MATERNAL CARDIOVASCULAR DYSFUNCTION AND REMODELLING FOLLOWING PRETERM PRE-ECLAMPSIA

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List of Abbreviations

2D	2-dimensional
3D	3-dimensional
A2C	Apical 2 chamber
A3C	Apical 3 chamber
A4C	Apical 4 chamber
A5C	Apical 5 chamber
ACE	Angiotensin converting enzyme
ACh	Acetylcholine
AHA	American Heart Association
AIx	Augmentation index
AGA	Appropriate for gestational age
AHA	American Heart Association
APS	Antiphospholipid syndrome
ARB	Angiotensin II Receptor Blocker
ASE	American Society of Echocardiography
ASPRE	Aspirin for Evidence-Based Preeclampsia Prevention trial
AV	Atrioventricular
AVC	Aortic valve closure
AWTd	Anterior wall thickness in end-diastole
AWTs	Anterior wall thickness in end-systole
BMI	Body mass index
BNP	Brain natriuretic hormone
BP	Blood pressure
bpm	Beats per minute
BSA	Body surface area
BSE	British Society of Echocardiography
C1qKO	C1qa-deficient mice
C57BL/6J	Common inbred strain of laboratory mouse
CI	Cardiac index
C.I.	Confidence interval
CKD	Chronic kidney disease
CO	Cardiac output
CoV	Coefficient of variation
C-section	Caesarean section
dBP	Diastolic blood pressure
DCM	Dilated cardiomyopathy

DPA	Data Protection Act
EDTA	Ethylenediaminetetraacetic acid
E/A	Early to late diastolic filling ratio
EACI	European Association of Cardiovascular Imaging
EBB	Earle's bicarbonate buffer
EBL	Estimated blood loss
ECG	Electrocardiogram
ED	End-diastolic
ED_{VOL}	End-diastolic volume
E/E'	Early diastolic filling to early diastolic mitral annular velocity ratio
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
EME	Efficacy and Mechanism Evaluation
ES	End-systolic
ES_{VOL}	End-systolic volume
Fc	Carrier-free
FGR	Fetal growth restriction
Flt1	Membrane-bound fms-like tyrosine kinase 1
GD	Gestational day
gDM	Gestational diabetes mellitus
GDPR	General Data Protection Regulation
GLS	Global longitudinal strain
GP	General practitioner
GROW	Gestation Related Optimal Weight
HCl	Hydrogen Chloride
HDP	Hypertensive disorders of pregnancy
HELLP	Haemolysis, elevated liver enzymes, and a low platelet count
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HIF	Hypoxia-inducible factors
HOPE	Heart Outcomes Prevention Evaluation
HP LC-MS/MS	High-performance liquid chromatography-tandem mass spectrometry
HR	Heart rate
HRA	Health Research Authority
HREC	Human Research Ethics Committees
HS-cTnT	High sensitivity cardiac troponin T
HTN	Hypertension
ICC	Intraclass correlation coefficient

ICD	International statistical classification of diseases and related health problems
ICT	Isovolumetric contraction time
ID	Identity
IgG	Immunoglobulin G
IOA	Interobserver agreement
IRAS	Integrated Research Application System
ISSHP	International Society of the Study of Hypertension in Pregnancy
IU	International units
IVH	Intraventricular haemorrhage
IVR	Isovolumetric relaxation
IVRT	Isovolumetric relaxation time
IVSd	Interventricular septal thickness in end-diastole
IVSs	Interventricular septal thickness in end-systole
LA	Left atrium
LAV	Left atrial volume
LAVi	Left atrial volume index
LV	Left ventricular
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVIDd	Left ventricular internal diameter in end-diastole
LVIDs	Left ventricular internal diameter in end-systole
LVM	Left ventricular mass
LVMi	Left ventricular mass index
LVOT	Left ventricular outflow tract
MACE	Major adverse cardiac event
MAP	Mean arterial pressure
MHRA	Medicines and Healthcare products Regulatory Agency
mmHg	Millimetres of mercury
M-mode	Motion mode
NaOH	Sodium hydroxide
NEC	Necrotising enterocolitis
NHS	National Health Service
NICE	The National Institute for Health and Care Excellence
NICU	Neonatal intensive care unit
NO	Nitric oxide
NND	Neonatal death
NP	Non-pregnant
NTproBNP	N-terminal pro B-type natriuretic peptide

Nullip	Nulliparous
NYHA	New York Heart Association
OR	Odds ratio
PDVF	Polyvinylidene fluoride
PE	Pre-eclampsia
PICk-UP	Postnatal enalapril to Improve Cardiovascular fUnction following preterm Pre-eclampsia
pPE	Preterm pre-eclampsia
PLAX	Parasternal long axis
PlGF	Placental growth factor
PND	Postnatal day 1
PPCM	Peripartum cardiomyopathy
PPROM	Premature prolonged rupture of membranes
PSAX	Parasternal short axis
PWd	Posterior wall thickness in end-diastole
PWs	Posterior wall thickness in end-systole
PWV	Pulse wave velocity
RAAS	Renin-angiotensin-aldosterone system
RDS	Respiratory distress syndrome
RUPP	Reduced uterine perfusion pressure
RWT	Relative wall thickness
SAE	Serious adverse event
sBP	Systolic blood pressure
sBPao	Aortic systolic blood pressure
SDS	Sodium dodecyl sulphate
sENG	Soluble endoglin
sFlt	Soluble fms-like tyrosine kinase-1
SGA	Small for gestational age
SOLVD	Studies Of Left Ventricular Dysfunction
SR	Strain rate
SR_A	Late diastolic strain rate
SR_E	Early diastolic strain rate
$SR_{E/A}$	Early to late diastolic strain rate ratio
SR_{IVR}	Strain rate during isovolumetric relaxation
STE	Speckle tracking echocardiography
STOX1	Storkhead Box 1 gene
STRIDER	Maternal sildenafil for severe fetal growth restriction
SV	Stroke volume
TAPSE	Tricuspid annular plane systolic excursion

TBS	Tris-buffered saline
TDI	Tissue Doppler imaging
TGA	Transposition of the great arteries
TRUFFLE	Trial of Randomized Umbilical and Fetal Flow in Europe
TR Vmax	Tricuspid regurgitation maximum velocity
TVR	Total vascular resistance
UK	United Kingdom
uPCR	Urinary protein:creatinine
VEGF	Vascular endothelial growth factor
VTE	Venous thromboembolism
VTI	Velocity time integral
WHO	World Health Organization

Scientific abstract

Pre-eclampsia, a condition that affects 5% of pregnancies, is associated with postnatal cardiovascular dysfunction, remodelling and long-term cardiovascular risk. Women with preterm pre-eclampsia (requiring delivery <37 weeks) are at particular risk, with up to an eightfold risk of death from cardiovascular disease, compared to those with a normotensive pregnancy. Despite this association, the mechanism linking pre-eclampsia and cardiovascular morbidity is not known and interventional studies to reduce cardiovascular morbidity are lacking.

I hypothesised that pre-eclampsia (mediated by soluble fms-like tyrosine kinase-1 [sFlt]) causes maternal cardiovascular dysfunction, and that this can be ameliorated by postnatal administration of enalapril. In order to test this hypothesis I firstly aimed to explore the mechanistic link between preterm pre-eclampsia and maternal cardiovascular dysfunction, using multicentre retrospective clinical data, single centre prospective clinical data and an established sFlt-induced pre-eclampsia-like rodent model. My second aim was to explore the reversibility of cardiovascular dysfunction following preterm pre-eclampsia with six months' postnatal treatment with enalapril in a feasibility double-blind randomised controlled trial.

Attempts to recapitulate the animal model were unsuccessful and therefore mechanistic insights were extrapolated from clinical data. Pre-eclampsia prevalence in women with pre-existing cardiac dysfunction was comparable with the general population. Pre-pregnancy left ventricular function did not correlate with pregnancy outcome, however aspects of preterm pre-eclampsia severity (including birthweight centile and gestation at delivery) correlated with severity of postnatal cardiovascular dysfunction. Six months' postnatal treatment with enalapril was acceptable to women and was associated with significant improvement in diastolic dysfunction and left ventricular remodelling, compared to placebo.

Data from this thesis support the hypothesis that pre-eclampsia poses a direct insult on the cardiovascular system, although the mechanism linking the two requires further investigation. This body of work has demonstrated potential for postnatal enalapril to improve cardiovascular function and remodelling in the early postnatal period. Further work is needed to determine the longevity of improvement following treatment cessation and its long-term impact on cardiovascular risk.

Lay Abstract

Pre-eclampsia is a life-threatening pregnancy complication, which puts women at a significantly higher risk of death from future heart disease. Women who develop preterm pre-eclampsia (birth before 37 weeks), are at particular risk and are up to eight times more likely to die from heart disease. Despite this association, it is unclear whether heart disease is a cause or consequence of pre-eclampsia and efforts to improve heart health in women who have had preterm pre-eclampsia are currently lacking.

This body of work is based on the theory that pre-eclampsia causes a direct insult on the heart and that this can be treated after birth with enalapril (a commonly prescribed heart medication). In order to test this theory, I firstly aimed to explore the link between preterm pre-eclampsia and maternal heart injury, using routinely collected clinical information from multiple hospitals, prospective observational information from the clinical trial (PICk-UP) and an established rat model of pre-eclampsia. My second aim was to determine if treatment with enalapril after birth could improve heart health in women who have had preterm pre-eclampsia. This was assessed via a feasibility double-blind randomised controlled trial, in which women were blindly randomised to take enalapril or placebo for six months after birth.

The multicentre study showed that pre-eclampsia was not more common in women with pre-existing heart disease compared with the general population, implying that the relationship cannot be entirely explained by pre-existing disease or risk factors. The observational part of PICk-UP showed that some measures of pre-eclampsia severity (including birthweight and timing of birth) were associated with some aspects of worse heart health, highlighting a potential dose-effect of pre-eclampsia. Six months' treatment with enalapril after birth was acceptable to women and was associated with significant improvement in heart stiffness and thickness, compared to placebo.

These results support the theory that pre-eclampsia poses a direct insult on the heart, although the mechanism remains unclear. This body of work has demonstrated potential for enalapril to improve heart health after birth. Further work is needed to determine if the improvement lasts beyond stopping the medication and whether it infers any long-term impact on heart disease risk.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

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Publications arising from this thesis

Peer reviewed papers:

1. Ormesher L, Higson S, Luckie M, Roberts SA, Glossop H, Trafford A, Cottrell E, Johnstone ED, Myers JE. Postnatal Enalapril to Improve Cardiovascular Function following Preterm Preeclampsia (PICk-UP): A Randomized Double-Blind Placebo-Controlled Feasibility Trial. Hypertension. 2020;76:1828-1837. doi: 10.1161/HYPERTENSIONAHA.120.15875.

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2. Ormesher L, Higson S, Glossop H, Cottrell E, Trafford A, Luckie M, Johnstone E, Myers J. Do Features of Severe Preterm Pre-eclampsia Correlate with Magnitude of Postnatal Cardiac Dysfunction? Reproductive Sciences. 2020; 27:330A. doi: 10.1007/s43032-020-00176-9.

Preface

LO is a clinical trainee in Obstetrics and Gynaecology (ST3) who is currently Out of Programme for Research (OOPR). At the start of her OOPR she took up a split clinical/research role funded by Manchester University NHS Foundation Trust, during which she worked on two feasibility randomised controlled trials (investigating nutritional supplements in pregnancy) and two cohort studies (investigating the value of placental growth factor and pregnancy associated plasma protein-A in obstetric management). In that time she secured funding from the Medical Research Council for her current Clinical Research Training Fellowship, which has led to the work described in this thesis.

CHAPTER 1: INTRODUCTION

1.1. Overview

Pre-eclampsia is a condition in pregnancy, characterised by hypertension and proteinuria. It complicates ~5% of pregnancies globally[1] and is associated with significant perinatal and maternal morbidity and mortality[2]. A popular hypothesis is that failed maternal cardiovascular adaptation to pregnancy[3,4] contributes to impaired placental development and therefore the pathogenesis of pre-eclampsia. Furthermore, there is abundant observational data linking pre-eclampsia with postnatal maternal cardiovascular dysfunction[5–7] and long-term cardiovascular risk[8–17]. Long-term risk is particularly increased following preterm pre-eclampsia (requiring delivery before 37 weeks)[9,11,13]. Not only is cardiovascular disease more common in these women, but it tends to occur earlier and with a higher fatality rate[10]. Additionally, postnatal cardiovascular dysfunction is associated with an increased risk of pre-eclampsia recurrence[18]. Despite this wealth of data, clinicians' awareness of the long-term sequelae of pre-eclampsia is variable and risk-reduction counselling or intervention is often neglected[19].

Pre-eclampsia has also been linked with increased cardiovascular risk in the offspring[20]. This includes an increase in systolic blood pressure (sBP), diastolic blood pressure (dBP) and body mass index (BMI) in childhood[21] and a twofold increase in stroke[22] risk in adulthood, compared with those not exposed to pre-eclampsia *in utero*. Although the effect of *in utero* exposure to pre-eclampsia on the offspring's cardiovascular system warrants further investigation, this is beyond the scope of this doctoral thesis, which focuses on maternal cardiovascular function and remodelling.

Angiotensin II converting enzyme (ACE) inhibitors protect against ischaemia/reperfusion injury[23] and consequently reduce long-term cardiovascular risk when initiated soon after cardiovascular insult[24,25]. It is therefore plausible that postnatal ACE inhibitors will protect against cardiovascular injury following preterm pre-eclampsia. Since abnormal maternal cardiovascular function and adaptation to pregnancy might have aetiological roles in pre-eclampsia[3,18,26], improvement in postnatal cardiovascular function could also reduce the risk of pre-eclampsia recurrence.

The postnatal period provides an ideal window for intervention, with less pharmacological restrictions than antepartum. Despite this, the potential for postnatal intervention to correct cardiovascular impairment, and thereby influence long-term cardiovascular risk, has not been investigated[27].

1.2. Pre-eclampsia

Pre-eclampsia is a heterogeneous syndrome that is defined by clinical manifestations of endothelial dysfunction (proteinuria and hypertension) rather than the underlying pathology, which is poorly understood. The International Society for the Study of Hypertension in Pregnancy (ISSHP) has defined pre-eclampsia by the presence of the following clinical end-point: new or worsening hypertension after 20 weeks' gestation with proteinuria or other features suggestive of pre-eclampsia[28]. These other features include abnormal haematological or biochemical parameters or fetal growth restriction (FGR)[28]. Although abnormal angiogenic biomarkers are not included in the ISSHP definition, they are endorsed by the National Institute for Health and Care Excellence (NICE) guidance[29], due to their association with earlier pre-eclampsia diagnosis and improved maternal outcomes[30]. These definitions result in significant heterogeneity[31]. Pre-eclampsia is therefore increasingly recognised as at least two distinct phenotypes: preterm and term disease. These differ not only in gestational age at onset and delivery, but also fetal and maternal characteristics (including birthweight centile[32–34], maternal comorbidities[32,33], maternal cardiovascular dysfunction[35–37] and long-term maternal cardiovascular risk[9,11,13,14]). These phenotypic differences likely point toward at least two distinct pathophysiologies, as supported by Leavey *et al*.'s microarray analyses[38,39]. In their work, Leavey *et al*. have identified three subclasses of pre-eclampsia, including term pre-eclampsia (with mild disease and largely normal placentas); preterm pre-eclampsia (with FGR and placental dysfunction); and a novel third "immunologic" subclass (with FGR, less severe maternal disease and evidence of maternal-fetal incompatibility)[39]. Given the increased cardiovascular risk associated with the clinically-defined subclass preterm pre-eclampsia[9,11,13,14], this thesis will largely focus on preterm disease.

Multiple theories exist for the pathophysiology of pre-eclampsia. Probably the most widely accepted theory is the two-stage model, proposed by Redman more than 25 years ago[40]. In this model, the first stage of pre-eclampsia comprises of placental malperfusion[41], oxidative stress[40] and release of inflammatory factors into the maternal circulation[42]. This is followed by the second stage, in which placental-derived toxins cause maternal endothelial dysfunction[40]. Unlike term disease, preterm pre-eclampsia is more consistently associated with placental pathology[43] and lower birthweight[32]. Preterm pre-eclampsia also appears to have a distinct maternal cardiovascular phenotype, characterised by low-output, high-resistance haemodynamic status[26], mid-gestational diastolic dysfunction[3] and higher uterine artery resistance[33]. The increased prevalence of gestational diabetes[32] and long-term cardiovascular disease[8–17] in women with preterm pre-eclampsia might also reflect a pre-pregnancy maternal phenotype associated with preterm pre-eclampsia. This maternal phenotype likely contributes to both stages of the traditional two-stage hypothesis: impaired maternal cardiovascular performance leads to shallow trophoblast invasion and therefore a poorly perfused placenta (stage one)[44,45]; these constitutional factors then increase maternal sensitivity to placental oxidative stress (stage two: endothelial dysfunction)[46]. Conversely, term pre-eclampsia likely encompasses a variety of disease processes (with and without placental involvement) that result in maternal proteinuria and hypertension[47]. For those with term placental dysfunction, microvillous overcrowding and subsequent impaired intervillous perfusion[48] have been postulated as a cause. In this way, the functional demands of a previously healthy placenta surpass placental growth. These are less likely influenced by maternal cardiovascular phenotype.

1.3. Normal cardiac structure and function

The adult heart is a complex four-chambered muscle, derived from looping, twisting and segmentation of the primitive heart tube. For this reason, left ventricular muscle fibres change orientation from clockwise helices in the subendocardium to anticlockwise helices in the subepicardium[49]. This leads to an efficient “wringing” motion of the left ventricle during contraction (systole)[49]. The cardiac cycle, comprising of sequential ventricular filling and ejection, is a result of electrical excitation, myocardial contraction and pressure gradients. A clear understanding of the relationship between these components is key to

interpreting clinical measures of cardiac function. Although normal electrical communication and valvular structure are also required for cardiac muscle to perform efficiently, the proposed studies aim to modulate cardiovascular performance in terms of cardiac structure (remodelling), haemodynamics +/- contractility. Therefore, this thesis will largely focus on these properties.

The cardiac cycle comprises of seven phases[50], as illustrated in Figure 1.1. In phase one, atrial contraction (triggered by atrial depolarisation; P-wave on the electrocardiogram [ECG]), leads to a pressure gradient forcing blood into the ventricles. As the atria empty, pressure falls causing reversal of the pressure gradient and subsequent closure of the atrioventricular valves. Ventricular repolarisation (QRS complex on the ECG) triggers phase two (isovolumetric contraction), which leads to a rapid rise in pressure with a constant volume (as all valves are closed). Once the intraventricular pressures exceed aortic/pulmonary pressures, the respective valves open leading to rapid ejection of blood from the ventricles (rapid ejection; phase three). Subsequent ventricular repolarisation (T-wave on the ECG) reduces ventricular tension, thereby slowing ejection of blood (reduced ejection; phase four). Isovolumetric relaxation (phase five) then occurs as the aortic and pulmonary valves close due to a drop in intraventricular pressures[51]. Once the intraventricular pressures fall below atrial pressures, the atrioventricular valves open and rapid ventricular filling (phase six) occurs down the pressure gradients. The ventricles then become less compliant as they enlarge leading to reduced filling (phase seven).

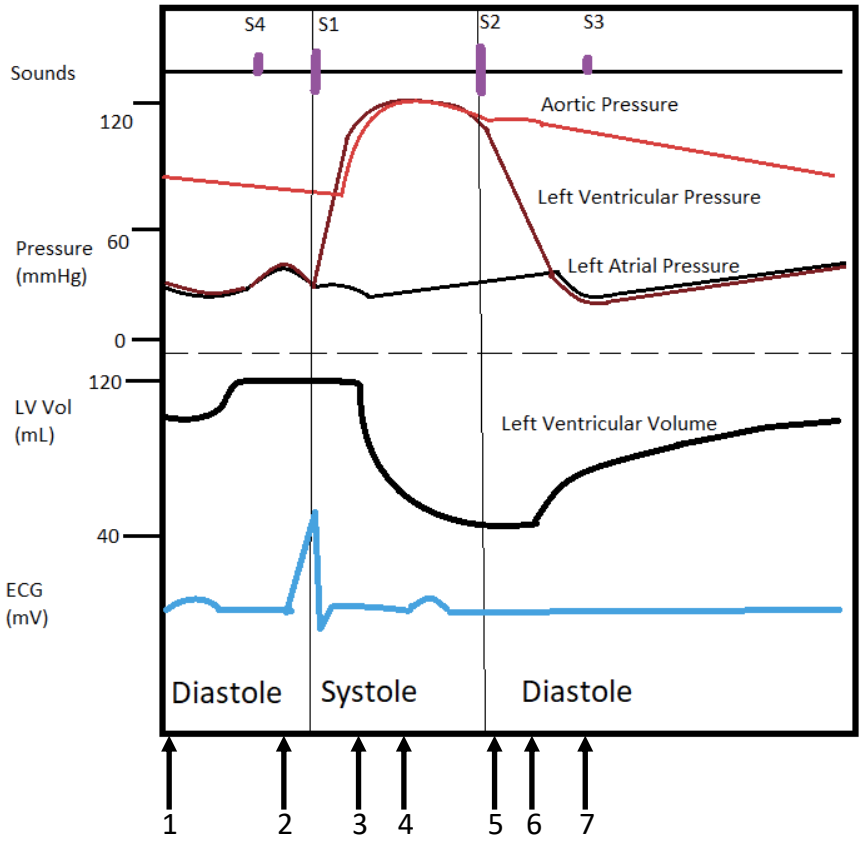

Figure 1.1: The seven stages of the cardiac cycle.
S1-S4 indicate the four heart sounds.
1-7 indicate the seven stages of the cardiac cycle.
mmHg, millimetres of mercury; LV Vol, left ventricular volume; ECG, electrocardiogram.
Adapted from Cardiovascular Physiology Concepts Second Edition published by Lippincott Williams & Wilkins, 2012[50].

Frank-Starling's law describes the positive relationship between myocardial fibre length and the force of contraction[52]. In this way, an increase in preload (left ventricular end-diastolic pressure and therefore stretch) leads to an increase in stroke volume (SV; volume of blood ejected by the left ventricle). LaPlace's law states that wall stress of any given chamber is determined by pressure, radius and wall thickness[53]. Accordingly, at a given pressure, afterload (the force that impedes left ventricular contraction / wall tension) is affected by the degree of left ventricular hypertrophy and dilatation. Therefore, myocardial performance (often clinically referred to as left ventricular ejection fraction [LVEF]) is dependent on intrinsic cardiac structure and contractility, and loading conditions[54].

Combining newer echocardiographic techniques (like speckle-tracking echocardiography [STE]) with cardiac biomarkers allows us to assess if alterations in cardiac performance are a direct effect (i.e. by altering contractility or remodelling) or indirect effect (i.e. by altering blood pressure (BP) and therefore afterload) of the disease or intervention on the heart.

1.4. Abnormal cardiac structure and function

1.4.1. Left ventricular remodelling

Left ventricular remodelling is the change in size and shape of the left ventricle in response to injury or load[55]. This definition comprises physiological and pathological remodelling, which differ in terms of stimuli and phenotype. As per LaPlace's law, wall stress is determined by pressure, radius and wall thickness[53]. In this way, increased haemodynamic load triggers a compensatory increase in left ventricular wall thickness aiming to reduce wall stress and optimise cardiac efficiency. In this early remodelling process, parallel addition of sarcomeres initially improves contractility[56]. Volume overload can trigger physiological remodelling, in which cardiomyocytes grow in length and width, thereby increasing left ventricular cavity and wall thickness proportionally[57] (eccentric hypertrophy; Figure 1.2). Physiological remodelling can also manifest as concentric hypertrophy (increase in left ventricular wall thickness relative to cavity size) as a result of pressure overload (i.e. strength training). Unlike pathological remodelling, physiological remodelling is accompanied by a proportional increase in angiogenesis and reversibility following stimulus removal[58]. Pathological remodelling has a number of potential triggers, including hypoxic injury and pressure overload due to hypertension[59]. It is initially characterised by a disproportionate increase in cardiomyocyte thickness, thereby increasing left ventricular wall thickness relative to cavity size (concentric remodelling / hypertrophy; Figure 1.2). Contrary to physiological remodelling, capillary density is not maintained, resulting in deficient oxygenation and nutrient transfer to the growing myocardium[58]. Pathological remodelling is also accompanied by interstitial fibrosis, upregulation of fetal genes, cardiomyocyte death and can subsequently progress to left ventricular dilatation, wall thinning and cardiac dysfunction[56].

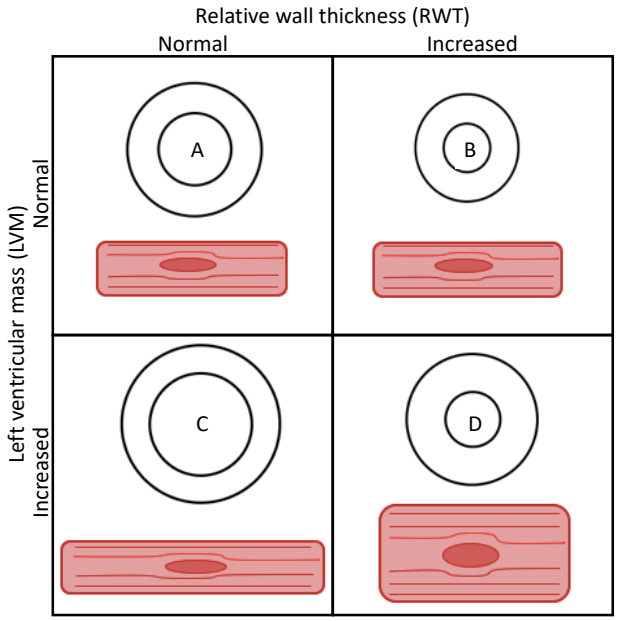

Figure 1.2: Categories of left ventricular remodelling.
A. No remodelling; **B.** Concentric remodelling (precursor to concentric hypertrophy; driven by pressure overload); **C.** Eccentric hypertrophy (secondary to volume overload); **D.** Concentric hypertrophy (driven by pressure overload).

1.4.2. Left ventricular diastolic dysfunction

Diastolic dysfunction describes a condition characterised by reduced left ventricular compliance, in which there is incomplete or delayed myocardial relaxation. It is quantified by left ventricular filling and pressure indices and can be attributed to abnormalities in cardiomyocytes (e.g. abnormal calcium homeostasis), extracellular matrix (e.g. fibrosis), neurohormonal activation (e.g. renin-angiotensin-aldosterone system [RAAS] overactivation) and endothelial function[60]. Endothelial dysfunction is thought to contribute to the pathogenesis of diastolic dysfunction via two potential mechanisms: 1) dysregulation of nitric oxide (NO) production and subsequent prolonged myocardial contraction, and 2) coronary endothelial dysfunction leading to subendocardial ischaemia[61]. Left ventricular hypertrophy (often accompanied by excess non-myocyte elements, including collagen) contributes to and correlates with impaired left ventricular filling[62,63]. Additionally, diastolic dysfunction is known to precede overt left ventricular remodelling[62,64,65], thereby highlighting a cyclical relationship between the two. Prolonged diastolic dysfunction and subsequent remodelling then have a negative impact on myocardial contractility, thereby progressing to systolic dysfunction[66]. On the other hand, a sufficient rise in left atrial pressure, as a result of diastolic dysfunction can

manifest as heart failure in the absence of systolic dysfunction. This is known as heart failure with preserved ejection fraction (HFpEF)[67].

1.4.3. Left ventricular systolic dysfunction

Systolic dysfunction describes impairment in myocardial contractility and can result from abnormal signal transduction mechanisms[68], loss of functional myocardium or myocardial structural changes (remodelling and fibrosis)[69,70]. Impaired contractility leads to a reduction in SV and subsequent incomplete ventricular emptying. This then contributes to an increase in end-systolic and end-diastolic (preload) volumes[71]. Compensatory mechanisms include increase in left ventricular cavity size, RAAS and sympathetic nervous system activation, aiming to preserve cardiac output (CO)[72]. Although initially compensatory mechanisms, these alterations in turn become pathological, leading to an increase in left ventricular wall stress and myocardial oxygen demand[71,73]. Left ventricular systolic dysfunction can then progress into heart failure with reduced ejection fraction (HFrEF) in which typical symptoms, including breathlessness and orthopnoea, prevail[74].

1.4.4. Right ventricular dysfunction

This body of work largely focuses on the left ventricle due to its role in supplying oxygenated blood to the systemic circulation and consequently the placenta. The left ventricle is also the focus of this thesis as persistent left ventricular dysfunction is more commonly associated with pre-eclampsia than its right counterpart[5]. A complete review of the pathophysiology of right ventricular dysfunction is beyond the scope of this report, however given the interdependence between the two ventricles, a brief summary of its causes and consequences is required. Left ventricular traction contributes ~30% of right ventricular output[75]; in this way left ventricular function directly impacts on right ventricular function. The right ventricle supplies the relatively low resistance pulmonary system, thereby requiring less myocardial work and subsequently a thinner wall than the high resistance left ventricle[76]. As a result, the right ventricle is less tolerant of pressure overload[77]. Failure to adapt to pressure overload (i.e. in pulmonary hypertension) results in ballooning of the right ventricle[78] and subsequent tricuspid regurgitation, further exacerbating the problem and leading to venous congestion[76,78]. Most commonly, however, right ventricular dysfunction co-exists with left ventricular dysfunction, either as

a direct consequence (secondary to left ventricular failure-induced pulmonary hypertension, decreased coronary perfusion or left ventricular dilatation constraining right ventricular filling) or due to shared aetiology (e.g. myocardial ischaemia or cardiomyopathy)[76]. Right ventricular failure is therefore commonly referred to as the "common final pathway", associated with poor prognosis and typically presenting with systemic venous congestion, resulting in peripheral oedema, ascites and weight gain[76,79–81].

1.5 Cardiovascular changes in normal pregnancy

1.5.1. Haemodynamics

The cardiovascular system rapidly adapts during pregnancy to meet the demands of the growing fetus[82]. For this reason, pregnancy has been described as a 'cardiovascular stress test'[83]: in those whose cardiovascular systems fail to adapt sufficiently, pregnancy complications, such as pre-eclampsia, can occur.

In a healthy pregnancy, maternal CO increases by ~45% by 24 weeks' gestation[84]. In the first trimester this is mostly secondary to reduced vascular resistance[85], which decreases until mid-second trimester to ~40% below baseline[84]. The resultant reduction in afterload stimulates the sympathetic nervous system, leading to an increase in heart rate (HR)[86]. This continues to rise throughout pregnancy, reaching 24% above baseline by late third trimester[85]. CO then decreases towards term, likely due to inferior vena caval compression and diversion of blood flow (up to 12% of the total CO) to the growing uterus[87]. The compensatory increase in HR minimises the functional effects of these two phenomena.

Maternal BP falls in early pregnancy with the biggest decline occurring at six to eight weeks' gestation[86]. It reaches a nadir of 5 to 10mmHg below baseline at 22 to 24 weeks' gestation[86]. BP then increases towards term, approaching pre-pregnancy levels[88]. Renal vasodilation stimulates the release of antidiuretic hormone and the RAAS[89]. The subsequent rise in blood volume contributes to an increase in glomerular filtration rate by 50% in the first trimester[86,90].

Overall, these changes contribute to a high-output, low-resistance state, which is critical for the increasing metabolic demands of the fetus. Failure to adapt in this way is associated with pregnancy complications, including pre-eclampsia[91].

After delivery, maternal haemodynamics return to baseline within six months[84,92], with most changes (including CO and LVEF) nearly normalising by as early as two weeks postpartum[93]. Although breastfeeding is associated with reduced maternal cardiovascular risk[94,95], evidence for its effect on postnatal haemodynamics is conflicting[96–98].

1.5.2. Cardiac remodelling

The heart adapts its morphology during pregnancy in response to the haemodynamic changes described above. In order to define "normal" gestational cardiac remodelling, de Haas *et al.* performed a meta-analysis of 48 longitudinal studies[6]. All measures were obtained using echocardiography, however there was significant heterogeneity in terms of the reference groups used (ranging from non-pregnant controls, pre-pregnancy and postpartum data from the same women)[6]. Since cardiac chamber dimensions do not return to baseline immediately postpartum, this led to relatively lower differences in studies using postpartum data as reference[6]. Contrary to previous belief, they found that normotensive pregnancies were associated with concentric cardiac remodelling, beginning in the second trimester and reaching a plateau in the third trimester[6]. Concentric remodelling, a precursor to concentric hypertrophy, is defined by an increase in relative wall thickness (RWT) and normal left ventriclar mass index (LVMi; Figure 1.2) and is an independent predictor of cardiovascular risk[99,100].

Gestational increases in left ventricular and left atrial volumes (13.6% and 4.6% respectively, compared with reference)[6] accommodate the physiological rise in preload. As previously mentioned, LaPlace's law[53] states that in order to maintain stable wall stress and myocardial oxygen demand, a compensatory increase in left ventricular wall thickness is required[6]. This is disproportionately increased in normal pregnancy, leading to a rise in RWT by approximately 10%, consistent with concentric remodelling[6]. Postnatal studies show that following normotensive pregnancies, these morphological alterations return to baseline by three to six months postpartum[92,101,102].

1.5.3. Cardiac contractility

Despite the demands of altered loading conditions and cardiac geometry, intrinsic cardiac function is not impaired during normal pregnancy[103]. In fact, Gilson *et al.* demonstrated enhanced contractility antenatally[104].

1.6. The role of NO in cardiovascular adaptations to pregnancy

NO is a potent vasodilator[105], which is raised in pregnancy[106], but reduced in pre-eclampsia[107]. It is essential for uteroplacental[108] and extra-uterine vascular adaptations, through vasodilation-induced BP reduction, and maintenance of vascular compliance and structure[105]. NO prevents platelet and leucocyte adherence to the vascular endothelium and proliferation of the smooth muscle cells that constitute the vascular wall[109]. These inhibitory processes prevent vascular thickening and atherosclerosis, which are key pathogenic steps in cardiovascular disease[110]. NO also plays an important role in optimising intrinsic cardiac function, by improving myocardial oxygen supply, limiting remodelling[111] and regulating contractility[112].

1.7. Cardiovascular changes in pre-eclampsia

1.7.1. Haemodynamics

As described above, preterm pre-eclampsia is associated with altered haemodynamics (relatively low-output, high-resistance) compared with normal pregnancy. The differences between cardiovascular changes in pre-eclampsia and uncomplicated pregnancies is summarised in Table 1.1. Melchiorre *et al*.[3] prospectively investigated subclinical cardiovascular changes in nulliparous normotensive women who later developed pre-eclampsia compared with those who did not. They found that increased total vascular resistance (TVR) and reduced cardiac index (CI) were particularly associated with preterm pre-eclampsia[3]. This demonstrates a subclinical high-impedance low-volume haemodynamic state in preterm pre-eclampsia. Similar, albeit milder, cardiovascular changes have also been seen in normotensive severe FGR[113]. Since the two disease processes share placental aetiology, this highlights the potential causative role of cardiovascular maladaptation in poor placentation and therefore preterm pre-

eclampsia[26]. Mid-gestation diastolic dysfunction, which affects a third of women destined to develop preterm pre-eclampsia[3], could be attributed to increased afterload and/or endothelial dysfunction (both of which are pathognomonic of pre-eclampsia).

Interestingly two recent observational studies found that fetoplacental characteristics, including birthweight, correlate more consistently with maternal haemodynamics than timing of pre-eclampsia onset / delivery[114,115]. They demonstrated a reduction in CO and increase in peripheral and total vascular resistance in small for gestational age (SGA) / FGR-associated pre-eclampsia, however in the absence of SGA/FGR, pre-eclampsia was associated with unchanged[115] or even increased[114] CO. A limitation of these studies is the use of non-invasive CO monitoring devices rather than echocardiography or cardiac magnetic resonance imaging[116,117].

Table 1.1: Cardiovascular changes in normotensive and pre-eclamptic pregnancy.

	Normotensive pregnancy	Pre-eclamptic pregnancy	Significance
Haemodynamics			
TVR	↓ by 40% by mid-second trimester[84]	↑ prior to the onset of PE[26]	p < 0.0001 (HDP with SGA) & p = 0.008 (HDP & AGA)[115]
CO	↑ by 45% by 24 weeks; then ↓ towards term[84]	↑ by less[3]	p = 0.8 (HDP AGA) & p = 0.007 (HDP SGA)[115]
SV	↑ 15-25% by 8 weeks	↑ by less[118]	p = 0.8 (HDP AGA) & p = 0.3 (HDP SGA)[115]
HR	↑ by 24% by late 3rd trimester[85]	↑ by less in HDP with SGA[115]	p = 0.40 (HDP AGA) & p = 0.09 (HDP SGA)[115]
BP	↓ by 5 - 10mmHg at 22-24 weeks; then increases towards term, approaching pre-pregnancy levels[86]	↑ prior to the onset of PE[88]	p < 0.01 (9-12 weeks)[88]
Blood volume	↑ plasma volume by 45.6%[6]	↑ by less[119,120]	p < 0.001[119]
GFR	↑ by 50% in 1st trimester[86,90]	↑ by less[121]	p = 0.0001[122]
Cardiac remodelling			
IVSd	↑ by 11.21% by 35/40 then 3.93% by term[6]	↑ by 19.74%[6]	p < 0.0001[6]
PWd	↑ by 9.71% by 35/40 then 9.01% by term[6]	↑ by 18.49%[6]	p = 0.0017[6]
RWT	↑ by 10%[6]	↑ by 56.0%[6]	p < 0.0001[6]
LV Mass	↑ by 23.6%[6]	↑ by 94.85%[6]	p < 0.0001[6]
LV remodelling	Concentric remodelling, beginning in 2nd trimester and reaching a plateau in 3rd trimester[6]	Majority have concentric hypertrophy; pPE is associated with severe LV hypertrophy in nearly 20% of cases[37]	p < 0.0001[123]
LVIDd	↑ by 4.55%[6]	↑ by 7.47%[6]	p = 0.96[6]
LVIDs	↑ by 4.38%[6]	↑ by 11.40%[6]	p = 0.15[6]
LA diameter	↑ by 13.62%[6]	↑ by 26.89%[6]	p = 0.02[6]
Intrinsic cardiac function			
Contractility	Preserved[103] perhaps enhanced[104]	↓ myocardial strain (impaired myocardial contractility) in pPE[124,125]; preserved contractility in term PE[124,125]	Longitudinal strain: p = 0.0009[126] Radial strain: p = 0.007[126] Circumferential strain: p = 0.03-0.04[126,127]
LVEF	Mixed reports[128]	No difference[126]	p = 0.52[126]
Diastolic function	Mixed reports[129–132]	Mid-gestation diastolic dysfunction affects a 1/3 women destined to develop pPE; biventricular global diastolic dysfunction is more common in pPE[3,37]	p < 0.05[133]

TVR, total vascular resistance; HDP, hypertensive disorders of pregnancy; SGA, small for gestational age; AGA, appropriate for gestational age; CO, cardiac output; SV, stroke volume; HR, heart rate; BP, blood pressure; mmHg, millimetres of mercury; GFR, glomerular filtration rate; IVSd, interventricular septal wall thickness in end-diastole; PWd, posterior wall thickness in end-diastole; RWT, relative wall thickness; LV, left ventricular; pPE, preterm pre-eclampsia; PE, pre-

eclampsia; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; LA, left atrium; LVEF, left ventricular ejection fraction.

1.7.2. Cardiac remodelling

De Haas *et al.*'s meta-analysis[6] attempted to define the difference between morphological alterations seen in hypertensive and healthy pregnancies. Their data for hypertensive disorders of pregnancy (n=739) comprised of ten studies reporting pre-eclamptic pregnancies and one reporting pregnancy-induced hypertension[134]. These studies only provided data from the time of diagnosis onwards. Therefore, we cannot know if these differences existed pre-pregnancy, nor can we explore gestational pre-disease remodelling. Despite these limitations, there are distinct differences between the two groups (Table 1.1). Women with hypertensive disorders of pregnancy have a disproportionate increase in left ventricular mass (LVM) and RWT (95% and 56%, respectively)[6]. This is likely secondary to increased afterload, as per LaPlace's law. Similarly, the left atrium is more enlarged than in healthy pregnancies[6], likely due to increased left ventricular thickness and subsequent reduced compliance. Cardiac remodelling deviates further from the norm in pregnancies complicated by preterm pre-eclampsia, which can be associated with severe left ventricular hypertrophy in nearly a fifth of cases[37].

1.7.3. Cardiac contractility

Preterm pre-eclampsia appears to be associated with impaired myocardial contractility, as demonstrated by reduced myocardial strain[37,126]. Under normal loading conditions, this is a precursor to diastolic and systolic dysfunction[37]. In term pre-eclampsia, contractility is preserved[124,125], indicating that the concentric remodelling in term disease is appropriately adaptive to the increased afterload. Compared with term disease, preterm pre-eclampsia is more frequently associated with biventricular global diastolic dysfunction and is uniquely associated with global biventricular systo-diastolic dysfunction[37]. This indicates maladaptive remodelling, in which cardiac performance is negatively affected. These phenomena could be explained by increased afterload, altered angiogenic profile and/or pre-pregnancy impairment (i.e. aberrant cardiovascular remodelling could be a cause and/or consequence of pre-eclampsia).

1.8. Cardiovascular dysfunction following pre-eclampsia

In normotensive pregnancies, maternal cardiovascular function and structure return to pre-pregnancy levels within 6 months postpartum[84,92,135–138]. Conversely, there is a plethora of observational data demonstrating persistent cardiovascular dysfunction and remodelling following pre-eclampsia[5,7,36,139].

Boardman *et al.*[139] highlighted distinct differences in cardiac remodelling and function five to ten years after pregnancies complicated by pre-eclampsia, compared with normotensive pregnancies. Pre-eclampsia was associated with a higher LVMi (49.9±7.1 versus 46.0±6.5g/m^2, p=0.001), worse global longitudinal strain (GLS; -18.3±4.5 versus -19.9±3.6%, p=0.02) and worse diastolic function (E/A [early to late diastolic filling ratio]: 1.34±0.34 versus 1.52±0.45, p=0.003). Although Boardman *et al*.[139] did not compare cardiac functional measures between women with early- and late-onset pre-eclampsia, they demonstrated no difference in cardiac remodelling between groups. On the other hand, both Melchiorre and Soma Pillay demonstrated clear functional differences between groups at one year postpartum[5,36]. Preterm pre-eclampsia (delivery <37 weeks) was associated with a higher prevalence of diastolic dysfunction (51.9% versus 16.2%, p=0.006) and a trend towards a higher prevalence of altered left ventricular geometry (40.7% versus 18.9%, p=0.1)[5]. The lack of statistical significance is likely due to insufficient numbers (n=64). Similarly, in Soma-Pillay *et al*.'s study (n=96)[36], early-onset pre-eclampsia (delivery <34 weeks) had a relative risk of diastolic dysfunction of 3.41 (1.1-10.5) compared with late-onset (delivery >34 weeks) pre-eclampsia. Importantly, postnatal differences in left ventricular remodelling and diastolic function are predictive of later development of hypertension[140]. For this reason, they are likely important indices to target in the postnatal period.

1.9. Mechanistic link between pre-eclampsia and cardiovascular dysfunction

In support of the hypothesis that cardiovascular dysfunction is a cause of pre-eclampsia[141], women identified as having cardiovascular dysfunction in the interval between pregnancies are at an increased risk of pre-eclampsia recurrence[18,142]. A postnatal case control study of 75 normotensive women with previous preterm pre-eclampsia demonstrated a difference in cardiovascular function between those who went

on to develop recurrent pre-eclampsia and those who did not[18]. TVR was the best independent predictive factor of recurrent pre-eclampsia (77% positive predictive value for adverse outcome with a cut-off of 1400 dyne.$s^{-1}cm^{-5}$)[18]. Given this data, it is plausible that the risk of pre-eclampsia recurrence could be reduced by correcting postnatal cardiovascular dysfunction, in particular, TVR. Pre-eclampsia and cardiovascular disease share common risk factors, including increased maternal BMI and age, ethnic origin, diabetes and pre-existing hypertension[143]. However, the fact that pre-eclampsia increases long-term cardiovascular risk, independent of these mutual risk factors[144,145] points towards both a causal and consequential relationship between the two.

An increased prevalence of pre-eclampsia in women with pre-existing cardiac dysfunction would also support a causal role of cardiac dysfunction in the development of pre-eclampsia. Despite a number of large retrospective registry studies[146–152] investigating obstetric outcomes in women with known cardiac disease, pre-eclampsia prevalence was not the primary focus of these studies. For this reason, pre-eclampsia prevalence was not correlated with cardiac functional measures nor adjusted for known risk factors, making it difficult to explore the nature of the relationship between the two. Additionally, results are conflicting, with some studies demonstrating an increased prevalence[146,148–151] and others not[147,153–155].

The mechanism by which pre-eclampsia might be a cause of cardiovascular dysfunction is poorly understood. Shahul *et al*.[156] proposed a mechanistic link through soluble fms-like tyrosine kinase-1 (sFlt). sFlt is a truncated splice variant of the main vascular endothelial growth factor (VEGF) receptor. VEGF is pro-angiogenic and is a promoter of NO signalling [157,158]. It also has a role in glomerular healing[159–161], inhibiting leakage of protein from the glomerular capillaries. sFlt inhibits VEGF and placental growth factor (PlGF) activity by binding to them in circulation, preventing their interaction with membrane-bound fms-like tyrosine kinase 1 (Flt1)[162]. It also forms a heterodimer with the VEGF receptor, thereby inhibiting post-receptor signal transduction[163]. By antagonising VEGF and PlGF, sFlt induces a pre-eclampsia-like phenotype with endothelial dysfunction, hypertension and proteinuria[164]. sFlt is primarily produced by villous and extravillous placental trophoblasts[165], but in smaller amounts by extra-placental endothelial cells[166] and

monocytes[167,168]. It is directly upregulated by placental hypoxia[169] and therefore pre-eclampsia[164], due to hypoxia-inducible factors (HIF) binding to the promoter region of the Flt1 gene. Evidence also suggests sFlt upregulation via hypoxia-independent pathways[170–172], which are less well understood.

In non-pregnant animals, sFlt administration causes deficient cardiac microvasculature and subsequent hypoxia-induced cardiac remodelling[173]. In this way, sFlt appears to be both a cause and consequence of hypoxia. It is predictive of heart failure[174–176] and has been positively linked with arterial aging[177]. Given its role in endothelial dysfunction and arterial aging, it is plausible that sFlt could be the mediator of cardiovascular dysfunction in pre-eclampsia. In Shahul *et al.*'s[156] prospective study of hypertensive disorders of pregnancy, third trimester sFlt levels were independently correlated to GLS (a sensitive technique for assessing subtle changes in left ventricular function). This correlation persisted after adjusting for age and other medical confounders[156], demonstrating a plausible aetiological role of sFlt in preterm pre-eclampsia-related cardiovascular dysfunction. More recently, Garrido-Gimenez *et al*.[178] demonstrated a correlation between antenatal sFlt levels with cardiovascular risk factors, including lipid profile, left ventricular remodelling and carotid intima-media thickness, ~12 years after delivery. This further supports an aetiological role of pre-eclampsia-related anti-angiogenic factors in the development of long-term cardiovascular risk.

1.10. Long-term cardiovascular risk in women with pre-eclampsia

There is a plethora of data demonstrating increased short-[179] and long-term[8–17] cardiovascular risk associated with pre-eclampsia, with 40% of women with preterm pre-eclampsia developing hypertension within one to two years postpartum[5]. Women with pre-eclampsia also have increased arterial aging[177], with micro- and macro-vascular changes being seen during pregnancy and up to 25 years later[7,180,181]. These changes represent early markers of cardiovascular risk[182]. Other associated risks include: type II diabetes, venous thromboembolism and a twofold risk of ischaemic heart disease and cerebrovascular accident[8–10,16,183]. These associations persist despite accounting for mutual risk factors, including age, obesity and pre-pregnancy hypertension[144].

Cardiovascular risk is graded in terms of severity and recurrence of pre-eclampsia. Presence of severe features[8,10,12,15–17], prematurity[9,11,13,14], SGA[15,184] and pre-eclampsia recurrence[17,185] are associated with particularly increased cardiovascular risk. Despite this, women with gestational hypertension alone[12], are still at increased risk of cardiovascular disease, compared to women with previous normotensive pregnancies. Compared with normotensive term pregnancies, term and preterm pre-eclampsia are associated with 1.65-fold and 8.12-fold risk of death from cardiovascular disease, respectively[11]. This may reflect mutual risk factors however the dose-effect suggests a direct insult of pre-eclampsia on the cardiovascular system.

