in the early third trimester, 1.5 L/min (31%) above non-pregnant values. The observed results from this study indicated a non-linear rise to this point. In the early postpartum, cardiac output had returned to non-pregnant values.

Conclusion The present results suggest that cardiac output peaks in the early third trimester, following a non-linear pattern of adaptation; however, this must be confirmed using longitudinal studies. The findings provide new insight into the normal progression of cardiac output during pregnancy.

INTRODUCTION

During pregnancy, progressive adaptation of the maternal cardiovascular system is necessary for fetal development and growth. As part of the many physiological adaptations occurring during pregnancy, the maternal heart undergoes major structural and functional changes. These changes occur to ensure adequate oxygen and nutrient delivery to the fetus. It is known that changes in cardiac function typically precede structural remodelling and, therefore, may be early markers of adaptation during pregnancy.[1 2] Cardiac output ($\dot{Q}$), a fundamental functional parameter, reflects the total demand placed on the maternal cardiovascular system. During pregnancy, this is increased due to the additional requirement for blood flow to the uterus/placenta, kidneys, breasts, skin and the heart itself.[1–3]

Despite a wealth of literature describing $\dot{Q}$ during healthy gestation, there is a lack of consensus in published literature regarding the time course of adaptation.[1 2 4–11] Previous reviews agree that $\dot{Q}$ increases across pregnancy; however, there are discrepancies regarding the magnitude and pattern of change after the second trimester.[2 11–13] Specifically, $\dot{Q}$ has been reported to follow three different patterns of change throughout pregnancy, namely: (1) a continued increase until term;[1 4 5] (2) a continued increase to peak in the latter half of pregnancy, after which $\dot{Q}$ decreases towards term[6 7] and (3) a continued increase to peak in the latter half of pregnancy, after which $\dot{Q}$ plateaus until term.[8–10] The contribution of the determinants of $\dot{Q}$ to the pregnancy-related adaptation also remains unclear.[11] The adaptation of $\dot{Q}$ may be driven by increases in blood volume and heart rate (HR), altered regulation of the autonomic nervous system or as a result of changes within the peripheral vasculature.[1 4–11 14–16]

Presently, the lack of certainty in the haemodynamic adaptation during healthy pregnancy impairs the understanding and, therefore, the early diagnosis of pregnancy-related cardiovascular complications, such as pre-eclampsia and gestational hypertension. To improve the current understanding of normal cardiac adaption to pregnancy, insight from larger cohorts with greater statistical power than typically possible within pregnancy research is required.[17] Therefore, the aim of this study was to perform a series of meta-analyses to determine the time course of adaptation in $\dot{Q}$ and related haemodynamics in response to healthy pregnancy.

METHODS

Ethical approval and search strategy

This study received ethical approval from the Cardiff Metropolitan University ethics board. A comprehensive literature search of the PubMed and Scopus databases for peer-reviewed publications examining the maternal cardiovascular responses to pregnancy was conducted. The pre-set search engine criteria, both on PubMed and Scopus, were restricted to studies using humans, women and publications written in the English language. Reviews, editorials, case reports and unpublished data were excluded. The keywords and phrases used in the online search included combinations of the words *cardiac output*, *maternal*, *cardiovascular*, *pregnancy*, *haemodynamic/hemodynamic*, *normotensive*, *and*

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HYPOTHESES

MATERNAL CARDIAC TWIST PRE-PREGNANCY: POTENTIAL AS A NOVEL MARKER OF PRE-ECLAMPSIA

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BACKGROUND

Cardiovascular function during normotensive vs. pre-eclamptic pregnancy

Healthy pregnancy is characterised by progressive physiological adaptation of the maternal cardiovascular (CV) system that facilitates optimal fetal development. The adaptations that constitute a healthy or normal progression are not always evident, and, in particular, CV adaptation to pregnancy is highly individualised. Some women develop pregnancy-related CV dysfunction such as pre-eclampsia (PE). Typically, PE is diagnosed by the development of hypertension and proteinuria after 20 weeks of pregnancy[1,2] and is the leading cause of maternal and perinatal mortality and morbidity[3]. Despite continued efforts to improve the understanding of the aetiology, pathophysiology and subsequently treatment for the disease, CV changes in PE are not well understood. PE before 34 weeks (early onset PE) is believed to differ in pathogenesis from late onset PE (>34 weeks) and can be characterised by a haemodynamic profile of increased systemic vascular resistance (SVR) and lower cardiac output (CO). Early onset PE is more often associated with uteroplacental insufficiency and significant adverse maternal and perinatal outcomes. In contrast, late onset PE (>34 weeks) involves an increased CO and lower SVR and is less likely to be associated with uteroplacental insufficiency and adverse perinatal outcomes[4]. It is not known if PE develops secondary to the CV maladaptation in pregnancy or if a preexisting CV dysfunction predisposes some women to develop PE[5]. Screening, diagnosis and disease management would be vastly improved if more were known about the onset of the maladaptive process associated with PE. To date, a combination of maternal factors including medical history, body mass index[6], age, parity[7] and

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