1.11. Cardioprotective role of ACE inhibitors

There is extensive evidence to support the cardioprotective effects of ACE inhibitors. Importantly cardioprotection has been proven even in normotensive subjects with normal LVEF[186]. The HOPE study[187], which randomised patients at high risk of cardiovascular disease to ramipril or placebo, was stopped prematurely due to the 22% reduction in myocardial infarction / cerebrovascular accident / death from cardiovascular disease. This was irrespective of hypertension or other confounders[187]. ACE inhibitors provide cardioprotection through a variety of mechanisms, including anti-inflammatory effects[188], increased NO bioavailability[189] and diminished fibrosis[190]. These are all relevant to pre-eclampsia which has an inflammatory component[46] and is associated with reduced NO bioavailability[191] and vascular fibrosis[192]. ACE inhibitors increase NO production 1) directly (by inhibiting the depleting effect of angiotensin) and 2) indirectly (by inhibiting breakdown of bradykinin, which stimulates NO release)[193]. In canine studies, enalapril increased NO production in coronary vessels, leading to vasodilation and reduced myocardial oxygen consumption[194]. Given the role of NO in cardiovascular remodelling and function described earlier, many therapeutic trials have targeted the NO pathway to improve (cardio)vascular function in[195] and outside of[196] pregnancy.

ACE inhibitors consequently improve endothelial-dependent vasodilation, inhibit atherogenesis and protect against ischaemia/reperfusion injury. Since sFlt causes endothelial dysfunction and microvascular vasoconstriction, it is plausible that ACE inhibitors would ameliorate sFlt (and therefore preterm pre-eclampsia)-induced

cardiovascular dysfunction, in part by increasing NO bioavailability. Since preterm pre-eclampsia is associated with excessive concentric remodelling[6], it is important to note that ACE inhibitors have been shown to reduce left ventricular mass by 40% in hypertensive patients with left ventricular hypertrophy, into a normal range[197]. This could be explained by a combination of reduced afterload[198] and an antigrowth effect of bradykinin on the adult heart[199,200]. By lowering arterial pressures, ACE inhibitors reduce afterload, which is raised and contributes to cardiovascular dysfunction in preterm pre-eclampsia. Finally, ACE inhibitors improve myocardial contractility[201], which can be reduced in preterm pre-eclampsia[5,37,126]. They do this by preserving myocardial collagen matrix and improving myocyte relaxation properties[201].

Alternative cardioprotective agents exist, including ß blockers, antiplatelets and statins[202]. Each of these agents provide cardioprotection via different mechanisms. ß blockers reduce HR and sBP, thereby reducing myocardial oxygen demand. Similar to ACE inhibitors, they are safe in breastfeeding[29]; however they are inferior to ACE inhibitors in terms of reversal of left ventricular remodelling[203]. Antiplatelets prevent platelet aggregation and subsequently reduce the risk of vascular thrombosis; they are frequently used in secondary prevention and less commonly in primary prevention[204]. Statins, commonly known for their lipid-modulating properties, improve endothelial function via lipid-independent and dependent mechanisms[205]. However, evidence for the effectiveness of statins in primary prevention of cardiovascular disease is conflicted[206]. Despite these alternatives, an ACE inhibitor was deemed the most appropriate first-line cardioprotective drug to trial in the context of preterm pre-eclampsia-related postnatal cardiovascular morbidity. This is due to a superior effect of ACE inhibitors on left ventricular mass[197,203]; proven cardioprotection in normotensive patients with normal cardiac function[186]; and multiple mechanistic actions that target pathological processes[188–190] known to occur in preterm pre-eclampsia[46,191,192]. ACE inhibitors are safe[207], affordable and widely used for their antihypertensive properties in the postnatal period[29,208]. For this reason, they could be readily established into clinical practice, if effective cardioprotection is demonstrated following preterm pre-eclampsia.

1.12. Animal models of pre-eclampsia

Animal models have long been used to explore disease and therapeutic mechanisms. They offer the advantage of reduced biological variability compared with humans, due to environmental controls and reduced genetic variation[209]. Although each species has distinct physiologies, there is often enough overlap to translate animal model discoveries to human research[210,211]. Pre-eclampsia is unique to primates[212], but given the financial and ethical limitations in primate research[213], rodent models have frequently been used in their place.

Current experimental models of pre-eclampsia involve a variety of pathogenic mechanisms, aiming to recapitulate only a part of the pre-eclampsia-like phenomenon. These include abnormal trophoblast invasion[214], immune response[215], placental insult[172,216] and anti-angiogenic models, including elevated levels of sFlt[135–138] or soluble endoglin (sENG)[217]. As a result, these models do not mimic human pre-eclampsia in its entirety and so findings cannot always be directly translated to humans.

Rodents follow a similar haemodynamic antepartum and postpartum course as humans, with an antenatal rise in CO, left ventricular volume and blood volume and decrease in BP[218–220]; however, evidence for an increase in HR is conflicting[218,219]. Postpartum, haemodynamic changes return to pre-pregnancy levels by approximately 17 days postpartum[219]. These similarities highlight the potential value of rodent models in pre-eclampsia-related cardiovascular research. Several animal models have investigated potential causality of pre-eclampsia for long-term cardiovascular risk, with mixed results[221–225]. Garrett *et al.*[223] demonstrated that C57BL/6 wild-type mice, who developed a pre-eclampsia-like phenotype during pregnancy following mating with immunological impaired transgenic males (C1qKO), had persistent endothelial damage, raised angiotensin II and aortic stiffness postpartum. Similarly, Miralles *et al.*[225] sought to explore the mechanism responsible for pre-eclampsia-related long-term cardiovascular risk, using the Storkhead Box 1 (STOX1) mouse model. STOX1 is a protein-coding gene, known to regulate reactive oxidative and nitrogen species in placental mitochondria[226]. Mutations of this gene have been linked to pre-eclampsia[226]. In this model, wild-type females are mated with STOX1-overexpressing males, to produce a pre-eclampsia-like

phenotype. They demonstrated that eight months postpartum, female mice had persistent left ventricular hypertrophy and fibrosis[225]. These findings were associated with genetic deregulation in endothelial cells and the heart and discrete differences in eight cytokines, indicating long-term cardiovascular consequences following STOX1-induced pre-eclampsia[225]. Additionally Pruthi[221] and Bytautiene[222] demonstrated increased vascular reactivity and altered proteome indicative of cardiovascular disease, following sFlt-induced pre-eclampsia, respectively. On the other hand, using the same model, Bytautiene *et al.*[224] demonstrated no difference in vascular function six to eight months postpartum, as measured by telemetry-derived BP and wire myography-derived carotid artery reactivity. In this thesis, I have focused on three of the most widely used pre-eclampsia-like rodent models, including the reduced uterine perfusion pressure (RUPP)[227], endothelial NO synthase (eNOS) knockout[191] and sFlt models[135–138].

1.12.1. RUPP

The RUPP model has been used in many species, although most commonly in the rat[228]. This model was motivated by the hypothesis that uteroplacental malperfusion causes the characteristic features of pre-eclampsia. In the pregnant rat, RUPP is induced by clipping the infrarenal aorta and bilateral ovarian arteries[227], leading to a ~40% reduction in uterine blood flow[229]. This model is associated with the typical pre-eclampsia-like syndrome of hypertension, proteinuria, impaired renal function and FGR[228]. Interestingly it also mimics human pre-eclampsia-related cardiovascular dysfunction (increased TVR and reduced CI, LVEF and fractional shortening)[230,231] and angiogenic imbalance (reduced PlGF and increased sFlt)[172].

1.12.2. eNOS knockout

Human pre-eclampsia is associated with reduced NO bioavailability[191]. For this reason, pregnant mice deficient for the eNOS gene were developed to attempt to phenotypically mimic pre-eclampsia[232]. They demonstrated impaired cardiovascular and uteroplacental adaptation to pregnancy[218], however the classic pre-eclampsia phenotype of gestational hypertension and proteinuria is not reliably reproduced with this model[232].

1.12.3. sFlt-induced pre-eclampsia-like models

sFlt is raised in pre-eclampsia and successfully induces a pre-eclampsia-like phenotype in rodents[135–138]. sFlt can be administered via adenoviral vector[138] or osmotic minipumps[135–137]. An infusion rate of 3.7µg/kg/h[136,137] via osmotic minipumps has been associated with a threefold increase in circulating sFlt levels in pregnant Sprague-Dawley rats[137], which is comparable with human pre-eclampsia[164]. Using the sFlt-induced pre-eclampsia-like mouse model, Pruthi *et al.*[221] demonstrated increased vascular reactivity, compared with normotensive controls. This was evident by a relative increase in smooth muscle cell proliferation, vessel medial area and fibrosis in response to left carotid artery injury by endothelial denudation[221]. Using the same model, Bytautiene *et al.*[222] investigated the effect of experimental pre-eclampsia on the maternal proteome. They analysed 105 peptides, which were categorised by function into 75 groups[222]. They demonstrated that the category most affected by exposure to experimental pre-eclampsia was cardiovascular disease (in particular, vascular disease and atherosclerosis)[222]. As previously mentioned, di Marco *et al.*[173] linked sFlt and cardiovascular disease in non-pregnant Sprague-Dawley rats. Their study supported the potential contribution of sFlt to cardiovascular dysfunction and demonstrated feasible methods of interrogating cardiovascular sequelae of sFlt in this species. These include assessment of cardiac microvascular morphology using electron microscopy, and quantification of capillary density and fibrosis using Isolectin B4 and Sirius Red staining, respectively[173]. Neither this model, nor Granger's model of pre-eclampsia[136], which uses sFlt-secreting minipumps in pregnant Sprague-Dawley rats, have explored long-term cardiovascular sequelae.

Rats offer many advantages over mice for *in vivo* experiments: they have a larger blood volume, enabling serial biomarker measurements; their cardiovascular physiology is closer to that of humans; and their size and slower HR facilitate studies including echocardiography[233]. The sFlt-induced pre-eclampsia-like model in Sprague-Dawley rats[135–137] has been used in this thesis, aiming to test the hypothesis that sFlt is a mediator of cardiovascular dysfunction in preterm pre-eclampsia. The investigative techniques used by Di Marco *et al.,* combined with echocardiography and cardiovascular biomarkers were planned to assess the direct effect of sFlt on the heart, in a pre-eclampsia-like model.

1.13. Investigative techniques

Cardiovascular structure and function can be non-invasively assessed in numerous ways. The most widely used non-invasive imaging technique is echocardiography, which is readily available and affordable. Direct assessment of intrinsic cardiac function is difficult as echocardiographic measurements are largely affected by loading conditions[234]. Use of specific cardiovascular biomarkers along with newer imaging techniques like STE allows us to explore the possible mechanisms of altered cardiac morphology and performance in the context of preterm pre-eclampsia.

1.13.1. Biomarkers of cardiovascular function

1.13.1.1. PlGF

PlGF is a pro-angiogenic factor secreted by the placenta[235]. It is also expressed in skeletal muscle, thyroid, heart and lungs[236]. Outside of pregnancy, evidence for PlGF's role in cardiovascular disease is conflicting: some studies support its protective role[237–239], whilst others have identified it as a risk factor[240–242]. PlGF is increased in pregnancy[243], but relatively reduced in pre-eclampsia[243–247]. PlGF levels decline after pregnancy, with a half-life of 3.7 days[248]. Postnatal longitudinal PlGF data is lacking and little is known about the effect of ACE inhibitor use on PlGF levels.

1.13.1.2. sFlt

sFlt is a potent antiangiogenic factor. During pregnancy, sFlt levels increase from 33 weeks until delivery[243]. This rise is exaggerated in pre-eclampsia[243,244,246]. Although sFlt rapidly declines postpartum[248], women with previous pre-eclampsia have persistently raised sFlt compared to those with a normotensive pregnancy [181,249]. Wolf *et al.*[249] compared postnatal sFlt levels in women with previous pre-eclampsia with those with an uncomplicated pregnancy. Women with previous pre-eclampsia had persistently higher sFlt levels ~18 months postpartum (41.6±6.7 versus 30.4±10.2pg/mL; $p<0.01$)[249]. Similarly, Kvehaugen *et al.*[181] measured circulating sFlt five to eight years postpartum and demonstrated a trend towards higher sFlt levels in those with a history of pre-eclampsia, compared to those with a normotensive pregnancy (79.7±15.0 versus 70.9±11.2pg/mL;

p=0.05)[181]. As discussed previously, sFlt has been linked to cardiovascular risk[174–176] and could be the mediator of cardiovascular dysfunction in the context of preterm pre-eclampsia.

1.13.1.3. N-terminal pro B-type natriuretic peptide (NTproBNP)

Ventricular production of NTproBNP (a prohormone of brain natriuretic peptide [BNP]) is upregulated in response to wall stress[250]. Levels therefore reflect cardiac remodelling[250], pressure[251] and volume[252] status and are predictive of heart failure prognosis in general and at-risk populations[253]. BNP[254,255] and NTproBNP[256] levels are relatively raised in pre-eclampsia and remain so for three to six months postpartum[257]. This is likely due to pressure overload and secondary remodelling. ACE inhibitors are known to reduce BNP in heart failure patients[258,259] as early as six to twenty-four hours post-administration[259]. In elderly patients with heart failure, eight month treatment with quinapril was associated with 67.4% reduction in BNP[258]. It is therefore plausible that postnatal enalapril could reduce NTproBNP following preterm pre-eclampsia.

1.13.1.4. High sensitivity cardiac troponin T (HS-cTnT)

Cardiac troponin T is a regulatory protein specific to the myocardium[260]. For this reason, varying sensitivity troponin assays are used clinically to detect myocardial damage[260]. HS-cTnT is detectable in 78% of people with essential hypertension and correlates with left ventricular hypertrophy[261]. Although troponin is raised in pre-eclampsia[262], no longitudinal or postnatal data currently exist. Inclusion of HS-cTnT in the postnatal assessment of women with preterm pre-eclampsia has never been done, despite enabling detection of subtle cardiomyocyte damage and potentially, like in myocardial infarction[263,264], amelioration with ACE inhibitors.

1.13.2. Clinical cardiovascular measurements

1.13.2.1. BP

The diagnosis or surveillance of hypertension and therefore pre-eclampsia, is complicated by the differing accuracy of devices[265,266], diurnal variation[267,268] and the effect of white coat hypertension[269]. Therefore, a single BP reading has significant limitations[270,271].

The oscillometric technique of brachial BP measurement, which has been used for the purposes of this work, appears to be most representative of central BP[272]. This technique relies on transduction of electrical signals from oscillations in the vessel wall. Both Microlife 3AS1-2[273] and Microlife 3BTO-A[274] are oscillatory devices that have been validated for use in and out of pregnancy and in pre-eclampsia, according to the British Hypertension Society protocol.

1.13.2.2. Arteriography

Arteriography is a validated method of assessing pulse wave velocity (PWV) and augmentation index (AIx)[275,276]. It uses oscillometry to assess pulsatile pressure changes in the brachial artery, whilst a continuous suprasystolic pressure is applied via the upper arm cuff[275]. These measures of arterial stiffness accurately correlate with endothelial dysfunction[277,278] and are therefore useful tools in the assessment of pre-eclampsia and cardiovascular risk. Particular advantages of oscillometric arteriography are that it is operator-independent and relatively affordable given the lack of consumables and need for expertise[278].

1.13.2.3. Echocardiography

Echocardiography is well-established in the assessment of cardiac structure and function. It is safe and affordable; however, its reliability depends on what is being measured and how. The left ventricle comprises of three continuous layers of muscle fibres with changing orientation from radial to circumferential to longitudinal[279]. Given this myoarchitecture, the left ventricle shortens, thickens and rotates during systole. Therefore, in order to accurately assess left ventricular function, segmental wall motion needs to be visualised in each direction throughout the cardiac cycle. In-built algorithms are then required to convert the 2-dimensional (2D) images to 3-dimensional (3D) measures. To account for potential algorithm errors, an integrative assessment of left ventricular function should include several different methods.

Such methods include qualitative and quantitative assessment of 2D, 3D, STE, Doppler and motion mode (M-mode) ultrasound. All segments of the left ventricular wall can be visualised by a combination of parasternal long- and short-axis (PLAX, PSAX) and apical 2-,

3-, 4- and 5-chamber (A2C, A3C, A4C, A5C) views. Combining pulse wave Doppler with 2D images permits haemodynamic measurements including SV and CO. These endpoints require measurement of the left ventricular outflow tract (LVOT) diameter, velocity time integral (VTI) of the Doppler signal through the LVOT +/- HR. Since these measures are dependent on several steps, variability between measurements is significant (SV inter- and intra-observer intraclass coefficients (ICC) of 0.72 and 0.81, respectively[280]).

1.13.2.4. Systolic function

LVEF, which is the proportion of end-diastolic (ED) volume of blood ejected by the left ventricle ([(ED_{vol} - ES_{vol})/ ED_{vol}] x 100), is frequently used in clinical practice as a measure of cardiac performance and can be calculated using a variety of methods. These measures can be quite subjective, as they rely on tracing the left ventricular contours, which are often poorly defined. The preferred method is Simpson's biplane[281], which has an interobserver ICC of 0.87[282]. Such subjectivity has driven software development to recognise and label segments of the left ventricular wall. Ultrasound refraction, reflection and scattering lead to speckle artefacts in the myocardium[283]. Echocardiography software can now identify and track myocardial segments by their speckle pattern, allowing assessment of regional left ventricular wall motion, speed and thickening throughout the cardiac cycle. This is used to calculate the degree of deformation/strain over time, with negative strain referring to myocardial shortening (contraction) and positive to lengthening (relaxation). Using echocardiography loops in A2-, 3- and 4-C views, STE can assess longitudinal deformation of all left ventricular wall regions. The subendocardium, which largely governs longitudinal function, is most susceptible to ischaemic injury due to its relatively reduced blood supply[284]. This discrepancy in perfusion is further accentuated by left ventricular wall thickening[284], which occurs in pressure loaded conditions, like pre-eclampsia[6]. For this reason, longitudinal strain is a sensitive marker of subclinical left ventricular dysfunction, particularly in the context of increased afterload[285]. It is very reproducible, with inter- and intra-observer ICCs of 0.95-0.99[286,287] and 0.99[287] respectively.

1.13.2.5. Diastolic function

Longitudinal function can also be measured using a combination of pulse wave and tissue Doppler imaging (TDI). TDI has two main disadvantages compared with strain: angle dependence and reliance on a high frame rate for temporal resolution[288]. When used to assess diastolic dysfunction at the mitral annular level (E/E' and E/A), TDI has excellent inter- and intra-observer ICCs of 0.97-0.99 and 0.98-0.99 respectively[282]. E' represents the longitudinal motion of the mitral annulus towards the apex. The ratio of transmitral early (passive) filling velocity (E) to mitral annular velocity (E') provides an estimate of left ventricular filling pressure and left atrial pressure. The A wave is measured using transmitral pulse wave Doppler and represents late diastolic filling due to atrial contraction. Figure 1.3 illustrates the change in E/A during the different stages of diastolic dysfunction.

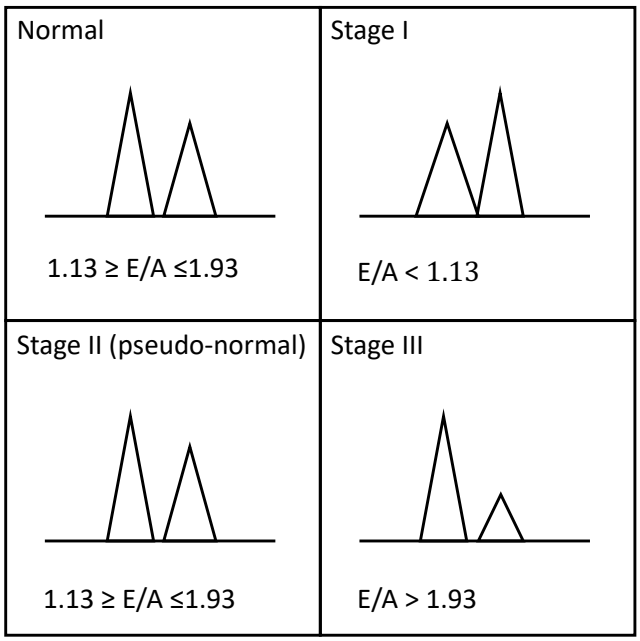

Figure 1.3: Change in transmitral flow velocity in diastolic dysfunction.
E/A, early to late diastolic filling ratio.
Quoted ranges are age-specific (41-60 years).

In a young healthy heart, the majority of ventricular filling occurs passively (E>A). In diastolic dysfunction, impaired left ventricular relaxation limits passive filling and therefore peak A wave velocity increases relative to E (Figure 1.3; stage I). As left ventricular compliance further reduces, left atrial pressures increase, leading to a steeper early diastolic transmitral pressure gradient, and thus pseudonormalisation of the E/A

ratio (stage II). Stage III diastolic dysfunction represents severely restrictive filling dynamics, associated with increased left atrial pressure and volume. It can be distinguished from Stage IV (fixed restrictive diastolic dysfunction) by its reversibility with the Valsalva manoeuvre. Cut-offs for the different stages of diastolic dysfunction are age-dependent[289]. STE and TDI are considered the most load independent measures and are therefore most representative of myocardial contractility[54,234,288,290].

Diastolic strain rate (SR) curves, which are derived from speckle-tracking software (Figure 1.4), have been proposed as a potential superior measure of diastolic function over mitral annular velocity[291]. Advantages include angle and load independence and representation of all myocardial segments, rather than basal alone[291,292]. Peak diastolic SRs are measured at three points in the cardiac cycle: isovolumetric relaxation (SR_{IVR}), early diastole (SR_E) and late diastole (SR_A). The following ratios combining pulse wave Doppler and SR have previously been correlated with left ventricular pressures and diastolic function: $SR_{E/A}$, E/SR_{IVR}, E/SR_E and E/SR_A[291–293].

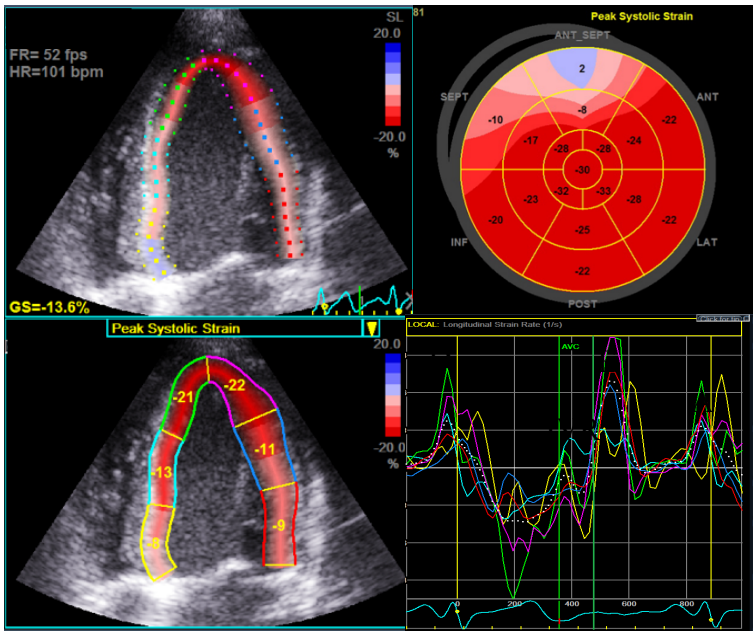

Figure 1.4: Peak systolic strain and diastolic strain rate derived from speckle-tracking software. **A.** Tracking of the left ventricular myocardium; **B.** Bull's eye plot of segmental longitudinal strain (combining A2C, A3C and A4C); **C.** Segmental longitudinal strain in one view; **D.** Strain rate curve in one view; each colour represents a different segment; the dotted line represents global strain rate.
AVC, aortic valve closure; IVR, isovolumetric relaxation; E, early diastole; A, late diastole.

1.13.2.6. Remodelling

Echocardiographic evaluation of cardiac remodelling includes 2D measurements of the left ventricle in PLAX to calculate LVM and RWT and 2D diameters of the left atrium in A2C and A4C to calculate left atrial volume. These measures can also be derived using M-mode, however this is limited by its fixed location[294]. For research purposes, reliability of echocardiography is optimised by the same observer using the same methods, equipment and settings, and recording at least three cardiac cycles for each cine-loop or image[295].

1.13.3. Rodent cardiovascular measurements

1.13.3.1. BP

BP can be measured in the rat both invasively (via intra-arterial methods / radio-telemetry) and non-invasively (using tail-cuff method)[296]. Intra-arterial BP measurement requires cannulation of the carotid artery under anaesthesia[296]. Although the most precise method[296], this is often reserved for acute studies as exteriorised catheters cannot be used for longer than three weeks[297]. Where longer-term BP studies are required, telemetry is gold-standard[297]. Once surgically implanted, telemetry devices enable continuous monitoring, without restraint or human interaction[298]. Like clinical ambulatory monitoring[299,300], this helps to account for biological variation and potential stress factors[297]. However, implantation of telemetry devices involves invasive surgery and the required equipment is expensive[297].

Non-invasive BP measurement is conventionally performed using the tail-cuff method. As in clinical studies, consistent equipment[301], methods[302] and conditions[303–305] are required to optimise reliability. Ibrahim *et al*.[301] sought to determine the validity of BP measurements using this method, by simultaneously comparing it with carotid artery measurements. They demonstrated no significant difference between the two methods[301] but both had poor reproducibility, contributed by biological variability and possible environmental factors, such as temperature and handling[301]. Therefore, to minimise restraint-induced BP alterations[301,303,304] rats can be trained prior to collection of any study BP measurements. The cuff is then placed at the base of the tail[301], to account for the 2mmHg drop in pressure per cm of tail length[306].

1.13.3.2. Echocardiography

In recent years, non-invasive cardiac imaging on small laboratory animals has advanced, in particular with the introduction of STE[307]. This has significant implications for longitudinal *in vivo* studies, enabling characterisation of the remodelling process in different disease models. Transthoracic echocardiography is performed under anaesthesia, with the rat in left lateral position[308]. Left ventricular remodelling and function can then be assessed from the PLAX, PSAX, A2C, A4C and A5C views, using 2D, M-mode, pulse wave Doppler, TDI and STE[308]. Reliability is again optimised by calculating each variable from an average of at least three cardiac cycles[308,309].

1.14. Summary

Preterm pre-eclampsia is associated with cardiovascular dysfunction, which persists beyond the pregnancy period. Evidence suggests that this could be mediated by increased levels of sFlt (largely from the placenta), via endothelial dysfunction and microvascular vasoconstriction. ACE inhibitors are widely used for cardioprotection in myocardial injury outside of pregnancy. They exert their effect through a variety of mechanisms, including increased NO bioavailability and subsequent vasodilation. It is therefore plausible that they could ameliorate cardiovascular injury mediated by sFlt. Women with preterm pre-eclampsia are at particular risk of long-term cardiovascular disease and women with cardiovascular impairment are at a higher risk of pre-eclampsia recurrence. Despite this, no interventional studies have investigated if the postnatal cardiovascular impairment associated with preterm pre-eclampsia is modifiable using a postnatal intervention (e.g. ACE inhibition). These studies are therefore the first of their kind to address whether postnatal treatment with enalapril could ameliorate cardiovascular dysfunction in women who have had preterm pre-eclampsia.

1.15. Rationale for the proposed studies

The proposed studies build on recent data, which have highlighted the relationship between pre-eclampsia and postnatal cardiovascular dysfunction[5,7,36] and long-term cardiovascular risk[8–10,12]. Enalapril is an ACE inhibitor whose use is well-established in cardioprotection[186,197,310,311]; however, its cardioprotective properties have never been

tested in the context of pre-eclampsia. The aim of this thesis is to explore the mechanism of preterm pre-eclampsia-associated cardiovascular dysfunction and its potential reversibility. The clinical trial is the first of its kind to address whether postnatal enalapril can ameliorate cardiovascular dysfunction/remodelling in women who have had preterm pre-eclampsia. The pre-clinical and retrospective cohort studies aim to elucidate the mechanism linking pre-eclampsia to cardiovascular dysfunction in the peripartum period.

Hypothesis

I hypothesise that sFlt mediates maternal cardiovascular dysfunction during/following preterm pre-eclampsia, and that this can be ameliorated by postnatal administration of enalapril.

Aims

In order to address this hypothesis, the aims of my doctoral thesis are:

1. To explore the mechanistic link between preterm pre-eclampsia and maternal cardiovascular dysfunction;
2. To explore the reversibility of postnatal cardiovascular dysfunction following preterm pre-eclampsia.

Objectives

1. To determine the prevalence of pre-eclampsia and placental dysfunction in women with pre-existing cardiac dysfunction (chapter two);
2. To explore the relationship between placental dysfunction and the severity and cause of pre-existing cardiac impairment (chapter two);
3. To use an established pre-eclampsia-like rodent model to characterise sFlt-induced postnatal cardiac dysfunction (chapter three);
4. To characterise the pathophysiology of maternal cardiovascular dysfunction following preterm pre-eclampsia (chapter four);
5. To determine if maternal cardiovascular dysfunction following preterm pre-eclampsia can be modified by postnatal treatment with enalapril (chapter four).

CHAPTER 2: PLACENTAL DYSFUNCTION IN WOMEN WITH PRE-EXISTING CARDIAC IMPAIRMENT: A RETROSPECTIVE COHORT STUDY

2.1. Introduction

As discussed in chapter one, there is abundant observational data linking pre-eclampsia with postnatal maternal cardiac dysfunction[5–7] and long-term cardiovascular risk[9–17,144,145,177]. However, the mechanistic link between the two remains inconclusive: it is unclear whether the association between cardiac and placental dysfunction is causal or consequential. There is some evidence to support a pre-eclampsia dose-effect: the more severe pre-eclampsia phenotypes (determined by presence of severe features[8,10,12,15–17], gestation[9,11,13,14], fetal size[15,184] and recurrence[17,185]) are associated with increased future cardiovascular risk. In the context of pre-eclampsia, prematurity is also associated with worse maternal diastolic dysfunction[5,36] and potentially worse cardiac remodelling[35], although the evidence for the latter is more conflicted[5,139]. These data support a causal role of pre-eclampsia in the development of cardiac dysfunction and long-term cardiovascular risk. Animal studies have also sought to investigate the direction of this relationship, again with mixed results[221–225]. This inconclusiveness could in part be explained by the fact that existing animal models only recapitulate part of the pre-eclampsia-like phenomenon. Pre-eclampsia-associated long-term cardiovascular risk could be attributed to mutual risk factors[312], endothelial and therefore vascular dysfunction[313], however this does not account for postnatal cardiac dysfunction[5,7,36] and the persistence of increased cardiovascular risk after adjusting for mutual risk factors[144]. An alternative approach is to examine pre-eclampsia rates in different subsets of women using routine clinical data. By investigating the rate of pre-eclampsia in women with pre-existing cardiac dysfunction, a potential causal role of cardiac dysfunction in the development of pre-eclampsia can be explored. A number of large retrospective registry studies[146–151,153–155] have previously investigated obstetric outcomes in women with known cardiac disease. However, pre-eclampsia prevalence was not the primary focus of these studies, and therefore to my knowledge, no one has correlated pre-pregnancy cardiac functional measures with pregnancy outcome and pre-eclampsia risk factors have largely been overlooked. Pre-eclampsia and FGR are presumed to be placental in

origin[314–317]. In this way, if inadequate cardiac function contributes significantly to impaired placental development, women with pre-existing cardiac dysfunction should have disproportionately increased pre-eclampsia and FGR risk. To date, the results from previous studies have been conflicting, with some demonstrating an increased prevalence[146,148–151] of pre-eclampsia and others not[147,153–155]. Additionally, only a few studies reviewed the risk specifically in women with cardiomyopathy (i.e. impaired cardiac systolic function)[147,148,150]. Of these studies, Koutrolou-Sotiopoulou *et al.*'s retrospective cohort study[147] provided the most detailed phenotypic data for both obstetric and cardiac outcomes; however this only included 37 cardiomyopathy cases, some of whom had normal LVEF and therefore were potentially mislabelled as cardiomyopathy. Although echocardiography data were included in this study, gestation at scan was not limited and the relationship between echocardiography parameters and pregnancy outcome was not investigated[147]. Lima *et al.*[148] and Owens *et al.*'s[150] studies consisted of much larger datasets (2078 and 676 cardiomyopathy cases, respectively). However data were extracted from national / regional registries and relied on International Statistical Classification of diseases and related health problems (ICD) coding and birth certificate-linkage; antenatal medication was not known; echocardiography data were not provided; and given the administrative nature of the data, verification and timing of cardiac diagnosis relative to pregnancy were not possible.

2.2. Aims

The aim of this study was to determine the prevalence of pre-eclampsia and FGR (clinical proxies for presumed placental dysfunction) in women with pre-existing cardiac dysfunction. A better understanding of the prevalence of pre-eclampsia and FGR in this group of women should improve:

1. Our understanding of the relationship between cardiac and placental function, with a view to inform future preventative and therapeutic strategies;
2. Counselling of women with cardiac disease and their families, before and during pregnancy.

2.3. Methods

This was a multicentre retrospective cohort study, including 11 United Kingdom (UK) sites and one Australian site. The protocol was approved by the UK Health Research Authority (HRA; IRAS ID 261380), the Australian Human Research Ethics Committees (HREC; HREC/60940/MonH-2020-203642) and each site's Research and Innovation team. Individual consent was not required, due to the use of routinely collected anonymised data.

2.3.1. Study population

All women aged 16 years and older in participating sites with pre-existing left ventricular systolic impairment (defined by LVEF <55%), who had a pregnancy from January 2008 to 2020, were included in the study. Women were excluded if they delivered before 22 weeks' gestation or insufficient data were available. Data were collated from 12 collaborating sites in England, Scotland and Australia (Figure 2.1).

Figure 2.1: Map of participating sites in **A.** UK and **B.** Australia.
Blue stars highlight the 12 hospitals that collected data for the study.

Eligible participants were identified using different methods across sites. These included use of cardiac obstetric databases, cardiac obstetric clinic lists and ICD-10 codes (including heart failure and cardiomyopathy). Eligibility was then checked following review of echocardiography reports, online clinical reporting systems, clinic letters and case notes.

Data were collected in accordance with the "Caldicott Principles", the Data Protection Act (DPA) and the General Data Protection Regulation (GDPR). Pseudonymisation keys were held separately at each site and no identifiable information was shared between sites. Each participating site was given identical excel spreadsheets with pre-determined data fields to complete (Table 2.1). Minimum data criteria included presence / absence of pre-eclampsia and evidence of LVEF <55% pre-pregnancy or within the first 12 weeks of pregnancy.

Table 2.1: Requested variables to be recorded from each case.

Baseline maternal characteristics	Age at delivery Ethnicity Booking height Booking weight Booking blood pressure Smoking status at booking Medical history (hypertension, renal disease, autoimmune disease, pre-existing proteinuria, pre-existing diabetes) Primary cardiac diagnosis NYHA functional status Presence and cause of dilated cardiomyopathy BNP / NTproBNP (within 1 year of conception) Medication before and during pregnancy (ACE inhibitors, ARB, ß blockers, calcium channel blockers, α blockers, diuretics, heparin, vitamin K antagonists, aspirin)
Obstetric history	Gravidity Parity Previous pregnancy complications (including preterm birth, SGA, FGR and pre-eclampsia)
Echocardiography parameters **(pre-pregnancy [most recent] and early pregnancy [<12 weeks' gestation])**	LVEF (discrete number / range / description) Presence of valvular disease: which valve, quantified if present Presence of HOCM Cardiac remodelling: presence of eccentric hypertrophy / concentric remodelling / concentric hypertrophy TAPSE LVIDd LVIDs PWd IVSd E/A E/E' LAV CO SV
Pregnancy outcome	Gestational age at delivery Birthweight Infant sex Mode of delivery Indication for delivery Outcome – livebirth / stillbirth / neonatal or infant death Neonatal admission to NICU (yes / no; number of days, if available) Pre-eclampsia/eclampsia/PIH (gestation at diagnosis; severity) gDM Placental abruption PPROM EBL
Cardiac outcome	Acute / worsening heart failure Pulmonary oedema Sustained arrhythmia Stroke / angina / myocardial infarction / cardiac arrest

NYHA, New York Heart Association; BNP, brain natriuretic peptide; NTproBNP, N-terminal-pro hormone BNP; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blockers; SGA, small for gestational age; FGR, fetal growth restriction; LVEF, left ventricular ejection fraction; HOCM, hypertrophic obstructive cardiomyopathy; LVIDd, left ventricular internal diameter end-

diastole; LVIDs, left ventricular internal diameter end-systole; PWd, posterior wall thickness end-diastole; IVSd, interventricular septum thickness end-diastole; E/A, early to late diastolic filling ratio (mitral inflow Doppler indices); E/E', early diastolic filling to early diastolic mitral annular velocity ratio (mitral inflow and annular Doppler indices); LAV, left atrial volume; CO, cardiac output; SV, stroke volume; NICU, neonatal intensive care unit; PIH, pregnancy induced hypertension; gDM, gestational diabetes; PPROM, premature prolonged rupture of membranes; EBL, estimated blood loss.

2.3.2. Cardiac classifications

LVEF impairment was categorised, as per the British Society of Echocardiography (BSE) guidelines[318], as borderline (50-54%), impaired (36-49%) and severely impaired (≤35%). Primary cardiac diagnoses were categorised as dilated cardiomyopathy (DCM), congenital, ischaemic, valvular, hypertensive, genetic and other acquired (including drug-induced, and previous peripartum cardiomyopathy [PPCM]) heart disease[319]. For the purpose of this study, cardiomyopathy was defined as LVEF <55%. DCM was defined as a combination of dilated left ventricle (left ventricular internal diameter in end-diastole [LVIDd] >5.2cm)[281] and systolic dysfunction, or evidence of DCM diagnosis in the case notes. DCM was further categorised into genetic, idiopathic, acquired and previous PPCM. As per the American Heart Association (AHA)[319] and BSE guidance[320], congenital, ischaemic, valvular and hypertensive heart disease were not included in the DCM definition, irrespective of left ventricular cavity size.

Where available, RWT was calculated in end-diastole by: (interventricular septal wall thickness [IVSd] + posterior wall thickness [PWd]) / LVIDd. LVM was derived from the following equation: $0.8(1.04[LVIDd + PWd + IVSd]^3 - [LVIDd]^3) + 0.6$. Remodelling measures were then indexed to body surface area (BSA). BSA was calculated using the Mosteller formula[321]: BSA = square root of (height (cm) x weight (kg) / 3600). Concentric remodelling was defined as RWT ≥0.42 and hypertrophy was defined as LVMi >95g/m^2 [281,318]. Left atrial dilatation was defined using the American and European 2015[281] (using indexed measures, if available) and 2006 guidelines[322] (when indexed measures were not available). This definition is summarised in Table 2.2. Echocardiography parameters were used from the most recent pre-pregnancy scan. When this was not available, early pregnancy (<12 weeks' gestation) echocardiography parameters were used instead.

Table 2.2: Classification of left atrial dilatation.

Classification	Left atrial diameter (cm)*	Left atrial volume (mL)*	Left atrial volume index (mL/m^2)
Normal	< 3.9	22 - 52	16 - 34
Mild	3.9 - 4.2	53 - 62	35 - 41
Moderate	4.3 - 4.6	63 - 72	42 - 48
Severe	≥ 4.7	≥ 73	> 48

*Derived from American and European 2006 recommendations[322].
†Derived from American and European 2015 recommendations[281].

2.3.3. Obstetric classifications

Presence of pre-eclampsia was confirmed by documented diagnosis in the case notes or clinic letters. All cases met the ISSHP criteria for diagnosis[323]: new or worsening hypertension >20 weeks and proteinuria or other features suggestive of pre-eclampsia (abnormal haematological parameters, abnormal biochemical parameters or FGR). Severe pre-eclampsia was defined as maximum BP ≥160/110mmHg, alanine aminotransferase >100U/L, creatinine >100µmol/L or platelets <100x10^9/L. Data for birthweight centile customisation was not available for all women. For this reason the World Health Organization (WHO) population Z score was used[324]. SGA (birthweight <10th centile) equated to a Z score ≤-1.282 and FGR (birthweight <3rd centile)[325] equated to a Z score ≤-1.881[324].

Presence of risk factors for pre-eclampsia, as defined by NICE[326], was recorded by each site. These include pre-existing hypertension, renal, vascular or autoimmune disease, diabetes, previous pre-eclampsia, nulliparity, age ≥40 years, multi-fetal pregnancy and BMI ≥35kg/m^2. Presence of pre-existing medical disease and risk factors were determined by the clinical care team at each site, following review of source data.

Population pre-eclampsia prevalence of 4.6% was derived from Abalos *et al.*'s systematic review[1]. Preterm pre-eclampsia prevalence was derived from the ASPRE trial[327] in which they identified pre-eclampsia requiring delivery <37 weeks in 0.7% of the population. Population rates of SGA (18.2%), FGR (9.5%), preterm delivery (<37 weeks; 8.2%) and preterm FGR (<37 weeks; 1.5%) were derived from 5-year data (2016-2020) from St Mary's Hospital, Manchester, UK.

2.3.4. Primary and secondary outcomes

The primary outcome was to determine the prevalence of pre-eclampsia in women with pre-existing cardiac impairment, compared with the general population. Pre-specified secondary outcomes included: 1) the prevalence of FGR (<3rd centile) and SGA (<10th centile) in women with pre-existing cardiac impairment, compared with the general population; 2) the prevalence of pre-eclampsia, FGR and SGA depending on primary cardiac diagnosis; 3) the relationship between pre-eclampsia, FGR and SGA prevalence and severity of LVEF impairment; 4) the relationship between gestation at birth and birthweight Z score and severity of LVEF impairment / primary cardiac diagnosis / echocardiography parameters of cardiac structure and function.

2.3.5. Maternal cardiac outcomes

Cardiac end points included acute heart failure, pulmonary oedema, sustained arrhythmia, stroke, angina, myocardial infarction and cardiac arrest. A major adverse cardiac event (MACE) was defined by a composite outcome of stroke, myocardial infarction or maternal death.

2.3.6. Statistical analysis

All statistical analyses were performed using Stata v.14.2. Baseline characteristics of the cohort were represented as mean ± standard deviation / median (range) as appropriate for continuous data, or counts (percentage) for categorical data. Prevalence of primary and secondary outcomes (including pre-eclampsia, SGA and FGR) were compared against quoted population prevalence, as described in the literature[1,327], using equality of proportions test. The prevalence of these outcomes were also compared between groups (i.e. categorised by primary cardiac diagnosis / LVEF impairment severity) using Chi-square test. Univariate analysis was used to identify those factors significantly associated with pregnancy outcome. Heavily skewed variables were log-transformed prior to analysis. Multivariable regression analyses were used to adjust for confounding factors. Analyses were performed for the whole cohort and repeated for the DCM subgroup, aiming to reduce heterogeneity.

2.3.7. Sample size calculation

The prevalence of pre-eclampsia in the general population is 4.6%[1]. In order to identify a twofold increase in pre-eclampsia in this cohort (≥9.2%) compared with the general population, a sample size of 245 women was required at 80% power, α 0.05. From the initial single centre cohort study at St Mary's Hospital 66 eligible cases were identified. It was therefore anticipated that 12 additional sites would be needed (recruiting 15 to 20 per site) to reach the target sample size.

2.4. Results

The study cohort included 247 pregnancies from 218 women (Figure 2.2). Table 2.3 describes the spread of participants from different sites.

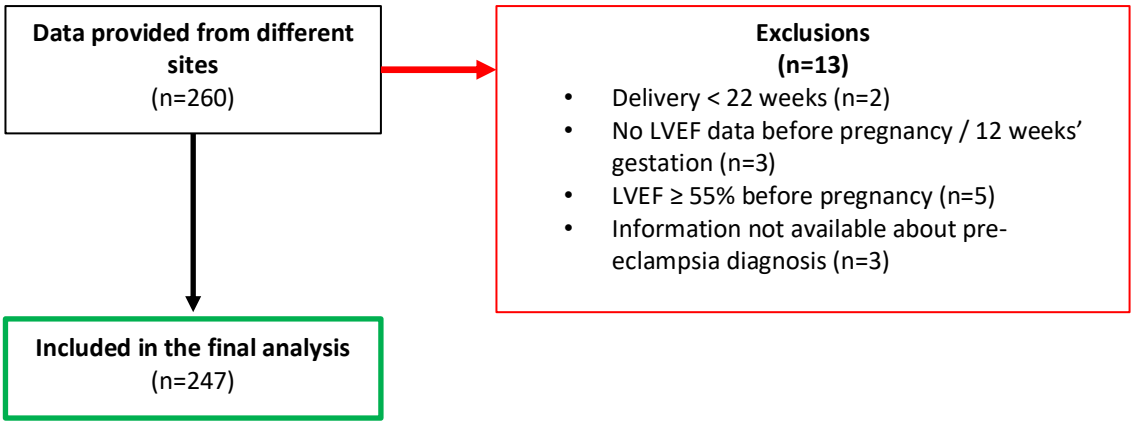

Figure 2.2: Consort diagram.
LVEF, left ventricular ejection fraction.

Table 2.3: Recruitment from different sites.

City	Participating Hospital	Number of pregnancies included
Birmingham	Birmingham Women's Hospital	3
Blackpool	Blackpool teaching Hospitals NHS Foundation Trust	1
Bristol	Bristol Royal Infirmary	51
Edinburgh	NHS Lothian	6
Glasgow	Queen Elizabeth University Hospital	10
London	St Thomas' Hospital	12
Manchester	St Mary's Hospital	99
Melbourne	Monash	11
Norwich	Norfolk and Norwich University Hospital NHS Foundation Trust	7
Nottingham	Nottingham University Hospitals NHS Trust	13
Oxford	John Radcliffe Hospital	19
Sheffield	Sheffield Teaching Hospitals NHS Foundation Trust	15

NHS, National Health Service.

2.4.1. Demographics and baseline characteristics

Distribution of baseline characteristics of the cohort are summarised in Table 2.4. The mean age was 30.4±6.1 years and the majority of the cohort were white (190/247, 76.9%). Sixty six (26.7%) women were nulliparous and 68/242 (28.1%) had risk factors for pre-eclampsia, as defined by NICE guidance[328].

Table 2.4: Baseline characteristics.

		DCM (n=151)	All (n=247)
Demographics			
Age at delivery (years)		30.4 ± 6.4	30.6 ± 6.1
Ethnicity	**White**	111/151 (73.5%)	190/247 (76.9%)
	Black	14/151 (9.3%)	18/247 (7.3%)
	Asian	8/151 (5.3%)	15/247 (6.1%)
	Other	5/151 (3.3%)	11/247 (4.5%)
	Unknown	13/151 (8.6%)	13/247 (5.3%)
Booking BMI (kg/m^2)*		25.9 (17.0 - 48.7)	25.6 (17.0 - 50.4)
Smoker during pregnancy		14/142 (9.9%)	38/228 (16.7%)
Medical History			
Chronic hypertension		12/148 (8.1%)	18/242 (7.4%)
Pre-existing renal disease		9/148 (6.1%)	15/242 (6.2%)
Pre-existing proteinuria		11/146 (7.5%)	15/235 (6.4%)
Pre-existing diabetes		6/150 (4.0%)	7/240 (2.9%)
Autoimmune disease		7/148 (4.7%)	9/242 (3.7%)
Booking sBP (mmHg)		109.9 ± 14.4	109.0 ± 14.0
Booking dBP (mmHg)		67.2 ± 10.3	67.0 ± 10.0
Obstetric history			
Nulliparous		35/151 (23.2%)	66/247 (26.7%)
High risk for pre-eclampsia		33/148 (22.3%)	68/242 (28.1%)
One moderate risk factor for pre-eclampsia		25/97 (25.8%)	45/182 (24.7%)
Previous pre-eclampsia (if multiparous)		11/114 (9.6%)	21/176 (11.9%)
Previous SGA < 10th centile (if multiparous)		19/87 (21.8%)	41/148 (27.7%)
Previous FGR < 3rd centile (if multiparous)		12/87 (13.8%)	25/148 (16.9%)

Frequencies: n/N (%)
Mean ± standard deviation
*Median (range)
Denominators vary between variables due to missing data.
†High risk for pre-eclampsia is defined by presence of: pre-existing hypertension, renal, vascular or autoimmune disease, diabetes, previous pre-eclampsia, or two moderate risk factors.
‡Moderate risk factors include: nulliparity, age ≥40 years, multi-fetal pregnancy, BMI ≥35kg/m^2.
BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure; mmHg, millimetres of mercury; SGA, small for gestational age; FGR, fetal growth restriction.

Table 2.5 summarises the different cardiac diagnoses amongst all included women with LVEF <55%. LVEF data were derived from early pregnancy (<12 weeks) in 16/247 (6.5%) women, where pre-pregnancy echocardiography data were not available. DCM affected 151/247 (61.1%) of the cohort. Forty (16.2%) women had cardiomyopathy related to previous PPCM (with or without DCM). Congenital heart disease encompassed structural

defects including coarctation of the aorta, patent ductus arteriosus, ventricular septal defect, pulmonary atresia, tetralogy of fallow, transposition of the great arteries and truncus arteriosus.

Table 2.5: Primary cardiac diagnoses.

Primary cardiac diagnosis		**All (n=247)**
DCM	**Genetic**	50 (20.2%)
	Idiopathic	3 (1.2%)
	Acquired	63 (25.5%)
	PPCM	35 (14.2%)
Congenital		23 (9.3%)
Ischaemic		12 (4.9%)
Hypertensive		3 (1.2%)
Valvular		17 (6.9%)
Genetic without DCM		19 (7.7%)
Other acquired without DCM		22 (8.9%)

Frequencies: N (%)
Genetic causes without DCM (dilated cardiomyopathy) include: hypertrophic obstructive cardiomyopathy, arrhythmogenic cardiomyopathy and left ventricular non-compaction cardiomyopathy.
Other acquired causes without DCM include: previous PPCM, drug-induced and inflammatory.
DCM, dilated cardiomyopathy; PPCM, peripartum cardiomyopathy.

The majority of women were New York Heart Association (NYHA) functional classification I (86/247 [34.8%]); 52/247 (21.1%) were class II; 14/247 (5.7%) were class III, and 95/247 (38.4%) were unknown. Table 2.6 summarises the echocardiography parameters of the cohort. Thirty two (13.0%) women had severe systolic dysfunction (LVEF ≤35%). Left ventricular geometry data was provided for 126/247 (51.0%) of pregnancies. Concentric remodelling / hypertrophy affected 14/126 (11.1%) and eccentric hypertrophy affected 49/126 (38.9%) of the cohort. Prevalence of left ventricular remodelling (concentric remodelling, or concentric / eccentric hypertrophy) did not differ between those with a history of previous pre-eclampsia and those without (8/17 [47.1%] versus 54/108 [50.0%], p=0.23). Similarly, left ventricular remodelling did not differ between those with and without chronic hypertension (9/13 [69.2%] versus 54/113 [47.8%], p=0.10). Echocardiography data, beyond LVEF was not available for every participant.

Table 2.6: Echocardiography measures of cardiac structure and function prior to pregnancy or before 12 weeks' gestation.

Echocardiography parameters		DCM (n=151)		All (n=247)	
		Mean ± SD / median (range) / N (%)	N	Mean ± SD / median (range) / N (%)	N
Cardiac remodelling	Concentric remodelling	2 (2.5%)	79	8 (6.3%)	126
	Concentric hypertrophy	3 (3.8%)	79	6 (4.8%)	126
	Eccentric hypertrophy	35 (44.3%)	79	49 (38.9%)	126
LVIDd (cm)		5.59 ± 0.58	90	5.32 ± 0.68	155
LVIDs (cm)		4.33 ± 0.70	78	4.05 ± 0.79	125
PWd (cm)		0.83 ± 0.18	79	0.84 ± 0.18	130
IVSd* (cm)		0.80 (0.40 - 1.40)	85	0.83 (0.40 - 2.50)	139
LVM* (g)		181.66 (72.34 - 286.04)	79	168.21 (52.09 - 376.33)	128
LVMi* (g/m^2)		94.09 (39.14 - 164.50)	79	90.81 (34.40 - 182.38)	126
RWT*		0.31 (0.13 - 0.59)	80	0.32 (0.13 - 0.64)	131
E/A*		1.30 (0.61 - 4.50)	55	1.46 (0.61 - 4.50)	91
E/E'*		8.00 (3.00 - 16.00)	31	8.00 (3.00 - 24.30)	46
Left atrial dilatation		12 (30.8%)	39	16 (24.2%)	66
Aortic stenosis		2 (1.6%)	126	3 (1.5%)	197
Aortic regurgitation		4 (3.2%)	126	16 (8.1%)	197
Mitral stenosis		3 (2.4%)	126	6 (3.0%)	199
Mitral regurgitation		12 (9.7%)	124	25 (12.9%)	194
Pulmonary stenosis		0 (0.0%)	126	1 (0.5%)	198
Pulmonary regurgitation		15 (12.3%)	122	26 (13.5%)	192
Tricuspid stenosis		0 (0.0%)	126	0 (0.0%)	197
Tricuspid regurgitation		20 (16.7%)	120	43 (22.8%)	189
Cardiac output (L/minute)		5.53	1	4.47 ± 1.12	5
Stroke volume (mL)		71 ± 65 - 90	5	60.4 ± 15.6	16
TAPSE* (cm)		2.02 (0.70 - 3.10)	40	2.0 (0.7 - 14.0)	68

Frequencies: N (%)
Mean ± standard deviation
*Median (range)
Mild, moderate and severe valvular abnormalities were included; trivial and physiological regurgitation / stenosis were excluded.
Echocardiography data was not available for all women, therefore the number included in the analysis (N) is recorded for each parameter.
SD, standard deviation; LVIDd, left ventricular internal diameter in end-diastole; LVIDs, left ventricular internal diameter in end-systole; PWd, posterior wall thickness in end-diastole; IVSd, interventricular septal wall thickness in end-diastole; LVM, left ventricular mass; LVMi, LVM indexed to body surface area; RWT, relative wall thickness; E/A, early to late diastolic filling ratio; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; TAPSE, tricuspid annular plane systolic excursion.

Table 2.7 summarises the classes of medication taken during pregnancy by the cohort. ß blockers were taken by 116/243 (47.7%) women antenatally. Information on the type of ß blocker was available for 77/116 (66.4%) women; bisoprolol was the most commonly prescribed ß blocker (68/77, 88.3%). Forty one (46.6%) of those taking antenatal aspirin were indicated due to pre-eclampsia risk factors, as per NICE guidelines[328]. All women with ischaemic heart disease (n=12) took aspirin during pregnancy.

Table 2.7: Concomitant medication during pregnancy.

Medication	DCM (n=151)		All (n=247)	
	Before pregnancy	During pregnancy	Before pregnancy	During pregnancy
ACE inhibitor / ARB	71/140 (50.7%)	-	101/236 (42.8%)	-
ß blockers	75/144 (52.1%)	82/149 (55.0%)	112/235 (47.7%)	116/243 (47.7%)
Ca channel blockers	4/148 (2.7%)	4/148 (2.7%)	8/243 (3.3%)	8/243 (3.3%)
α blockers	3/148 (2.0%)	4/148 (2.7%)	4/243 (1.6%)	5/243 (2.1%)
Diuretics	25/147 (17.0%)	31/146 (21.2%)	31/242 (12.8%)	39/241 (16.2%)
Heparin	14/145 (9.7%)	41/146 (28.0%)	20/239 (8.4%)	62/238 (26.0%)
Vitamin K antagonists	4/147 (2.7%)	2/150 (1%.3)	13/241 (5.4%)	3/244 (1.2%)
Aspirin	27/149 (18.1%)	55/131 (42.0%)	48/243 (19.8%)	89/222 (40.1%)

n/ N (%)
Denominators vary between variables due to missing data.
ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; Ca, calcium.

2.4.2. Pregnancy outcomes

As demonstrated in Table 2.8, 8/247 (3.2%) of the cohort developed pre-eclampsia. Of those, 4/8 (50.0%) delivered before 37 weeks' gestation, 3/8 (37.5%) delivered before 34 weeks' gestation and 7/8 (87.5%) had risk factors for pre-eclampsia (including hypertension, renal disease, antiphospholipid syndrome and a combination of obesity and nulliparity). Of those who were indicated antenatal aspirin due to pre-eclampsia risk factors[328] and for whom pre-eclampsia risk factors and antenatal aspirin use were known, 41/64 (64.1%) took aspirin antenatally. Table 2.9 summarises the baseline characteristics of those who later developed pre-eclampsia. The prevalence seen in this cohort was not significantly increased compared with the general population[1] (3.2% [95%C.I. 1.0-5.4] versus 4.6% [95% C.I. 2.7-8.2], p=0.29). Similarly, preterm pre-eclampsia (<37 weeks) prevalence was not significantly increased relative to population prevalence[327] (1.6% [95% C.I. 0.5-3.2] versus 0.7% [95% C.I. 0.6-0.8], p=0.08). Three of the four women with preterm pre-eclampsia had co-existent FGR. Prevalence of FGR (<3rd centile) and SGA

(<10th centile) in women with pre-existing cardiac impairment were higher than that of the background population (FGR: 17.1% [95% C.I. 12.3-21.9] versus 5.5% [95% C.I. 5.3-5.7], p<0.001; SGA: 34.6% [95% C.I. 28.5-40.7] versus 18.2% [95% C.I. 17.9-18.6], p<0.001).

Table 2.8: Pregnancy outcomes of the cohort.

Pregnancy outcome		**DCM (n=151)**	**All (n=247)**
Gestation at delivery* (weeks + days)		38+0 (27+2 - 42+1)	38+0 (22+0 - 42+1)
Delivery < 37 weeks		43/147 (29%)	73/243 (30%)
Delivery < 34 weeks		12/147 (8%)	30/243 (12%)
Iatrogenic delivery < 34 weeks		11/147 (7%)	21/243 (9%)
Female sex		46/102 (45%)	99/199 (50%)
Mode of delivery	**Emergency C-section**	21/142 (15%)	37/238 (16%)
	Elective C-section	44/142 (31%)	82/238 (34%)
	Operative vaginal delivery	9/142 (6%)	16/238 (7%)
	Breech vaginal delivery	0/142 (0%)	4/238 (2%)
	Spontaneous vaginal delivery	68/142 (48%)	99/238 (42%)
Indication for delivery	**Spontaneous**	67/151 (44.4%)	106/247 (42.9%)
	Routine	41/151 (27.2%)	79/247 (32.0%)
	Fetal concerns	4/151 (2.7%)	6/247 (2.4%)
	Worsening maternal cardiac disease	29/151 (19.2%)	35/247 (14.2%)
	Pre-eclampsia	4/151 (2.7%)	7/247 (2.8%)
	Other maternal disease	0/151 (0%)	2/247 (0.8%)
	Unknown	6/151 (4.0%)	12/247 (4.9%)
EBL*		400 (50 - 4000)	400 (40 - 4000)
Multiple pregnancy		0/151 (0%)	2/247 (1%)
Perinatal outcomes			
Birthweight Z score*		-0.70 (-3.83 - 4.66)	-0.81 (-3.83 - 4.66)
Birthweight centile < 10th		42/139 (30%)	81/234 (35%)
Birthweight centile < 3rd		21/139 (15%)	40/234 (17%)
NICU admission		28/106 (26%)	57/197 (29%)
Stillbirth		0 (0%)	0 (0%)
NND		1/151 (1%)	2/247 (1%)
Maternal outcomes			
Pre-eclampsia		5/151 (3%)	8/247 (3%)
Severe pre-eclampsia		4/151 (3%)	5/247 (2%)
Early-onset pre-eclampsia (delivery < 34 weeks)		2/151 (1%)	3/247 (1%)
Preterm pre-eclampsia (delivery < 37 weeks)		3/151 (2%)	4/247 (2%)
Eclampsia		1/151 (1%)	1/247 (0.4%)
Gestation at pre-eclampsia diagnosis* (weeks + days)		33+0 (31+0 - 35+5)	33+0 (31+0 - 36+5)
Gestational diabetes		7/113 (6%)	10/186 (5%)
Placental abruption		2/114 (2%)	4/187 (2%)
PPROM		3/113 (3%)	7/186 (4%)

Frequencies: n/N (%)

*Median (range)

Denominators vary between variables due to missing data.

EBL, estimated blood loss; NICU, neonatal intensive care; NND, neonatal death; HELLP, haemolysis, elevated liver enzymes and low platelets; PPROM, premature rupture of membranes.

Table 2.9: Baseline characteristics of the women who developed pre-eclampsia.

Cardiac diagnosis	LVEF impairment	Age (years)	BMI (kg/m^2)	Booking BP (mmHg)	PE risk factors	Obstetric history
Congenital heart disease (TGA)	Borderline	28	37.2	120/78	2 moderate risk factors (nullip, ↑ BMI)	Nullip
Hypertensive cardiomyopathy	Impaired	25	25.3	112/72	Hypertension CKD	Nullip
Ischaemic heart disease	Borderline	40	21.2	145/77	Previous PE Hypertension CKD Age	Para 2 Previous PE & FGR
DCM (post-viral)	Borderline	41	44.8	130/88	Age Hypertension	Para 1; no previous PE/FGR
DCM secondary to chemotherapy	Borderline	19	23.7	90/67	None	Nullip
DCM secondary to Libman-Sacks endocarditis linked to APS	Severe	36	22.7	132/77	Previous PE APS CKD	Para 1 Previous PE
DCM secondary to sickle cell disease	Borderline	33	17.9	114/70	CKD	Nullip
DCM; cardiac transplant for restrictive cardiomyopathy	Borderline	29	24.1	106/70	CKD	Para 1 Previous FGR

Each row represents an individual woman.
LVEF, left ventricular ejection fraction; BMI, body mass index; BP, blood pressure; mmHg, millimetres of mercury; PE, pre-eclampsia; TGA, transposition of the great arteries; nullip, nulliparous; CKD, chronic kidney disease; FGR, fetal growth restriction; DCM, dilated cardiomyopathy; APS, antiphospholipid syndrome.

Preterm delivery (<37 weeks) and preterm FGR were also more prevalent than in the background population (preterm delivery: 30.0% [95% C.I. 24.3-35.8] versus 8.2% [8.0-8.5], p<0.001; preterm FGR: 8.5% [95% C.I. 5.0-1.2] versus 1.5% [95% C.I. 1.4-0.2], p<0.001). Pre-eclampsia affected 3/20 (15.0%) women with preterm (<37 weeks) FGR and 2/6 (33.3%) women with early-onset (<34 weeks) FGR. Twenty-one (8.6%) women underwent iatrogenic delivery <34 weeks and 57/243 (23.5%) had iatrogenic delivery <37 weeks, of whom 37/57 (64.9%) were indicated by routine obstetric factors / maternal disease only. Half (n=119) of the women delivered by Caesarean section. Four women suffered a placental abruption, none of whom had evidence of pre-eclampsia. Figure 2.3 illustrates the distribution of birthweight Z score and gestation at delivery in this cohort compared with the background population.

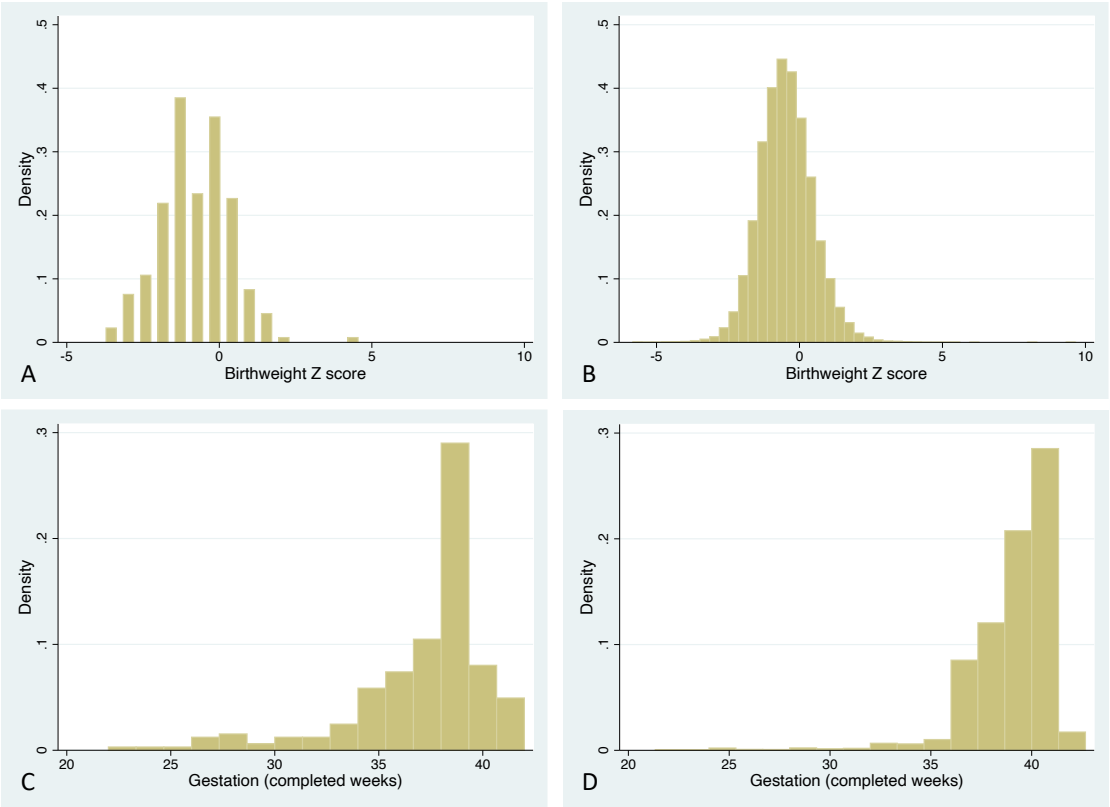

Figure 2.3: Histograms of **A.** birthweight Z score in this cohort; **B.** birthweight Z score in the background population; **C.** gestation at delivery (completed weeks) in this cohort; and **D.** gestation at delivery (completed weeks) in the background population.
Background population distributions were derived from 5-year data (2016-2020) from St Mary's Hospital, Manchester, UK.

2.4.3. Relationship between cardiac parameters and pregnancy outcome

Table 2.10 summarises the prevalence of adverse pregnancy outcome depending on severity of LVEF impairment and primary cardiac diagnosis. Severity of LVEF impairment did not correlate with prevalence of pre-eclampsia (p=0.23), SGA (p=0.38), FGR (p=0.73), or preterm delivery <34 weeks (p=0.47; Table 2.10). LVEF impairment severity also did not correlate with birthweight Z score or gestation at delivery (Tables 2.11 and 2.12, respectively).

Table 2.10: Prevalence of adverse pregnancy outcome depending on severity of LVEF impairment.

Severity of LVEF impairment	Pre-eclampsia	SGA < 10th centile	FGR < 3rd centile	Delivery < 34 weeks
DCM				
Borderline (n=63)	4/63 (6.3%)	19/56 (33.9%)	9/56 (16.1%)	4/60 (6.7%)
Impaired (n=62)	0/62 (0.0%)	13/58 (22.4%)	8/58 (13.8%)	4/61 (6.6%)
Severely impaired (n=26)	1/26 (3.8%)	10/25 (40.0%)	4/25 (16.0%)	4/26 (15.4%)
P value	0.14	0.20	0.94	0.33
All				
Borderline (n=116)	6/116 (5.2%)	37/108 (34.3%)	20/108 (18.5%)	12/113 (10.6%)
Impaired (n=99)	1/99 (1.0%)	30/95 (31.6%)	14/95 (14,7%)	12/98 (12.2%)
Severely impaired (n=32)	1/32 (3.1%)	14/31(45.2%)	6/31 (19.3%)	6/32 (18.8%)
P value	0.23	0.38	0.73	0.47

Frequencies: n/N (%)
Denominators vary between variables due to missing data.
Severity of LVEF impairment was classified as: borderline (50-54%), impaired (36-49%) and severe (≤35%)[318].
P values represent comparison between LVEF impairment categories using Chi-square test.
LVEF, left ventricular ejection fraction; SGA, small for gestational age; FGR, fetal growth restriction; DCM, dilated cardiomyopathy.

Hypertensive cardiomyopathy was associated with an increased prevalence of pre-eclampsia (odds ratio [OR] 16.93 [95% C.I. 1.37-209.43], p=0.03). Ischaemic heart disease was not associated with pre-eclampsia prevalence but was associated with earlier gestation at delivery and lower birthweight Z score (difference 20.44 days [95% C.I. -33.97- -6.91], p=0.003 and -0.76 [95% C.I. -1.43- -0.09], p=0.03, respectively). No other cardiac diagnoses correlated with pregnancy outcome (Tables 2.11 and 2.12). Other acquired causes of cardiomyopathy (including chemotherapy, inflammatory and previous PPCM) were associated with higher birthweight Z score (Table 2.11).

Table 2.11: Relationship between severity of LVEF impairment / primary cardiac diagnosis and birthweight Z score.

	DCM (n=139)		All (n=234)	
	Coefficient (95% C.I.)	**P value**	**Coefficient (95% C.I.)**	**P value**
Severity of LVEF impairment				
Borderline (n=116)	-	-	-	-
Impaired (n=99)	0.00 (-0.44 - 0.44)	0.99	0.00 (-0.44 - 0.44)	0.99
Severely impaired (n=32)	-0.29 (-0.85 - 0.28)	0.32	-0.29 (-0.85 - 0.28)	0.32
Primary cardiac diagnosis				
DCM (n=151)	-	-	0.20 (-0.11 - 0.50)	0.20
Congenital (n=23)	-	-	-0.23 (-0.73 - 0.27)	0.37
Ischaemic (n=12)	-	-	**-0.76 (-1.43 - -0.09)**	**0.03**
Hypertensive (n=3)	-	-	-0.11 (-1.44 - 1.21)	0.86
Valvular (n=17)	-	-	0.03 (-0.55 - 0.60)	0.93
Genetic without DCM (n=19)	-	-	-0.49 (-1.03 - 0.05)	0.08
Other acquired without DCM (n=22)	-	-	**0.57 (0.05 - 1.08)**	**0.03**

Borderline LVEF impairment was used as the comparator for impaired and severely impaired LVEF.
Each cardiac diagnosis was compared against all other cohort participants.
Birthweight Z score was available for 234 women (of whom, 139 had DCM).
Bold text indicates statistical significance ($p<0.05$).
LVEF, left ventricular ejection fraction; C.I., confidence interval; DCM, dilated cardiomyopathy.

Table 2.12: Relationship between severity of LVEF impairment / primary cardiac diagnosis and gestation at delivery (log-transformed).

	DCM (n=147)		All (n=243)	
	Coefficient (95% C.I.)	**P value**	**Coefficient (95% C.I.)**	**P value**
Severity of LVEF impairment				
Borderline (n=116)	-	-	-	-
Impaired (n=99)	-0.01 (-0.03 - 0.02)	0.72	-3.60 (-9.99 - 2.79)	0.27
Severely impaired (n=32)	-0.03 (-0.07 - 0.01)	0.10	-8.54 (-17.81 - 0.73)	0.07
Primary cardiac diagnosis				
DCM (n=151)	-	-	0.03 (0.00 - 0.05)	0.05
Congenital (n=23)	-	-	0.00 (-0.05 - 0.04)	0.83
Ischaemic (n=12)	-	-	**-0.08 (-0.14 - -0.03)**	**0.005**
Hypertensive (n=3)	-	-	0.01 (-0.11 - 0.12)	0.89
Valvular (n=17)	-	-	-0.03 (-0.08 - 0.02)	0.17
Genetic without DCM (n=19)	-	-	-0.02 (-0.07 - 0.03)	0.40
Other acquired without DCM (n=22)	-	-	0.02 (-0.02 - 0.07)	0.33

Borderline LVEF impairment was used as the comparator for impaired and severely impaired LVEF.
Each cardiac diagnosis was compared against all other cohort participants.
Gestation at delivery (days) was available for 243 women (of whom, 147 had DCM).
Gestation at delivery was skewed and therefore log-transformed for the purpose of these analyses.
Bold text indicates statistical significance ($p<0.05$).
LVEF, left ventricular ejection fraction; C.I., confidence interval; DCM, dilated cardiomyopathy.

Tables 2.13, 2.14 and 2.15 summarise the relationship between pre-pregnancy echocardiography parameters and pregnancy outcome. LVEF, diastolic function (E/A and E/E') and left atrial dilatation did not correlate with any pregnancy outcome. Increased LVMi was weakly associated with an increase in pre-eclampsia prevalence ($5g/m^2$ increase in LVMi: OR 1.18 [95% C.I. 1.01-1.38], p=0.04). Statistical significance was preserved after adjustment for pre-existing hypertension but not booking mean arterial pressure (hypertension: adjusted OR 1.03 [95% C.I. 1.00-1.07], p=0.04; mean arterial pressure [MAP]: adjusted OR 1.03 [95% C.I. 1.00-1.07], p=0.05). Aortic and mitral stenosis were also associated with an increase in pre-eclampsia prevalence (OR 18.90 [95% C.I. 1.46-244.35], p=0.02, and OR 23.63 [95% C.I. 3.31-168.59], p=0.002, respectively). Right systolic dysfunction, measured by tricuspid annular plane systolic excursion (TAPSE) was also associated with higher pre-eclampsia rates (1mm decline in TAPSE: OR 1.32 [95% C.I. 1.03-1.71]). No pre- / early pregnancy echocardiography parameters correlated with

birthweight Z score or gestation at delivery, except presence of concentric hypertrophy, which was associated with earlier gestation at delivery (log-transformed difference: -0.10 [95% C.I. -0.18- -0.02], p=0.01; Tables 2.14 and 2.15).

Table 2.13: Relationship between echocardiography parameters and pre-eclampsia prevalence.

Echocardiography parameters	DCM			All		
	N	OR (95% C.I.)	P value	N	OR (95% C.I.)	P value
LVM (increment 10g)	79	1.06 (0.86 - 1.30)	0.58	128	1.05 (0.99 - 1.23)	0.53
LVMi (increment 5g/m^2)	79	1.16 (0.98 - 1.38)	0.09	126	**1.18 (1.01 - 1.38)**	**0.04**
RWT (increment 0.1)	80	1.73 (0.51 - 5.85)	0.38	131	1.60 (0.60 - 4.23)	0.35
E/A (increment 0.2)	55	1.04 (0.80 - 1.34)	0.79	91	1.03 (0.79 - 1.33)	0.84
E/E' (increment 1)	31	0.92 (0.60 - 1.40)	0.70	46	0.90 (0.64 - 1.27)	0.56
LV remodelling (compared to normal)	76	-	-	126	-	-
Concentric hypertrophy (n=6)		-	-		12.40 (0.67 - 229.39)	0.09
Concentric remodelling (n=8)		**38.00 (1.26 - 1149.64)**	**0.04**		8.86 (0.50 - 0.76)	0.14
Eccentric hypertrophy (n=49)		2.30 (0.20 - 26.56)	0.50		2.64 (0.23 - 29.97)	0.43
Aortic stenosis* (n=3)	126	**40.33 (2.01 - 809.46)**	**0.02**	197	**18.90 (1.46 - 244.35)**	**0.02**
Aortic regurgitation* (n=16)	126	**13.22 (1.05 - 167.10)**	**0.046**	197	2.35 (0.26 - 21.41)	0.45
Mitral stenosis* (n=6)	126	**121.00 (7.53 - 1945.59)**	**0.001**	199	**23.63 (3.31 - 168.59)**	**0.002**
Mitral regurgitation* (n=25)	124	3.30 (0.32 - 34.52)	0.32	194	1.37 (0.15 - 12.20)	0.78
Pulmonary stenosis* (n=1)	-	-	-	-	-	-
Pulmonary regurgitation* (n=22)	122	**8.08 (1.05 - 62.30)**	**0.045**	192	3.38 (0.59 - 19.43)	0.17
Tricuspid stenosis*(n=0)	-	-	-	-	-	-
Tricuspid regurgitation* (n=43)	120	5.44 (0.72 - 41.18)	0.10	189	1.73 (0.31 - 9.79)	0.53
TAPSE (increment 1mm)	40	**0.74 (0.55 - 0.997)**	**0.048**	68	**0.76 (0.59 - 0.98)**	**0.03**

*Compared to none / physiological valvular regurgitation / stenosis.
N describes the number of observations included in the analysis.
n describes the number of pregnancies affected by the condition.
Bold text indicates statistical significance ($p<0.05$).
DCM, dilated cardiomyopathy; C.I., confidence interval; LVM, left ventricular mass; LVMi, LVM indexed to body surface area; RWT, relative wall thickness; E/A, early to late diastolic filling ratio; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; LA, left atrium; TAPSE, tricuspid annular plane systolic excursion.

Table 2.14: Relationship between echocardiography parameters and birthweight Z score.

Echocardiography parameters	DCM (n=151)			All (n=247)		
	N	Coefficient (95% C.I.)	P value	N	Coefficient (95% C.I.)	P value
LVM (g)	76	-0.00 (-0.01 - 0.00)	0.47	124	0.00 (0.00 - 0.00)	0.998
LVMi (g/m^2)	76	-0.01 (-0.02 - 0.00)	0.08	122	0.00 (-0.01 - 0.00)	0.29
RWT	77	-0.63 (-4.23 - 2.98)	0.73	127	-0.40 (-2.88 - 2.09)	0.75
E/A	52	-0.32 (-0.81 - 0.16)	0.18	87	-0.20 (-0.58 - 0.19)	0.31
E/E'	28	-0.08 (-0.22 - 0.07)	0.28	43	-0.01 (-0.09 - 0.06)	0.70
LV remodelling (compared to normal)	76			122		
Concentric hypertrophy (n=6)		-1.08 (-2.54 - 0.38)	0.15		-0.70 (-1.69 - 0.29)	0.17
Concentric remodelling (n=8)		0.59 (-1.18 - 2.35)	0.51		0.24 (-0.64 - 1.11)	0.60
Eccentric hypertrophy (n=49)		0.08 (-0.50 - 0.66)	0.79		0.04 (-0.41 - 0.49)	0.85
Aortic stenosis* (n=3)	114	0.98 (-0.74 - 2.71)	0.26	184	0.56 (-0.77 - 1.90)	0.41
Aortic regurgitation* (n=16)	114	-0.49 (-1.73 - 0.75)	0.44	184	-0.10 (-0.70 - 0.50)	0.75
Mitral stenosis* (n=6)	114	-0.85 (-2.26 - 0.57)	0.24	186	-0.51 (-1.45 - 0.44)	0.30
Mitral regurgitation* (n=25)	112	0.05 (-0.70 - 0.79)	0.91	181	0.08 ('-0.42 - 0.57)	0.76
Pulmonary stenosis* (n=1)	114	-		185	-0.03 (-2.33 - 2.27)	0.98
Pulmonary regurgitation* (n=22)	110	-0.22 (-0.92 - 0.47)	0.52	179	-0.11 (-0.60 - 0.39)	0.67
Tricuspid stenosis*(n=0)	-	-		-	-	
Tricuspid regurgitation* (n=43)	108	-0.46 (-1.09 - 0.17)	0.15	176	-0.33 (-0.74 - 0.08)	0.11
TAPSE (cm)	36	0.31 (-0.65 - 1.27)	0.52	64	0.02 (-0.19 - 0.23)	0.83

*Compared to none / physiological valvular regurgitation / stenosis.
N describes the number of observations included in the analysis.
n describes the number of pregnancies affected by the condition.
DCM, dilated cardiomyopathy; C.I., confidence interval; LVM, left ventricular mass; LVMi, LVM indexed to body surface area; RWT, relative wall thickness; E/A, early to late diastolic filling ratio; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; LA, left atrium; TAPSE, tricuspid annular plane systolic excursion.

Table 2.15: Relationship between echocardiography parameters and gestation at delivery (log-transformed).

Echocardiography parameters	DCM (n=151)			All (n=247)		
	N	Coefficient (95% C.I.)	P value	N	Coefficient (95% C.I.)	P value
LVM (g)	78	0.00 (0.00-0.00)	0.41	127	0.00 (0.00 - 0.00)	0.07
LVMi (g/m^2)	78	0.00 (0.00 -0.00)	0.82	125	0.00 (0.00 - 0.00)	0.59
RWT	79	0.06 (-0.13 - 0.24)	0.55	130	-0.19 (-0.39 - 0.01)	0.06
E/A	54	0.00 (-0.02 - 0.02)	0.78	90	-0.01 (-0.04 - 0.02)	0.60
E/E'	30	0.00 (-0.01 - 0.00)	0.56	45	0.00 (-0.01 - 0.01)	0.83
LV remodelling (compared to normal)	78	-	-	125	-	-
Concentric hypertrophy (n=6)		-0.02 (-0.09 - 0.06)	0.64		**-0.10 (-0.18 - -0.02)**	**0.01**
Concentric remodelling (n=8)		0.02 (-0.07 - 0.11)	0.62		-0.03 (-0.10 - 0.04)	0.40
Eccentric hypertrophy (n=49)		0.01 (-0.02 - 0.04)	0.46		-0.01 (-0.04 - 0.02)	0.59
Aortic stenosis* (n=3)	122	-0.02 (-0.14 - 0.09)	0.68	193	0.00 (-0.10 - 0.11)	0.95
Aortic regurgitation* (n=16)	122	0.04 (-0.05 - 0.12)	0.39	193	0.01 (-0.04 - 0.05)	0.83
Mitral stenosis* (n=6)	122	0.00 (-0.09 - 0.09)	0.97	195	-0.07 (-0.15 - 0.01)	0.07
Mitral regurgitation* (n=25)	120	0.00 (-0.05 - 0.05)	0.92	190	0.00 (-0.04 - 0.04)	0.87
Pulmonary stenosis* (n=1)	-	-	-	194	0.03 (-0.16 - 0.22)	0.79
Pulmonary regurgitation* (n=22)	118	0.03 (-0.01 - 0.08)	0.17	188	0.01 (-0.03 - 0.05)	0.59
Tricuspid stenosis* (n=0)	-	-	-	-	-	-
Tricuspid regurgitation* (n=43)	116	0.00 (-0.04 - 0.04)	0.92	185	-0.01 (-0.04 - 0.02)	0.55
TAPSE (cm)	39	0.02 (-0.03 - 0.07)	0.45	67	0.01 (0.00 - 0.02)	0.40

Gestation at delivery was skewed and therefore log-transformed for the purpose of these analyses.

N describes the number of observations included in the analysis.

n describes the number of pregnancies affected by the condition.

Bold text indicates statistical significance ($p<0.05$).

DCM, dilated cardiomyopathy; C.I., confidence interval; LVM, left ventricular mass; LVMi, LVM indexed to body surface area; RWT, relative wall thickness; E/A, early to late diastolic filling ratio; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; LA, left atrium; TAPSE, tricuspid annular plane systolic excursion.

Table 2.16 summarises the relationship between antenatal medication and adverse pregnancy outcomes. Pre-eclampsia prevalence was increased in women taking α blockers antenatally (1/5 [20.0%] versus 7/238 [2.9%], p=0.03). SGA (<10th centile) and FGR (<3rd centile) were more prevalent in women taking ß blockers antenatally (SGA: 47/109 [43.1%] versus 34/1229 [27.7%], p=0.02; FGR: 27/109 [24.8%] versus 13/122 [10.7%], p=0.005). Antenatal exposure to heparin and vitamin K antagonists were similarly associated with a higher prevalence of FGR (heparin: 15/59 [25.4%] versus 22/167 [13.2%], p=0.03; vitamin K antagonists: 2/3 [66.7%] versus 37/229 [16.2%], p=0.02). The relationship between heparin and fetal growth persisted after adjustment for potential confounders, including ischaemic heart disease (adjusted difference in birthweight Z score: -0.35 [95% C.I. -0.68- -0.01], p=0.04). This was not the case for vitamin K antagonists (adjusted difference in birthweight Z score: -0.94 [95% C.I. -2.24- 0.36], p=0.16).

Antenatal use of ß blockers was associated with lower birthweight Z score (difference: -0.47 [95% C.I. -0.76- -0.17], p=0.002) and lower gestation at delivery (log-transformed difference: -0.03 days [95% C.I. -0.05- 0.00], p=0.03). A similar relationship was also demonstrated for antenatal use of heparin (difference in birthweight Z score: -0.38 [95% C.I. -0.71- -0.04], p=0.03; difference in log-transformed gestation at delivery: -0.08 days [95% C.I. -0.11- -0.05], p<0.001). Although antenatal use of diuretics and calcium channel blockers were associated with earlier gestation at delivery (log-transformed difference -0.08 days [95% C.I. -0.11- -0.04], p<0.001 and log-transformed difference -0.12 days [95% C.I. -0.19- -0.05], p=0.001, respectively), they were not correlated with birthweight Z score.

Table 2.16: Prevalence of adverse pregnancy outcome depending on antenatal medication.

Antenatal medication		Pre-eclampsia	SGA < 10th centile	FGR < 3rd centile	Delivery < 34 weeks
DCM					
ß blockers	**Yes**	3/82 (4%)	28/75 (37%)	15/75 (20%)	**10/80 (13%)**
	No	2/67 (3%)	14/63 (22%)	6/63 (10%)	**2/65 (3%)**
Ca channel blockers	**Yes**	0/4 (0%)	2/4 (50%)	1/4 (25%)	1/4 (25%)
	No	5/144 (3%)	40/133 (30%)	20/133 (15%)	11/140 (8%)
α blockers	**Yes**	0/4 (0%)	1/3 (33%)	1/3 (33%)	1/4 (25%)
	No	5/144 (3%)	41/134 (31%)	20/134 (15%)	11/140 (8%)
Diuretics	**Yes**	1/31 (3%)	8/27 (30%)	5/27 (19%)	**9/30 (30%)**
	No	4/115 (3%)	34/108 (31%)	16/108 (15%)	**3/112 (3%)**
Heparin	**Yes**	3/41 (7%)	16/38 (42%)	**11/38 (29%)**	**8/41 (20%)**
	No	2/105 (2%)	25/97 (26%)	**9/97 (9%)**	**4/101 (4%)**
Vitamin K antagonists	**Yes**	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/2 (0%)
	No	5/143 (3%)	41/137 (30%	20/137 (15%)	12/144 (8%)
Aspirin	**Yes**	3/55 (5%)	17/51 (33%)	**12/51 (24%)**	**8/55 (15%)**
	No	2/76 (3%)	18/69 (26%)	**6/69 (9%)**	**2/73 (3%)**
All					
ß blockers	**Yes**	4/116 (3%)	**47/109 (43%)**	**27/109 (25%)**	19/114 (17%)
	No	4/127 (3%)	**34/122 (29%)**	**13/122 (11%)**	11/125 (9%)
Ca channel blockers	**Yes**	1/8 (13%)	3/8 (38%)	2/8 (25%)	**4/8 (50%)**
	No	7/235 (3%)	77/223 (35%)	37/223 (17%)	**26/231 (11%)**
α blockers	**Yes**	**1/5 (20%)**	2/4 (50%)	1/4 (25%)	1/5 (20%)
	No	**7/238 (3%)**	78/227 (34%)	38/227 (17%)	29/234 (12%)
Diuretics	**Yes**	1/39 (3%)	11/35 (31%)	6/35 (17%)	**10/38 (26%)**
	No	7/202 (3%)	69/194 (36%)	33/194 (17%)	**20/199 (10%)**
Heparin	**Yes**	4/62 (6%)	**27/59 (46%)**	**15/59 (25%)**	**18/62 (29%)**
	No	4/176 (2%)	**50/167 (30%)**	**22/167 (13%)**	**12/172 (7%)**
Vitamin K antagonists	**Yes**	0/3 (0%)	2/3 (67%)	**2/3 (67%)**	1/3 (33%)
	No	8/241 (3%)	78/229 (34%)	**37/229 (16%)**	29/237 (12%)
Aspirin	**Yes**	5/89 (6%)	34/84 (40%)	18/84 (21%)	15/89 (17%)
	No	3/133 (2%)	37/126 (29%)	17/126 (14%)	13/130 (10%)

Frequencies: n/ N (%)
Denominators vary between variables due to missing data.
Some women were exposed to several types of medication antenatally and therefore are represented more than once in this table.
Bold text indicates statistical significance derived using Chi-square test ($p<0.05$).

In order to explore the relationship between ß blocker exposure and pregnancy outcome, cardiovascular characteristics were compared between those exposed to antenatal ß blockers and those not. Severe LVEF impairment was more prevalent in those taking ß blockers antenatally (21/116 [18.1%] versus 11/127 [8.7%], $p<0.001$), as were chronic

hypertension (13/113 [11.5%] versus 5/125 [4.0%], p=0.03), DCM (82.116 [70.7%] versus 67/127 [52.8%], p=0.004) and ischaemic heart disease (10/116 [8.6%] versus 2/127 [1.6%], p=0.01). However, the relationship between antenatal ß blocker exposure and birthweight Z score persisted after adjustment for pre-existing hypertension, underlying cardiac diagnosis and severity of LVEF impairment (Table 2.17). Furthermore, the relationship between antenatal ß blocker exposure and birthweight Z score persisted, when only those known to have taken bisoprolol were included in the analysis (difference -0.48 [95% C.I. -0.83- -0.14], p=0.006).

Table 2.17: Multivariate regression coefficients for birthweight Z score.

Variables	Difference / coefficient	95% C.I.	(P value)
ß blocker exposure (n=116)	**-0.48**	**-0.81 - -0.16**	**0.006**
Chronic hypertension (n=18)	0.07	-0.56 - 0.69	0.83
Congenital (n=23)	-0.50	-1.03 - 0.03	0.06
Ischaemic (n=12)	-0.65	-1.35 - 0.06	0.07
Hypertensive (n=3)	-1.00	-3.33 - 1.33	0.40
Valvular (n=17)	-0.26	-0.86 - 0.34	0.39
Genetic without DCM (n=19)	-0.55	-1.10 - 0.01	0.05
Other acquired without DCM (n=22)	0.30	-0.25 - 0.84	0.29
Impaired LVEF (n=99)	-0.01	-0.34 - 0.32	0.95
Severely impaired LVEF (n=32)	-0.18	-0.66 - 0.30	0.45
Constant	**-0.43**	**-0.73 - -0.13**	**0.005**

Borderline LVEF impairment was used as the comparator for impaired and severely impaired LVEF and DCM (dilated cardiomyopathy) was used as the comparator for cardiac diagnoses.
Bold text indicates statistical significance (p<0.05).
C.I., confidence interval; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction.

2.4.4. Cardiac outcomes

MACE occurred in 3/247 (1.2%) pregnancies: one woman with left ventricular non-compaction had a transient ischaemic accident and there were two maternal deaths (secondary to 1) a drug overdose and 2) valvular thrombosis one month postpartum associated with a metal aortic valve). Thirty five (14.2%) women developed acute heart failure and 13/247 (5.3%) developed pulmonary oedema. Sustained arrhythmia complicated 10/247 (4.0%) pregnancies. Table 2.18 summarises the prevalence of adverse cardiac outcomes according to severity of LVEF impairment and underlying

cardiac diagnosis. Worse LVEF impairment was associated with a higher rate of acute heart failure (p<0.001).

Table 2.18: Prevalence of adverse cardiac outcome depending on severity of LVEF impairment and cardiac diagnosis.

Severity of LVEF impairment	MACE	Acute heart failure	Pulmonary oedema	Sustained arrhythmia
Borderline	2/116 (1.7%)	**7/115 (6.1%)**	1/115 (0.9%)	6/115 (5.2%)
Impaired	0/98 (0.0%)	**12/96 (12.5%)**	0/95 (0.0%)	2/96 (2.1%)
Severely impaired	1/31 (3.2%)	**13/31 (41.9%)**	0/31 (0.0%)	2/31 (6.5%)
P value	0.29	**<0.001**	0.58	0.41
Primary cardiac diagnosis				
DCM	**0/149 (0.0%)**	21/121 (17.4%)	1/147 (0.7%)	9/148 (6.1%)
Congenital	**0/23 (0.0%)**	0/23 (0.0%)	0/21 (0.0%)	1/21 (4.8%)
Ischaemic	**0/12 (0.0%)**	0/12 (0.0%)	0/12 (0.0%)	0/12 (0.0%)
Hypertensive	**0/3 (0.0%)**	0/3 (0.0%)	0/3 (0.0%)	0/3 (0.0%)
Valvular	**3/17 (17.6%)**	3/17 (17.6%)	0/17 (0.0%)	0/17 (0.0%)
Genetic without DCM	**0/19 (0.0%)**	0/19 (0.0%)	0/19 (0.0%)	0/19 (0.0%)
Other acquired without DCM	**0/22 (0.0%)**	0/22 (0.0%)	0/22 (0.0%)	0/22 (0.0%)
P value	**<0.001**	0.14	0.996	0.60

Frequencies: n/N (%)
Denominators vary between variables due to missing data.
Severity of LVEF impairment was classified as: borderline (50-54%), impaired (36-49%) and severe (≤35%)[318].
P values represent comparison between LVEF impairment categories and cardiac diagnoses using Chi-square test.
Bold text indicates statistical significance (p<0.05).
LVEF, left ventricular ejection fraction; SGA, small for gestational age; FGR, fetal growth restriction; DCM, dilated cardiomyopathy.

2.5. Discussion

This study describes a large retrospective cohort of pregnancies affected by pre-existing maternal cardiomyopathy. Cardiovascular disease is the leading cause of indirect maternal deaths in the UK[2]. For this reason, combined with evidence linking pre-existing cardiovascular disease with poor obstetric outcomes[154], this represents a high risk obstetric population. Despite this, pre-eclampsia prevalence was not increased compared to the general population and neither was preterm (<37 weeks) pre-eclampsia. On the other hand, there were high rates of SGA (34.6%), FGR (17.1%), preterm delivery <37 weeks (30.0%) and iatrogenic delivery <34 weeks (8.6%). The severity of LVEF impairment

did not correlate with any pregnancy outcome and there was only one case of pre-eclampsia amongst the pregnancies complicated by the most severely impaired baseline cardiac function (LVEF ≤35%). The only cardiac diagnoses that correlated with pregnancy outcome were hypertensive and ischaemic heart disease, supporting a potential vascular mechanistic link between cardiovascular and placental disease.

Despite a lack of association with LVEF impairment severity, a number of correlations were demonstrated between other echocardiography parameters and pre-eclampsia prevalence. Increased LVMi, aortic and mitral stenosis and reduced right ventricular systolic function (measured by TAPSE) were all associated with an increased prevalence of pre-eclampsia. No correlation was demonstrated between echocardiography parameters and birthweight Z score.

Both expected and unexpected associations between antenatal medication use and pregnancy outcome were demonstrated in this cohort. ß blockers were consistently associated with adverse pregnancy outcome, including an increased prevalence of SGA and FGR and a negative correlation with birthweight centile and gestation at delivery. In those who were prescribed ß blockers, bisoprolol was the most commonly used agent (88.3%). Antenatal heparin use was surprisingly associated with lower birthweight centile, gestation at delivery and a higher prevalence of FGR.

Adverse cardiac events, although less frequent than previous reports in the literature[148,149], were by no means uncommon, thereby endorsing close antenatal and postnatal surveillance in this high risk group.

In summary, pre-eclampsia rates were not increased in this group of women with pre-existing left ventricular systolic dysfunction. However adverse obstetric and cardiac outcomes were not uncommon, highlighting the need for close multidisciplinary care during and after pregnancy. Some associations between cardiac diagnoses and pregnancy outcome indicate a potential vascular link between cardiovascular and placental disease. However, the absence of association between the severity of left ventricular functional impairment and pre-eclampsia prevalence supports the theory that postnatal

cardiovascular dysfunction is at least in part a consequence rather than cause of pre-eclampsia.

2.5.1. Mechanistic link between pre-eclampsia and maternal cardiovascular dysfunction

The relationship between pre-eclampsia and maternal cardiovascular dysfunction is well evidenced in the literature[5–7]. Persistence of this relationship after adjustment for mutual risk factors[144,145] indicates a direct mechanistic link between the two. However, the direction of this remains elusive. It has been proposed that placental malperfusion and subsequent pre-eclampsia are a consequence rather than cause of maternal cardiovascular maladaptation to pregnancy and thereby represent end-organ dysfunction[329]. This is supported by the equivalent predictive nature of ophthalmic and uterine artery Dopplers for pre-eclampsia, despite no anatomical link between the former and trophoblast invasion[330]. If cardiovascular dysfunction precedes placental dysfunction, one would anticipate a high prevalence of pre-eclampsia in women with pre-existing cardiovascular disease. This then lends the question of what cardiovascular parameters influence placental function. Hypertension is a vascular disease[331] that is associated with a high prevalence of pre-eclampsia (~1/4 women)[332–335]. It is therefore plausible that vascular function has a causal role in placental dysfunction and subsequent pre-eclampsia; this is in keeping with the previously described predictive nature of non-uterine vascular function for pre-eclampsia[330]. On the other hand, postnatal cardiac dysfunction frequently persists after pre-eclampsia in the absence of hypertension[5], potentiating a pathological process beyond vascular disease. Pre-eclampsia is associated with left ventricular diastolic and systolic dysfunction, remodelling and reduced CO postpartum[5,7,139,140,336]. The presence of these abnormalities prior to pre-eclampsia-affected pregnancies is less certain.

In a healthy pregnancy, maternal CO increases by ~45%[84] in order to accommodate the diversion of blood flow to the growing uterus, which receives up to 12% of the total CO[87]. In this way, an insufficient rise in CO could negatively impact on uterine and therefore placental perfusion[87]. Diastolic dysfunction is considered a precursor to systolic dysfunction[66], which inevitably leads to a reduction in CO[337]. In this cohort of women with

impaired systolic function (defined by LVEF <55%), pre-eclampsia prevalence was comparable with the general population. Additionally, only hypertensive and ischaemic heart disease were associated with worse obstetric outcome, when compared to other cardiac diagnoses. Given the observational nature of this study, causality cannot be confirmed or excluded, however these findings indicate that vascular but not cardiac dysfunction confers a significant additional risk for placental disease. In this way, these findings support a causal rather than consequential role of pre-eclampsia in the development of postnatal cardiac dysfunction.

2.5.2. Strengths and limitations

This was a relatively large multicentre study comprising of data from 12 different sites across the UK and Australia. This enabled 151 pregnancies complicated by DCM to be included in the study, despite the rarity of this condition. Inclusion of 247 pregnancies affected by maternal cardiomyopathy allowed correlation of pre-existing cardiac parameters with pregnancy outcome. To my knowledge, this is the first study of women with pre-existing cardiac disease, in which pre-eclampsia is the primary outcome. The pre-specified aims of exploring the link between pre-eclampsia and cardiac dysfunction, ensured appropriate capture of pre-eclampsia risk factors and adjustment for these where appropriate. Although the retrospective nature of the study has its limitations, variables for collection were pre-specified and confirmed by the clinical care team following careful review of echocardiography reports, online clinical reporting systems, clinic letters and case notes.

One limitation of this study is that echocardiography data was incomplete for a number of women. This was due to transfer of care from other hospitals and differences in reporting protocols depending on the hospital and underlying diagnoses. Incomplete echocardiography data limited statistical power when correlating pre-pregnancy cardiac parameters with pregnancy outcomes. In particular, CO was not routinely reported and therefore there was insufficient data to explore the link between CO and pregnancy outcome. Similarly E/E', a sensitive measure of diastolic function, which is commonly raised following pre-eclampsia[36], was infrequently recorded. If the link between cardiac dysfunction and pre-eclampsia is due to a problem with cardiovascular supply rather than

demand, CO would be a useful pre-pregnancy parameter to determine this. Unfortunately, confirmation or exclusion of CO as the mechanistic link between cardiac and placental dysfunction is currently beyond the scope of this study.

Where pre-pregnancy echocardiography data were not available, first trimester data were used in their place. A gestation cut-off of 12 weeks was used to maximise available data whilst limiting the potential effect of pregnancy on cardiac structure and function. Despite this, it is possible that inclusion of early pregnancy echocardiography data could mask and therefore underestimate pre-existing cardiac morbidity, as a result of pregnancy-related cardiovascular adaptation[84–86].

LVEF was categorised into borderline, impaired and severely impaired[318]. This in part aimed to compensate for the variation in 1) measurement techniques (including Simpson's biplane and subjective visual estimation) and 2) reporting (including discrete numbers, ranges and categorisations as above). However, categorisation of LVEF inevitably reduced statistical power and may have contributed to a type II statistical error.

Another consideration is routinely indicated preterm and early term delivery in women with cardiomyopathy could have masked a potential for increased prevalence of term pre-eclampsia. However, since preterm pre-eclampsia is associated with the worst long-term cardiovascular risk[9,11,13], the comparable prevalence of preterm pre-eclampsia with the general population is an important finding.

Finally, for the purpose of this study, pre-eclampsia and FGR were considered clinical proxies for placental dysfunction, in the absence of confirmatory placental histology. This is due to the widely accepted theory of their mutual placental origin[314–317], however this limits the ability to link pre-pregnancy cardiac parameters with distinct placental pathologies.

2.5.3. Adverse pregnancy outcomes

The results of the primary outcome demonstrated no increased prevalence in pre-eclampsia in women with pre-existing cardiomyopathy, compared with the general

population. As mentioned above, iatrogenic preterm delivery was common in this cohort, potentially masking the prevalence of term pre-eclampsia if these women were to be conservatively managed. Importantly, prevalence of preterm pre-eclampsia was similarly not increased.

The high rates of SGA and FGR could be a consequence of reduced uteroplacental blood supply in women with cardiomyopathy[338]. This has been proposed as a potential link between cardiovascular disease and pre-eclampsia[339]. However, this was not demonstrated in this study, in which only eight cases of pre-eclampsia (compared with 40 cases of FGR) were seen. The high rates of SGA and FGR could also be attributed to antenatal drug exposure, in particular, ß blockers (see section 2.5.5). The high prevalence of preterm births was largely attributed to routinely indicated delivery, spontaneous preterm labour and worsening maternal cardiac disease. Since the majority of births were iatrogenic, a biological link is hard to ascertain. Decisions on timing of delivery are complex, incorporating both maternal and fetal factors; in this way they can be significantly influenced by underlying maternal diagnoses and presumed associated risks. As mentioned previously, the high prevalence of iatrogenic preterm births (23.5%), of which more than half were indicated by non-placental disease, limits the ability to investigate the prevalence of term pre-eclampsia in this cohort. Additionally, the frequent use of antenatal ß blockers could have masked late hypertension, thereby preventing a diagnosis of pre-eclampsia being made.

2.5.4. Relationship between cardiac parameters and pregnancy outcome

No correlation was demonstrated between severity of left ventricular systolic (LVEF) or diastolic (E/A and E/E') dysfunction and pregnancy outcome. This suggests that left ventricular dysfunction following pre-eclampsia is unlikely to be solely a consequence of pre-existing impairment. In this way, it is plausible that postnatal left ventricular dysfunction develops during or after the onset of pre-eclampsia, thereby supporting the hypothesis that pre-eclampsia poses a direct deleterious effect on the heart. In contrast, increased LVMi was weakly associated with a higher rate of pre-eclampsia. This is in keeping with de Haas *et al.*'s meta-analysis which demonstrated a disproportionate increase in LVM in hypertensive disorders of pregnancy (95% compared with 24% in

normotensive pregnancies)[6]. On the other hand, pre-eclampsia is typically associated with concentric remodelling during[6] and after[5] pregnancy, whereas hypertrophy was predominantly eccentric in this cohort. The correlation between LVMi and pre-eclampsia in this cohort indicates that unlike postnatal left ventricular systolic and diastolic dysfunction, remodelling likely precedes and potentially predicts the development of pre-eclampsia. This correlation could therefore reflect pre-existing comorbidities such as hypertension, supported by a weakening or loss of this relationship after adjustment for pre-existing hypertension and booking MAP, respectively.

Both aortic and mitral stenosis were associated with an increased prevalence of pre-eclampsia. Both conditions contribute to a reduction in CO[340] thereby supporting a potential role of reduced CO and subsequent uteroplacental supply in the pathogenesis of pre-eclampsia. On the other hand, valvular stenosis was not associated with SGA, FGR or birthweight Z score, adding uncertainty to this hypothesis. Additionally, valvular pathology was dichotomised for the purpose of this study and therefore combined mild, moderate and severe disease in one group, thereby limiting the ability to investigate a potential dose-effect.

Right ventricular systolic dysfunction, as measured by TAPSE, was associated with a higher prevalence of pre-eclampsia, however this should be interpreted with caution, given the small numbers involved (n=68). Right ventricular function has not been the focus of this thesis and is less well represented in the literature; however, given the findings in this study, the link between right ventricular function and pre-eclampsia warrants further investigation.

Hypertensive cardiomyopathy was the only cardiac diagnosis to be associated with pre-eclampsia prevalence. This is not surprising as hypertension is a well-established risk factor for pre-eclampsia (pre-eclampsia prevalence is 17-25% in those with pre-existing hypertension)[332–334], and hypertensive cardiomyopathy likely represents a severe end of the hypertensive spectrum. Presence of ischaemic heart disease was associated with reduced birthweight Z score and gestation at delivery. Ischaemic heart disease is a consequence of coronary microvascular disease, which results in hypoperfusion of the

myocardium. Cauldwell *et al.*[341] demonstrated an increased prevalence of SGA (27%) and pre-eclampsia (15%) in those with a history of ischaemic heart disease, however mutual risk factors were not adjusted for, despite a high prevalence of smoking, hypertension and diabetes in their cohort (14%, 26% and 23%, respectively). In this cohort, pre-eclampsia rates were not increased in women with ischaemic heart disease, however the numbers are small. Additionally the relationship between ischaemic heart disease and birthweight Z score did not persist after adjustment for smoking, a known mutual risk factor[342,343]. These findings do not support a direct mechanistic link between prior ischaemic heart disease and adverse pregnancy outcome.

As previously mentioned, there was a disproportionately high prevalence of FGR (both term and preterm) in this cohort of women with pre-existing cardiac dysfunction. Like pre-eclampsia, FGR is thought to be of placental origin[314–317]; therefore the present data may add uncertainty to the current hypothesis that placental dysfunction is not a direct consequence of cardiac dysfunction. However, the lack of association between any measure of pre-pregnancy cardiac impairment and birthweight Z score / FGR prevalence makes a causal role of cardiac dysfunction in the development of FGR unlikely. Additionally, if the preterm FGR seen in this cohort shared the presumed aetiology of preterm pre-eclampsia, in which early placentation is affected by defective spiral artery remodelling[314], the prevalence of co-existing hypertension (i.e. pre-eclampsia) should also be higher. In this cohort only 33% of women with early-onset FGR developed pre-eclampsia, compared with 52-60% women in the early-onset FGR cohorts in TRUFFLE[344] and STRIDER[345]. It is therefore unlikely that reduced uteroplacental perfusion and subsequent poor placentation is the cause of FGR in this cohort. However in order to explore this further, measures of early placentation (including uterine artery Doppler, pregnancy-associated plasma protein-A and placental pathology) need to be investigated in this or a similar cohort. Given the inconsistencies described above, the high FGR prevalence seen in this study could instead be attributed to concomitant antenatal medication (see below, section 2.5.5).

2.5.5. Relationship between antenatal medication and pregnancy outcome

Only five women were taking α blockers and three women were taking vitamin K antagonists antenatally. It is therefore likely that the associations demonstrated between antenatal exposure to these medications and adverse pregnancy outcomes constitute a type I statistical error.

Heparin has previously been proposed as a preventative strategy against placenta-mediated complications[346–350], including pre-eclampsia and FGR. This is due to the association of thrombophilias[351,352] and placental microvascular thrombi[353,354] with these pregnancy complications. However, findings from several meta-analyses have been conflicting, with a modest reduction in placenta-mediated pregnancy complications (including pre-eclampsia and FGR) shown in some[346–348] and not in others[349,350]. Given these inconsistencies, antenatal heparin is not currently recommended as a preventative strategy by NICE guidelines[328]. However, the inverse relationship seen between heparin use and fetal growth in this cohort was unexpected. It is unlikely to represent a causal relationship, given the wealth of existing data demonstrating no harmful effect of antenatal heparin on fetal growth[346–348,350]. It is therefore plausible that this relationship is a result of confounding factors, including vascular and prothrombotic disorders, that trigger the use of antenatal heparin and potentiate the development of pre-eclampsia. Unfortunately, adjustment for such confounders was not possible in this dataset.

The potential negative effect of antenatal ß blocker use on fetal growth has long been considered[355–359]. A recent meta-analysis including 13 cohort studies (in which 14,010/3,281,239 pregnancies were exposed to ß blockers antenatally), demonstrated a significant increase in SGA associated with antenatal ß blocker use (OR 1.72 [95% C.I. 1.59-1.85], $p<0.001$)[355]. It has been proposed that ß blocker subtypes are associated with varying risk[356,357]. Labetalol, which is an α and ß antagonist and partial ß2 agonist[360–362], is commonly used as a first-line antihypertensive in pregnancy[328]. It is possible that the partial ß2 agonistic properties of labetalol induce vasodilation in placental and umbilical vasculature, thereby favourably increasing placental blood flow[360,363,364]. However atenolol, which selectively blocks ß1 adrenergic receptors, is not recommended in pregnancy[328] due to negative associations with fetal growth[358,365,366]. The impact of

bisoprolol (a selective ß1 receptor blocker[367]), which was the most commonly prescribed ß blocker in this cohort, on fetal growth is less understood.

The mechanism behind the association between ß blockers and fetal growth is not known, but could relate to reduced CO and subsequently reduced uteroplacental blood flow[357,368]. Alternatively, despite its ß1 cardio-selective nature[367], bisoprolol could have a direct effect on the placental vasculature. Placental vasculature has α, ß1, ß2 and ß3 receptors[369,370], however their relative activity is not known. It is possible that some of these receptors are down-regulated as a result of an evolutionary mechanism to protect the fetus against maternal adrenergic response, thereby potentiating varying placental responses to different ß blockers, depending on which receptors they target. There is some evidence from *in vitro* studies that ß blockers can induce vasoconstriction in placental vessels[360,371], thereby proposing a potential mechanistic link between ß blocker exposure and FGR.

The rationale for ß blocker use in the context of cardiac dysfunction is to protect the heart against the deleterious effects of increased adrenergic activity, by reducing HR, BP and myocardial oxygen demand[372]. As a result, ß blockers have been shown to improve LVEF, heart failure symptoms and long-term prognosis[373–375]. It is therefore likely that continued antenatal use of ß blockers indicates a particular cardiac phenotype or degree of severity. This is supported by the increased prevalence of hypertension, severe LVEF impairment and DCM in those taking antenatal ß blockers in this cohort. However despite this, the relationship between ß blockers and birthweight Z score persisted after adjustment for FGR risk factors and cardiac phenotype, indicating a direct mechanistic link between the two.

Antenatal exposure to diuretics was associated with lower gestation at delivery. It was not associated with other measures of pregnancy outcome and therefore a biologically plausible causal link is unlikely. Instead, this association probably reflects a more severe or worsening cardiac phenotype indicating early delivery. As mentioned previously, it is possible that associations between antenatal medication use and pregnancy outcome reflect underlying cardiac pathology rather than direct causal links. However, the lack of

association demonstrated between cardiac diagnosis and degree of impairment does not support this theory.

2.5.6. Implications of these findings

This study provides valuable information to aid clinicians with pre-conception and antenatal counselling of patients with cardiomyopathy. Women with cardiomyopathy can be reassured that their risk of pre-eclampsia does not appear to be significantly increased, however serial ultrasound scanning is likely warranted to monitor for FGR. Women with pre-existing cardiomyopathy have a high chance of early delivery and delivery by Caesarean section. Data from this study support previous concerns about ß blocker use in pregnancy; further study is required to explore the effect of bisoprolol on the placenta to guide clinical decision-making. Finally, the absence of dose-effect demonstrated by lack of correlation between severity of left ventricular dysfunction and pregnancy outcome does not support a causal role of cardiovascular dysfunction in the development of pre-eclampsia. This highlights the need for further mechanistic exploration into pre-eclampsia as a cause of cardiovascular dysfunction.

2.6. Conclusion

The results of this study should be interpreted within the context of several previously discussed limitations. However, current findings indicate that pre-existing impaired cardiac function confers no significant additional risk of pre-eclampsia. This contrasts with the prevalence of FGR, which was high in this cohort. The mechanism linking an increased prevalence of FGR and cardiac dysfunction remains inconclusive, however it could be attributed to reduced uteroplacental perfusion as a consequence of the underlying cardiac disease or concomitant medication (in particular, ß blockers). Given the observational nature of this study, causality cannot be confirmed or excluded. However, the lack of association between pre-pregnancy left ventricular systolic and diastolic dysfunction and pre-eclampsia supports the hypothesis that postnatal left ventricular dysfunction is at least in part a consequence rather than cause of pre-eclampsia. The association between ischaemic and hypertensive cardiomyopathy and pregnancy outcome indicate that in cases where pre-existing cardiovascular impairment contributes to the development of pre-eclampsia, the mechanistic link is more likely

vascular/endothelial than purely cardiac. To conclude, observational findings from this study support a causal rather than consequential role of pre-eclampsia in the development of postnatal cardiac dysfunction. Further study is needed to explore the mechanistic link.

CHAPTER 3: THE USE OF A PRE-ECLAMPSIA-LIKE ANIMAL MODEL TO CHARACTERISE SFLT-INDUCED CARDIOVASCULAR DYSFUNCTION.

3.1. Introduction

There is a plethora of observational data linking pre-eclampsia with postnatal maternal cardiac dysfunction[5–7] and long-term maternal cardiovascular risk[8–17]. The persistence of this association after adjustment for mutual risk factors[144,376] indicates a causal relationship; however the direction of causality is not known. The animal experiments outlined in this chapter were designed to establish a previously reported pre-eclampsia-like rodent model in our unit, with a view to further exploring the mechanistic link between pre-eclampsia and cardiac dysfunction. Having explored this link in the opposing direction in chapter two, the purpose of this chapter was to investigate the hypothesis that pre-eclampsia is a cause, rather than consequence of maternal cardiac dysfunction. This hypothesis is supported by previous clinical studies demonstrating associations between sFlt levels and cardiac function and cardiovascular prognosis both in[156,178] and outside[174] of pregnancy. If a maternal cardiac phenotype was demonstrated, a postnatal interventional study using oral enalapril was planned to test its reversibility.

Pre-eclampsia is a condition unique to primates[212], who share the deep trophoblast invasion and spiral artery remodelling characteristic of human placentas[377]. Although pre-eclampsia is not known to develop in rodents, they have frequently been used to model aspects of pre-eclampsia, given the cost, ethical and legal issues associated with primate research[213]. An ideal rodent model would include all aspects of pre-eclampsia including poor trophoblast invasion, maternal hypertension, proteinuria, endothelial dysfunction, FGR, and angiogenic imbalance[210]. Unfortunately, existing rodent models are only able to recapitulate part of the pre-eclampsia-like phenomenon. Typically, these models focus on either stage of Redman's[40] two-stage model of pre-eclampsia. In "stage one models", abnormal trophoblast invasion, due to STOX1-overexpression, induced gestational hypertension, proteinuria, renal and placental histological changes and raised sFlt[214]. Excess immune response, induced by chronic infusion of interleukin-17, has also been used for stage one; this was associated with gestational hypertension and reduced placental and fetal weight[215]. Placental insult has been surgically induced in the RUPP

model[172]. Along with the triad of maternal hypertension, proteinuria and FGR, the RUPP model was associated with a threefold increase in sFlt, comparable with human pre-eclampsia[378]. The association between stage one models and raised antiangiogenic markers triggered "stage two models" to be developed, in which sFlt has been directly administered, either via adenoviral vector[138] or minipump infusion[135–137].

The present work has focussed on a previously-reported anti-angiogenic model of pre-eclampsia[135–137], in which exogenous sFlt was infused into pregnant Sprague-Dawley rats via osmotic minipumps in order to cause the triad of maternal hypertension, proteinuria and FGR. This model[137] has previously mirrored the threefold increase in maternal sFlt, as seen in human pre-eclampsia[164]. It therefore provides the opportunity to test the hypothesis that sFlt mediates cardiac dysfunction in the context of pre-eclampsia, in the absence of human-related confounders. To my knowledge, maternal cardiac function and remodelling has not previously been investigated in this model.

In a rat model of chronic kidney disease, induced by 5/6th resection of renal tissue, Di Marco *et al*.[173] infused sFlt via subcutaneous osmotic minipumps into male Sprague-Dawley rats. Compared with vehicle controls, sFlt-infused animals exhibited cardiac dysfunction (determined using echocardiography), reduced myocardial capillary density and increased myocardial fibrosis[173]. This study demonstrated the feasibility of using echocardiography and histological markers to investigate cardiac dysfunction in a sFlt-infused rat model. Along with the known association between third trimester sFlt levels and postnatal maternal cardiac dysfunction[156], Di Marco's model supports the hypothesis that sFlt has a causal role in the development of maternal cardiac dysfunction in the context of pre-eclampsia.

As a first step in exploring this hypothesis, a series of experiments was planned to establish and subsequently validate the use of the model in our unit.

3.2. Aim

To assess if elevated sFlt causes maternal cardiac dysfunction in a pre-eclampsia-like rat model.

3.3. Methods

In order to test the hypothesis that raised sFlt is a mediator of cardiac dysfunction in pre-eclampsia, a previously reported pre-eclampsia model[135–137] was used, in which pregnant Sprague-Dawley rats were treated with exogenous sFlt, infused via osmotic minipumps from gestational day (GD) 13 to 19 (term is ~GD 22). Paired allocation (1:1 block randomisation to sFlt or normal saline [0.9% sodium chloride]) and blinding were applied to minimise the risk of bias. A pre-eclampsia-like phenotype was defined previously as significantly higher BP and proteinuria levels in the treatment group, compared with saline controls[135]. Measurements used to assess maternal cardiac function are outlined below.

3.3.1. Ethics

This work was carried out under existing Home Office institutional (50/2506; University of Manchester), project (P9755892D; holder Dr Mark Dilworth) and personal licenses (IF5788936; holder Dr Laura Ormesher).

3.3.2. General housing and husbandry

All procedures were performed in accordance with the Animals (Scientific) Procedures Act 1986. Female rats were housed together and sources for enrichment, including wooden sticks, tubes and nesting materials were provided. Individual rats were identified using tail markings. Group housing was used, to minimise behavioural disturbance and stress[379]. Cage dividers were used following pump insertion, to allow two animals to share a cage but to prevent any potential contamination between treatment groups. Female rats were paired with stud males overnight and replaced into their home cages the following morning. Successful timed mating was presumed following identification of a vaginal plug that morning (designated GD 1). Maternal weight gain was measured between GD 1, GD 13 and GD 17 to 18 to monitor the pregnancy.

3.3.3. Protocol one

Figure 3.1 illustrates the experimental design of protocol one. Rats were trained to acclimatise to the tail-cuff BP measurement system (section 3.3.3.3) prior to mating. This provided non-pregnant BP data. The minipumps were inserted on GD 13 (section 3.3.3.1) and pre- and on-treatment BP and proteinuria measurements were taken on GD 10 to 12 and GD 17 to 19, respectively. Assuming an average gestation length of 22.5 days[380], cages were checked for pups twice a day (morning and evening) from GD 21 to 23. Characterisation of pregnancy outcomes included litter size and pup weight, on postnatal day (PND) 1. Pups demonstrating no signs of life on PND 1 were defined as stillborn. Female rats were euthanised by cervical dislocation following anaesthesia (isoflurane 2% in oxygen at 2L/min) on PND 1.

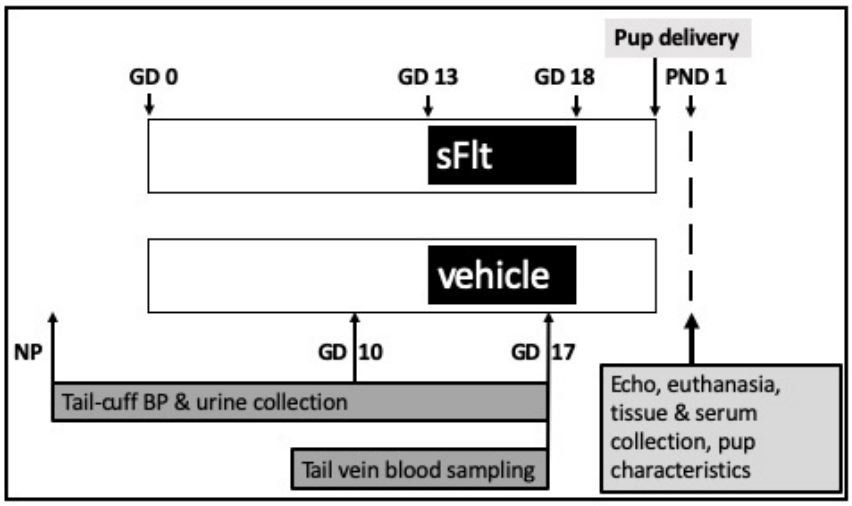

Figure 3.1: Experimental design of protocol one.
Schematic of protocol one illustrating the timing of regulated procedures.
GD, gestational day; PND, postnatal day; sFlt, soluble fms-like tyrosine kinase; vehicle was normal saline; NP, non-pregnant; BP, blood pressure; echo, echocardiography; pup characteristics were litter size and pup weight.

3.3.3.1. sFlt rat model

Sprague-Dawley female rats were purchased from Charles River at 200 to 225g, approximately two weeks prior to timed mating. The recombinant mouse sFlt/Fc Chimera (471-F1-100, carrier-free, R&D Systems, UK) and placebo (normal saline) solutions for the minipumps were made in a single batch, by an unblinded colleague, and aliquots stored at -80°C until the day of minipump insertion. A dose of 3.7µg/kg/day[136,137] was used in the treatment arm. The dose of 3.7µg/kg/day was achieved using the Alzet micro-osmotic pump model 1007D for subcutaneous infusion, with a pre-determined infusion rate of

0.5µL/h. sFlt solution (reconstituted with normal saline to a concentration of 100ng/µL) or vehicle was inserted into the minipumps under sterile conditions at the time of surgery. Assuming a maternal weight of 320g at GD 13 (mean GD 13 weight was 315±21g), this equates to 3.7µg/kg/day[136,137] sFlt in the treatment arm. Rats were anaesthetised at GD 13 using inhalational isoflurane (4% for induction; 2% maintenance in oxygen at 2L/minute) and positioned prone on the operating table. A 1cm interscapular incision was made and the pump was inserted[173] into a subcutaneous pocket large enough to facilitate free movement. The skin was then closed using subcuticular absorbable sutures. Subcutaneous buprenorphine (0.19mg/kg) and sterile saline (1mL) were administered intraoperatively. The animals were then monitored post-operatively until normal behaviours resumed.

3.3.3.2. Osmotic minipump

Alzet osmotic minipumps function via negative osmotic pressure from the surrounding environment (the subcuticular space) to the high osmolality 'osmotic layer' of the minipump[381]. This causes water to diffuse into the pump through a semipermeable membrane, compressing the flexible reservoir, which contains the solution to be infused (here, reconstituted sFlt/saline). In turn, sFlt/saline solution is secreted through the flow moderator at a predetermined rate (Figure 3.2).

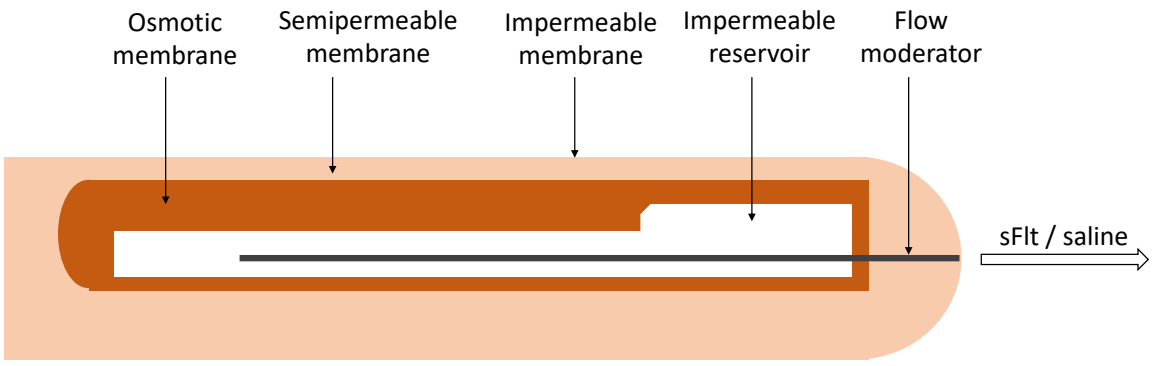

Figure 3.2: Mechanism of Alzet osmotic minipump.
Illustration of the different compartments of the Alzet minipump.

3.3.3.3. Tail-cuff BP protocol

BP was measured non-invasively using the PanLab BP system (model LE5001) on non-restrained rats at room temperature (Figure 3.3).

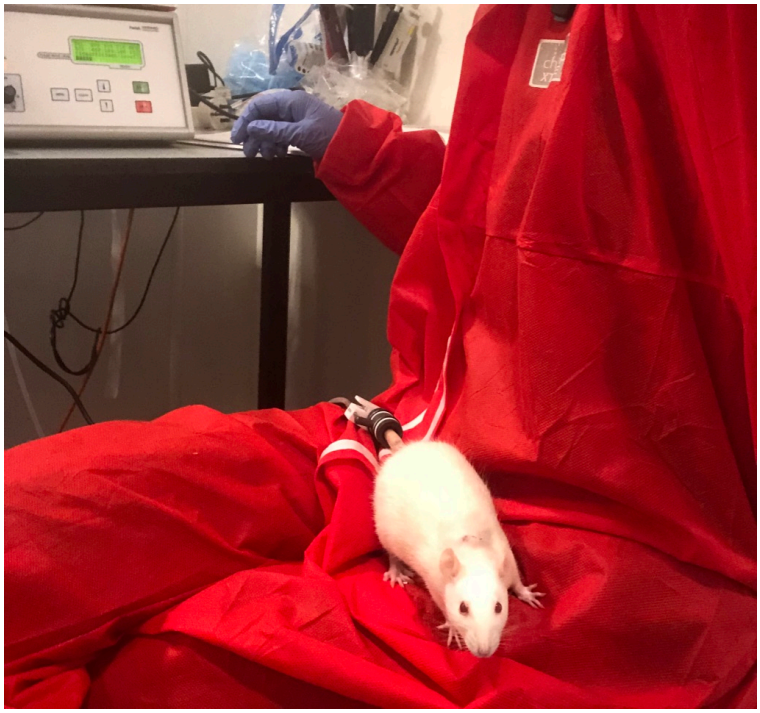

Figure 3.3: Unrestrained tail cuff BP measurement.
The unrestrained rat is positioned on the researcher's lap. The tail cuff is connected to the PanLab BP system and results are recorded in real-time.
BP, blood pressure.

Each rat was placed on the researcher's lap and given a minimum of ten minutes to acclimatise prior to cuff-positioning. The cuff was then placed at the base of the tail[301], to avoid the 2mmHg drop in pressure per cm of tail length[306]. To account for significant biological variability in BP, the cuff was inflated repetitively (~20 to 40 times) over ~60 minutes for each rat. sBP was determined by the pressure at which the pulse signal was lost. The mean sBP was calculated from the middle 11 to 12 readings. At the time of BP measurement (pre- and post-pump insertion), rat urine was collected non-invasively by immediate aspiration of voided urine. Urine samples were then frozen at -80°C for future analysis.

3.3.3.4. Echocardiography protocol

In order to assess cardiac function, echocardiography was performed under anaesthesia on PND 1 in the left lateral position, using an Acuson Sequoia C256 ultrasound system fitted with a 14-MHz transducer (Siemens). Hair was removed from the overlying area using hair-removal cream to optimise visualisation. A2C, A4C, A5C, PSAX and PLAX views were obtained. All measurements were made post-hoc from three separate cardiac cycles

using OsiriX v.11 software. Left ventricular geometry was measured in PLAX using 2D and M-Mode settings (Figure 3.4).

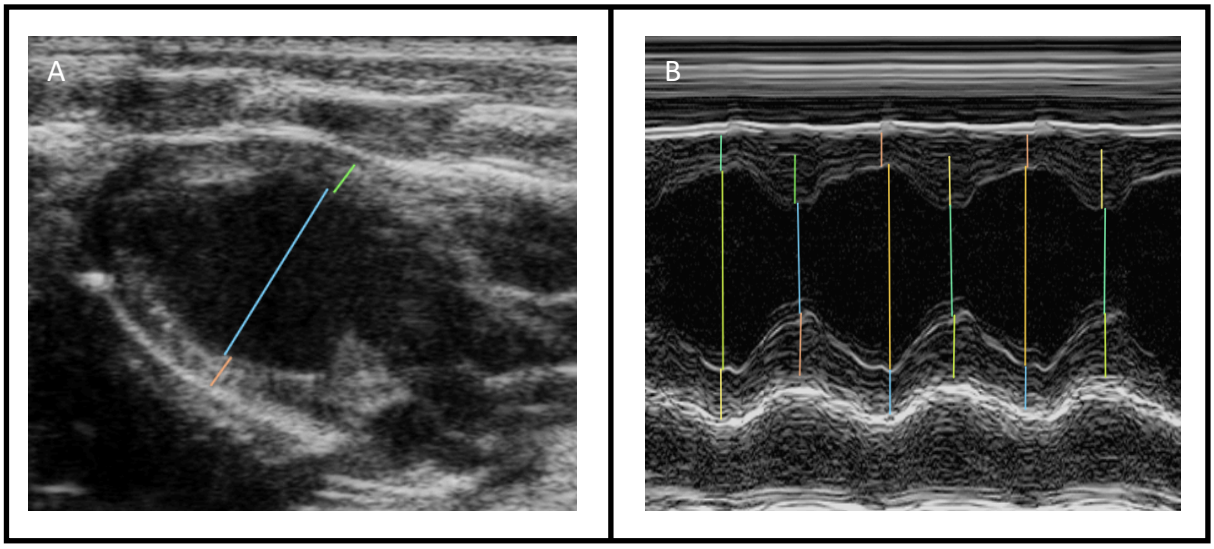

Figure 3.4: Left ventricular geometry measured in triplicate in PLAX.
A. Echocardiography image of the left ventricle in end-diastole, derived from a 2-dimensional mode cine-loop; measurements include septal wall thickness, internal diameter and posterior wall thickness. **B.** Triplicate measurements over three cardiac cycles of left ventricular septal wall thickness, internal diameter and posterior wall thickness in end-systole and end-diastole using M-mode.
PLAX, parasternal long axis; M-mode, motion mode.

Left atrial volume was measured by tracing the left atrial endocardial border in end-systole in A2C and A4C (Figure 3.5).

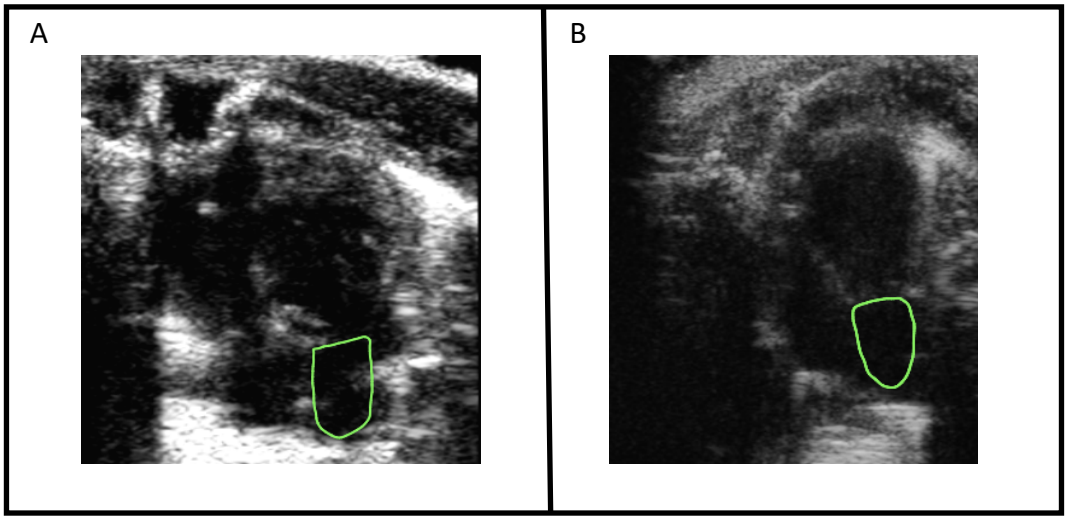

Figure 3.5: A4C and A2C views for measurement of left atrial volume.
A. Echocardiography image of the left atrium in end-systole, derived from a 2-dimensional mode cine-loop in apical 4-chamber view. **B.** echocardiography image of the left atrium in end-systole, derived from a 2-dimensional mode cine-loop in apical 2-chamber view. Tracing of the left atrial endocardial border is illustrated in green.
A4C, apical 4-chamber; A2C, apical 2-chamber.

LVEF was measured by tracing the left ventricular endocardial border in end-diastole and end-systole in PSAX view (Figure 3.6).

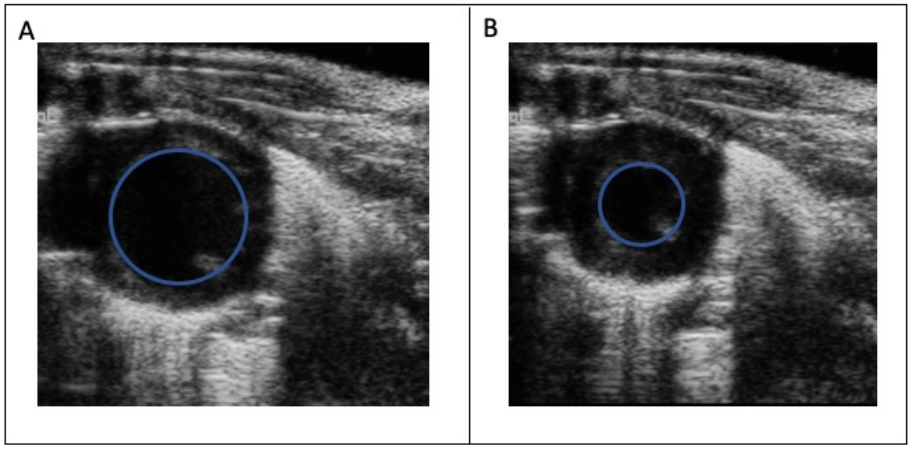

Figure 3.6: PSAX view for measurement of LVEF.
A. Echocardiography 2-dimensional image of the left ventricle in end-diastole. **B.** echocardiography 2-dimensional image of the left ventricle in end-systole. Tracing of the left ventricular endocardial border is illustrated in blue.
PSAX, parasternal short axis; LVEF, left ventricular ejection fraction.

LVM was indexed to GD 1 maternal weight. LVM, RWT and left atrial volume were calculated using the following formulae[281,382,383]:

- $LVM = 0.8(1.04[LVIDd + PWd + IVSd]^3 - [LVIDd]^3) + 0.6$
- LVMi = LVM / maternal weight at GD 1
- $RWT = (IVSd + PWd) / LVIDd$
- Left atrial volume = $0.85 \times (A_1 + A_2 / L)$

LVM; left ventricular mass; LVIDd, left ventricular internal diameter in end-diastole; PWd, posterior wall thickness in end-diastole; IVSd, interventricular septal wall thickness in end-diastole; A_1, left atrial area in AC2 view; A_2, left atrial area in A4C view; L, shortest left atrial length in A2C or A4C view.

Simultaneous pulse wave Doppler traces of aortic outflow and mitral inflow were obtained in the A5C view. This allowed measurement of isovolumetric relaxation and contraction time (IVRT and IVCT, respectively; Figure 3.7).

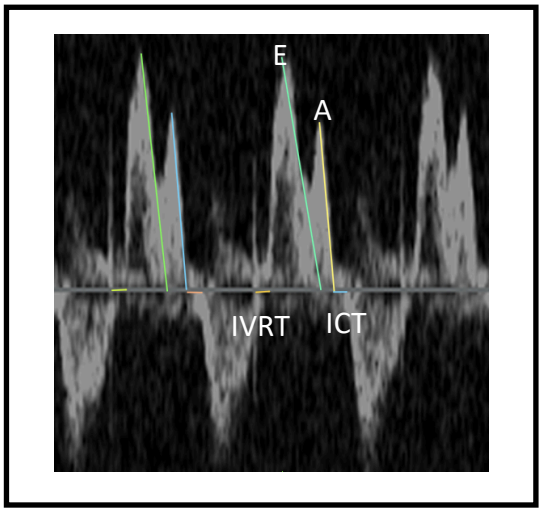

Figure 3.7: Pulse wave Doppler at aortic outflow and mitral inflow.
Measurement of systolic and diastolic function using pulse wave Doppler to measure time intervals and peak velocities at the aortic outflow and mitral inflow.
E, early left ventricular filling peak velocity; A, late left ventricular filling peak velocity; IVRT, isovolumetric relaxation time; ICT isovolumetric contraction time.

3.3.3.5. Blood sampling

On-treatment maternal blood samples were collected post-minipump insertion via the lateral tail vein (between GD 17-19), approximately 5cm from the tip of the tail. Prior to sampling, rats were placed in a warming cabinet (40°C) for up to ten minutes. A maximum of three attempts to access a patent vein were made, to minimise animal distress. At the

end of the experiment, following euthanasia, terminal maternal postnatal plasma and serum samples were collected from trunk blood.

3.3.3.6. Protein Assay

Urinary protein concentration was determined using the Bio-Rad protein assay kit (Bio-Rad, UK). The reference standard, bovine serum albumin stock solution, was achieved by diluting bovine serum albumin with 0.3M NaOH to give a standard concentration of 0.25mg/mL, and a standard curve prepared by serial dilution (standard range 0.39-250µg/mL). Samples (rat urine / reconstituted sFlt) and standards were added to microplate wells. A volume of 180µL of neutralising solution (1:1.25 mix of 0.3M NaOH with 0.3M HCl) was added to each well, using a multichannel pipette. A final volume of 50µL of Bio-Rad dye reagent was added and immediately mixed into each well. Absorbance was measured using a microplate reader (BMG abtech, Ayelsbury, UK) at 595nm, within ten minutes.

3.3.3.7. Measurement of urinary creatinine

Urinary creatinine was measured using Sentinel Diagnostics' creatinine liquid kinetic CREA Jaffe assay (Alpha Laboratories, 17609H; serially diluted with Earle's bicarbonate buffer [EBB]). Undiluted EBB perfusate was included as a zero. Microplate wells were loaded with 20µL of standard (creatinine 25µg/mL to 200µg/mL; Thermo Fisher Scientific, UK), sample or zero. At time zero (T=0 seconds), 125µL of reagent 1 (28.8mM picric acid) was added to each well. Equal volumes of reagent 2 (NaOH) were added to the wells at T=300 seconds. Using the microplate reader (BMG abtech, Ayelsbury, UK), absorbance was determined at 500nm, with readings taken at 360 and 420 seconds. Creatinine concentrations were derived from the following formula:

- Calculated absorbance = (Read 2 - Read 1) - (Blank 2 - Blank 1).

3.3.3.8. Enzyme-linked immunosorbent assay (ELISA)

The R&D systems MVR100 Quantikine ELISA was used to measure recombinant mouse sFlt concentrations in rat serum and plasma. This immunoassay is calibrated against the R&D recombinant mouse sFlt/Fc Chimera used in the animal model. All samples and standards were measured in duplicate, according to the manufacturer's instructions, and

absorbance determined at 450nm on a microplate reader (BMG abtech, Ayelsbury, UK). Sample concentrations were determined against the assay standard curve (detection range 125-8000pg/mL; sensitivity 9.8pg/mL; Figure 3.8). A commercial internal control was included in every assay. The coefficient of variation (CoV) for duplicates across all assays ranged from 0.7-7.7%.

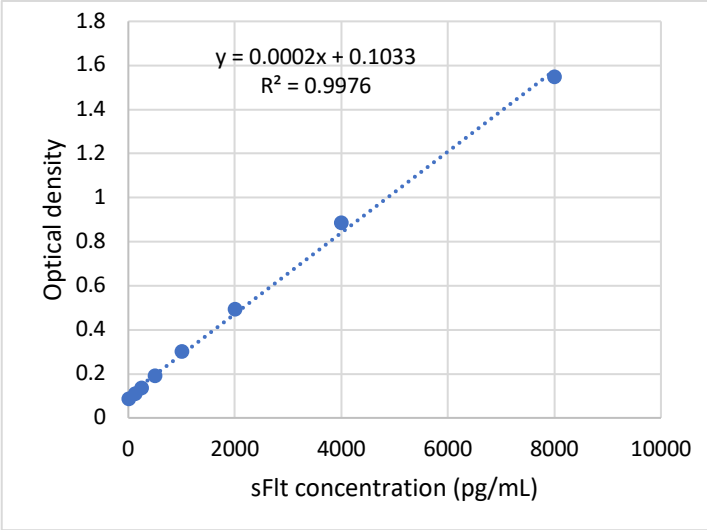

Figure 3.8: Example standard curve from sFlt MVR100 ELISA.

3.3.3.9. Western blotting

Due to inconclusive ELISA results (see section 3.4.1.5), Western blotting was carried out as an alternative method to measure sFlt in rat samples. Samples, containing 30µg of protein or 100ng of recombinant mouse sFlt, were prepared in water, with 10% reducing agent (Novex by Life Technologies, Thermo Fisher, UK) and 1 x sample buffer (Laemmli, Bio-Rad, UK). Samples were boiled for 5 minutes at 95°C prior to loading on 4-15% polyacrylamide gels (Bio-Rad, UK), and run at 100V for approximately one hour. Positive controls constituted mouse brain homogenate (as recommended on the antibody datasheet) and reconstituted sFlt solution. Normal saline, that had been infused via the minipumps in the vehicle arm, was used as a negative control. Proteins were transferred to polyvinylidene fluoride (PDVF) membranes at 200mAmps for 70 to 80 minutes. The membranes were blocked with 1 x Tris-buffered saline (TBS), 5% milk and 0.1% bovine serum albumin for one hour. Following this, the membranes were incubated overnight in primary antibody (ab32152 rabbit monoclonal antibody, 1:300; abcam, Cambridge, UK), diluted with TBS, 5% milk and 0.1% tween. Membranes underwent 4 x 5-minute washes,

in TBS / 0.2% tween, prior to 1-hour incubation in the secondary antibody (donkey anti-rabbit 800; 1:20,000). Finally, the membranes were washed in TBS/0.2% tween in 6 x 5-minute washes, followed by tween-free TBS, prior to imaging (LI-COR Odyssey Sa).

3.3.3.10. Sample size

PND 1 maternal echocardiography measurements were not available at the time of protocol development, and therefore protocol one sample size was initially powered to determine a 20% rise in NTproBNP in the treatment group, compared with placebo. There is ~20% difference between NTproBNP postpartum in women with pre-eclampsia (76±94.7ng/L) and those without (61±32.9ng/L)[257]. The mean serum NTproBNP in non-pregnant rat controls is 0.19±0.03ng/mL[384]; this is likely to be higher in pregnancy (no data available). Therefore, a sample size of 22 animals was determined to detect a 20% increase in NTproBNP at 80% power, α 0.05. An interim analysis was carried out after 11 animals.

3.3.3.11. Statistical analysis

Categorical data were presented using counts (percentages). Continuous data were presented as mean ± standard deviation and median (range), as appropriate. Continuous variables were compared between the two groups, using paired t-test.

3.3.4. Protocol two

As a result of the negative findings from protocol one (see section 3.4.1), the subsequent batch of sFlt was quality-checked before embarking on the second *in vivo* study. A new batch of recombinant sFlt was reconstituted with normal saline to a concentration of 1μg/μL before being diluted to a final calculated concentration of 500μg/mL. Protein concentration was determined by NanoDrop (NanoDrop 2000 Spectrophotometer, Thermo Scientific, UK), due to concerns about the accuracy of the Bio-Rad assay, which measured 62% of the anticipated recombinant protein in the first batch. Four vials of recombinant sFlt were reconstituted as above. Measured concentrations were 437-457μg/mL (87-91% of anticipated). These were then pooled to make a single aliquot that was used for all sub-aliquots (calculated concentration 446μg/mL; 89% of anticipated).

Checks of recombinant protein quality of this second batch were also assessed by sodium dodecyl sulphate (SDS)-page, followed by Coomassie staining, as described in section 3.4.2. This demonstrated that the majority of the protein was likely to be recombinant sFlt (molecular weight ~150kDa), however there was evidence of some impurities, or degradation, as demonstrated by the faint bands or smearing at lower molecular weights (Figure 3.9).

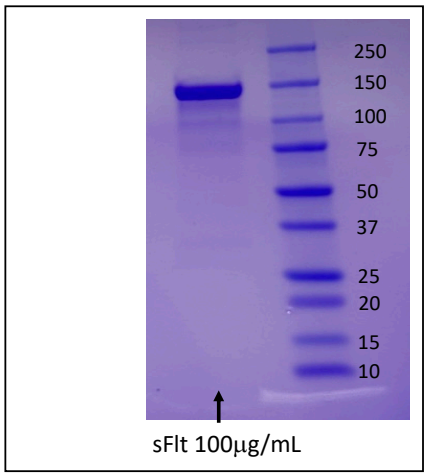

Figure 3.9: Coomassie staining shows predicted molecular weight of the second batch of reconstituted sFlt.
~150kDA protein detected in the 100μg/mL reconstituted sFlt.
sFlt, soluble fms-like tyrosine kinase.

The sFlt infusion model used in this thesis has been published in the literature by the same group at the University of Mississippi Medical Center with two different doses: 49ng/h (assuming a maternal weight of ~320g at the time of minipump insertion)[136,137] and 500ng/h[135]. The second dose is ~tenfold the original, yet the publications state that both models result in an equivalent fold increase in plasma sFlt[135,137]. For this reason, having confirmed 1) the quality and quantity of the next batch of reconstituted sFlt and 2) adequate minipump function, the second *in vivo* protocol planned to use the higher sFlt dose of 500ng/h. This dose was achieved by filling the Alzet 2001 micro-osmotic pumps (with a pre-determined infusion rate of 1μL/h for seven days) with 200μL of sFlt solution (reconstituted with normal saline to a concentration of 500μg/mL). Figure 3.10 illustrates the experimental design of protocol two. The osmotic minipumps were inserted on GD 13, as per protocol one. The endpoint was moved to GD 19 (whilst sFlt infusion should still be active), aiming to reduce some biological variability due to timing postpartum and

obtain a measurement of on-treatment circulating sFlt levels. BP in this experiment was measured via carotid cannulation, again intending to reduce variability seen with the tail cuff BP measurements. Blood was collected via the jugular cannula under anaesthesia (intraperitoneal sodium thiobutabarbital 100mg/kg [Inactin hydrate T133-aG, Sigma Aldrich]), prior to euthanasia by cervical dislocation. Pregnancy outcomes were as for protocol one (litter size/fetal weight), with placental weights also being obtained. Due to undetectable levels of serum sFlt in protocol one (see section 3.4.1.5), the osmotic minipumps and adjacent subcutaneous fat were also removed at the time of tissue harvest, to enable assessment of any potential problems with pump function and/or transfer of sFlt into maternal circulation from the subcutaneous space.

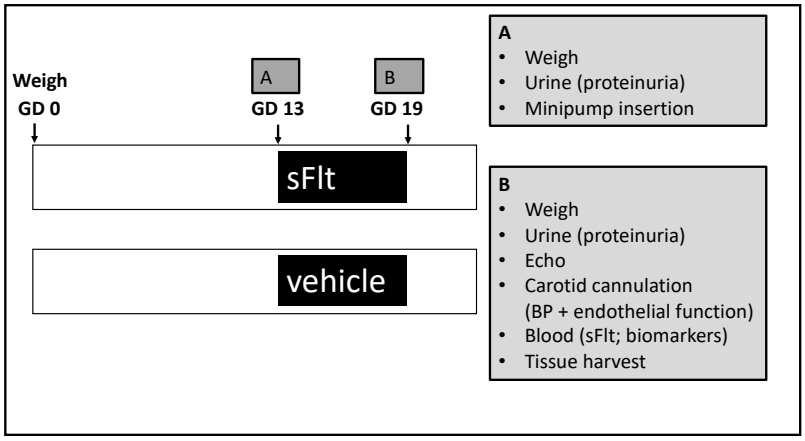

Figure 3.10: Experimental design of protocol two.
GD, gestational day; PND, postnatal day; sFlt, soluble fms-like tyrosine kinase; vehicle was normal saline; BP, blood pressure; echo, echocardiography; pup characteristics were litter size and fetus weight.

3.3.4.1. Carotid BP measurement

BP was measured invasively via carotid artery cannulation[296]. Rats were anaesthetised using intraperitoneal sodium thiobutabarbital 100mg/kg (Inactin hydrate T133-aG, Sigma Aldrich). They were transferred to a heated surgical table, where a 2cm midline incision was made in the ventral neck region, followed by blunt dissection laterally to expose the jugular vein. An incision was made distal to the bifurcation point using iris scissors. The catheter (Portex polythene tubing, 0.58mm internal and 0.96mm external diameter, Portex Ltd) was then inserted and secured within the vessel. It was then flushed with 1mL of heparinised saline (10,000 international units [IU]/100mL) to ensure patency. Blunt dissection was performed to separate the platysma muscles and expose the trachea. The

carotid artery, lateral to the trachea, was separated from the vagus nerve by blunt dissection. The cardiac end of the carotid artery was then perforated using iris scissors and the catheter was inserted and secured.

After cannulation, the catheter was connected to the PowerLab/4SP transducer and PowerLab data acquisition system (Power Lab, AD Instruments Ltd, Chalgrove, Oxfordshire, UK, Chart 5 software), which was manually calibrated before each experiment. Baseline measurements of mean sBP, dBP, MAP and HR were recorded over a five minute period following ten minutes' stabilisation. In order to test endothelial function, acetylcholine (ACh) was given through the jugular vein in a series of doses: 0.35µg/mL, 0.035µg/mL, 3.5µg/mL, followed by 35µg/mL. The BP was given adequate time (more than five minutes) to recover to baseline between doses. The minimum dBP following each dose was recorded.

3.3.4.2. Echocardiography protocol

The echocardiography protocol was unchanged from protocol one, except rats were anaesthetised using intraperitoneal sodium thiobutabarbital 100mg/kg (Inactin hydrate T133-aG, Sigma Aldrich) and the examination was performed on GD 19.

3.3.4.3. Blood sampling

Rat blood samples were collected at GD 19 via the jugular cannula prior to terminal euthanasia.

3.3.4.4. ELISA

The MVR100 ELISA was used as the initial method for quantifying recombinant sFlt in rat samples, as per section 3.3.3.8.

3.3.4.5. Western blotting

Given the inconsistencies in ELISA results, Western blotting was carried out as an alternative method for measuring recombinant sFlt in rat samples (including serum and homogenised subcutaneous adipose tissue). RINO tubes (1.5mL; Thistle Scientific Ltd., Cheshire, UK) were filled with 100mg of adipose tissue, equal volumes of zirconium oxide

beads (0.5 and 2.0mm; Thistle Scientific Ltd., Cheshire, UK) and diluted (4:1) with RIPA buffer (R0278, Sigma-Aldrich, UK). Samples were then homogenised in the Bullet Blender Gold (BB24-AU, BB5E-AU, Next Advance, Troy, USA) at 4°C, speed 10 for 3 x 3 minute cycles. Plasma and homogenised adipose samples, containing 30µg of protein or 100ng of recombinant mouse sFlt were prepared, as described in section 3.3.3.9. Positive controls included reconstituted sFlt solution spiked into saline, rat plasma and homogenised adipose tissue. The purpose of this Western blotting experiment was to detect recombinant mouse sFlt; for this reason alternative primary and secondary antibodies were used (AF471 goat polyclonal IgG 0.1µg/mL 1:1000 and sc-2020 donkey anti-goat IgG-horseradish peroxidase 1:5000, respectively). Imaging was carried out using the ChemiDoc XRS+ system (Bio-Rad, UK) for this second experiment, following a 5-minute exposure to detection reagent (1:1 ECL chemiluminescent, GE Healthcare). The remainder of the Western blotting protocol is exactly as described in section 3.3.3.9.

3.3.4.6. Reproducibility and variability of echocardiography measurements

Reproducibility and variability data were collected to guide future sample size calculations. Each echocardiography variable was measured in triplicate by a single investigator. The CoV was calculated using the following formula: CoV = ([standard deviation/mean] x 100). The mean CoV for triplicate measures represented reproducibility and the CoV for the mean measures in the control group represented population variability.

3.4. Results

3.4.1. Protocol one

3.4.1.1. Maternal wellbeing

All rats recovered well following their minipump insertion and resumed regular feeding and grooming behaviour within 90 minutes of the surgery. There was no difference in weight gain across pregnancy between groups (Figure 3.11).

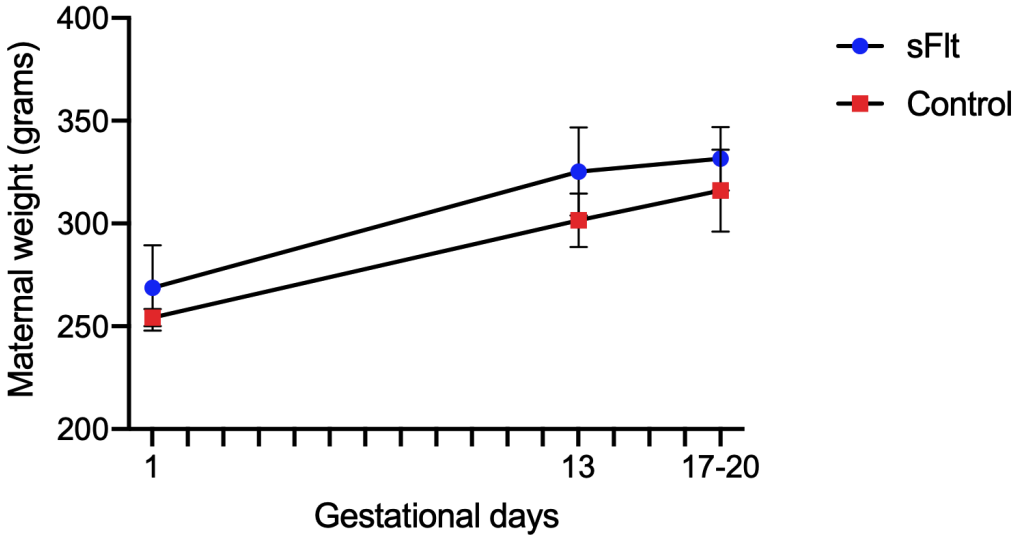

Figure 3.11: Maternal weight gain through gestation.
Change in maternal weight from GD 1-17 in the sFlt and control groups.
GD, gestational day; sFlt, soluble fms-like tyrosine kinase 1.

3.4.1.2. Pre-eclampsia-like phenotype

3.4.1.2.1. BP

There was no difference in BP on-treatment between the two groups (sFlt mean sBP: 130.4±15.5mmHg versus control: 123.4±18.0mmHg; p=0.52; Figure 3.12). There was also no difference in the change in BP between groups from pre-treatment (GD 10 to 12) to on-treatment (GD 17 to 19) timepoints: sFlt: 11.4±17.3mmHg versus 12.1±31.4mmHg, p=0.96). However, there was significant variation between pre- and post-treatment sBP measurements within both groups (Figure 3.12).

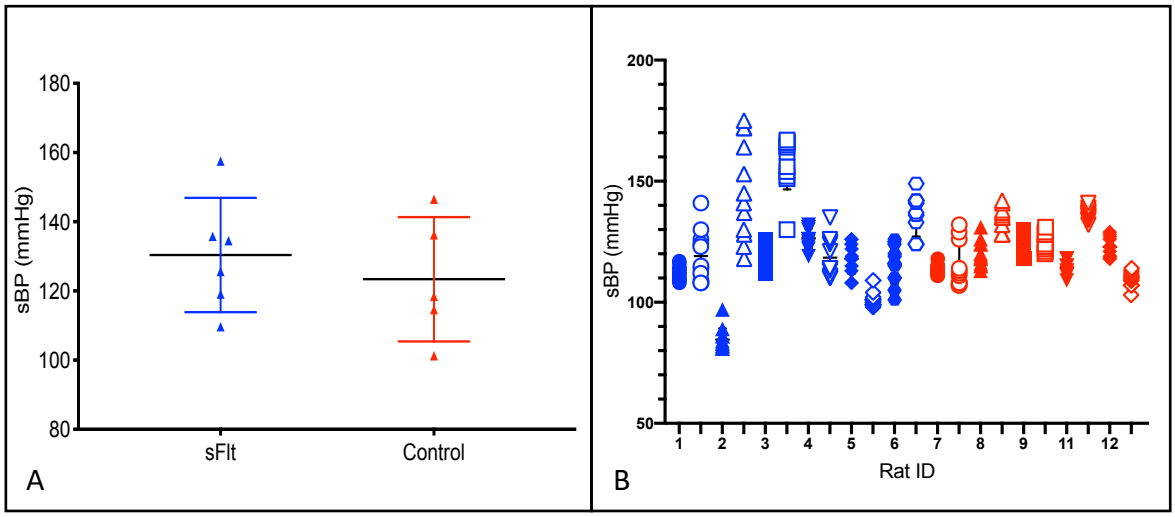

Figure 3.12: Maternal BP readings on treatment.
A. Dot plot comparing mean on-treatment (GD17-19) sBP between groups. **B.** Dot plot illustrating the middle 11-12 pre- (closed symbols) and on-treatment (open symbols) sBP readings in each rat in the sFlt (blue) and control (red) groups.
BP, blood pressure, sBP, systolic blood pressure; mmHg, millimetres of mercury; sFlt, soluble fms-like tyrosine kinase 1; ID, identity.

3.4.1.2.2. Proteinuria

Proteinuria was quantified as urinary protein:creatinine (uPCR). There was no difference in on-treatment uPCR between groups (0.88±0.41 in the sFlt group versus 0.95±0.24 in the control group; p=0.753). There was also no change in uPCR in the sFlt group from pre- and on-treatment measures (pre-treatment: 0.81±0.28 versus on treatment: 0.95±0.24; p=0.450).

3.4.1.3. Litter outcomes and neonatal body weights

There were no differences in litter characteristics between groups, as demonstrated in Table 3.1.

Table 3.1: Litter outcomes and neonatal body weights

Litter outcome	sFlt	Control	Significance (P value)
Number of pups	14.2 ± 1.9	12.0 ± 3.1	0.19
Weight of pups (grams)	6.04 ± 0.25	6.53 ± 0.20	0.17
Number of stillborn pups	0.5 ± 0.2	0.0 ± 0.0	0.11

Mean ± standard deviation.

3.4.1.4. Maternal cardiac phenotype

There was also no difference in any echocardiographic measure of cardiac function or morphology between groups, as shown in Table 3.2.

Table 3.2: Cardiac phenotype in sFlt versus control dams.

Echocardiography measure	sFlt	Control	Statistical significance (P value)
LVIDd (mm)	7.73 (0.84)	7.62 (0.51)	0.80
AWTd (mm)	1.18 (0.28)	1.28 (0.33)	0.60
PWd (mm)	1.25 (0.38)	1.48 (0.27)	0.27
LVM (g)	0.56 (0.07)	0.59 (0.08)	0.77
RWT	0.32 (0.10)	0.36 (0.06)	0.50
Fractional shortening (%)	48.16 (7.89)	46.30 (8.10)	0.71
LVEF	77.35 (7.90)	74.18 (9.56)	0.56
HR (bpm)	334.86 (39.36)	311.43 (65.94)	0.48
E/A	1.20 (0.29)	1.73 (0.63)	0.21
IVRT (ms)	19.06 (3.12)	22.87 (10.73)	0.42
ICT (ms)	15.94 (2.43)	17.93 (1.69)	0.16
ET (ms)	46.11 (4.73)	43.08 (10.81)	0.67
MPI	0.52 (0.07)	0.63 (0.17)	0.16

Mean ± standard deviation.
Left ventricular geometry was measured in PSAX using M-mode.
MPI = (IVRT + ICT)/ET
PSAX, parasternal short axis; M-mode; motion mode; LVIDd, left ventricular internal dimension in end-diastole; LVIDs, left ventricular internal dimension in end-systole; AWTd, anterior wall thickness in end-diastole; PWd, posterior wall thickness in end-diastole; LVM, left ventricular mass; RWT, relative wall thickness; HR, heart rate; bpm, beats per minute; E/A, early to late diastolic filling ratio; IVRT, isovolumetric relaxation time; ICT isovolumetric contraction time; ET, ejection time; MPI, myocardial performance index.

3.4.1.5. Serum sFlt levels

The minipumps were expected to infuse over seven days (commencing GD 13) and therefore the endpoint procedures at PND 1 were carried out ~2-3 days after active sFlt treatment would have stopped. Serum samples quantified using the MVR100 ELISA determined that there was no measurable sFlt in any samples, either during treatment (GD 17 to 19) or at post-treatment (PND 1) timepoints, despite the ELISA standard curve and internal control samples being within the expected range (Figure 3.8).

The ELISA was then repeated on plasma, serum and assay diluent, spiked with recombinant mouse sFlt (expected concentrations between 500-10^7pg/mL), to determine whether the recombinant mouse sFlt protein was measurable using the ELISA, and whether measurements were affected by the sample type (i.e. rat serum / plasma versus

diluent). In spiked samples, sFlt was detected by the ELISA, however interpolated sample concentrations were lower than expected concentrations and increasing sFlt amounts demonstrated non-linearity in measured concentrations (see Table 3.3). The discrepancy between measured and expected concentrations was greater in plasma and serum samples than with assay diluent, suggesting a matrix effect.

Table 3.3: sFlt concentrations as measured by MVR100 ELISA.

Sample	Calculated concentration (pg/mL)	Expected concentration (pg/mL)
6μL sFlt in serum	253	500
6μL sFlt in plasma	151	500
6μL sFlt in diluent	461	500
12μL sFlt in diluent	686	1000
24μL sFlt in diluent	1161	2000
Neat sFlt (100μg/mL)	13381*	10^7

*Concentration outside of the detection range.
sFlt; soluble fms-like tyrosine kinase-1.

Western blotting was subsequently performed on a subset of rat serum samples and reconstituted sFlt solutions. Serum samples included those randomised to sFlt and vehicle, on- and post-treatment. Figure 3.13 illustrates non-specificity of the antibody; the very faint bands at ~150kDa could represent endogenous sFlt, but this remains inconclusive. sFlt was not measurable in the reconstituted recombinant sFlt solution, indicating potential antibody incompatibility due to the mouse origin of the recombinant protein.

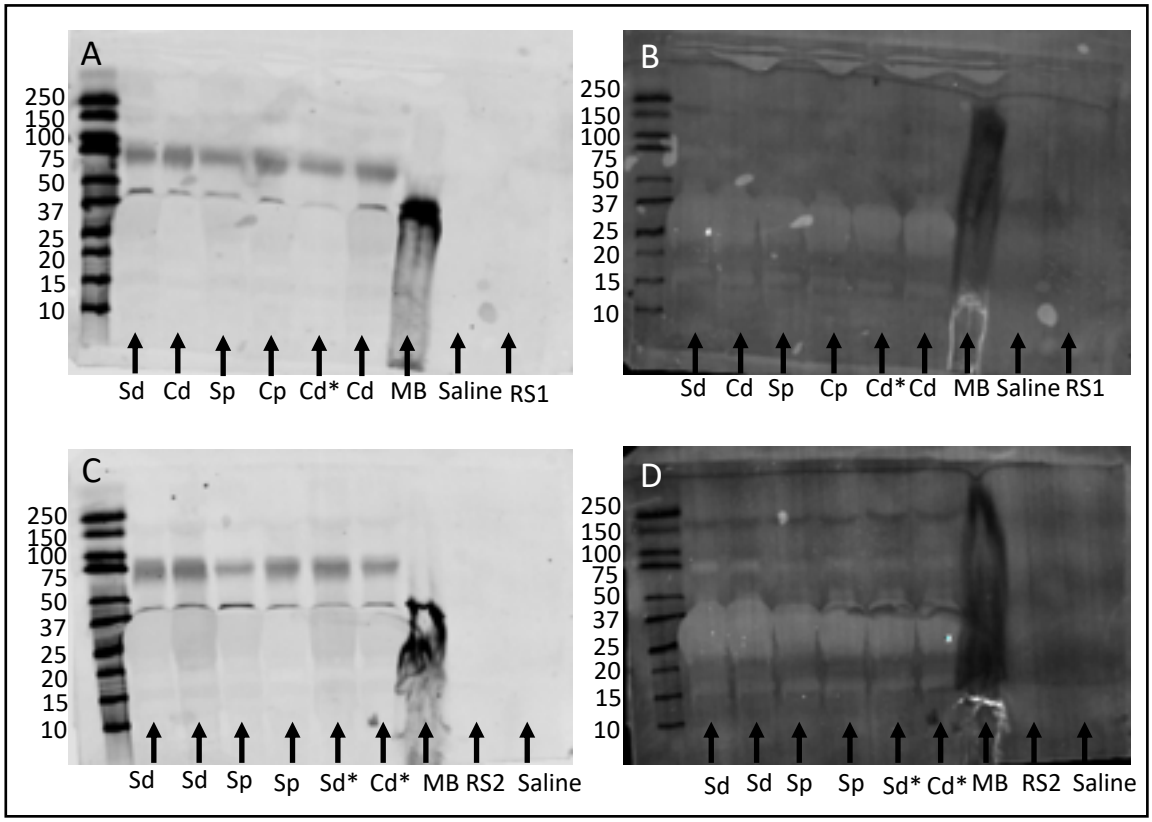

Figure 3.13: Detection of sFlt protein using Western blotting.
A. gel 1 β-actin; **B.** gel 1 sFlt; **C.** gel 2 β-actin; **D.** gel 2 sFlt.
Sd, sFlt serum during treatment; Cd, control serum during treatment; Sp, sFlt serum post-treatment; Cp, control serum post-treatment; Cd* control plasma during treatment; MB, mouse brain homogenate (C5718); RS1, reconstituted sFlt 100 μg/mL; Sd*, sFlt plasma during treatment; RS2, reconstituted sFlt 50 μg/mL.

3.4.2. Recombinant sFlt quality

To verify the presence of a recombinant protein of expected molecular weight (150-170kDa under reducing conditions), a separate SDS-PAGE gel was run (as described in section 3.3.3.9). This was subsequently stained with Coomassie blue R-250 (Bio-Rad, UK). Following overnight soaking with Coomassie stain, the gel was immersed in destain (30% methanol, 10% acetic acid and 60% water) for 3 x 20 minutes, prior to imaging. This confirmed the presence of ~150kDa protein in both reconstituted sFlt solutions; however it did not detect a protein of a similar molecular weight in the ELISA MVR100 standard sample used to make the standard curve (Figure 3.14).

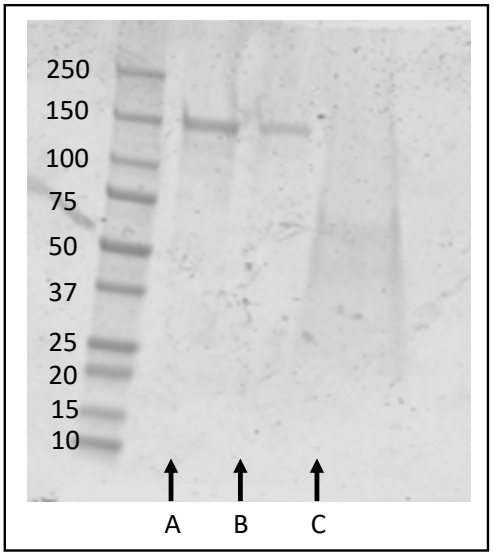

Figure 3.14: Coomassie staining shows predicted molecular weight of recombinant sFlt protein. **A.** 100μg/mL reconstituted sFlt. **B.** 50μg/mL reconstituted sFlt. **C.** Standard sample from MVR100 ELISA used to generate the standard curve (62.5μg/mL).

Protein concentration was then determined by Bio-Rad protein assay (Bio-Rad, UK, as described in section 3.3.3.6). The two reconstituted recombinant protein samples should have had protein concentrations of 100μg/mL and 50μg/mL; however measured concentrations were 42.6μg/mL and 12.4μg/mL respectively.

3.4.3. Minipump function

Having established that the recombinant protein was the appropriate size for recombinant mouse sFlt (150-170kDa under reducing conditions), blinded experiments were set up to test the function of the osmotic minipumps. Two Alzet 2001 minipumps were filled with either 250μL of reconstituted sFlt (50μg/mL) or normal saline. sFlt (50μg/mL) / normal saline was added either directly (48 or 96μL via pipette) or via the pre-filled minipump to 15mL of normal saline. This volume was chosen to mimic rat circulating blood volume (64mL/kg)[385]. All procedures were carried out blinded to treatment allocation. The solutions were then stored in a warming oven at 37^{o}C. Forty-eight hours later, 200μL of solution was removed for subsequent sFlt quantification. Blunt Alzet filling tubes were used to extract the residual solution from the pumps on day six (equivalent to *in vivo* experimental endpoint). sFlt concentrations were measured using the MVR100 ELISA, as described previously.

The results, as demonstrated in Table 3.4, were conflicting. The commercial internal control was out of range (measured at 258pg/mL but should have been 476-793pg/mL). sFlt was detected in the solutions that contained pipetted / minipump-infused sFlt. However, the doses were not comparable with what was expected. The residual solutions in the minipumps had detectable sFlt but this was lower than expected (1028pg/mL compared with 5000pg/mL).

Table 3.4: sFlt concentrations in *ex vivo* experiment.

Sample (all in 15mL saline)	**Diluted sample**		**Un-diluted sample**	
	Interpolated concentration (pg/mL)	**Calculated expected concentration (pg/mL)**	**Interpolated concentration (pg/mL)**	**Calculated expected (pg/mL)**
48µL saline	Out of range < 125	0	Out of range < 125	0
48µL sFlt	Out of range < 125	3200	193	160,000
96µL saline	Out of range < 125	0	Out of range < 125	0
96µL sFlt	318	3200	Out of range > 8000	320,000
2001 minipump saline	Out of range < 125	0	Out of range < 125	0
2001 minipump sFlt	Out of range < 125	3200	1728	160,000
Pump residual (control)	128	0	138	0
Pump residual (sFlt)	1028	5000	Out of range > 8000	50,000,000

The ELISA detection range was 125-8000pg/mL.
sFlt, soluble fms-like tyrosine kinase 1.

3.4.4. Protocol two

3.4.4.1. Maternal well-being

There was no difference in weight gain between the groups (sFlt mean weight gain 117±13.87g versus control 106.40±14.86g; p=0.34; Figure 3.15).

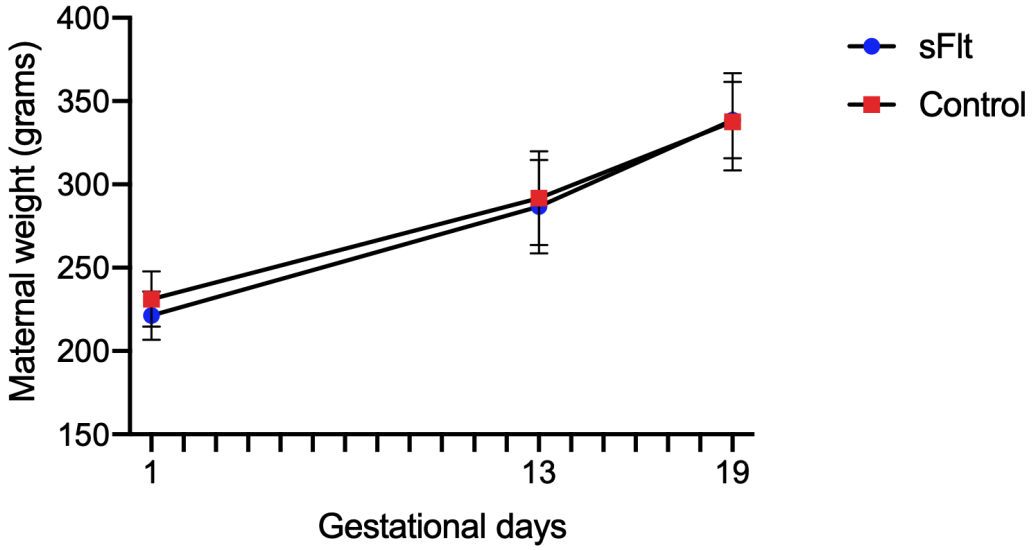

Figure 3.15: Maternal weight gain through gestation.
Change in maternal weight from GD 1-19 in the sFlt and control groups.
GD, gestational day; sFlt, soluble fms-like tyrosine kinase 1.

3.4.4.2. Pre-eclampsia-like phenotype

The carotid BP protocol was successfully completed on 6/8 rats. One rat in the control group had a cardiac arrest following ~1mL blood loss from the jugular vein. Although CO was restored, the rat remained hypotensive and therefore the procedure was abandoned. The final control rat did not generate any BP readings, despite seemingly appropriate positioning of the carotid and jugular cannulas. There was a non-significant trend towards a higher MAP in the control group, compared with the sFlt group (p=0.09, Figure 3.16).

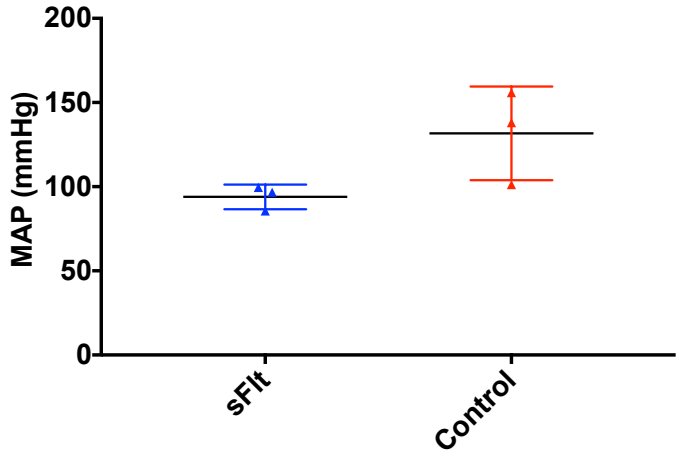

Figure 3.16: MAP measured via carotid cannulation on GD 19.
Dot plot comparing the MAP between sFlt and control groups.
MAP, mean arterial pressure; GD, gestational day; mmHg, millimetres of mercury; sFlt, soluble fms-like tyrosine kinase-1.

Response to intravenous ACh was used as a measure of endothelial function. The percentage decline in BP (as defined by [(MAP - minimum dBP)/MAP] x 100) was expected to have a positive correlation with endothelial function; i.e. the larger the decline, the better the endothelial function. There was no difference in endothelial function between groups (Figure 3.17).

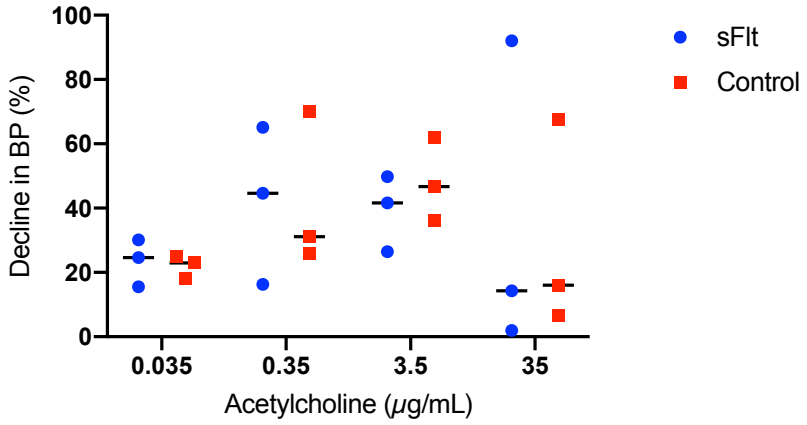

Figure 3.17: Percentage decline in BP following ACh boluses.
Comparison of ACh dose-dependent percentage decline in BP (as defined by [(MAP - minimum dBP)/MAP] x 100) between sFlt and control groups.
ACh, acetylcholine; BP, blood pressure; MAP, mean arterial pressure; dBP, diastolic blood pressure; sFlt, soluble fms-like tyrosine kinase-1.

3.4.4.3. Litter outcomes

As shown for protocol one, there was no difference in offspring characteristics between groups at GD 19 (Table 3.5).

Table 3.5: Offspring characteristics between groups at GD 19.

Litter outcome	sFlt	Control	Significance (P value)
Number of pups	14.33 ± 0.58	13.20 ± 2.39	0.46
Pup weight (grams)	1.52 ± 0.07	1.57 ± 0.12	0.60
Number of pup resorptions	0	0	-
Placental weight (grams)	0.41 ± 0.04	0.42 ± 0.09	0.74

Mean ± standard deviation.

3.4.4.4. Maternal cardiac phenotype

Similar to protocol one, there was also no difference in any echocardiographic measure of cardiac function or morphology between groups, as demonstrated in Table 3.6.

Table 3.6: Cardiac phenotype in sFlt versus control dams.

Echocardiography measure	sFlt	Control	Statistical significance (P value)
LVIDd (mm)	0.77 ± 0.06	0.75 ± 0.04	0.55
AWTd (mm)	0.12 ± 0.02	0.11 ± 0.04	0.88
PWd (mm)	0.14 ± 0.03	0.13 ± 0.02	0.55
LVM (g)	0.52 ± 0.06	0.47 ± 0.13	0.59
LVMi (g/kg)	2.36 ± 0.42	2.04 ± 0.54	0.41
RWT	0.34 ± 0.07	0.33 ± 0.07	0.83
Fractional shortening	52.33 ± 5.54	57.62 ± 4.42	0.18
LVEF	87.30 ± 3.96	90.98 ± 2.60	0.16
HR (bpm)	360.67 ± 34.12	358.20 ± 28.92	0.92
E deceleration time	0.04 ± 0.01	0.04 ± 0.01	0.57
E/A	1.18 ± 0.06	1.12 ± 0.08	0.32
IVRT (ms)	0.02 ± 0.00	0.02 ± 0.00	0.70
ICT (ms)	0.01 ± 0.00	0.01 ± 0.00	0.77
ET (ms)	0.07 ± 0.00	0.07 ±0 .01	0.54
MPI	0.43 ± 0.11	0.51 ± 0.04	0.19
LA volume	0.19 ± 0.01	0.20 ± 0.01	0.44

Mean ± standard deviation.
Left ventricular geometry was measured in PSAX using M-mode.
PSAX, parasternal short axis; M-mode; motion mode; LVIDd, left ventricular internal dimension in end-diastole; LVIDs, left ventricular internal dimension in end-systole; AWTd, anterior wall thickness in end-diastole; AWTs, anterior wall thickness in end-systole; PWd, posterior wall thickness in end-diastole; PWs, posterior wall thickness in end-systole; LVM, left ventricular mass; RWT, relative wall thickness; HR, heart rate; bpm, beats per minute; IVRT, isovolumetric relaxation time; ICT isovolumetric contraction time; ET, ejection time; MPI, myocardial performance index; LA, left atrial.

3.4.4.5. Reproducibility

Several cardiac function and morphological measures were repeated in different views and modes to compare reproducibility. Tables 3.7 and 3.8 summarise the reproducibility (within subject CoV) and variability (within control group CoV) of each echocardiography measure. M-Mode was superior to 2D measures of left ventricular geometry, in terms of reproducibility. Despite acceptable reproducibility of M-mode measures (CoV <10), there was significant variability within the control group, necessitating a larger sample size to determine any difference between groups.

Table 3.7: CoV within subject and within population (control group only) for 2D and M-mode echocardiography measures.

Echocardiography measure	Within subject CoV (%)				Population CoV (%)			
	PSAX 2d	PSAX M-mode	PLAX 2d	PLAX M-mode	PSAX 2d	PSAX M-mode	PLAX 2d	PLAX M-mode
LVIDd (mm)	3.18	2.21	2.91	1.65	5.63	5.18	5.73	6.74
LVIDs (mm)	3.25	4.20	5.60	4.55	11.77	11.45	14.20	13.73
AWTd / IVSd (mm)	7.70	7.36	7.22	7.06	14.01	31.76	17.89	5.25
AWTs / IVSs (mm)	9.95	5.28	7.04	4.74	26.32	21.02	9.46	17.00
PWd (mm)	7.71	4.64	9.83	4.43	11.28	13.43	9.08	7.05
PWs (mm)	5.09	4.26	9.49	4.64	10.44	11.84	12.47	7.46
LVM (g)	8.91	3.20	10.78	3.31	22.35	28.21	8.22	10.35
RWT	7.45	3.56	8.33	4.63	8.26	20.28	15.12	10.02
Fractional shortening	7.27	0.53	8.49	0.16	18.79	7.67	14.88	8.84

CoV, coefficient of variation; 2D, 2-dimensional; M-mode, motion mode; PSAX, parasternal short axis; PLAX, parasternal long axis; LVIDd, left ventricular internal dimension in end-diastole; LVIDs, left ventricular internal dimension in end-systole; AWTd, anterior wall thickness in end-diastole; IVSd, interventricular septal wall thickness in end-diastole; AWTs, anterior wall thickness in end-systole; IVSs, interventricular septal wall thickness in end-systole; PWd, posterior wall thickness in end-diastole; PWs, posterior wall thickness in end-systole; LVM, left ventricular mass; RWT, relative wall thickness.

Table 3.8: CoV within subject and within population (control group only) for Doppler echocardiography measures.

Echocardiography measure	Within subject CoV (%)	Population CoV (%)
IVRT	6.24	22.05
ICT	11.56	17.86
MPI	8.53	8.30
E/A	8.59	7.15
E deceleration time	9.76	23.51

CoV, coefficient of variation; IVRT, isovolumetric relaxation time; ICT, isovolumetric contraction time; MPI, myocardial perfusion index; E/A, early to late diastolic filling ratio.

Data from the PLAX M-mode measurements of LVM in the control group were used to determine sample sizes for potential future work. Using a mean of 0.52±0.05g, 18 rats would be needed to determine a 15%[386] increase in LVM at 80% power, α 0.05.

3.4.4.6. sFlt levels

To more closely reproduce the previous studies using this model[135–137], sFlt was measured in plasma, rather than serum, for this second experiment. Positive controls (spiked plasma and homogenised adipose tissue) were clearly detectable by the ELISA. On the other hand, wells containing negative controls (e.g. saline only) and some empty wells (containing only assay diluent) generated absorbance within the detectable range (176-

376pg/mL), implying that the ELISA was picking up signal where there should be none. The ELISA was repeated due to concern about potential contamination; however, the repeat ELISA data were consistent with the first measurements, and no contamination step was identified during the process. Measured sFlt levels were 179-534pg/mL and 416-564pg/mL in the control arm plasma and homogenised adipose samples, respectively. One homogenised adipose sample in the sFlt arm had measured sFlt concentration of 2894pg/mL; even with inclusion of this suspected outlier, sFlt levels in both rat plasma and adipose tissue were comparable between groups (p=0.86 and p=0.18, respectively).

Since the ELISA results were inconclusive, Western blotting was performed (see sections 3.3.3.9 and 3.3.4.5 for details). Alternative antibodies and imaging system were used in this protocol. The primary antibody (R&D polyclonal goat IgG, AF471) is reportedly the same as that used in the R&D MVR100 ELISA. Results confirmed that this antibody could detect the recombinant mouse sFlt (in spiked saline, rat plasma and subcutaneous adipose tissue homogenates) but there was some evidence of potential cross-reactivity as proteins of other sizes were also detected in the reconstituted sFlt. There was no measurable mouse recombinant sFlt in the rat plasma or subcutaneous adipose tissue homogenates (Figure 3.18).

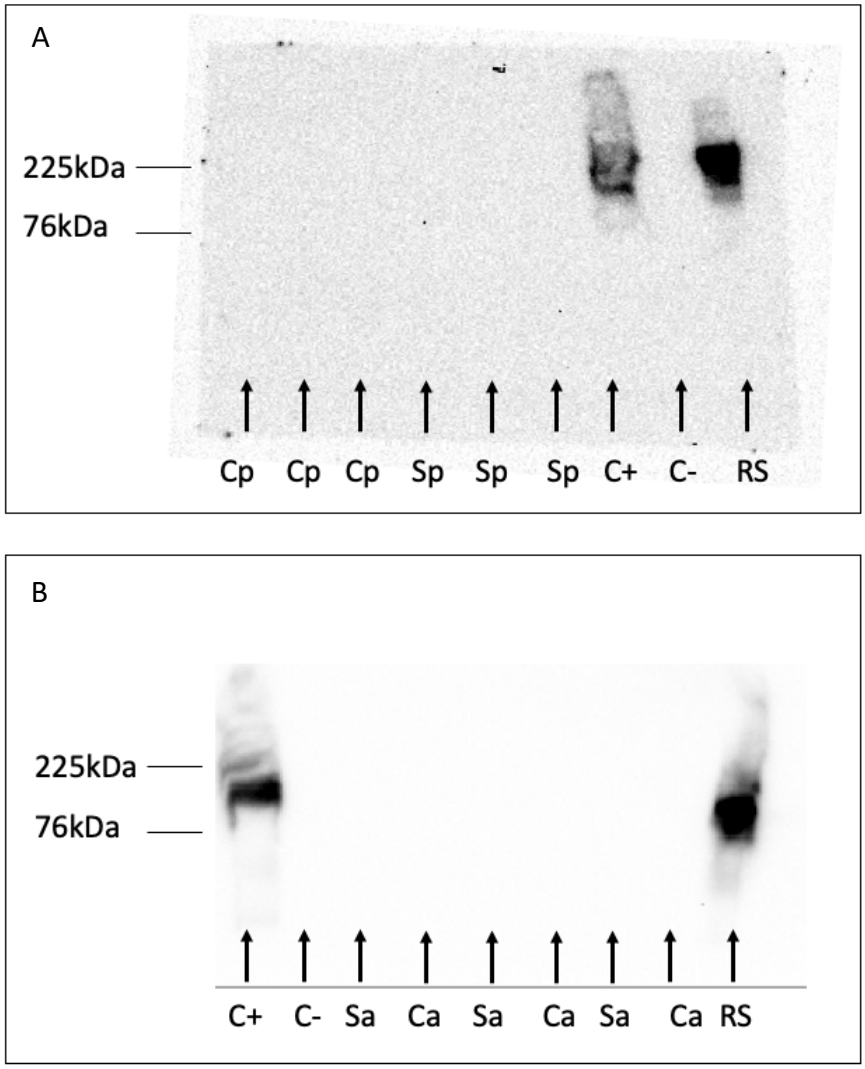

Figure 3.18: Detection of sFlt protein using Western blotting .
A. Western blotting of rat plasma samples with positive and negative controls; **B**. Western blotting of rat subcutaneous adipose homogenate samples with positive and negative controls. Cp, plasma sample from rat randomised to control; Sp, plasma sample from rat randomised to sFlt; C+, plasma / adipose homogenate from rat randomised to control, spiked with recombinant sFlt; C-, unspiked plasma / adipose homogenate from same rat as C+; RS, reconstituted sFlt; Sa, adipose homogenate from rat randomised to sFlt; Ca, adipose homogenate from rat randomised to control. Following dilution, reconstituted sFlt had an anticipated concentration of 50μg/mL.

Given the lack of measurable sFlt in the plasma and protein from homogenised subcutaneous adipose tissue, an additional Western blot was carried out to assess potential adherence of sFlt to the reservoir walls of the minipump. In order to do this, residual reconstituted sFlt / saline solutions were extracted carefully using a 1mL syringe from the minipumps. This method has previously been used to calculate Alzet minipump infusion rates[387]. These samples were diluted to an anticipated concentration of 50ng/mL (~1:10 dilution with saline). The pumps were then flushed with 200μL heparinised saline

(5000 IU/mL) in order to displace any sequestered sFlt[388]. Minipump 2 had an additional 200μL flush with heparinised saline, as indicated by the weaker signal in sF2 (Figure 3.19). The Western demonstrated a stronger signal in the heparinised flush samples than the residual samples (sF1 and sF3 versus sR1 and sR3; Figure 3.19), indicating that an incomplete dose of sFlt was being released from the minipump and suggesting there had been adherence of the recombinant protein to the inside of the minipumps. Additionally, the residual sFlt solutions were observed to have a cloudy appearance, which was not seen in the residual volume recovered from the saline minipumps, potentially indicating incomplete solubility of sFlt.

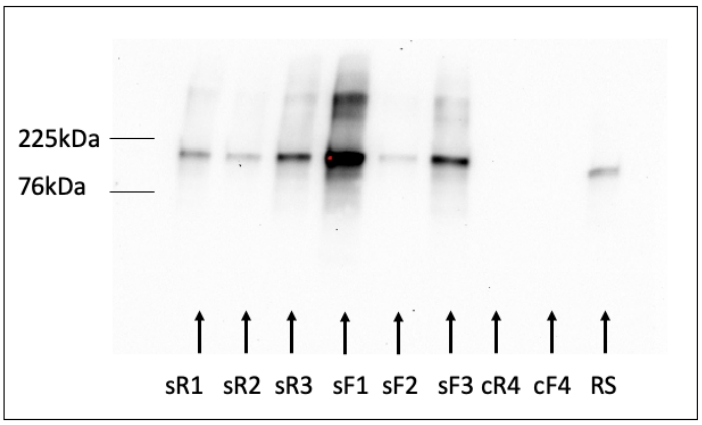

Figure 3.19: Detection of sFlt protein in residual and flushed minipump solutions using Western blotting.
Western blotting of the residual solutions extracted from minipumps 1-4 (R) and heparinised saline-flushed solutions from the corresponding minipumps after emptying (F).
sR, residual sample from a minipump containing reconstituted sFlt; sF, heparinised saline-flushed sample from a minipump previously containing reconstituted sFlt; cR, residual sample from a minipump containing saline; cF, heparinised saline-flushed sample from a minipump previously containing saline; RS, reconstituted sFlt (500 ng/μL); 1-4 represent 4 different minipumps.

3.5. Discussion

The experiments performed in this chapter aimed to replicate the findings of a previously published pre-eclampsia-like model in order to explore a potential sFlt-induced cardiac phenotype. Unfortunately no pre-eclampsia-like phenotype was demonstrated, with comparable BP between groups in both protocols. Despite an approximate tenfold increase in sFlt dose in protocol two, sFlt remained undetectable in the rat serum and local subcutaneous adipose tissue. The lack of difference in phenotype between groups was possibly attributable to an insufficient rise in circulating sFlt in the treatment group. Given the absence of measurable recombinant sFlt in plasma or serum, despite previous

reported success in the literature with the doses used[136,137,173], potential explanations were explored, including the method of sFlt measurement and local sFlt sequestration both in and outside of the minipumps.

3.5.1. sFlt measurement

The Quantikine MVR100 ELISA uses an antibody specific for mouse sFlt and is calibrated against the recombinant mouse sFlt/Fc Chimera used in the *in* vivo experiments described in protocols one and two. For this reason and due to its use in previous publications[135,137], it was chosen as the initial detection method for sFlt measurement in these experiments, anticipating minimal cross-reactivity with endogenous rat sFlt. Despite this, in our hands each of the ELISA experiments generated inconclusive and inconsistent results. The standard curves were always satisfactory; however spiked saline, serum and plasma samples at different doses demonstrated a lack of linearity and the kit control was out of range on several occasions. On two separate ELISA plates, sFlt was detected in multiple samples that should have been sFlt-deplete (from saline control animals) as well as in empty wells of the microplate. The detection of sFlt in the plasma or subcutaneous samples could be explained by cross-reactivity with endogenous rat sFlt or other endogenous proteins. However, this does not explain the sFlt measured in the saline controls or empty wells. The spiked plasma and subcutaneous samples had comparable sFlt concentrations with the unspiked samples, indicating potential degradation of the pooled reconstituted sFlt that was used for spiking. Measured sFlt levels of 176-376pg/mL in the saline samples of the repeat ELISA indicate unreliability of the ELISA at the lower end of the detection range. On the other hand, there were very strong signals in the three reconstituted sFlt samples and one homogenised subcutaneous adipose sample from one of the rats randomised to sFlt. Contamination or cross-reactivity is less likely to cause such high measurements and therefore it was speculated that these measurements, despite being quantitatively inaccurate, reflected the presence of recombinant mouse sFlt.

The Western blotting experiments confirmed that the antibody in the ELISA was able to detect the recombinant mouse sFlt. It did not, however, mimic the ELISA findings seen in the one subcutaneous adipose sample, nor did it detect sFlt in any of the other rat

samples. This highlights potential cross-reactivity within the ELISA reagents, and therefore suggests that the ELISA did not produce results that were reliable. Data from the Western blotting confirmed that inadequate sFlt levels reached the maternal circulation.

3.5.2. sFlt sequestration outside of the minipump

The original group to use this model implanted the minipumps intraperitoneally[135–137]. This could explain a difference in measured plasma / serum sFlt using the same minipump dose. Even if the minipumps worked effectively, it is possible that the recombinant sFlt did not reach the vasculature from the subcutaneous tissues. This could be attributed to local retention of the exogenous sFlt, due to binding to ubiquitous heparan sulphate proteoglycans on the cell surface and in the extracellular matrix[388,389]. Alternatively, given the large molecular size of recombinant sFlt (110kDa), failure to reach the vasculature could be attributed to inability of the protein to permeate the capillary wall.

In pregnancy, sFlt is thought to be released by the placenta directly into the maternal circulation via syncytiotrophoblast extracellular vesicles[390]. In contrast, from the subcutaneous minipump position, sFlt needs to permeate the tight junctions of the capillary endothelium[391] to reach the maternal circulation. It is therefore possible that this step is limited under physiological conditions, due to protein size. Given the variance in endothelial permeability between different vascular beds[392], permeability could be reduced in capillaries in the interscapular subcutaneous space, compared with intraperitoneal capillaries. On the other hand, Di Marco *et al.*[173] demonstrated a rise in plasma sFlt using the same recombinant sFlt in comparably positioned subcutaneous minipumps in a chronic kidney disease rat model. This implies that minipump position should not impede sFlt distribution. However, this non-pregnant nephrectomised rat model[173] was associated with endothelial dysfunction prior to exogenous sFlt infusion[393] and therefore could have been accompanied by altered endothelial structure and subsequent increased vascular permeability. The lack of sFlt measured in the subcutaneous adipose tissue in the present studies adds uncertainty to the local accumulation hypothesis, but does not preclude it as a possibility.

3.5.3. sFlt sequestration in the minipump

Di Marco *et al.*[173] used a dose of 300ng/h for a longer duration (14 days) and found a peak in plasma sFlt concentration at approximately seven days. This may indicate that in a pregnant model with a larger circulating blood volume, 500ng/h for six days was an insufficient dose and/or exposure time to elevate plasma levels accordingly. Another theory is that the insufficient rise in sFlt could be secondary to minipump failure. Although my *ex vivo* experiment had inconsistent results, they did confirm that sFlt was able to exit from the minipumps, initially making minipump failure a less likely explanation. On the other hand, the Western results revealed that a significant amount of sFlt was sequestered in the seemingly empty minipumps. This indicates that a lower dose of sFlt was released from the minipumps than intended. Unfortunately it was not possible to confirm the *ex vivo* ELISA findings with Western blotting, due to insufficient samples. Given the inconsistencies of the ELISA results, minipump failure cannot be excluded. However, it is more likely that the minipumps infused the solution at the appropriate rate in terms of volume, but not dose. This is supported by the minimal residual volume in the pumps (≤30μL) yet strong sFlt signals in the flushed samples, indicating potential sFlt adherence to the minipump walls. It is also possible that a combination of these factors (including sFlt sequestration in- and outside of the minipumps) resulted in an insufficient dose of sFlt reaching the maternal circulation.

3.5.4. Reproducibility

Although these studies have not addressed the original aim, they have provided useful echocardiography and BP pilot data to power future work. Since concentric remodelling is a key feature of human pre-eclampsia[6] and a prognostic indicator of future cardiovascular disease[394–396], LVM is an appropriate measure on which to power future animal studies. M-mode provided the most reproducible measures of LVM, in both PLAX and PSAX views; however within population variability was significantly reduced when calculated using PLAX views (CoV 10.35 compared with 28.21). Kraker *et al*.[386] demonstrated a 15% increase in LVM in a transgenic Sprague-Dawley rat model of pre-eclampsia four weeks postpartum, compared with controls. Although they did not replicate this in their human cohort[386], de Haas *et al.*'s meta-analysis[6] demonstrated a 24% antenatal increase in LVM in human pre-eclampsia, compared with normotensive controls. Using variability data

from the control group of the second protocol, 18 rats (9 per group) would be needed to determine a conservative 15% increase in LVM[386] at 80% power, α 0.05. This is an achievable and acceptable sample size for future work.

In order to simulate a pre-eclampsia-affected pregnancy, a significant rise in BP needs to be demonstrated in the model. There was substantial variability in BP within the control group, using both tail-cuff and invasive measures. Therefore, it is worth considering alternative methods of BP measurement for future work, including telemetry which is the gold-standard for real-time measurement of BP in rat models[297,397]. Telemetry provides continuous BP monitoring over a longer time period (weeks rather than minutes or hours), thereby reducing variability attributable to diurnal and stress factors[297]. Although invasive and expensive[297], telemetry is established in our institution and is therefore a feasible option for future work.

3.5.5. Alternative models

In order to test the hypothesis that sFlt mediates cardiac dysfunction following pre-eclampsia, it is likely that an alternative model is required, for which there are currently two main candidates. The STOX1 transgenic mouse model consists of mating wild-type females with males over-expressing the human STOX1 gene, a candidate gene for pre-eclampsia development[214]. In this model, pregnant dams develop gestational hypertension, proteinuria and exhibit increased circulating sFlt levels[214]. This model has already been associated with maternal left ventricular hypertrophy and fibrosis in pregnancy[398], which has subsequently been shown to persist up to eight months postpartum[225], findings that were not available at the outset of this project. It is possible that these findings are in-part mediated by raised sFlt levels, however this is yet to be investigated in this model. Two potential methods of investigation include statistical correlation between sFlt levels and degree of cardiac impairment, or co-administration of VEGF[399] to assess the impact of sFlt reduction on cardiac phenotype. The first method is limited by the requirement of a large sample size and inability to confer causation.

Another model that has potential to test the original hypothesis uses injection of adenoviral vectors encoding the murine sFlt gene into pregnant rats[164]. This model is

associated with an increase in circulating sFlt and induces a pre-eclampsia-like phenotype with gestational hypertension, proteinuria and glomerular endotheliosis. The long-term[222,224] but not early postnatal cardiovascular phenotype has been investigated for this model. Initially Bytautiene *et al.*[224] demonstrated no difference in BP or vascular function at six to eight months postpartum. However the same group went on to explore the maternal plasma proteome six months postpartum and demonstrated significant enrichment of proteins linked to cardiovascular disease[222]. These studies suggest that long-term cardiovascular disease may in part be a consequence of pre-eclampsia and highlight the potential of these models to explore the relationship between pre-eclampsia and postnatal cardiovascular phenotype and risk. They also highlight the potential for these models to explore the translational question of postnatal ACE inhibitor treatment and longer-term effects on maternal cardiovascular function.

Use of an effective animal model has the potential to address both aims of this doctoral thesis: 1) to explore the mechanistic link between pre-eclampsia and maternal cardiac dysfunction; and 2) to explore the reversibility of postnatal cardiac dysfunction following pre-eclampsia. A future animal model therefore needs to demonstrate a clear pre-eclampsia-like phenotype and persistent postnatal cardiac phenotype, neither of which have been achieved with this current model.

3.6. Conclusion

To conclude, there was no consistently measurable recombinant mouse sFlt protein in the serum or plasma of rats implanted with sFlt-filled minipumps, either during or post-treatment. There are several potential explanations for this, but I hypothesise that this relates either to an inability of exogenous sFlt to reach the maternal vasculature from the subcutaneous space and/or inadequate dosing due to sequestration of the recombinant protein within the minipumps. In order to explore the hypothesis that sFlt mediates cardiac dysfunction in the context of pre-eclampsia, alternative models need to be considered.

CHAPTER 4: POSTNATAL ENALAPRIL TO IMPROVE CARDIOVASCULAR FUNCTION FOLLOWING PRETERM PRE-ECLAMSPIA (PICk-UP): A RANDOMISED DOUBLE-BLIND PLACEBO-CONTROLLED FEASIBILITY TRIAL

4.1. Introduction

Cardiovascular disease is the leading cause of mortality worldwide, accounting for more than 80,000 deaths in women in the UK per annum[400]. It is increasingly recognised that primary prevention is more effective than treating established cardiovascular disease[401]; however this requires identification of at-risk individuals prior to the onset of disease.

For many asymptomatic women, antenatal care is their first adult engagement with the healthcare system. Consequently, pregnancy and the early postnatal period provide an ideal window for risk screening and primary prevention. Pre-eclampsia is a pregnancy-specific condition, cured by delivery of the baby and placenta[314]. Despite this, maternal health implications persist well beyond the pregnancy[5,7–12]. In particular, pre-eclampsia is associated with maternal postnatal cardiovascular dysfunction[5,7,35,36] and long-term cardiovascular risk[8–14,17,184,185,376]. The association between pre-eclampsia and future cardiovascular disease persists despite accounting for mutual risk factors[8]. Women with preterm pre-eclampsia (delivery before 37 weeks) are at particular risk: compared with normotensive term pregnancies, preterm pre-eclampsia is associated with two- to three-fold and three- to eight-fold risks of cardiovascular events[8,9,13,14] and deaths[9,11,184], respectively.

Not only is cardiovascular disease more common in women with pre-eclampsia, but it tends to occur earlier and with a higher fatality rate[10]. Most recent studies demonstrating increased cardiovascular risk following pre-eclampsia, had a median follow-up less than 20 years, with some presenting as early as one year postpartum[9,11,13–17]. Despite cardiovascular impairment likely being a consequence as well as a trigger of pre-eclampsia, research to date has mainly focused on antenatal screening and treatment[3,26,402]. These include cardiovascular phenotyping prior to the onset of pre-

eclampsia[3,26], BP management to reduce maternal and neonatal complications[402] and targeting the NO pathway to improve maternal vascular and placental function in those at risk of, or with established, pre-eclampsia[403,404]. However, the early postnatal period provides an ideal window for intervention to improve long-term cardiovascular health and future pregnancy outcomes, with less pharmacological restrictions than the antenatal period. For example, ACE inhibitors are contraindicated in pregnancy, due to associated fetopathy[405], yet they are considered safe first-line antihypertensives postpartum, irrespective of breastfeeding status[208,328,406].

Women identified as having cardiovascular dysfunction in the interval between pregnancies are at an increased risk of pre-eclampsia recurrence[18]. In particular, TVR is the best independent predictor of recurrent pre-eclampsia [18]. It is therefore plausible that the risk of pre-eclampsia recurrence could be reduced by correcting postnatal cardiovascular dysfunction. There is also some evidence supporting the association between raised TVR and long-term cardiovascular risk[407], indicating the potential to reduce long-term risk in the early postnatal period. Understanding and preventing the long-term health implications of pre-eclampsia were highlighted by the James Lind Alliance priority setting partnership as the first and fourth research priority for hypertensive disorders of pregnancy, respectively[408]. Despite this, the potential for a postnatal therapeutic intervention to correct cardiovascular impairment, and thereby influence long-term cardiovascular risk following preterm pre-eclampsia, has not yet been investigated. As discussed in section 1.11, ACE inhibitors provide cardioprotection[187] through a variety of mechanisms, including anti-inflammatory effects[188], increased NO bioavailability[189] and diminished fibrosis[190], all of which are relevant to pre-eclampsia[46,107,192]. Importantly, ACE inhibitors provide cardioprotection even in normotensive subjects with normal LVEF[186] and are particularly effective at reversing concentric remodelling[197], which is a key feature of preterm pre-eclampsia-related cardiovascular morbidity[6]. For these reasons, they are an ideal candidate for potential reversal of cardiovascular morbidity associated with preterm pre-eclampsia.

Despite a plethora of data associating preterm pre-eclampsia with postnatal cardiovascular dysfunction and remodelling[5,7] the mechanism linking the two is not

known. Improved understanding of the relationship between preterm pre-eclampsia and postnatal cardiovascular morbidity could aid appropriate counselling for affected women and potentially identify important subgroups who could benefit from intervention targeted in the postnatal period.

4.2. Aims

This study aimed to 1) explore the natural history of preterm pre-eclampsia-related postnatal cardiovascular dysfunction and 2) assess the feasibility of an early postnatal intervention in women who have had preterm pre-eclampsia to improve cardiovascular function and remodelling.

4.3. Methods

4.3.1. Trial design

The PICk-UP study was a single centre feasibility randomised double-blind placebo-controlled trial of six months' treatment with enalapril to improve postnatal cardiovascular function and remodelling in women with preterm pre-eclampsia. Enalapril was the chosen intervention in this study since most of the safety data relating to ACE inhibitors when breastfeeding relates to enalapril and captopril[208,406]. Given associated fetopathy, women were advised not to conceive during the trial, and to stop taking the study medication if found to be pregnant. The trial was funded by the Medical Research Council and prospectively registered at clinicaltrials.gov (study identifier: NCT03466333). The protocol and all participant-facing information were approved by Haydock Research Ethics Committee (18/NW/0253), HRA and Medicines and Healthcare products Regulatory Agency (MHRA). All study procedures were carried out at St Mary's Hospital, Manchester, UK, in accordance with institutional guidelines. All participants gave written informed consent prior to randomisation.

4.3.2. Eligibility criteria

Postnatal women aged 18 and over, with no known cardiac disease and creatinine <100µmol/L, who had preterm pre-eclampsia (requiring delivery <37 weeks' gestation) were eligible for enrolment. Women were excluded if they were unable to consent; had

known cardiac disease; had a contraindication to ACE inhibitors; were currently taking an ACE inhibitor / Angiotensin II Receptor Blocker (ARB) or had known renal artery stenosis. As per the ISSHP definition, pre-eclampsia was defined as new or worsening hypertension >20 weeks and proteinuria or other features suggestive of pre-eclampsia (abnormal haematological parameters, abnormal biochemical parameters, FGR)[323]. Abnormal angiogenic markers (sFlt:PlGF >85) in combination with new or worsening hypertension were also included in the definition[409].

4.3.3. Randomisation and study procedures

Participants were allocated to enalapril or placebo using block randomisation in a 1:1 ratio. Following consent and randomisation, postnatal baseline investigations were performed within 3 days of delivery. These included echocardiography (to measure left ventricular remodelling, systolic and diastolic function), arteriography (to measure arterial stiffness using PWV and AIx), BP[410] and cardiovascular and placental biomarkers (HS-cTnT, NTproBNP, PlGF and sFlt). Enalapril dose was titrated as follows: 5mg once daily for one week, then 10mg for two weeks then 20mg maintenance dose[310]. Dose titration visits (at 1 week ±3 days and 3 weeks ±3 days) comprised of BP measurement, renal function and verbal check of side effects. Baseline cardiovascular measurements were then repeated at visits four and five (6±1 weeks and 6 months ±2 weeks, respectively). Between visits four and five, women received monthly phone calls to review compliance and wellbeing.

Women who declined participation were invited to take part in the observational arm. This invitation was extended to all eligible women, following completion of recruitment to the interventional arm. Women in the observational arm underwent the same cardiovascular measurements at baseline (within three days of delivery), six weeks and six months. They were not randomised to treatment and did not require dose-titration visits or monthly phone calls. Figure 4.1 provides an overview of the study design.

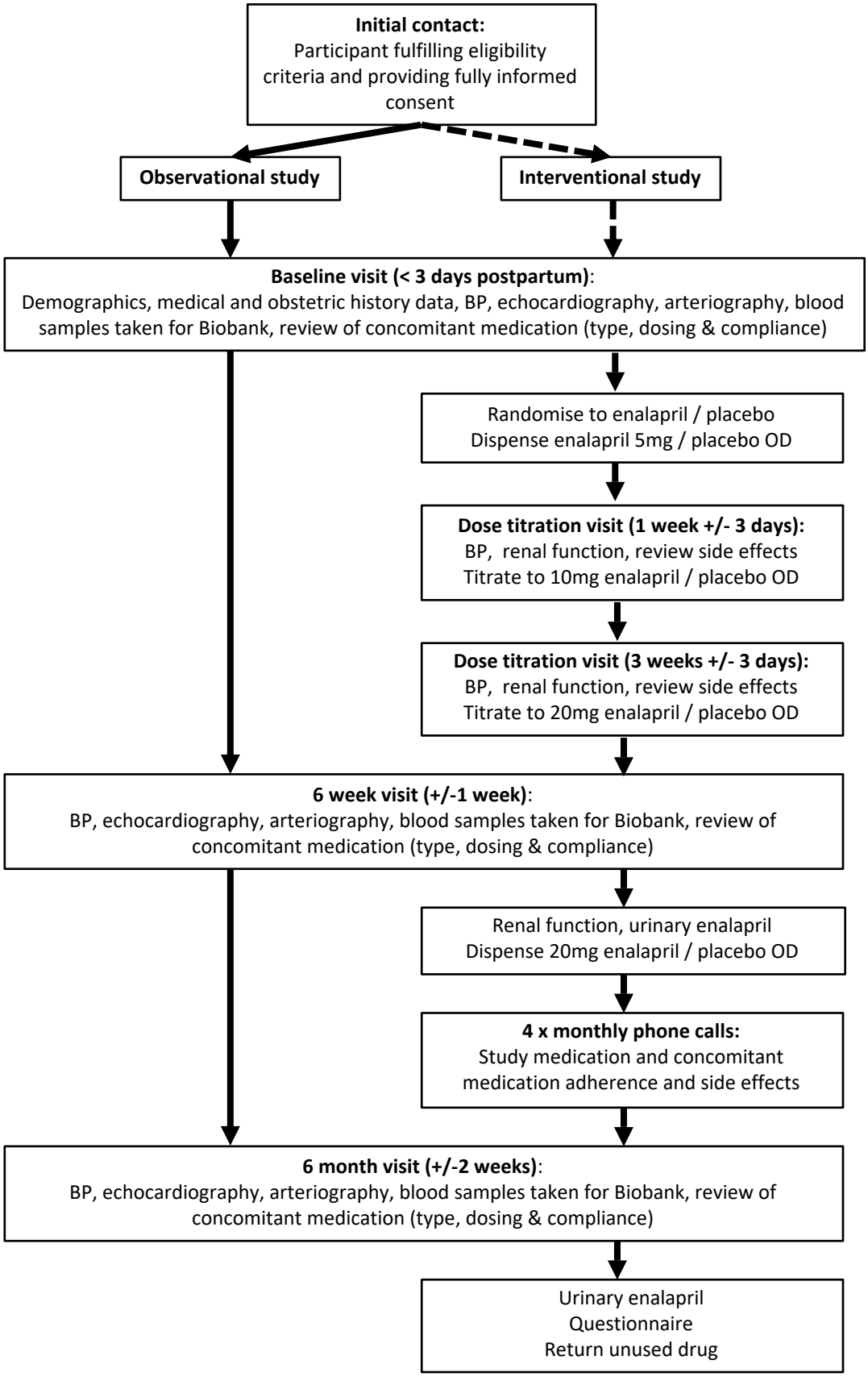

Figure 4.1: Study schematic.
BP, blood pressure; OD, once daily.

Postnatal hypertension was treated as per NICE guidance (with calcium channel blockers, ß blockers and/or α blockers)[328], irrespective of treatment allocation. Changes to antihypertensive medication were made based on standard measurements of BP, using targets defined by the clinical team.

Adherence was measured using three different methodologies: verbal recall during each visit and phone call; pill counts by pharmacy and high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS)[411] of urine samples from visits four and five.

4.3.4. Intervention

The enalapril and placebo (pharmaceutical grade lactose monohydrate) were over-encapsulated in identical hard gelatin sized 000 capsules. Over-encapsulation and manufacture of the placebo was carried out by Stockport Pharmaceuticals (Stockport, UK). The drugs were dispensed in identical packaging (100mL white high-density polyethylene screw neck containers) by Pharmacy using a pre-prepared computer-generated randomisation list.

4.3.5. Database

Data were inputted in real-time onto paper source documents, then transcribed onto the online COLLECT database[412], which was modified for the purpose of this study. Data fields included demographics, obstetric and medical history, early pregnancy data (including first recorded BMI and BP), pregnancy outcome, maximum pre-randomisation BP, antenatal bloods, concomitant medication, study drug adherence, echocardiography, arteriography and BP. Concordance between source data and the database were monitored by the Sponsor (Manchester University NHS Foundation Trust) on a regular basis.

4.3.6. Echocardiography

Echocardiography was performed in the left lateral position during quiet respiration, using a VIVID S70 scanner (GE Healthcare, UK). All measurements were made in triplicate

and analysed post-hoc using GE EchoPAC v.201 software by one investigator (blinded to treatment allocation).

TVR was derived from the LVOT VTI, HR and MAP by the following equation: 80 x (MAP/CO). Raised TVR was defined as two standard deviations above the mean (1355 dyne.$s^{-1}cm^{-5}$)[413].

Systolic function was measured using LVEF (Simpson's biplane method; Figure 4.2), mitral annular tissue Doppler (S'; Figure 4.3) and GLS (using EchoPAC speckle-tracking, with a frame-rate of 50-80 frames/second; Figure 4.4). Systolic dysfunction was pre-defined as LVEF <55%[318], GLS >-18%[414] and S' <0.064m/s[318].

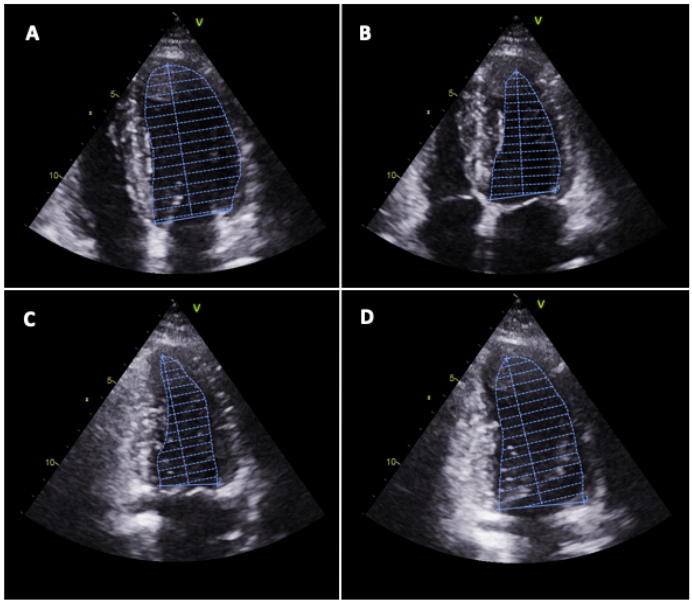

Figure 4.2: Simpson's biplane method of measuring LVEF.
The left ventricular endocardial border was manually traced in A4C in **A.** end-diastole and **B.** end-systole; and in A2C in **C.** end-systole and **D.** end-diastole. Software divided the left ventricle into 16 discs on which volumetric measures were based, allowing calculation of the proportion of end-diastolic volume of blood ejected by the left ventricle (LVEF).
A4C, apical 4 chamber; A2C, apical 2 chamber; LVEF, left ventricular ejection fraction.

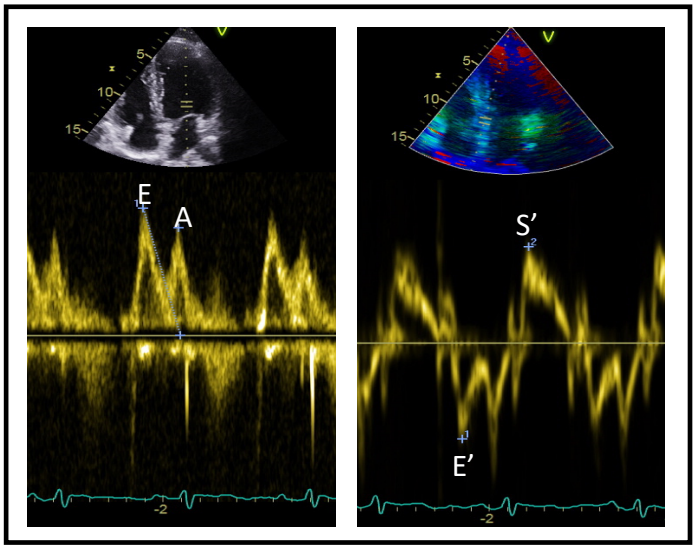

Figure 4.3: Echocardiography measurement of E/A, E/E' and S'.
A. Pulse wave Doppler at the mitral inlet; **B.** tissue Doppler at the medial mitral annulus.
E, early diastolic filling; A, late diastolic filling; S', peak systolic velocity; E', early diastolic mitral annulus velocity.

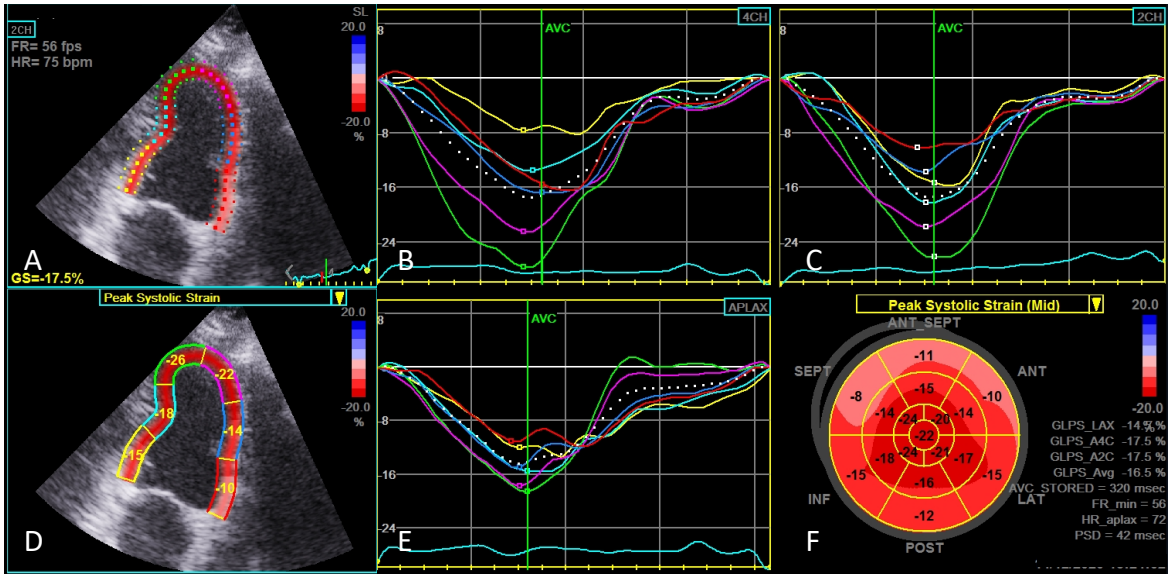

Figure 4.4: Speckle-tracking-derived measurement of longitudinal strain.
A. Tracking of cardiomyocyte deformation in A2C; **B.** longitudinal strain curves in A4C view (each colour represents a different segment of the left ventricle) **C.** longitudinal strain curves in A2C view; **D.** peak longitudinal strain (%) in the six segments of the left ventricle in A2C; **E.** longitudinal strain curves in A3C view; **F.** bullseye view summarising the peak longitudinal strain in all 17 segments of the left ventricle (acquired from A2C, A4C and A3C).
FR, frame rate; HR, heart rate; AVC, aortic valve closure; GLPS_LAX, global longitudinal strain in A3C; GLPS_A4C, global longitudinal strain in A4C; GLPS_A2C, global longitudinal strain in A2C; PSD, peak strain dispersion.

Diastolic function was measured using mitral annular tissue Doppler (E/E'), mitral pulse wave Doppler (E/A; Figure 4.3) and strain rate (early-to-late strain rate ratio; $SR_{E/A}$; Figure 4.5).

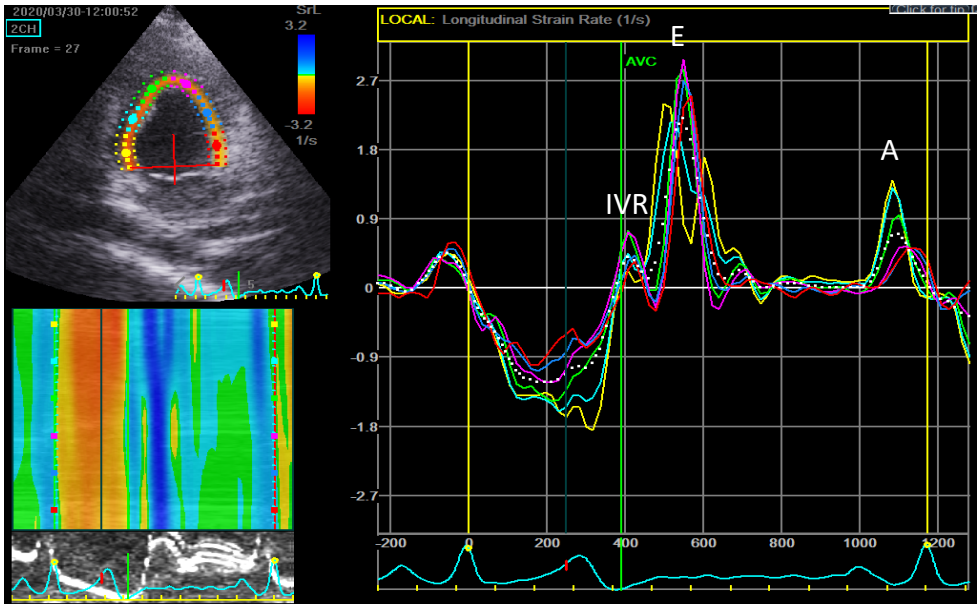

Figure 4.5: Speckle-tracking-derived strain rate curves.
IVR, peak strain rate during isovolumetric relaxation; E, peak strain rate during early diastolic filling; A, peak strain rate during late diastolic filling.

Global and segmental SR curves were derived using the EchoPAC speckle-tracking software (Figure 4.5). SR_E and SR_A were defined as the peaks on the global SR curve after aortic valve closure and before mitral valve closure, respectively. Diastolic dysfunction was defined using the BSE clinical flow chart[289], which used discrete cut-offs for pulse wave Doppler measures (E/A and deceleration time) and age-adjusted reference ranges for tissue Doppler (Figure 4.6). Given the relatively young age of this cohort, inclusion of criteria using age-adjusted reference ranges was considered most suitable.

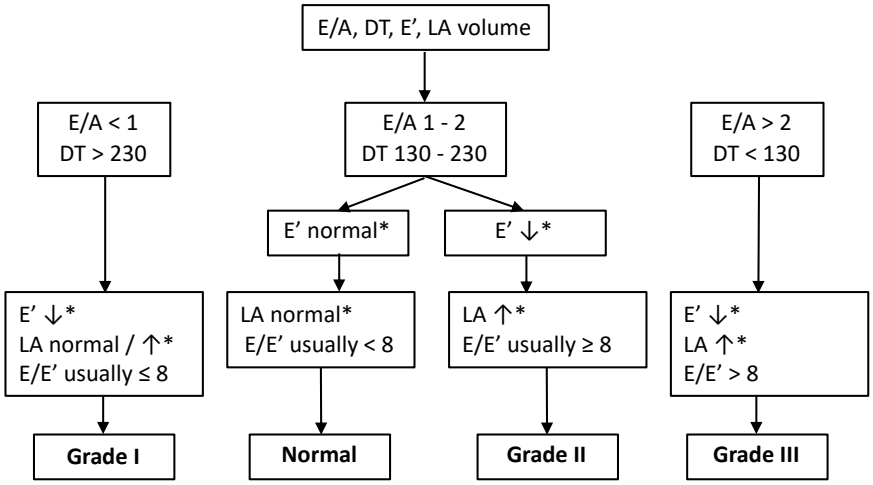

Figure 4.6: Flowchart indicating the definition of diastolic dysfunction, as adapted from BSE 2013 guidelines[289].
*Age-adjusted reference range (mean ± standard deviation [95% confidence interval]) derived from Nagueh *et al.*[289,415].
E/A, early to late diastolic filling ratio; DT, deceleration time; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; LA, left atrial volume; BSE, British Society of Echocardiography.

RWT was calculated in the PLAX view (Figure 4.7) in diastole by: (IVSd + PWd) / LVIDd. LVM was derived from the following equation: $0.8(1.04[LVIDd + PWd + IVSd]^3 - [LVIDd]^3) + 0.6$. Remodelling measures were then indexed to BSA. BSA was calculated using the Mosteller formula[321]: BSA = square root of (height (cm) x weight (kg) / 3600). Concentric remodelling was defined as RWT ≥0.42 and hypertrophy was defined as LVMi >95g/m^2[2281,318].

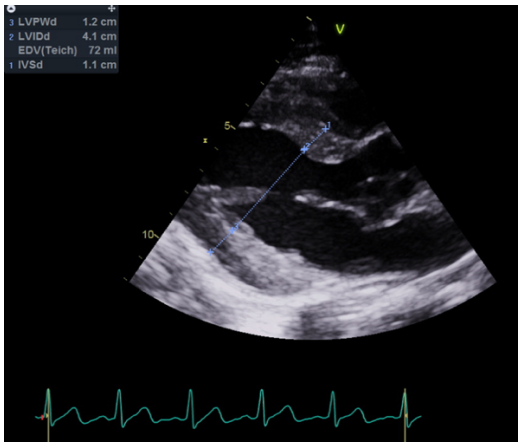

Figure 4.7: 2D echocardiography-derived PLAX view for the measurement of left ventricular remodelling.
2D, 2-dimensional; PLAX, parasternal long axis; LV PWd, left ventricular posterior wall thickness in end-diastole; LVIDd, left ventricular internal diameter in end-diastole; IVSd, interventricular septal thickness in end-diastole.

4.3.7. BP

Standard clinical BP measurements[410] were obtained in the sitting position using an automated BP machine (Alere Microlife BP Monitors; Cheshire, UK). Three measurements were taken in the right arm, at least two minutes apart, after having been seated for a minimum of ten minutes. BP targets were defined by the clinical team, in accordance with NICE[328] and institutional guidelines[416] (individualised BP targets of ≤135-140/80-90mmHg and triggers for treatment of 140-150/90-100mmHg, depending on underlying diagnoses).

4.3.8. Arteriography

PWV and AIx measurements (indicators of vessel compliance), HR, peripheral and central BP were obtained using a Tensioclinic Arteriograph (Tensiomed, Budapest, Hungary). Measurements were taken in the right arm, in the sitting position using an appropriately sized cuff.

4.3.9. Biomarkers

Hs-cTnT and NTproBNP were measured in real-time, by the clinical biochemistry laboratory, using the Roche Diagnostics Cobas e801 module. Results were coded and then reviewed at the end of the study, to avoid clinical intervention on the basis of these results. sFlt and PlGF were measured in two batches using the Roche Diagnostics Cobas e601 module.

4.3.10. Urinary enalapril

Urinary enalapril and enalaprilat were measured via HP LC-MS/MS in a single batch by the University of Leicester Pathology services. This method is effective at ruling out ingestion of an antihypertensive medication ≤4 times its half-life[411]. The elimination half-life of enalapril is 36 hours[417], thereby equating to a snapshot of adherence / non-adherence over six days.

4.3.11. Reproducibility

A subset of 20 participants had echocardiography exams performed by two observers to assess interobserver agreement (IOA). Both observers analysed their own scans, blinded

to the other's results. MAP was not repeated for assessment of TVR reproducibility; therefore this was assessed using repeat measurements of CO alone. ICCs were calculated using a two-way mixed effects model. Reproducibility was assessed in terms of image acquisition (image acquisition was repeated by two different observers; one observer analysed them all), image analysis (one observer acquired all images; two different observers analysed the same images) and both (image acquisition repeated by two different observers; each observer analysed their own images). Reproducibility was classified as poor (ICC <0.4), fair-to-good (ICC =0.4-0.75) and excellent (ICC ≥0.75)[282].

4.3.12. Primary and secondary outcomes

4.3.12.1. Interventional trial

The primary process outcome of the interventional trial was recruitment rate (number of women eligible, recruited and completing the study per month). The primary clinical outcome was reduction in TVR from baseline to six months post randomisation following treatment with enalapril, compared with placebo.

The secondary process outcome was the acceptability of the intervention in postnatal women, which was based on treatment adherence[411] and questionnaire feedback[418,419]. Prespecified secondary clinical outcomes included a change in measures of cardiac structure and function and biomarkers from baseline to six months post randomisation following treatment with enalapril, compared with placebo. The echocardiography measures of cardiac structure and function comprised of E/E' and E/A ratios, tricuspid valve regurgitation, left atrial volume index, LVEF, CO, SV, RWT, LVMi, concentric/eccentric remodelling/hypertrophy, GLS, left ventricular basal, mid and apical strain, and $SR_{E/A}$. The measured biomarkers were HS-cTnT, PlGF, sFlt and NTproBNP.

4.3.12.2. Observational trial

The primary outcome of the observational trial was to describe the change in cardiovascular structure and function over six months following preterm pre-eclampsia. Cardiovascular measures comprised of echocardiography, BP, arteriography and biomarkers at baseline (within three days of delivery), six weeks and six months. Secondary outcomes included correlation of 1) pregnancy and pre-eclampsia phenotypes,

2) maternal characteristics and 3) biomarkers with maternal cardiovascular phenotype at six months postpartum. An additional aim was to assess the correlation between the above factors and change in cardiovascular parameters over time.

4.3.13. Obstetric classifications

Birthweight centiles were customised for maternal ethnicity, height, weight, parity and infant sex, using the Gestation Related Optimal Weight (GROW) software[420]. FGR was defined as birthweight <3rd centile and SGA was defined as birthweight <10th centile. Risk factors for pre-eclampsia were defined as pre-existing hypertension, renal, vascular or autoimmune disease, diabetes, previous pre-eclampsia, nulliparity, age ≥40 years, BMI ≥35kg/m^2 and multi-fetal pregnancy[328]. Severe maternal features of pre-eclampsia were defined as maximum BP ≥160/110mmHg / alanine aminotransferase >100U/L / creatinine >100µmol/L / platelets <100x10^9/L.

4.3.14. Statistical analysis

A statistical analysis plan was agreed by the Trial Management Team and Trial Statistician prior to analysis. The principle of intention-to-treat was adopted for the primary and secondary outcomes in the interventional trial. These analyses included all randomised participants as allocated, for whom the outcome(s) of interest (and any covariates) were available. All statistical analyses were performed using Stata v.14.2. Categorical data were presented using counts (percentage). Continuous data were presented as mean ± standard deviation and median (range), as appropriate. Continuous variables were compared between treatment groups at six weeks and six months, using standard Analysis of Covariance with the baseline measurement included as a covariate. Analyses were repeated without adjustment for baseline measures for exploratory purposes only. At the same timepoints, categorical data were compared between groups using logistic regression (adjusted for baseline) and Chi-square test. Statistical significance was defined as $p<0.05$ for all analyses.

For the observational analysis, the observational and placebo arms were pooled together. Skewness of continuous variables was assessed using the Jarque-Bera skewness-kurtosis test and histograms. Continuous variables were compared between timepoints and

groups using paired t-test, following log-transformation if required. Correlations between continuous variables were assessed using Spearman's correlation coefficient. The relationship between baseline variables and change in cardiovascular parameters (from baseline to six months) was assessed using Spearman's correlation coefficient. Correlations between baseline biomarkers and cardiovascular parameters were assessed using a linear regression model, with number of days postpartum as a covariate for placental biomarkers. Intermodality and interobserver agreement was assessed using ICC, Bland Altman plots and linear regression analyses, as appropriate.

4.3.15. Sample size calculation

Previous studies investigating baseline to six-month changes in echocardiographic measurements were not available at the time of study development; the interventional study was therefore powered to identify a reduction in TVR of 255 dyne.$s^{-1}cm^{-5}$ in the enalapril group compared with placebo at six months postpartum. This outcome was selected as a previous study had demonstrated this magnitude of difference between postnatal women with pre-eclampsia recurrence and those with non-recurrence[18]. Using a mean ± standard deviation of 1638±261 dyne.$s^{-1}cm^{-5}$ with a between group difference of 255 dyne.$s^{-1}cm^{-5}$, a sample size calculation determined a minimum sample size of 36 women in total (1:1 allocation). Following review of the non-completion rate, the original target sample size of 40 was increased to 60 to ensure complete data sets on a minimum of 36 women. The observational study was exploratory in nature, investigating natural history with no prior data to inform the correlation between baseline and six-month cardiovascular parameters and therefore no *a priori* sample size calculation was possible.

4.4. Results

4.4.1. Process outcomes

Recruitment to completion rate was 3.5 eligible women per month. The proportion of eligible women that recruited to PICk-UP was 99/123 (80%). The proportion of eligible women who completed the study was 63/123 (51%; Figure 4.8). Ten women were lost to follow-up (10%) and four conceived before the end of the study (4%). One of the most common reasons for non-completion in the interventional study was postnatal life stressors (8/60, 13%), including neonatal transfers and readmissions. The majority (80%)

of non-completions in the interventional study were within the first six weeks, during which participants had four compulsory hospital visits. Follow-up to the observational arm was also affected by the Covid-19 global pandemic which prevented four of the six-week and eight of the six-month follow-up visits.

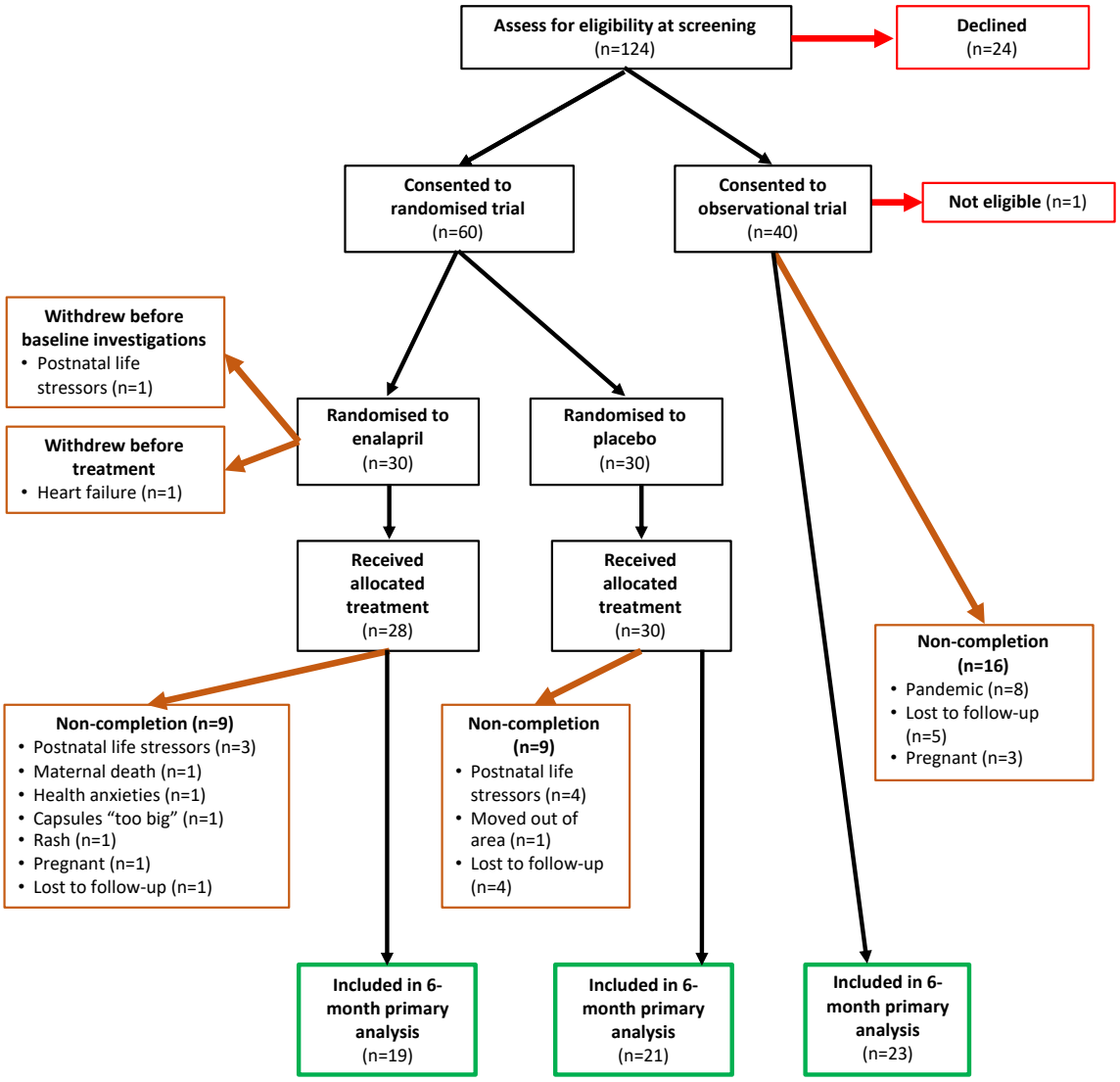

Figure 4.8: Consort diagram.

Of those who completed the interventional study, verbal recall of missed doses was comparable between the two groups (at six weeks: enalapril median 0 [0-6], placebo 0 [0-3]; at six months: enalapril median 4 [0-55], placebo median 6 [0-75]). Only 12/60 (20%) women returned all their drug bottles; therefore, pharmacy pill counting was not considered a reliable measure of adherence. Urinary enalapril and enalaprilat were detectable in 17/20 (85%) women in the enalapril arm at six weeks and 12/19 (63%) at six

months. Two of the women, in whom urinary enalapril was undetectable at six months, postponed their final appointment, reportedly causing them to run out of medication in the preceding week. Verbal recall of missed doses was higher in those without detectable urinary enalapril compared to those with (median missed doses at six months: 24 [4-55] versus 4 [0-18] respectively; p<0.01).

All the women who completed the interventional study completed the acceptability questionnaire. The majority of women (33/40, 83%) found it easy to take the allocated treatment; 6/40 (15%) women found it neither easy nor difficult and 1/40 (3%) found it difficult (Table 4.1). In the last month, 20/40 (50%) women recalled missing one to five doses and 2/40 (5%) recalled missing more than 20 doses. Of those that missed ten or more doses in the last month (6/40, 15%), the most common reasons for missing doses included a change in daily routine (3/6, 50% attributed it to this sometimes/often), being busy with other things (4/6, 67% attributed it to this sometimes/often) and simply forgetting (4/6, 67% attributed it to this sometimes/often). No women attributed missed doses to having too many pills. In terms of overall acceptability, all women who completed the study (n=40) said they would be interested in taking it in the future if it was found to be effective.

Table 4.1: Acceptability questionnaire results.

	Enalapril (n=19)	**Placebo (n=21)**	**All (n=40)**
Women found taking the allocated treatment			
Easy	15 (79%)	18 (86%)	33 (83%)
Neither easy / difficult	4 (21%)	2 (10%)	6 (15%)
difficult	0 (0%)	1 (5%)	1 (3%)
In the last month, women missed doses			
Never	6 (32%)	6 (29%)	12 (30%)
1 - 5	8 (42%)	12 (57%)	20 (50%)
6 - 10	1 (5%)	2 (10%)	3 (8%)
10 - 20	2 (11%)	0 (0%)	2 (5%)
> 20	2 (11%)	0 (0%)	2 (5%)
Unsure	0 (0%)	1 (5%)	1 (3%)
Things about the study that were particularly difficult			
Attending appointments due to childcare	1 (5%)	2 (10%)	3 (8%)
Size of capsules	1 (5%)	0 (0%)	1 (3%)

Frequencies: N (%).

4.4.2. Clinical outcomes

4.4.2.1. Demographics and pregnancy outcome

Baseline characteristics and pregnancy outcome data are summarised in Tables 4.2 and 4.3, respectively. One woman withdrew before her baseline echocardiogram and one was excluded due to an incorrect diagnosis of pre-eclampsia, leaving baseline echocardiography data for 98 women in the three arms. At randomisation, 85/98 (87%) had diastolic dysfunction, 20/98 (20%) had systolic dysfunction and 76/98 (78%) had concentric remodelling or hypertrophy (RWT ≥0.42)[289,318,414].

Table 4.2: Baseline characteristics.

		Enalapril (n=30)	Placebo (n=30)	Observational arm (n=39)	All (n=99)
Demographics					
Age at enrolment (years)		34.5 ± 6.0	30.9 ± 6.6	31.7 ± 6.5	32.3 ± 6.5
Ethnicity	**White**	21 (70%)	17 (57%)	18 (46%)	55 (56%)
	Black	4 (13%)	4 (13%)	6 (15%)	14 (14%)
	Asian	4 (13%)	9 (30%)	11 (28%)	24 (24%)
	Other	1 (3%)	0 (0%)	4 (10%)	5 (5%)
Booking BMI*(kg/m^2)		28.0 (19.4 - 37.3)	27.6 (19.3 - 51.0)	26.9 (19.0 - 61.4)	27.4 (19.0 - 61.4)
BMI > 30kg/m^2 at randomisation		12 (40%)	11 (37%)	14 (36%)	37 (37%)
Current smoker		5 (17%)	4 (13%)	4 (10%)	13 (13%)
Medical history					
Essential hypertension		6 (20%)	6 (20%)	6 (15%)	18 (18%)
Renal hypertension		3 (10%)	0 (0%)	0 (0%)	3 (3%)
Pre-existing renal disease		3 (10%)	0 (0%)	2 (5%)	5 (5%)
Antihypertensive medication at study entry		24 (80%)	20 (66%)	29 (74%)	73 (74%)
Booking systolic BP* (mmHg)		118 (100-163)	117 (90-152)	116 (90-146)	116 (90-163)
Booking diastolic BP* (mmHg)		70 (60-101)	70 (58-100)	68 (54-99)	70 (54-101)
Diabetes		3 (10%)	2 (7%)	2 (5%)	7 (7%)
Previous VTE		2 (7%)	1 (3%)	0 (0%)	3 (3%)
Antiphospholipid syndrome		0 (0%)	1 (3%)	0 (0%)	1 (1%)
Systemic lupus erythematosus		0 (0%)	0 (0%)	0 (0%)	0 (0%)
Obstetric history					
Primiparous women		16 (53%)	15 (50%)	22 (56%)	53 (54%)
High risk for PE†		21 (70%)	13 (43%)	18 (46%)	52 (53%)
One moderate risk factor for PE‡		7 (23%)	14 (47%)	14 (36%)	35 (35%)
High risk for PE (if multiparous)†		12/14 (86%)	8/15 (53%)	8/17 (47%)	28/46 (61%)
Previous PE (if multiparous)		10/14 (71%)	5/15 (33%)	7/17 (41%)	22/46 (48%)
Previous SGA < 10th centile (if multiparous)		10/14 (71%)	6/15 (40%)	11/17 (65%)	27/46 (59%)

Frequencies: N (%)
Mean ± standard deviation
*Median (range)
†High risk for pre-eclampsia is defined by presence of: pre-existing hypertension, renal, vascular or autoimmune disease, diabetes previous pre-eclampsia, or two moderate risk factors[328].
‡Moderate risk factors include: nulliparity, age ≥40 years, BMI ≥35kg/m^2, multi-fetal pregnancy[328].
BMI, body mass index; BP, blood pressure; mmHg, millimetres of mercury; VTE, venous thromboembolism; PE, pre-eclampsia; SGA, small for gestational age.

Table 4.3: Pregnancy outcomes.

Pregnancy outcome		Enalapril (n=30)	Placebo (n=30)	Observational (n=39)	All (n=99)
Gestation at delivery (weeks + days)*		35+0 (22+2 - 36+5)	35+2 (26+6 - 36+6)	34+3 (23+2 - 36+6)	35+0 (22+2 - 36+6)
Gestation at pre-eclampsia diagnosis*		34+1 (21+5 - 36+4)	34+4 (24+5 - 36+5)	33+6 (23+1 - 36+6)	34+1 (21+5 - 36+6)
Infant sex	**Male**	25 (69%)	16 (48%)	25 (56%)	66 (58%)
	Female	11 (31%)	17 (52%)	20 (44%)	48 (42%)
Mode of delivery	**Elective C-section**	18 (60%)	13 (43%)	24 (62%)	55 (56%)
	Emergency C-section	4 (13%)	6 (20%)	4 (10%)	14 (14%)
	Operative vaginal delivery	0 (0%)	2 (7%)	1 (3%)	3 (3%)
	Vaginal breech	0 (0%)	1 (3%)	0 (0%)	1 (1%)
	Spontaneous vaginal delivery	8 (27%)	8 (27%)	10 (26%)	26 (26%)
Indication for delivery	**Spontaneous**	1 (3%)	2 (7%)	3 (8%)	6 (6%)
	Maternal	19 (63%)	15 (50%)	22 (56%)	56 (57%)
	Fetal	6 (20%)	9 (30%)	11 (28%)	26 (26%)
	Both	4 (13%)	4 (13%)	3 (8%)	11 (11%)
Delivery <34 weeks		9 (30%)	8 (27%)	15 (38%)	32 (32%)
Multi-fetal pregnancy		6 (20%)	3 (10%)	5 (13%)	14 (14%)
Perinatal outcomes					
Birthweight centile*	**Singleton pregnancy (n=51)**	1.9 (0.0 - 23.0)	2.2 (0.0 - 96.8)	3.2 (0.0 - 94.3)	2.2 (0.0 - 96.8)
	Multi-fetal pregnancy (n=18)	10.6 (0.0 - 68.5)	21.6 (0.8 - 48.0)	21.6 (2.1 - 39.6)	18.1 (0.0 - 68.5)
Birthweight centile <10th	**Singleton pregnancy**	20/24 (83%)	17/27 (63%)	23/34 (67%)	60/85 (71%)
	Multi-fetal pregnancy	6/12 (50%)	2/6 (33%)	5/11 (45%)	13/29 (45%)
Birthweight centile <3rd	**Singleton pregnancy**	13/24 (54%)	14/27 (52%)	17/34 (50%)	44/85 (52%)
	Multi-fetal pregnancy	3/12 (25%)	2/6 (33%)	1/11 (9%)	6/29 (21%)
NICU admission (days)*	**Singleton pregnancy**	12 (0 - 64)	6 (0 - 98)	7 (0 - 90)	7 (0 - 98)
	Multi-fetal pregnancy	10 (0 - 22)	4 (0 - 5)	6 (0 - 33)	5 (0 - 33)
RDS	**Singleton pregnancy**	5/22 (23%)	6/25 (24%)	9/33 (27%)	20/80 (25%)
	Multi-fetal pregnancy	0/12 (0%)	4/6 (67%)	3/11 (27%)	7/29 (24%)
IVH	**Singleton pregnancy**	1/22 (5%)	0/25 (0%)	3/33 (9%)	4/80 (5%)
	Multi-fetal pregnancy	0/12 (0%)	0/6 (0%)	0/11 (0%)	0/29 (0%)
Seizure	**Singleton pregnancy**	0/22 (0%)	0/25 (0%)	1/33 (3%)	1/80 (1%)

	Multi-fetal pregnancy	0/12 (0%)	0/6 (0%)	0/11 (0%)	0/29 (0%)
NEC	**Singleton pregnancy**	1/22 (5%)	1/25 (4%)	3/33 (9%)	5/80 (6%)
	Multi-fetal pregnancy	0/12 (0%)	0/6 (0%)	0/11 (0%)	0/29 (0%)
Adverse perinatal outcome†	**Singleton pregnancy**	7/24 (29%)	8/27 (30%)	11/34 (32%)	26/85 (31%)
	Multi-fetal pregnancy	0/12 (0%)	4/6 (67%)	3/11 (27%)	7/29 (24%)
Stillbirth	**Singleton pregnancy**	2/24 (8%)	2/27 (7%)	1/34 (3%)	5/85 (6%)
	Multi-fetal pregnancy	0/12 (0%)	0/6 (0%)	0/11 (0%)	0/29 (0%)
NND	**Singleton pregnancy**	0/24 (0%)	0/27 (0%)	2/34 (6%)	2/85 (2%)
	Multi-fetal pregnancy	0/12 (0%)	0/6 (0%)	0/11 (0%)	0/29 (0%)
Maternal outcomes					
Maximum sBP (mmHg)		164.3 ± 14.0	164.2 ± 11.5	163.9 ± 12.2	164.1 ± 12.4
Maximum dBP (mmHg)		108.8 ± 9.7	107.1 ± 9.4	105.7 ± 9.0	107.1 ± 9.3
Eclampsia / HELLP syndrome		0 (0%)	0 (0%)	0 (0%)	0 (0%)
Abruption		0 (0%)	1(3%)	0 (0%)	1 (1%)
Maternal death		1 (3%)	0 (0%)	0 (0%)	1 (1%)
Gestational diabetes		1 (3%)	1 (3%)	4 (10%)	6 (6%)
Pre-eclampsia with severe features‡		20 (67%)	24 (80%)	29 (74%)	73 (74%)
Spontaneous preterm birth		1 (3%)	2 (7%)	3 (8%)	6 (6%)
Antenatal Steroids (lung maturity)		21 (70%)	17 (57%)	30 (77%)	68 (69%)

Frequencies: N (%).
Mean ± standard deviation.
*Median (range).
†Composite neonatal outcome: respiratory distress syndrome / intraventricular haemorrhage / necrotising enterocolitis.
‡Definition of pre-eclampsia with severe features: maximum BP ≥160/110mmHg / alanine aminotransferase >100U/L / creatinine >100µmol/L / platelets <100x10^9/L.
C-section, Caesarean section; NICU, neonatal intensive care unit; RDS, respiratory distress syndrome; IVH, intraventricular haemorrhage; NEC, necrotising enterocolitis; NND, neonatal death; BP, blood pressure; sBP, systolic BP; dBP, diastolic BP; mmHg, millimetres of mercury; HELLP, haemolysis, elevated liver enzymes and a low platelet count.

4.4.2.2. Change in cardiac structure and function over time

At six months, diastolic and systolic dysfunction affected 61% and 7% of women, respectively (Table 4.4). When using age-adjusted reference ranges for all diastolic functional measures[289] including E/A and deceleration time, diastolic dysfunction affected 32 (73%) women.

Table 4.4: Prevalence of cardiovascular and echocardiographic abnormalities at baseline (within three days of delivery), six weeks and six months postpartum.

Cardiovascular abnormality	Baseline (n=69)	6 weeks (n=50)	6 months (n=44)
Raised TVR (> 1200)	34 (49%)	45 (90%)	33 (75%)
Systolic dysfunction	14 (20%)	13 (26%)	3 (7%)
Diastolic dysfunction (any grade)	58 (84%)	21 (42%)	27 (61%)
Grade I diastolic dysfunction	53 (77%)	21 (42%)	26 (59%)
Grade II diastolic dysfunction	4 (6%)	0 (0%)	0 (0%)
Grade III diastolic dysfunction	1 (1%)	0 (0%)	1 (2%)
No remodelling	13 (19%)	25 (50%)	26 (59%)
Concentric remodelling	33 (48%)	20 (40%)	16 (36%)
Concentric hypertrophy	22 (32%)	5 (10%)	1 (2%)
Eccentric hypertrophy	1 (1%)	0 (0%)	1 (2%)
Requiring antihypertensives	53 (77%)	19 (38%)	15 (34%)
Requiring antihypertensives or BP > 140/90mmHg	58 (84%)	24 (48%)	25 (57%)
Requiring antihypertensives or BP > 140/90mmHg in the absence of pre-existing hypertension	46/57 (81%)	15/41 (37%)	16/35 (46%)

Frequencies: N (%).
Data are pooled from the placebo and observational arms of the study.
Diastolic dysfunction defined using British Society of Echocardiography guideline flow chart[289].
TVR, total vascular resistance; BP, blood pressure; mmHg, millimetres of mercury.

Prevalence of diastolic dysfunction is significantly impacted by the classification used. For example, the 2016 American Society of Echocardiography and European Association of Cardiovascular Imaging guideline does not use age-adjusted reference ranges, in contrast with BSE[289,421]. For this reason, prevalence of diastolic dysfunction using the different definitions is summarised in Table 4.5.

Table 4.5: Prevalence of diastolic dysfunction using different classification systems.

Classification system	Baseline (n=69)	6 weeks (n=50)	6 months (n=44)
BSE 2013 guidelines, clinical flowchart* [289]	58 (84%)	21 (42%)	27 (61%)
BSE 2013 guidelines, using all available age-corrected values† [289]	61 (88%)	36 (72%)	32 (73%)
ASE EACI 2016 guidelines [421]	10 (14%)	2 (4%)	2 (5%)

Frequencies: N (%).
Data are pooled from the placebo and observational arms of the study.
*Discrete cut-offs used for E/A and deceleration time; age-corrected normal values used for septal and lateral E', as per the BSE "Practical approach to assessment and grading of diastolic dysfunction" flow chart[289].
†Age-corrected normal values used for E/A, deceleration time, septal E' and lateral E'.
BSE, British Society of Echocardiography; ASE, American Society of Echocardiography; EACI, European Association of Cardiovascular Imaging.

TVR was raised in 75% and 41% of women had persistent left ventricular remodelling at six months (Table 4.4). Of those who had no pre-existing hypertension (diagnosed before or during the first half of pregnancy), 6/35 (17%) required antihypertensives and 16/35 (46%) had a diagnosis of hypertension, defined by clinic BP ≥140/90[422] and/or need for antihypertensives, at six months. There was considerable overlap in echocardiography abnormalities and only two (5%) women had a completely normal echocardiogram at six months (Figure 4.9).

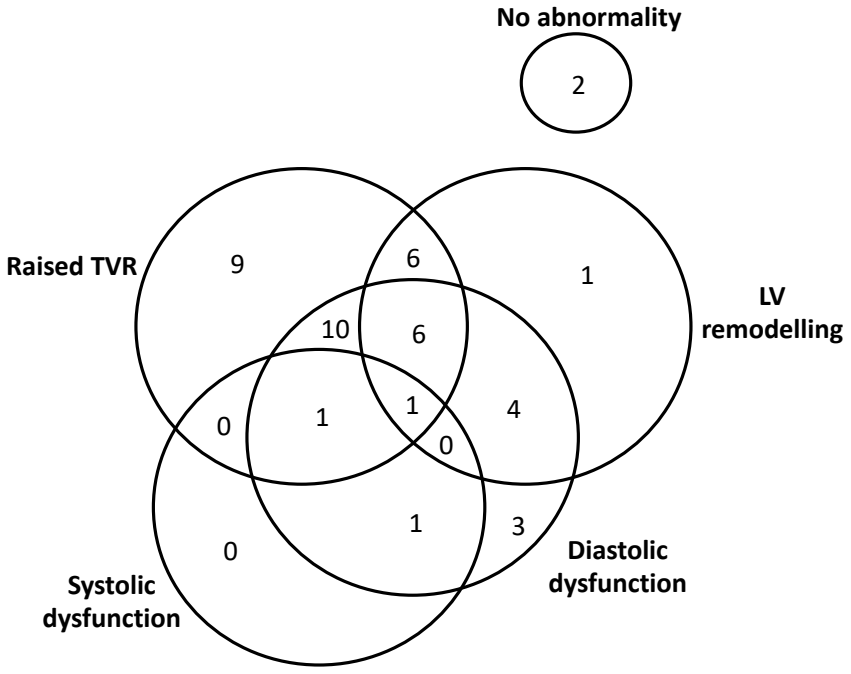

Figure 4.9: Venn diagram of six-month echocardiographic abnormalities.
Data are pooled from the placebo and observational arms of the study.
TVR, total vascular resistance; LV, left ventricular.

Details of echocardiography measures at each timepoint are summarised in Table 4.6. The majority of echocardiography parameters (including E/E' and left ventricular remodelling) significantly improved from baseline to six weeks; however there was no significant change in any echocardiography parameter from six weeks to six months. TVR increased from baseline to six weeks and systolic function (LVEF, GLS and S') did not significantly change over time (Table 4.6).

Table 4.6: Change in postnatal echocardiography measures over time.

	Baseline (n=69)	6 weeks (n=50)	6 months (n=44)	Mean difference between timepoints (95% C.I.)		
				Baseline to 6 weeks	Baseline to 6 months	6 weeks to 6 months
TVR (dyne.s^{-1}cm^{-5})	1427 ± 382	1749 ± 424	1662 ± 486	**322 (175 - 469)**	**235 (72 - 397)**	87 (-274 - 99)
HR (bpm)	85.3 (13.7)	75.3 (11.7)	78.2 ± 12.3	**-10.0 (-14.8 - -5.3)**	**-7.2 (-12.2 - -2.1)**	2.9 (-2.1 - 7.8)
SV (mL)	73.9 ± 16.1	63.1 ± 12.8	64.8 ± 13.3	**-10.8 (-16.3 - -5.4)**	**-9.1 (-14.8 - -3.3)**	1.7 (-3.6 - 7.1)
CO (L/minute)	6.3 ± 1.6	4.7 ± 1.0	5.0 ± 1.1	**-1.6 (-2.1 - -1.5)**	**-1.2 (-1.8 - -6.9)**	3.2 (-1.2 - 7.6)
LVEF (%)	63 ± 5	62 ± 4	62 ± 3	-1.1 (-2.7 - 0.5)	-1.0 (-2.5 - 0.6)	0.1 (-1.3 - 1.5)
Myocardial strain and strain rate						
LV basal strain (%)	-15.7 ± 3.3	-16.3 ± 2.2	-17.0 ± 2.5	-0.6 (-1.7 - 0.4)	**-1.3 (-2.5 - -0.2)**	-0.7 (-1.6 - 0.3)
LV mid strain (%)	-18.8 ± 2.3	-19.1 ± 1.8	-19.7 ± 1.9	-0.2 (-1.0 - 0.6)	**-0.9 (-1.7 - -0.1)**	-0.7 (-1.4 - 0.1)
LV apical strain (%)	-25.3 ± 3.8	-24.5 ± 3.0	-25.3 ± 2.6	0.8 (-0.5 - 2.1)	0.1 (-1.2 - 1.4)	-0.8 (-1.9 - 0.4)
GLS (%)	-19.9 ± 2.4	-20.0 ± 2.2	-20.7 ± 1.8	0.0 (-0.9 - 0.8)	-0.8 (-1.6 - 0.1)	-0.7 (-1.6 - 0.1)
$SR_{E/A}$	2.19 ± 0.72	2.38 ± 0.68	2.21 ± 0.73	0.19 (-0.07 - 0.46)	0.03 (-0.25 - 0.30)	-0.17 (-0.46 - 0.12)
Mitral inflow						
E deceleration time (ms)	192 ± 32	197 ± 29	189 ± 34	4.9 (-6.8 - 16.5)	-3.7 (-16.5 - 9.2)	-8.5 (-21.6 - 4.6)
E/A	1.22 ± 0.28	1.3 ± 0.27	1.24 ± 0.28	**0.12 (0.02 - 0.22)**	0.03 (-0.08 - 0.13)	-0.09 (-0.20 - 0.02)
Mitral annular motion						
Septal peak S' velocity (m/s)	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	-0.01 (-0.02 - 0.00)	-0.01 (-0.02 - 0.00)	0.00 (0.00-0.00)
Lateral peak S' velocity (m/s)	0.10 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.00 (-0.01 - 0.00)	0.00 (-0.01 - 0.01)	0.00 (-0.01 – 0.01)
E/E'	8.71 ± 2.00	7.37 ± 1.68	7.53 ± 1.51	**-1.35 (-2.04 - -0.66)**	**-1.19 (-1.89 - -0.49)**	0.16 (-0.40 - 0.81)
Tricuspid valve						
TR Vmax (cm/s)	0.98 ± 1.17	0.58 ± 0.95	0.55 ± 0.97	-0.40 (-0.80 - 0.00)	**-0.43 (-0.85 - -0.01)**	-0.03 (-0.42 - 0.36)
Cardiac morphology						
LVIDd (cm)	4.49 ± 0.45	4.32 ± 0.48	4.30 ± 0.42	-0.17 (-0.34 - 0.00)	**-0.18 (-0.35 - -0.01)**	-0.02 (-0.20 - 0.17)

PWd (cm)	1.12 ± 0.19	0.94 ± 0.19	0.89 ± 0.17	**-0.18 (-0.25 - -0.11)**	**-0.23 (-0.30 - -0.16)**	-0.05 (-0.13 - 0.02)
IVSd (cm)	1.01 ± 0.18	0.88 ± 0.17	0.8 ± 0.16	**-0.12 (-0.19 - -0.06)**	**-0.13 (-0.19 - -0.06)**	0.00 (-0.07 - 0.07)
LVM (g)	172.02 ± 48.50	132.40 ± 46.79	125.33 ± 39.32	**-39.62 (-57.20 - -22.04)**	**-46.69 (-63.96 - -29.43)**	-7.07 (-24.91 - 10.77)
LVMi (g/m^2)	89.58 ± 18.65	70.39 ± 18.73	66.92 ± 15.28	**-19.19 (-26.06 - -12.32)**	**-22.67 (-29.33 - -16.01)**	-3.47 (-10.54 - 3.59)
RWT	0.50 ± 0.10	0.44 ± 0.10	0.41 ± 0.09	**-0.06 (-0.10 - -0.03)**	**-0.09 (-0.12 - -0.05)**	-0.02 (-0.06 - 0.02)
LAV (mL)	47.3 ± 14.4	39.7 ± 11.3	38.9 ± 10.5	**-7.7 (-12.6 - -2.8)**	**-8.4 (-13.5 - -3.4)**	-0.8 (-5.3 - 3.8)
LAVi (mL/m^2)	24.7 ± 6.3	21.2 ± 4.7	20.9 ± 4.7	**-3.5 (-5.6 - -1.4)**	**-3.7 (-5.9 - -1.5)**	-0.3 (-2.2 - 1.7)

Frequencies: N (%).
Continuous data: mean ± standard deviation.
Variables were compared between time-points using paired t-test.
Data are pooled from the placebo and observational arms of the study.
Bold text indicates statistical significance ($p<0.05$).
C.I., confidence interval; TVR, total vascular resistance; HR, heart rate; SV, stroke volume; CO, cardiac output; LVEF, left ventricular ejection fraction; LV, left ventricular; GLS; global longitudinal strain; $SR_{E/A}$, early to late strain rate ratio; E/A, early to late diastolic filling ratio; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; LVIDd, left ventricular internal diameter in end-diastole; PWd, posterior wall diameter in end-diastole; IVSd, interventricular septal wall diameter in end-diastole; LVM, left ventricular mass; LVMi, LVM indexed to body surface area; RWT, relative wall thickness; LAV left atrial volume; LAVi, LAV indexed to body surface area; TR Vmax, tricuspid regurgitation maximum velocity.

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Six (8%) women had raised HS-cTnT (16-18ng/L) or NTproBNP (468-1259pg/mL) at baseline. All cardiac biomarkers had normalised by six weeks postpartum. The change in placental and cardiovascular biomarker levels over time is summarised in Table 4.7. Baseline placental biomarkers were significantly influenced by the number of days postpartum (PlGF: coefficient -21.5pg/mL/day [95% C.I. -38.6- -4.5], p=0.01 and sFlt: coefficient -1145.6pg/mL/day [95% C.I. -1717.8- -573.4], p<0.001, respectively). All biomarkers declined from baseline to six weeks; only sFlt continued to decline from six weeks to six months (88 [67-133] versus 80.0 [64-110], p=0.01; Table 4.7).

Table 4.7: Change in postnatal placental and cardiovascular biomarkers over time.

Biomarker	Baseline (n=69)	6 weeks (n=50)	6 months (n=44)	Difference in log-transformed biomarkers between timepoints (95% C.I.)		
				Baseline to 6 weeks	Baseline to 6 months	6 weeks to 6 months
sFlt (pg/mL)	1432 (262 - 11624)	88 (67 - 133)	80 (64 - 110)	**-2.92 (-3.16 - -2.67)**	**-2.99 (-3.25 - -2.73)**	**-0.07 (-0.13 - -0.02)**
PlGF (pg/mL)	22 (84 - 521)	10 (56- 20)	10 (5 - 17)	**-1.06 (-1.28 - -0.83)**	**-1.02 (-1.25 - -0.78)**	0.04 (-0.07 - 0.15)
sFlt:PlGF	57 (11 - 566)	10 (5 - 16)	8 (5 - 20)	**-1.86 (-2.09 - -1.63)**	**-1.97 (-2.21 - -1.74)**	-0.11 (-0.23 - 0.00)
HS-cTnT (ng/L)	5 (<3 - 28)	<3 (<3 - 14)	<3 (<3 - 7)	**-0.61 (-0.83 - -0.39)**	**-0.68 (-0.90 - -0.46)**	-0.06 (-0.23 - 0.10)
NTproBNP (pg/mL)	64 (<5 - 1259)	25.5 (<5 - 129)	30 (<5 - 162)	**-0.89 (-1.28 - -0.51)**	**-0.85 (-1.28 - -0.43)**	0.04 (-0.29 - 0.37)

Continuous data: median (range).
Log-transformed variables were compared between time-points using paired t-test.
Data are pooled from the placebo and observational arms of the study.
Bold text indicates statistical significance (p<0.05).
C.I., confidence interval; sFlt, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; HS-cTnT, high-sensitivity cardiac Troponin T; NTproBNP, N-terminal pro-brain natriuretic peptide.

Table 4.8 summarises the change in BP and arterial stiffness over time. There was no significant change in PWV, aortic or brachial AIx from baseline to six months. Central and peripheral sBP declined significantly from baseline to six months postpartum; however, there was no significant change in dBP. With the exception of arteriography-measured sBP and MAP, there was no significant difference in arteriography measures or peripheral BP between six weeks and six months.

Table 4.8: Change in postnatal BP and arterial stiffness over time.

	Baseline (n=69)	6 weeks (n=50)	6 months (n=44)	Mean difference between timepoints (95% C.I.)		
				Baseline to 6 weeks	Baseline to 6 months	6 weeks to 6 months
Arteriography						
PWV (m/s)	8.3 ± 1.8	7.4 ± 1.5	7.6 ± 1.8	**-0.9 (-1.6 - -0.2)**	-0.8 (-1.5 - 0.0)	0.2 (-0.6 - 0.9)
Heart rate (bpm)	86.4 ± 16.1	80.6 ± 13.6	84.4 ± 14.0	-5.8 (-11.8 - 0.2)	-1.9 (-8.1 - 4.3)	3.9 (-2.1 - 9.9)
sBP (mmHg)	137.6 ± 9.8	125.1 ± 11.5	132.1 ± 17.41	**-12.5 (-16.7 - -8.3)**	-5.5 (-10.9 - 0.0)	7.0 (0.6 - 13.4)
dBP (mmHg)	83.4 ± 8.8	78.4 ± 10.6	81.8 ± 13.6	**-5.0 (-8.9 - -1.2)**	-1.7 (-6.1 - 2.8)	3.3 (-2.0 - 8.6)
Augmentation index (aortic)	24.2 ± 15.1	23.1 ± 12.0	21.9 ± 12.2	-1.1 (-6.7 - 4.4)	-2.4 (-8.0 - 3.3)	-1.2 (-6.5 - 4.1)
Augmentation index (brachial)	-23.4 ± 28.8	-28.8 ± 23.8	-30.9 ± 24.2	-5.4 (-16.1 - 5.3)	-7.6 (-18.5 - 3.4)	-2.2 (-12.6 - 8.3)
Central sBP (mmHg)	132.6 ± 12.5	119.5 ± 13.3	125.8 ± 21.0	**-13.2 (-18.2 - -8.0)**	-6.7 (-13.4 - 0.0)	6.4 (-1.2 - 13.9)
Alere Microlife						
sBP (mmHg)	135.4 ± 9.0	126.4 ± 10.9	127.9 ± 13.7	**-8.9 (-12.6 - -5.3)**	**-7.5 (-11.7 - -3.2)**	1.5 (-3.6 - 6.5)
dBP (mmHg)	90.1 ± 7.6	84.6 ± 9.4	86.7 ± 12.2	**-5.5 (-8.6 - -2.4)**	-3.4 (-7.1 - 0.3)	2.1 (-2.4 - 6.5)
MAP (mmHg)	105.2 ± 7.4	98.6 ± 9.5	100.4 ± 12.4	**-6.6 (-97 - -3.6)**	**-4.8 (-8.4 - -1.1)**	1.9 (-2.6 - 6.4)

Continuous data: mean standard ± deviation.
Variables were compared between time-points using paired t-test.
Data are pooled from the placebo and observational arms of the study.
Bold text indicates statistical significance ($p<0.05$).
PWV, pulse wave velocity; BP, blood pressure; C.I., confidence interval; bpm, beats per minute; sBP, systolic BP; dBP, diastolic BP; mmHg, millimetres of mercury.

4.4.2.3. Relationship between features of pre-eclampsia and six-month cardiovascular phenotype

The presence of pre-existing hypertension did not influence systolic or diastolic function at six months (GLS: -19.96±2.34% versus -20.88±1.55%, p=0.16; E/E': 7.93±1.13 versus 7.43±1.59, p=0.38). However, women with pre-existing hypertension had worse left ventricular remodelling at all timepoints (six-month LVMi: 81.91±21.24g/m^2 versus 63.06±10.70g/m^2, p=0.001) and higher TVR at six months (2034±795 dyne.s^{-1}cm^{-5} versus 1567±323 dyne.s^{-1}cm^{-5}, p=0.009), compared to those without (Figure 4.10). Preterm pre-eclampsia with severe features (adapted from the NICE guidelines[328]) was not significantly associated with any six-month echocardiography parameter (Figure 4.10).

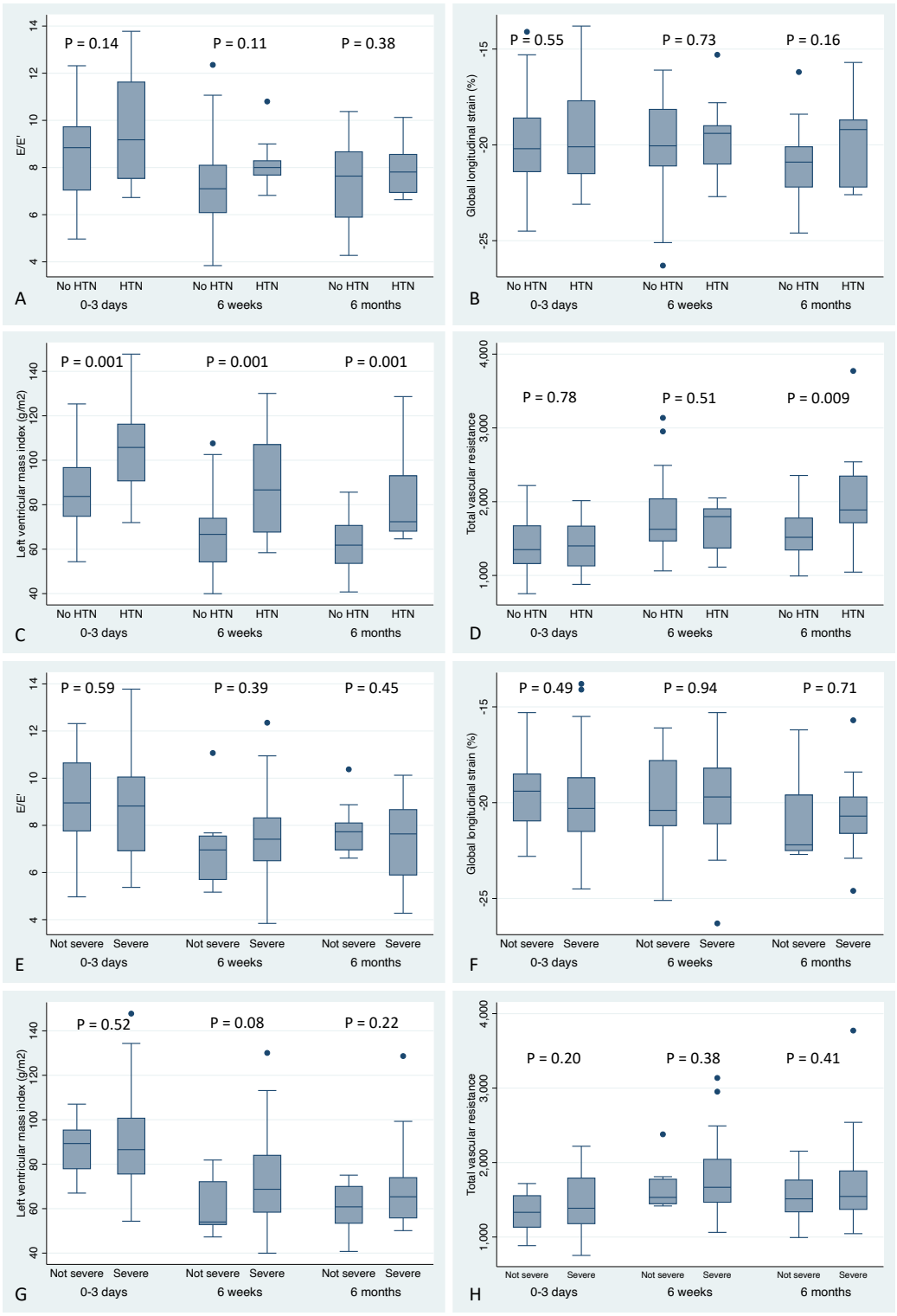

Figure 4.10: Influence of pre-existing hypertension and pre-eclampsia severity on change in echocardiography over time.
Box plots demonstrating the influence of pre-existing hypertension on **A.** E/E'; **B.** global longitudinal strain; **C.** left ventricular mass index; **D.** total vascular resistance; and the influence of severe features of pre-eclampsia on **E.** E/E'; **F.** global longitudinal strain; **G.** left ventricular mass index; **H.** total vascular resistance.
The line represents median; the box includes 25th to 75th percentile; the whiskers extend to the upper and lower adjacent values and the dots represent outliers.
P values are derived using paired t-test, comparing the two groups at different time-points.
Data are pooled from the placebo and observational arms of the study.

Definition of pre-eclampsia with severe features: maximum BP ≥160/110mmHg / alanine aminotransferase >100U/L / creatinine >100μmol/L / platelets <100x10^9/L.
HTN, pre-existing hypertension diagnosed <20 weeks' gestation; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; BP, blood pressure.

Earlier gestations at preterm pre-eclampsia diagnosis and delivery were associated with worse diastolic dysfunction (E/E': rho=-0.34, p=0.03 and rho=-0.39, p=0.001, respectively) and TVR (rho=-0.42, p=0.004 and rho=-0.34, p=0.02, respectively) at six months, as measured by Spearman's correlation coefficient (Figure 4.11). Prolonged preterm pre-eclampsia duration (ranging up to 48 days) was associated with increased TVR, but not E/E' at six months (rho=0.36, p=0.02 and rho=0.20, p=0.20, respectively; Figure 4.12). On the other hand, prolonged preterm pre-eclampsia duration was associated with reduced improvement in remodelling and a trend toward reduced improvement in diastolic dysfunction from baseline to six months (LVMi: rho=-0.33, p=0.03; E/E': rho=-0.27, p= 0.08). Lower birthweight centile was also associated with worse diastolic dysfunction and TVR at six months (Spearman's correlation E/E': rho=-0.42, p=0.005 and TVR: rho=-0.37, p=0.01; Figure 4.11). There was no correlation between birthweight centile, gestation at diagnosis or delivery, and left ventricular remodelling (LVMi and RWT) or systolic function (LVEF, GLS and S'). Correlations were unchanged by using WHO Z scores[324] instead of customised birthweight centile.

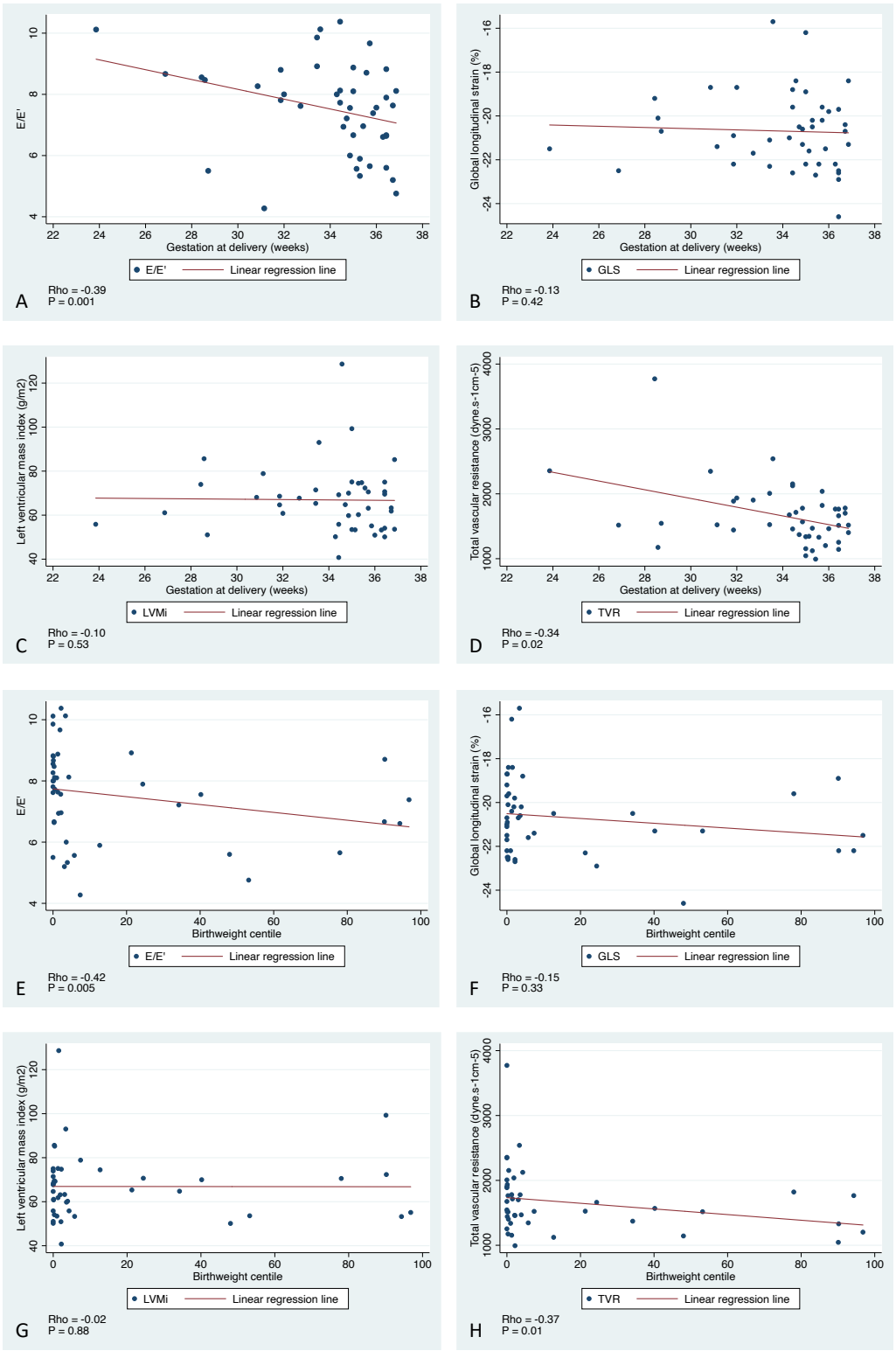

Figure 4.11: Relationship between gestation at delivery / birthweight centile and cardiovascular function and remodelling six months postpartum.
Scatter plots illustrating the relationship between gestation at delivery and A. E/E'; B. global longitudinal strain; C. left ventricular mass index; D. total vascular resistance; and birthweight centile and E. E/E'; F. global longitudinal strain; G. left ventricular mass index; H. total vascular resistance. Dots represent individual women; linear regression lines were added to aid interpretation.
Data are pooled from the placebo and observational arms of the study.
E/E', early diastolic filling to early diastolic mitral annular velocity ratio; GLS, global longitudinal strain, LVMi, left ventricular mass index; TVR, total vascular resistance.

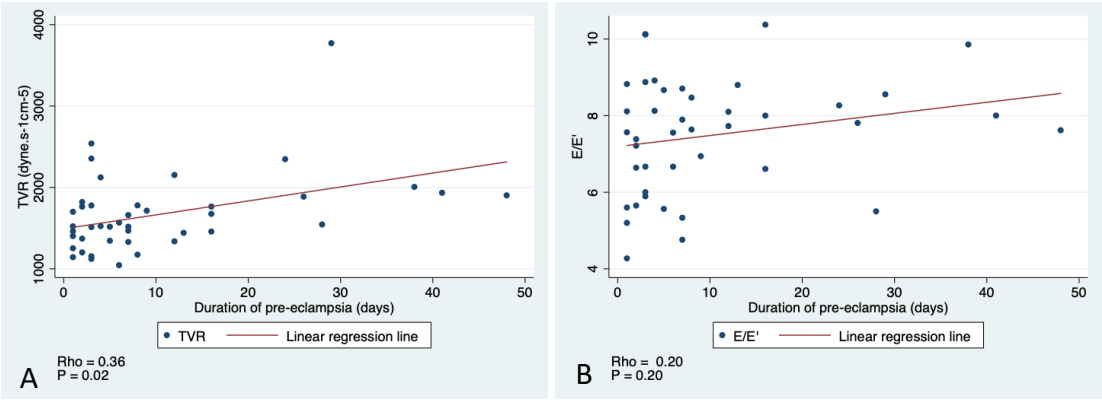

Figure 4.12: Relationship between pre-eclampsia duration and six-month echocardiography parameters.
Dots represent individual women; linear regression lines were added to aid interpretation.
Figure illustrates the relationship between pre-eclampsia duration (log-transformed) and **A.** total vascular resistance (TVR) and **B.** E/E'(early diastolic filling to early diastolic mitral annular velocity ratio) at six months postpartum.
Data are pooled from the placebo and observational arms of the study.

4.4.2.4. Relationship between biomarkers and cardiovascular function

Baseline placental biomarker levels were significantly affected by number of days postpartum; therefore number of days (but not hours) postpartum at visit one was included as a covariate in the linear regression. There were no correlations between baseline concentrations of PlGF, sFlt or sFlt:PlGF and baseline, six-week or six-month echocardiography measures, with the exception of baseline sFlt:PlGF (log-transformed) and baseline TVR (adjusted coefficient: 128.00 dyne.s^{-1}cm^{-5} [95% C.I. 10.17-247.83], p=0.03). However this did not persist at six weeks or six months (six-week adjusted coefficient: 84.97 dyne.s^{-1}cm^{-5} [95% C.I. -66.67-236.61], p=0.27; six-month adjusted coefficient: -78.00 dyne.s^{-1}cm^{-5} [95% C.I. -277.57-121.57], p=0.43).

Baseline NTproBNP (log-transformed) correlated with baseline E/E' (coefficient: 0.42 [95% C.I. 0.04-0.81], p=0.03) and six-week TVR (coefficient: 106.59 dyne.s^{-1}cm^{-5} [95% C.I. 6.05-207.13], p=0.04). Again, these associations did not retain statistical significance at six months (E/E' coefficient: 0.12 [95% C.I. -0.28-0.53], p=0.55; TVR coefficient: 51.04 dyne.s^{-1}cm^{-5} [95% C.I. -79.25-181.33], p=0.43). Baseline HS-cTnT (log-transformed) correlated with baseline RWT (coefficient: 0.05 [95% C.I. 0.01-0.08], p=0.009) but this did not remain statistically significant at six weeks or six months (six-week coefficient: 0.04 [95% C.I. 0.00-0.08], p=0.06; six-month coefficient: 0.02 [95% C.I. -0.02-0.06], p=0.31). Lastly, there was a modest association between baseline HS-cTnT (log-transformed) and six-week GLS

(coefficient: 0.96% [95% C.I. 0.07-1.85], p=0.04), which did not persist at six months (coefficient: 0.41% [95% C.I. -0.40-1.21], p=0.31). These associations are illustrated in Figure 4.13.

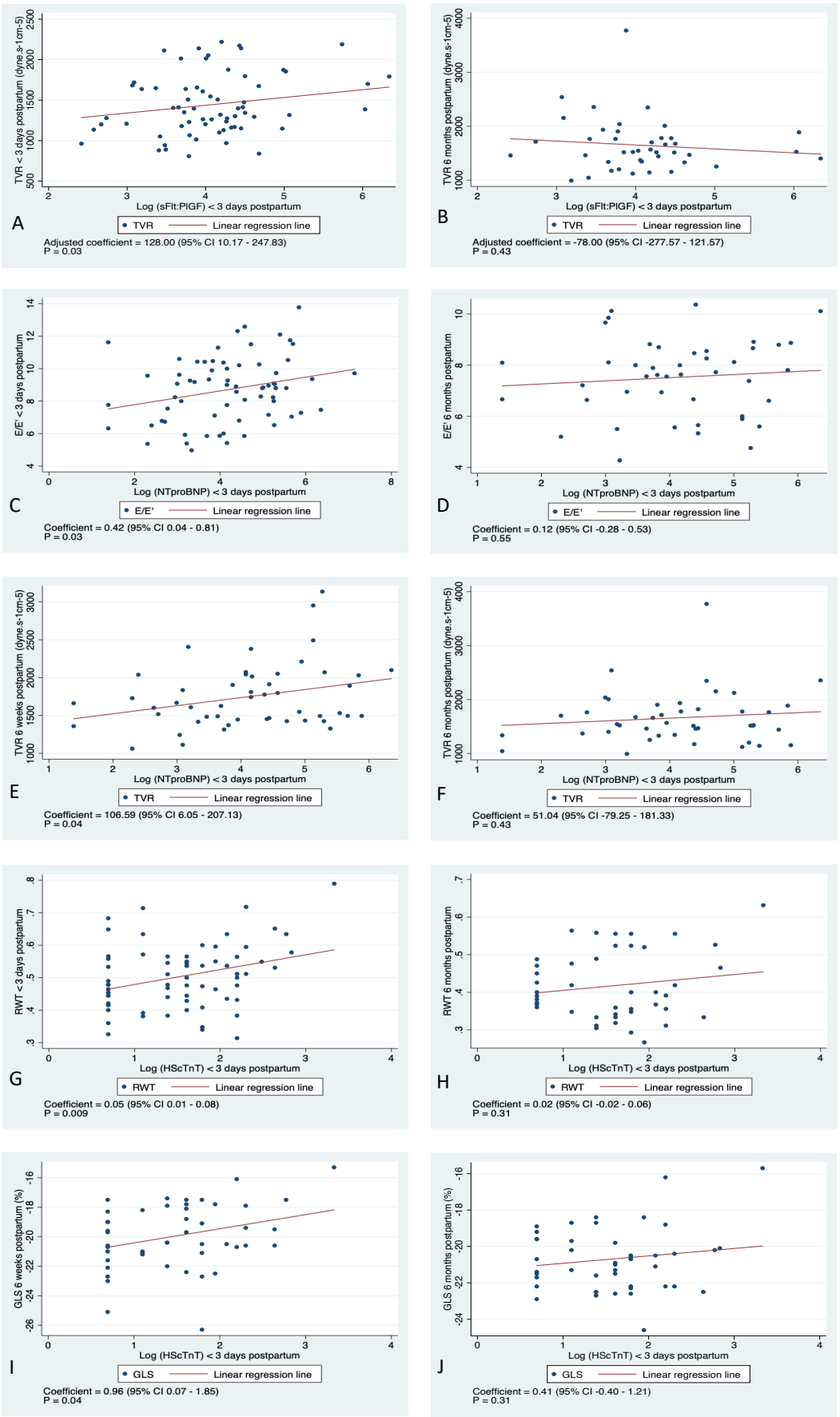

Figure 4.13: Relationship between baseline biomarkers and cardiovascular parameters at baseline, six weeks and six months postpartum.
sFlt:PlGF is adjusted for number of days postpartum.
Dots represent individual women and lines represent linear regression lines.

Figure illustrates the relationship between baseline sFlt:PlGF (log-transformed) and total vascular resistance (TVR) at dyne.$s^{-1}cm^{-5}$ **A.** baseline and **B.** six months; baseline NTproBNP (log-transformed) and E/E' (early diastolic filling to early diastolic mitral annular velocity ratio) at **C.** baseline and **D.** six months, and TVR at **E.** six weeks and **F.** six months; baseline HS-cTnT (log-transformed) and relative wall thickness (RWT) at **G.** baseline and **H.** six months, and global longitudinal strain (GLS) at **I.** six weeks and **J.** six months.
Data are pooled from the placebo and observational arms of the study.
NTproBNP, N-terminal pro-brain natriuretic peptide; HS-cTnT, high sensitivity troponin; RWT, relative wall thickness; sFlt, soluble fms-like tyrosine kinase-1.

4.4.2.5. Impact of breastfeeding status on cardiovascular and metabolic parameters

Figure 4.14 compares cardiovascular parameters between women who breastfed and those who did not. Breastfeeding status was not associated with any difference in systolic or diastolic function, BP or BMI. However, breastfeeding was associated with a trend towards a lower LVMi (mean difference 9.00g/m^2 [95% C.I. -0.03-18.02], p=0.05).

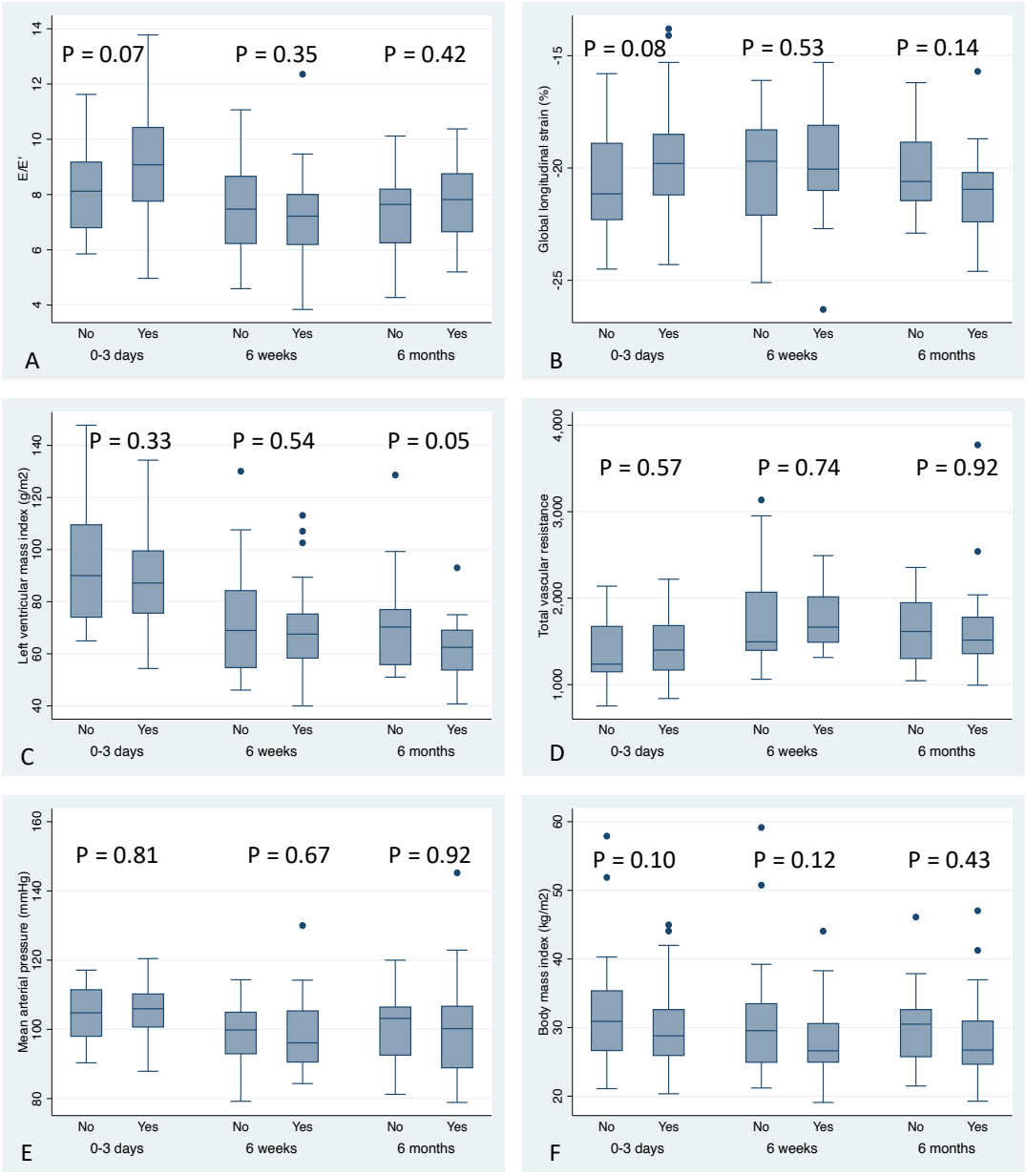

Figure 4.14: Influence of breastfeeding status on change on postnatal cardiovascular and metabolic parameters over time.
Box plots demonstrating the influence of breastfeeding status on **A.** E/E'; **B.** global longitudinal strain; **C.** left ventricular mass index; **D.** total vascular resistance; **E.** MAP; **F.** BMI.
The line represents median; the box includes 25th to 75th percentile; the whiskers extend to the upper and lower adjacent values and the dots represent outliers.
P values are derived using paired t-test, comparing the two groups at different time-points**.**
Data are pooled from the placebo and observational arms of the study.
E/E', early diastolic filling to early diastolic mitral annular velocity ratio; MAP, mean arterial pressure; BMI, body mass index.

4.4.2.6. Impact of enalapril on cardiovascular function and remodelling

As shown in Tables 4.9 and 4.10, there was no difference in the primary outcome (TVR) between groups at six months postpartum. Similarly, there was no difference in systolic function (measured by LVEF / GLS / S'). However, women who were treated with enalapril

had significantly better diastolic function at six months than those treated with placebo, as measured by E/E' (adjusted difference -1.07, 95% C.I. -2.08- -0.06, p=0.04). Allocation to enalapril was also associated with improved left ventricular remodelling at six months, compared with placebo (LVMi adjusted difference -9.23g/m^2, 95% C.I. -7.73- -0.71, p=0.03). No difference in echocardiography parameters was seen between groups at six weeks. Similarly, differences did not retain statistical significance when baseline measures were not adjusted for, except RWT (unadjusted difference -0.08, 95% C.I. -0.13- -0.02, p=0.01).

Table 4.9: Echocardiography measures of cardiac structure and function at baseline, six weeks and six months, depending on treatment allocation.

Indices	Enalapril			Placebo		
	Baseline (n=29)	6 weeks (n=22)	6 months (n=19)	Baseline (n=30)	6 weeks (n=22)	6 months (n=21)
TVR (dyne.$s^{-1}cm^{-5}$)	1451 ± 393	1619 ± 433	1516 ± 278	1510 ± 430	1774 ± 441	1579 ± 438
HR (bpm)	81.7 ± 15.5	73.5 ± 12.1	78.5 ± 12.0	87.4 ± 12.8	76.4 ± 11.6	82.1 ± 14.0
SV (mL)	74.1 ± 13.4	64.0 ± 13.5	64.6 ± 10.7	69.2 ± 15.7	60.6 ± 12.3	65.2 ± 14.1
CO (L/minute)*	6.0 (3.7 - 12.5)	4.5 (2.5 - 7.7)	4.9 (3.4 - 8.3)	5.6 (3.9 - 10.3)	4.7 (2.9 - 6.8)	5.2 (3.8 - 8.6)
LVEF (%)	64.0 ± 7.8	60.4 ± 4.5	61 .4 ± 4.3	63.2 ± 5.7	62.5 ± 4.7	61.5 ± 3.9
Myocardial strain and strain rate						
LV basal strain (%)	-15.5 ± 3.0	-16.7 ± 2.8	-17.4 ± 2.5	-14.9 ± 2.6	-16.2 ± 2.1	-16.6 ± 2.8
LV mid strain (%)	-18.9 ± 2.9	-19.0 ± 2.5	-19.5 ± 2.2	-18.1 ± 2.1	-18.7 ± 1.7	-19.1 ± 2.1
LV apical strain (%)	-25.7 ± 4.8	-23.0 ± 3.6	-24.4 ± 3.3	-24.6 ± 3.9	-23.9 ± 3.2	-24.5 ± 3.0
GLS (%)	-20.0 ± 3.0	-19.4 ± 2.8	-20.3 ± 1.9	-19.2 ± 2.2	-19.5 ± 2.1	-20.1 ± 2.1
$SR_{E/A}$	1.83 ± 0.69	2.16 ± 0.78	2.19 ± 0.54	2.11 ± 0.70	2.59 ± 0.79	2.17 ± 0.75
Mitral inflow						
E deceleration time (ms)	207 ± 39	211 ± 26	193.50 ± 34	199 ± 35	194 ± 26	194 ± 38
E/A ratio	1.13 ± 0.30	1.31 ± 0.32	1.24 ± 0.33	1.20 ± 0.28	1.29 ± 0.23	1.28 ± 0.34
Mitral annular motion						
Septal peak S' velocity (m/s)*	0.09 (0.06 - 0.14)	0.07 (0.06 - 0.11)	0.09 (0.06 - 0.12)	0.09 (0.06 - 0.14)	0.07 (0.05 - 0.10)	0.08 (0.05 - 0.12)
Lateral peak S' velocity (m/s)*	0.10 (0.08 - 0.14)	0.11 (0.05 - 0.16)	0.12 (0.06 - 0.16)	0.10 (0.07 - 0.18)	0.10 (0.06 - 0.12)	0.10 (0.05 - 0.16)
E/E' ratio	8.57 ± 2.07	6.90 ± 2.10	6.41 ± 2.03	8.27 ± 2.22	7.30 ± 1.50	7.48 ± 1.40
Tricuspid valve						
TR Vmax (cm/s)	0.69 ± 1.06	0.65 ± 0.98	0.31 ± 0.74	1.15 ± 1.15	0.61 ± 0.93	0.52 ± 0.96
Cardiac morphology						
LVIDd (cm)	4.58 ± 0.49	4.35 ± 0.46	4.34 ± 0.42	4.44 ± 0.47	4.29 ± 0.51	4.25 ± 0.49
PWd (cm)	1.12 ± 0.18	0.93 ± 0.19	0.81 ± 0.12	1.14 ± 0.22	1.02 ± 0.15	0.96 ± 0.19
IVSd (cm)	1.04 ± 0.16	1.00 ± 0.17	0.87 ± 0.19	1.01 ± 0.18	0.97 ± 0.14	0.95 ± 0.17
LVM (g)*	179.86 (112.20 - 267.95)	139.58 (87.44 - 215.32)	103.33 (72.34 - 189.31)	165.54 (95.13 - 340.24)	134.72 (80.26 - 277.71)	125.84 (73.16 - 265.83)

LVMi (g/m^2)	94.47 ± 17.39	77.35 ± 17.18	64.90 ± 14.93	90.72 ± 22.58	78.95 ± 18.47	71.91 ± 17.96
RWT	0.50 ± 0.11	0.43 ± 0.10	0.38 ± 0.07	0.52 ± 0.10	0.48 ± 0.09	0.45 ± 0.10
LAV (mL)	49.5 ± 12.3	40.7 ± 11.1	40.4 ± 9.8	42.2 ± 12.8	38.4 ± 11.3	36.3 ± 7.2
LAVi (mL/m^2)	25.9 ± 5.7	21.8 ± 4.6	21.8 ± 4.1	22.0 ± 5.3	20.6 ± 4.6	19.5 ± 3.6
No remodelling	7/29 (24%)	9 (41%)	14/19 (74%)	3/30 (10%)	7/22 (32%)	8/21 (38%)
Concentric remodelling	9/29 (31%)	9 (41%)	5/19 (26%)	17/30 (57%)	12/22 (55%)	11/21 (53%)
Concentric hypertrophy	12/29 (41%)	3/22 (14%)	0/19 (0%)	9/30 (30%)	3/22 (14%)	1/21 (5%)
Eccentric hypertrophy	1/29 (3%)	1/22 (5%)	0/19 (0%)	1/30 (3%)	0/22 (0%)	1/21 (5%)

Frequencies: N (%).

Mean ± standard deviation.

*Median (range).

TVR, total vascular resistance; HR, heart rate; bpm, beats per minute; SV, stroke volume; CO, cardiac output; LVEF, left ventricular ejection fraction; LV, left ventricular; GLS; global longitudinal strain; $SR_{E/A}$, early to late strain rate ratio; E/A, early to late diastolic filling ratio; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; TR Vmax, tricuspid regurgitation maximum velocity; LVIDd, left ventricular internal diameter in end-diastole; PWd, posterior wall diameter in end-diastole; IVSd, interventricular septal wall diameter in end-diastole; LVM, left ventricular mass; LVMi, LVM indexed to body surface area; RWT, relative wall thickness; LAV left atrial volume; LAVi, LAV indexed to body surface area.

Table 4.10: Regression coefficients comparing echocardiography measures of cardiac structure and function at six weeks and six months between enalapril and placebo arms.

Indices	6 weeks						6 months					
	Unadjusted (n=44)			Adjusted (n=44)			Unadjusted (n=40)			Adjusted (n=40)		
	Difference / OR	95% C.I.	P	Difference / OR	95% C.I.	P	Difference / OR	95% C.I.	P	Difference / OR	95% C.I.	P
Function												
TVR (dyne.s-1cm-5)	-154.36	-420.17 - 111.44	0.25	-151.45	-419.94 - 117.05	0.26	-62.74	-300.60 - 175.12	0.60	-63.21	-300.78 - 174.36	0.59
HR (bpm)	-2.91	-10.12 - 4.30	0.42	-0.73	-7.83 - 6.38	0.84	-3.52	-11.90 - 4.85	0.40	-1.83	-10.24 - 6.58	0.66
SV (mL)	3.32	-4.53 - 11.17	0.40	2.64	-5.35 - 10.62	0.51	-0.61	-8.71 - 7.49	0.88	-2.85	-10.05 - 4.35	0.43
CO (L/minute)	84.00	-565.33 - 733.33	0.80	92.97	-561.77 - 747.71	0.78	-261.32	-1055.85 - 533.22	0.51	-248.46	-1024.70 - 527.77	0.52
LVEF (%)	-2.09	-4.88 - 0.70	0.14	-2.58	-5.18 - 0.03	0.05	-0.16	-2.76 - 2.45	0.91	-1.12	-3.59 - 1.35	0.36
Myocardial strain and strain rate												
LV basal strain (%)*	-0.15	-1.76 - 1.47	0.86	0.26	-1.22 - 1.74	0.73	-0.06	-1.68 - 1.57	0.94	0.58	-1.05 - 2.21	0.47
LV mid strain (%)*	0.04	-1.35 - 1.44	0.95	0.57	-0.80 - 1.93	0.41	0.17	-1.16 - 1.50	0.80	0.88	-0.47 - 2.23	0.20
LV apical strain (%)*	1.18	-1.08 - 3.43	0.30	1.70	-0.53 - 3.94	0.13	0.16	-1.99 - 2.31	0.88	0.97	-1.06 - 2.99	0.34
GLS (%)*	0.29	-1.35 - 1.93	0.72	0.98	-0.59 - 2.55	0.21	0.16	-1.16 - 1.48	0.81	0.97	-0.33 - 2.26	0.14
$SR_{E/A}$†	**-0.65**	**-1.14 - -0.17**	**0.01**	-0.47	-0.96 - 0.02	0.06	-0.17	-0.56 - 0.23	0.39	-0.16	-0.57 - 0.25	0.44
Mitral inflow												
E deceleration time (cm	**17.19**	**1.00 - 33.39**	**0.04**	13.17	-3.79 - 30.14	0.12	-0.45	-24.47 - 23.57	0.97	-10.23	-34.37 - 13.91	0.40
E/A ratio	0.02	-0.15 - 0.19	0.82	0.04	-0.11 - 0.19	0.57	-0.04	-0.26 - 0.17	0.68	-0.03	-0.24 - 0.17	0.74
Mitral annular motion												
Septal peak S' velocity (cm/s)	0.00	-0.01 - 0.01	0.64	0.00	-0.01 - 0.01	0.67	0.00	-0.01 - 0.01	0.49	0.00	-0.01 - 0.01	0.52
Lateral peak S' velocity (cm/s)	**0.02**	**0.00 - 0.03**	**0.02**	**0.01**	**0.00 - 0.03**	**0.04**	0.02	0.00 - 0.03	0.11	0.01	-0.01 - 0.03	0.16
E/E' ratio	-0.40	-1.52 - 0.71	0.47	-0.56	-1.48 - 0.35	0.22	-1.07	-2.18 - 0.04	0.06	**-1.07**	**-2.08 - -0.06**	**0.04**

Tricuspid regurgitation												
TR Vmax (cm/s)	0.04	-0.54 - 0.62	0.90	0.31	-0.26 - 0.88	0.27	-0.21	-0.77 - 0.34	0.44	-0.11	-0.69 - 0.47	0.69
Cardiac morphology												
LVIDd (cm)	0.06	-0.23 - 0.36	0.67	-0.03	-0.27 - 0.22	0.83	0.09	-0.20 - 0.38	0.54	0.00	-0.20 - 0.19	0.96
PWd (cm)	-0.09	-0.19 - 0.02	0.10	-0.09	-0.19 - 0.01	0.09	**-0.15**	**-0.25 - -0.04**	**0.01**	**-0.14**	**-0.25 - -0.04**	**0.01**
IVSd (cm)	0.03	-0.07 - 0.12	0.56	0.03	-0.06 - 0.11	0.53	-0.07	-0.19 - 0.04	0.20	-0.07	-0.18 - 0.03	0.16
LVIDd (cm)	0.06	-0.23 - 0.36	0.67	-0.03	-0.27 - 0.22	0.83	0.09	-0.20 - 0.38	0.54	0.00	-0.20 - 0.19	0.96
LVM (g)	-3.16	-30.10 - 23.78	0.81	-6.86	-26.63 - 12.92	0.49	-17.69	-44.83 - 9.44	0.20	**-19.46**	**-37.34 - - 1.57**	**0.03**
LVMi (g/m^2)	-1.60	-12.45 - 9.25	0.77	-3.73	-13.49 - 6.03	0.45	-7.00	-17.64 - 3.63	0.19	**-9.23**	**-17.75 - - 0.71**	**0.03**
RWT	-0.05	-0.11 - 0.01	0.11	-0.05	-0.10 - 0.01	0.12	**-0.08**	**-0.13 - -0.02**	**0.01**	**-0.08**	**-0.13 - -0.02**	**0.01**
LAV (mL)	2.29	-4.69 - 9.26	0.51	-0.84	-7.43 - 5.75	0.80	4.06	-1.47 - 9.58	0.15	2.32	--3.26 - 7.90	0.40
LAVi (mL/m^2)	1.15	-1.73 - 4.04	0.42	0.34	-2.68 - 3.35	0.82	2.34	-0.16 - 4.83	0.07	2.00	-0.66 - 4.66	0.14
Concentric remodelling / hypertrophy	10.56†	0.16 - 1.91	0.36	0.57†	0.16 - 1.99	0.38	0.27†	0.07 - 1.02	0.05	0.26†	0.07 - 1.01	0.05

All regressions for six-week and six-month data are displayed as both unadjusted and adjusted for baseline measurements.

*Adjusted for mean arterial pressure (MAP)

†Odds ratio comparing the risk of concentric remodelling / hypertrophy (RWT>0.42) at six months, adjusting for the presence of concentric remodelling / hypertrophy at baseline.

Bold text indicates statistical significance ($p<0.05$).

OR, odds ratio; C.I., confidence interval; TVR, total vascular resistance; HR, heart rate; bpm, beats per minute; SV, stroke volume; CO, cardiac output; LVEF, left ventricular ejection fraction; LV, left ventricular; GLS; global longitudinal strain; $SR_{E/A}$, early to late strain rate ratio; E/A, early to late diastolic filling ratio; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; TR Vmax, tricuspid regurgitation maximum velocity; LVIDd, left ventricular internal diameter in end-diastole; PWd, posterior wall diameter in end-diastole; IVSd, interventricular septal wall diameter in end-diastole; LVM, left ventricular mass; LVMi, LVM indexed to body surface area; RWT, relative wall thickness; LAV left atrial volume; LAVi, LAV indexed to body surface area.

Twelve women in the placebo arm had persistent concentric remodelling / hypertrophy (RWT ≥0.42) at six months (57%), compared with 5/19 (26%) in the enalapril arm (adjusted OR 0.26 [95% C.I. 0.07-1.01], p=0.05). The association between enalapril and E/E' and LVMi persisted after adjustment for the presence of underlying risk factors (Table 4.11).

Table 4.11: Difference in diastolic function and left ventricular remodelling between treatment groups at six months, adjusted for maternal baseline risk factors.

Adjusted for maternal baseline characteristics	E/E'			LVMi		
	Adjusted regression coefficients (n=40)			Adjusted regression coefficients (n=40)		
	Coefficient	95% C.I.	P	Coefficient	95% C.I.	P
Chronic hypertension (n=11)	-1.12	-2.13 - -0.11	0.03	-9.92	-17.70 - -2.14	0.01
Any medical condition* (n=13)	-1.09	-2.12 - -0.06	0.04	-9.76	-17.98 - -1.55	0.02
High risk for pre-eclampsia risk factors† (n=21)	-1.17	-2.31 - -0.03	0.04	-10.36	-18.89- -1.83	0.02
Abnormal cardiac biomarkers at baseline (n=8)	-1.11	-2.16 - -0.05	0.04	-8.90	-17.76 - -0.04	0.05

*Medical conditions including hypertension, renal disease, diabetes, autoimmune disease (including antiphospholipid syndrome and systemic lupus erythematous).
†High risk for pre-eclampsia is defined by presence of: pre-existing hypertension, renal, vascular or autoimmune disease, diabetes previous pre-eclampsia, or two moderate risk factors[328]. Moderate risk factors include: nulliparity, age ≥40 years, BMI ≥35kg/m^2, multi-fetal pregnancy[328].
E/E', early diastolic filling to early diastolic mitral annular velocity ratio; LVMi, left ventricular mass index; C.I, 95% confidence interval; VTE, venous thromboembolism.

There was no difference in clinic sBP between groups at six months, however, allocation to enalapril was associated with a significant reduction in dBP at six weeks and six months (adjusted difference -6.2mmHg [95% C.I. -11.4- -1.1], p=0.02 and adjusted difference -7.3mmHg -95% C.I. -14.2-0.4], p=0.04, respectively; Tables 4.12 and 4.13). The differences in peripheral dBP at six weeks and six months were evident even without adjusting for baseline measures (Table 4.13). Conversely, arteriography demonstrated a significant difference between groups in peripheral and central sBP at six months (adjusted difference -13.2mmHg [95% C.I. -23.0- -3.5], p=0.01 and adjusted difference -13.4mmHg [95% C.I. -25.8- -9.4], p=0.04, respectively), but not dBP (Tables 4.14 and 4.15). Arteriograph equipment failure led to an incomplete arteriography dataset at each of the research visits. For this reason, six-month adjusted analyses comprised of data from 30/40 participants. On the other hand, unadjusted analyses (n=36) demonstrated a

reduction in arteriography-measured dBP in the enalapril group at six months (difference -8.2mmHg [95% C.I. -15.5- -0.8], p=0.03), compared with placebo. Fewer women were taking antihypertensive medication at six months in the enalapril group, but this difference did not reach statistical significance (11% versus 29%; OR 4.1 [95% C.I. 0.7-24.1], p=0.13).

Table 4.12: BP at baseline, six weeks and six months depending on treatment allocation.

Indices	**Enalapril**			**Placebo**		
Function	**Baseline (n=29)**	**6 weeks (n=22)**	**6 months (n=19)**	**Baseline (n=30)**	**6 weeks (n=22)**	**6 months (n=21)**
sBP (mmHg)	136.2 ± 10.2	119.7 ± 13.0	122.2 ± 12.3	135.3 ± 9.9	126.1 ± 11.1	128.0 ± 13.2
dBP (mmHg)	88.5 ± 8.0	77.0 ± 8.7	79.6 ± 10.9	90.3 ± 8.3	83.2 ± 8.2	86.9 ± 10.6
MAP (mmHg)	104.4 ± 7.8	91.2 ± 9.2	93.8 ± 11.0	105.3 ± 8.2	97.5 ± 8.8	100.6 ± 11.0
Taking antihypertensives	24 (80%)	9 (41%)	2 (11%)	20 (67%)	10 (45%)	6 (29%)

Frequencies: N (%)
Mean ± standard deviation
BP, blood pressure; sBP, systolic blood pressure; dBP, diastolic blood pressure; MAP, mean arterial pressure; mmHg, millimetres of mercury.

Table 4.13: Regression coefficients comparing BP six weeks and six months between enalapril and placebo arms.

Indices	6 weeks						6 months					
	Unadjusted (n=44)			Adjusted (n=44)			Unadjusted (n=40)			Adjusted (n=40)		
	Coefficient	95% C.I.	P	Coefficient	95% C.I.	P	Coefficient	95% C.I.	P	Coefficient	95% C.I.	P
sBP (mmHg)	-6.38	-13.74 - 0.98	0.09	-6.38	-13.74 - 0.98	0.09	-5.77	-13.97 - 2.42	0.16	-5.8	-14.0 - 2.4	0.16
dBP (mmHg)	**-6.23**	**-11.38 - -1.08**	**0.02**	**-6.23**	**-11.38 - -1.08**	**0.02**	**-7.28**	**-14.15 - -0.41**	**0.04**	**-7.3**	**-14.2 - 0.4**	**0.04**
MAP (mmHg)	**-6.28**	**-11.74 - -0.82**	**0.03**	**-6.22**	**-11.75 - -0.70**	**0.03**	-6.78	-13.83 - -0.27	0.06	-6.8	-13.9 - 0.4	0.06
Taking antihypertensives	0.83	0.25 - 2.74	0.76	0.54	0.14 - 2.06	0.37	0.29	0.05 - 1.68	0.17	0.25	0.04 - 1.48	0.13

All regressions for six-week and six-month data are displayed as both unadjusted and adjusted for baseline measurements.
Bold text indicates statistical significance ($p<0.05$).
OR, odds ratio (for categorical data only); C.I., confidence interval; sBP, systolic blood pressure; dBP, diastolic blood pressure; MAP, mean arterial pressure; mmHg, millimetres of mercury.

Table 4.14: Arteriography measures of BP and arterial compliance at baseline, six weeks and six months depending on treatment allocation.

Indices	Enalapril			Placebo		
	Baseline (n=25)	6 weeks (n=17)	6 months (n=18)	Baseline (n=21)	6 weeks (n=16)	6 months (n=18)
PWV (m/s)*	8.2 (5.4 - 16.6)	7.6 (4.9 - 11.9)	8.2 (5.7 - 11.2)	8.1 (5.9 - 11.1)	7.1 (4.6 - 11.3)	7.8 (4.7 - 11.4)
HR (bpm)	84.0 ± 16.9	79.9 ± 14.2	82.2 ± 14.4	90.9 ± 17.2	82.4 ± 14.6	89.7 ± 17.1
sBP (mmHg)	139.7 ± 13.4	118.1 ± 13.8	119.6 ± 13.6	138.8 ± 9.4	123.6 ± 9.0	134.8 ± 15.1
dBP (mmHg)	84.5 ± 9.6	69.9 ± 10.5	73.1 ± 11.6	84.1 ± 9.0	75.6 ± 8.2	81.3 ± 10.1
sBPao (mmHg)	136.0 ± 17.1	111.8 ± 16.0	112.4 ± 16.3	133.2 ± 10.8	117.5 ± 11.7	127.9 ± 17.6
AIx brachial	28.1 ± 13.2	22.6 ± 13.4	19.9 ± 9.4	22.3 ± 15.9	22.1 ± 13.2	20.9 ± 12.6
AIx aortic	-18.7 ± 26.1	-30.8 ± 26.3	-35.1 ± 18.6	-25.6 ± 28.3	-30.6 ± 26.1	-33.0 ± 25.0

Mean ± standard deviation.
*Median (range).
All BP measurements were performed using Tensioclinic arteriograph, on the right arm, in the sitting position.
Arteriography results were not obtained for every participant at every visit due to equipment failure.

BP, blood pressure; PWV, pulse wave velocity; HR, heart rate; bpm, beats per minute; sBP, systolic BP; dBP, diastolic BP; sBPao, aortic systolic BP; mmHg, millimetres of mercury; AIx, augmentation index.

Table 4.15: Regression coefficients comparing arteriography measures of BP and arterial compliance six weeks and six months between enalapril and placebo arms.

Indices	6 weeks						6 months					
	Unadjusted (n=33)			Adjusted (n=29)			Unadjusted (n=36)			Adjusted (n=30)		
	Difference	95% C.I.	P	Difference	95% C.I.	P	Difference	95% C.I.	P	Difference	95% C.I.	P
PWV (mmHg)*	0.77	-0.55 - 2.08	0.24	1.17	-0.09 - 2.43	0.07	0.75	-0.36 - 1.86	0.18	0.92	-0.32 - 2.16	0.14
HR (bpm)	-2.50	-12.71 - 7.71	0.62	-7.44	-18.15 - 3.26	0.17	-1.76	-13.25 - 9.72	0.76	-1.96	-15.08 - 11.16	0.76
sBP (mmHg)	-5.44	-13.76 - 2.87	0.19	-5.71	-13.84 - 3.41	0.21	**-15.22**	**-24.94 - -5.50**	**0.01**	**-13.22**	**-22.96 - -3.47**	**0.01**
dBP (mmHg)	-5.62	-12.35 - 1.11	0.10	-4.96	-12.54 - 2.50	0.18	**-8.17**	**-15.52 - -0.82**	**0.03**	-4.94	-12.69 - 2.81	0.20
sBPao (mmHg)	-5.68	-1569 - 4.32	0.26	-4.60	-14.36 - 5.16	0.34	**-15.48**	**-26.98 - -3.98**	**0.01**	**-13.36**	**-25.78 - -9.44**	**0.04**
AIx brachial	-3.15	-17.75 - 11.45	0.66	-4.16	-17.80 - 9.49	0.54	-0.05	-20.80 - 4.70	0.21	-6.64	-21.46 - 8.17	0.37
AIx aortic	-1.07	-8.41 - 6.27	0.77	-1.19	-8.18 - 5.79	0.73	-4.08	-10.54 - 2.37	0.21	-3.07	-10.73 - 4.60	0.42

All regressions for six-week and six-month data are displayed as both unadjusted and adjusted for baseline measurements.
Arteriography results were not obtained for every participant at every visit due to equipment failure.
Bold text indicates statistical significance ($p<0.05$).
C.I., confidence interval; PWV, pulse wave velocity; HR, heart rate; bpm, beats per minute; sBP, systolic blood pressure; dBP, diastolic blood pressure; sBPao, aortic systolic blood pressure; mmHg, millimetres of mercury; AIx, augmentation index.

4.4.2.7. Impact of enalapril on biomarkers

All women had HS-cTnT and NTproBNP levels within the normal range at six months. Four women (7%) had raised HS-cTnT and four different women had raised NTproBNP at baseline. There was no difference in HS-cTnT, NTproBNP, sFlt or PlGF between the two groups at six weeks or six months (Table 4.16).

Table 4.16: Placental and cardiovascular biomarkers at baseline, six weeks and six months, depending on treatment allocation.

Indices	**Enalapril**			**Placebo**			**Adjusted regression coefficients**		
Function	**Baseline (n=26)**	**6 weeks (n=22)**	**6 months (n=19)**	**Baseline (n=29)**	**6 weeks (n=22)**	**6 months (n=21)**	**Coefficient**	**95% C.I.**	**P**
sFlt (pg/mL)	1790 (542 - 22904)	84 (66 - 96)	84 (64 - 97)	1290 (262 - 9500)	87 (73 - 133)	84 (73 - 110)	0.0	-0.1 - 0.1	0.76
PlGF (pg/mL)	29 (13 - 152)	11 (4 - 19)	10 (5 - 15)	21 (8 - 133)	10 (6 - 20)	9 (6 - 15)	0.0	-0.2 - 0.2	0.75
sFlt:PlGF	54 (20 - 421)	7 (4 - 23)	8 (4 - 17)	56 (13 - 566)	10 (5 - 14)	8. (5 - 13)	0.0	-0.2 - 0.2	0.86
HS-cTnT (ng/L)	6 (2 - 62)	2 (2 - 13)	2 (2 - 9)	5 (2 - 28)	2 (2 - 14)	2 (2 - 7)	0.0	-0.2 - 0.2	0.94
NTproBNP (pg/mL)	102 (25 - 722)	22 (4 - 97)	30 (4 - 215)	51 (4 - 1259)	212 (12 - 129)	24 (4 - 162)	0.0	-0.7 - 0.8	0.91

Median (range).

Baseline measurements were up to 72 hours post-birth.

Measurements were log-transformed for all regression analyses.

All regressions are for six-month data, adjusted for baseline measurements.

C.I., confidence interval; sFlt, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; HS-cTnT, high-sensitivity troponin C; NTproBNP, N-terminal pro B-type natriuretic peptide.

4.4.3. Safety and tolerability

There was a 10% dry cough rate in the enalapril arm; all women reported resolution of symptoms, despite continuing the study treatment (Table 4.17). A serious adverse event (SAE) was pre-defined as any adverse event resulting in death, immediate risk of death, prolonged hospitalisation, persistent disability or any other important medical event that could require medical or surgical intervention to prevent one of the outcomes listed in this definition. Two SAEs were reported during the course of the study: one woman died secondary to acute coronary syndrome three months postpartum and one woman developed severe left ventricular failure and a subsequent watershed stroke. Neither SAE was deemed related to the allocated treatment. Two women in each group (7%) required treatment dose reduction due to a rise in creatinine by >20%. All these women later tolerated titration to the maximum dose (20mg enalapril / placebo), with stable renal function. Renal function was comparable between the two groups at six weeks (63 [39-103]µmol/L and 64 [43-81]µmol/L in the enalapril and placebo groups, respectively). Renal function was not repeated at six months and therefore could not be compared between groups at that timepoint. There were no reports of neonatal hypotension, negating any theoretical concern of adverse antihypertensive effect on breastfed neonates[328].

Table 4.17: Adverse events reported during the study period.

	Enalapril n=30	**Placebo n=30**	**Comment**
Dry cough / breathlessness	3/30 (10%)	0/30 (0%)	All resolved despite continuing drug
Rash	1/30 (3%)	0/30 (0%)	Withdrew following GP advice
Seizure	1/30 (3%)	0/30 (0%)	Unrelated - investigated for epilepsy
LV failure	1/30 (3%)	0/30 (0%)	Unrelated - did not take allocated drug
Maternal death	1/30 (3%)	0/30 (0%)	Unrelated - Acute coronary syndrome secondary to coronary thrombus

GP, general practitioner; LV, left ventricular.

4.4.4. Reproducibility

Interobserver reproducibility of the primary outcome, TVR, was excellent (ICC: 0.86 [95% C.I. 0.65-0.95]). Interobserver reproducibility of LVM and E/E' were 0.92 [95% C.I. 0.81-0.97] and 0.82 [95% C.I. 0.55-9.93] respectively. Interobserver reproducibility of other

echocardiography measures was comparable with previous studies[423,424], as summarised in Table 4.18.

Table 4.18: Interobserver reproducibility of echocardiography measures.

Echocardiography measure	Intraclass correlation coefficient (ICC)		
	Image acquisition only	Image analysis only	Image acquisition and analysis
Haemodynamics			
TVR	0.93 (0.82 - 0.97)	0.97 (0.92 - 0.99)	0.86 (0.65 - 0.95)
Systolic function			
LVEF	0.85 (0.61 - 0.94)	0.71 (0.25 - 0.89)	0.66 (0.14 - 0.87)
GLS	0.92 (0.78 - 0.97)	0.97 (0.93 - 0.99)	0.87 (0.67 - 0.95)
Diastolic function			
E/A	0.83 (0.58 - 0.93)	0.96 (0.91 – 0.99)	0.83 (0.57 - 0.93)
E/E'	0.65 (0.11 - 0.86)	0.67 (0.18 - 0.87)	0.82 (0.55 - 0.93)
$SR_{E/A}$	0.92 (0.80 - 0.97)	0.99 (0.96 - 0.99)	0.89 (0.72 - 0.96)
Morphology			
LVM	0.96 (0.90 - 0.98)	0.96 (0.91 - 0.99)	0.92 (0.81 - 0.97)
LVMi	0.95 (0.87 - 0.98)	0.96 (0.89 - 0.98)	0.90 (0.75 - 0.96)
RWT	0.84 (0.60 - 0.94)	0.89 (0.73 - 0.96)	0.78 (0.43 - 0.91)

ICC (95% confidence interval).
ICC, intraclass correlation coefficient; TVR, total vascular resistance; LVEF, left ventricular ejection fraction; GLS, global longitudinal strain; E/A, early to late diastolic filling ratio; E/E', early diastolic filling to early diastolic mitral annular velocity ratio; $SR_{E/A}$, early to late strain rate ratio; LVM, left ventricular mass; LVMi, left ventricular mass index; RWT, relative wall thickness.

There was good to excellent agreement between Alere Microlife and TensioClinic arteriograph in the measurement of sBP (ICC 0.87 [95% C.I. 0.83-0.91]) and dBP (ICC 0.92 [95% C.I. 0.89-0.95]; Figure 4.15). Although there was a significant linear relationship between E/A (measured by pulse wave Doppler at the mitral inlet) and $SR_{E/A}$ (measured using speckle-tracking software; coefficient 0.22 [95% C.I. 0.17-0.27], $p<0.001$; Figure 4.16), absolute values showed poor agreement (ICC 0.56 [95% C.I. 0.40-0.68]; Figure 4.15). In terms of different echocardiographic measures of systolic dysfunction, LVEF had a significant linear correlation with GLS (coefficient -0.82 [95% C.I. -1.07- -0.56], $p<0.001$; Figure 4.16).

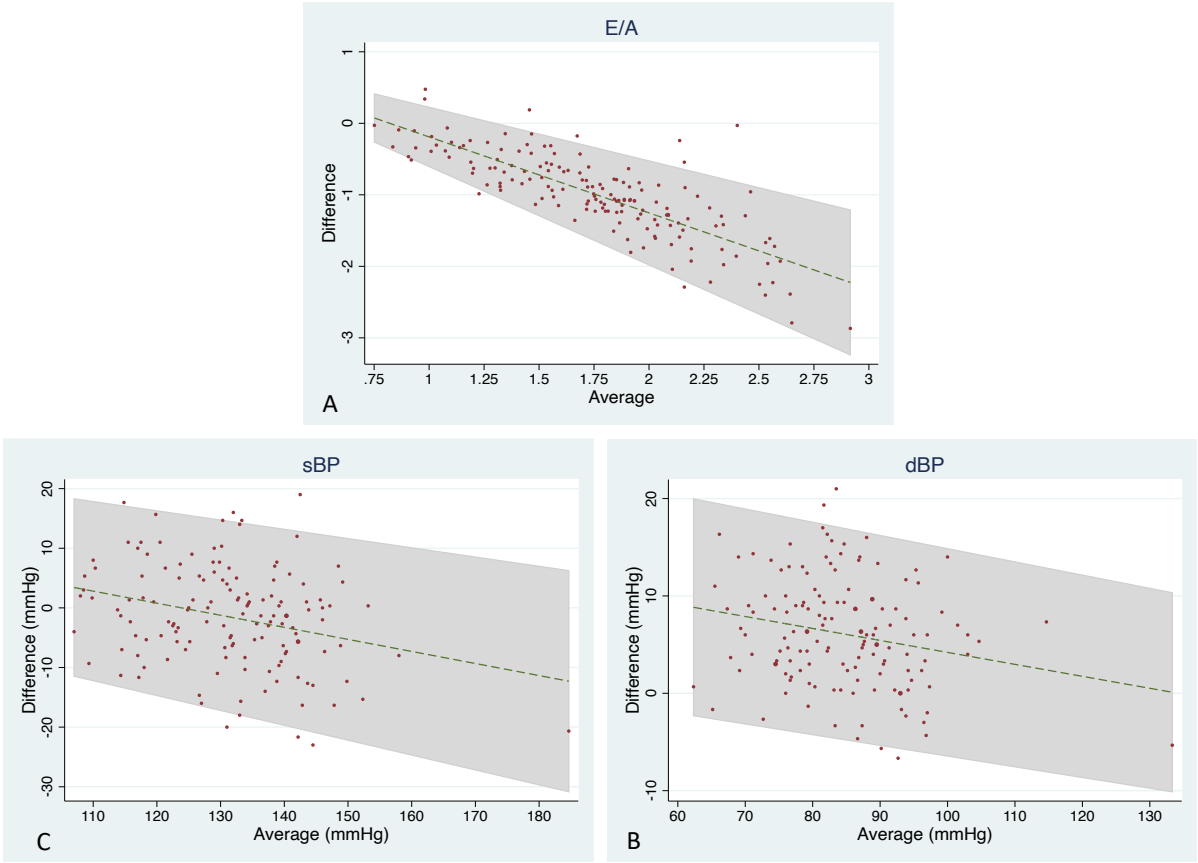

Figure 4.15: Intermodality agreement of cardiovascular measures.
Bland-Altman plots illustrating intermodality agreement in **A.** E/A (pulse wave Doppler compared with strain rate); **B.** sBP (Alere Microlife compared with TensioClinic arteriography); and **C.** dBP (Alere Microlife compared with TensioClinic arteriography).

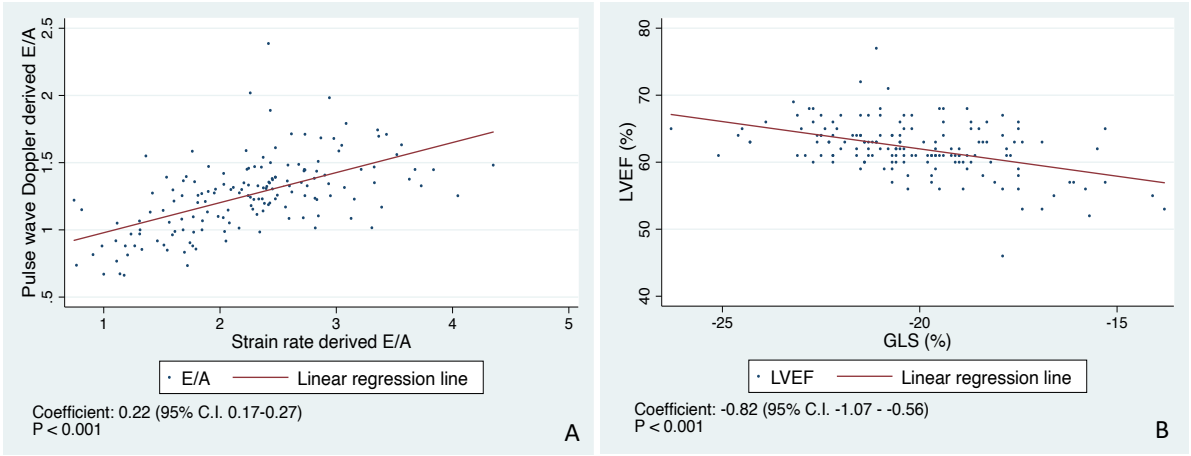

Figure 4.16: Correlations of cardiac function between modalities.
Scatter plot of **A.** pulse wave Doppler-derived E/A and speckle tracking-derived E/A strain rate ratio; and **B.** left ventricular ejection fraction and global longitudinal strain.
E/A, early to late diastolic filling ratio; LVEF, left ventricular ejection fraction; GLS, global logintudinal strain.

4.5. Discussion

This feasibility study, of six months treatment with enalapril, has confirmed that the study protocol is feasible and the intervention is acceptable to women. In the absence of cardioprotection, there was a high prevalence of persistent cardiovascular abnormalities at six months postpartum, following a pregnancy complicated by preterm pre-eclampsia. This is consistent with other studies[5–7], highlighting the importance of identifying this high risk group and investigating the impact of intervention in the postnatal period.

Correlations between preterm pre-eclampsia and cardiovascular phenotypes were investigated in order to explore a potential causal relationship between the two. None of the standard metrics defining maternal disease severity (including BP≥160/110 and abnormal haematological and biochemical parameters)[328] correlated with six-month postpartum cardiovascular phenotype. On the other hand, other markers of severity (including lower birthweight centile and earlier gestation at diagnosis / delivery) were associated with worse diastolic dysfunction (E/E') and TVR at six months. Longer duration of pre-eclampsia prior to delivery was also associated with higher six-month TVR, indicating a potential dose-effect.

This was the first study to investigate whether postnatal cardiovascular dysfunction in women with preterm pre-eclampsia is modifiable using a postnatal therapeutic intervention. Whilst there was no difference seen in the primary clinical outcome (change in TVR between treatment and placebo at six months), significant differences were observed in several secondary clinical outcomes related to cardiac remodelling and diastolic dysfunction. These observed differences indicate the potential for postnatal enalapril treatment to improve maternal cardiovascular health in this high risk group.

In summary, this study demonstrated a high prevalence of persistent cardiovascular morbidity six months following preterm pre-eclampsia. A possible pre-eclampsia dose-effect was indicated by an association between the severity of prematurity and FGR, and the severity of diastolic dysfunction and TVR at six months. Six months postnatal treatment with enalapril was acceptable to women and associated with improvement in left ventricular diastolic dysfunction and remodelling. In this way, findings from this

feasibility study support a larger RCT to confirm the effect of postnatal enalapril on cardiovascular dysfunction and remodelling and determine the longevity of effect beyond treatment cessation.

4.5.1. Strengths and limitations

To our knowledge, this is the largest longitudinal dataset describing postnatal cardiovascular structure and function following preterm pre-eclampsia. This study describes a multi-ethnic cohort with a severe preterm pre-eclampsia phenotype (74% had severe maternal features and 44% had FGR <3rd centile). All echocardiography scans were analysed by the same examiner to optimise reproducibility. A significant limitation of the study is the modest sample size, which was exacerbated by non-completion in the observational trial, due to the concurrent pandemic and early postnatal life stressors in the interventional trial.

In the observational trial, due to the lack of pre-pregnancy echocardiography data, it is not possible to confirm direction of causality between preterm pre-eclampsia and cardiovascular dysfunction. The lack of a contemporaneous matched control group is also a limitation, however it does not preclude the calculation of absolute prevalence in this cohort, which was undeniably high. Additionally, the three-day window for the baseline visit potentially limits the ability to relate baseline placental biomarkers with six-month cardiovascular outcomes, given the rapid decline in sFlt and PlGF in the first 48 hours postpartum[248].

This is the first interventional study of its kind to assess whether cardiovascular dysfunction following preterm pre-eclampsia is amenable to pharmacological treatment in the early postnatal period. Other strengths include the prospective randomised controlled study design and that all measurements were performed blinded to treatment allocation. Recruitment was inclusive to all women with preterm pre-eclampsia in a multi-ethnic population. The process outcomes included realistic assessments of recruitment, compliance and retention. On the other hand, this was a single centre feasibility study and therefore a larger multicentre study is required to confirm generalisability. The

positive findings of this study were in the secondary outcomes, and subject to potential type I error; results should therefore be interpreted with caution.

4.5.2. Feasibility

The interventional trial evaluated recruitment rate, acceptability, adherence and retention. Recruitment to the interventional study was achieved 12 months ahead of target and uptake to the study was good (71% of eligible women were recruited to the interventional study, equating to 5 per month). The intervention was acceptable to women, with 100% (n=40) of those completing the study responding positively to taking enalapril in the future. Adherence was acceptable: urinary enalapril was detected in 85% and 63% of the enalapril arm at six weeks and six months, respectively. It is possible that the latter measure of adherence is an underestimate, as two women with undetectable urinary enalapril at six months postponed their final appointment, reportedly causing them to run out of medication in the preceding week. Reassuringly, comparison of different adherence measures demonstrated concordance between verbal recall and urinary enalapril (missed doses were reportedly lower in women with detectable urinary enalapril compared to those without). This supports the use of verbal recall as a suitable adherence measure, when objective biochemical methods are not available.

Key barriers to trial continuation were identified; in particular, early postnatal life stressors (including neonatal readmissions and transfers) which accounted for seven non-completions in the first six weeks. This contributed to a high non-completion rate (33% of those recruited) and an overall completion rate of 48% of eligible women. It is important to note that 80% of the discontinuations occurred in the first six weeks. This has provided important information for the design of future postnatal studies; in particular, the potential benefit of limiting participant burden in the early postnatal period. Data from the feasibility study also demonstrated safety of enalapril in the early postnatal period: 10% of women taking enalapril reported a self-limiting dry cough (comparable with previous studies[425]) and renal function was equivalent between groups. All women were safely titrated up to maximum dose (20mg enalapril once daily) without persistence of side effects or renal impairment.

4.5.3. Natural history of preterm pre-eclampsia-related postnatal cardiovascular morbidity

In the absence of any cardioprotective intervention, women with preterm pre-eclampsia have a high prevalence of cardiovascular abnormalities at six months postpartum. This is consistent with previous studies[5,36]. Only two women (5%) had a completely normal echocardiogram at six months, with the majority of abnormalities attributable to raised TVR (75%), diastolic dysfunction (61%, as defined by the BSE[289]) and left ventricular remodelling (41%). Melchiorre *et al.*'s study demonstrated an 8% prevalence of diastolic dysfunction two years following a normotensive pregnancy, compared with 52% following preterm pre-eclampsia, using similar age-corrected definitions[5]. These findings have significant implications to long-term cardiovascular risk[394,426,427]. Diastolic dysfunction precedes and independently predicts left ventricular remodelling following myocardial infarction[428,429], indicating increased diastolic pressure as a sensitive functional measure of left ventricular injury and a precursor to adverse (concentric) remodelling. It is therefore likely that the prevalence of adverse remodelling will increase over time, despite already affecting 41% of the cohort. Remodelling is an independent predictor of cardiovascular events (hazard ratio 1.70 [95% C.I. 1.34-2.16] within ten years)[430] and overt hypertension[5,431], which is the leading modifiable risk factor for all-cause mortality[432]. Hypertension frequently requires lifelong therapy[433], thereby constituting an important outcome to clinicians and patients alike. In those who were not known to be hypertensive before 20 weeks' gestation, approximately one in six women required antihypertensives at six months and nearly half (46%) had *de novo* hypertension, defined by clinic BP ≥140/90[422] or need for antihypertensive treatment at six months postpartum. *De novo* / pre-existing hypertension affected over half (57%) of the total cohort at six months. This is higher than Melchiorre *et al.*'s finding of 40% hypertension prevalence at two years postpartum[5], potentially reflecting a different population or attenuation of hypertension over time. Although 8% of women had abnormal cardiac biomarkers in the early postnatal period, all had normalised by six weeks postpartum.

The absence of relationship between baseline HS-cTnT and NTproBNP and six-month cardiovascular parameters suggests that cardiovascular biomarkers in the early postnatal

period are unlikely to be effective in identifying women at particular risk of persistent cardiovascular morbidity.

These observational findings clearly demonstrate, in line with previous work[5,8–14,184], that women with preterm pre-eclampsia would benefit from appropriate counselling, lifestyle changes and therapeutic interventions to improve short- and long-term cardiovascular morbidity.

4.5.4. Mechanistic link between preterm pre-eclampsia and cardiovascular morbidity

The association between preterm pre-eclampsia phenotype (birthweight centile and gestation at diagnosis / delivery) and diastolic function, suggests a potential dose-effect and direction of causality. Although the lack of pre-pregnancy echocardiography data limits our ability to confirm direction of causation, the lack of relationship between pre-pregnancy cardiovascular risk factors (including hypertension) and postnatal diastolic dysfunction points away from preterm pre-eclampsia being solely a consequence of cardiovascular dysfunction. On the other hand, there was an association between remodelling and pre-existing hypertension, highlighting the possibility that women with pre-existing hypertension had some degree of pre-pregnancy cardiovascular changes. One could speculate that if preterm pre-eclampsia is a cause of cardiovascular dysfunction, duration of exposure should correlate with severity of cardiovascular dysfunction; this was only observed for TVR in this cohort. Longer exposure to preterm pre-eclampsia was associated with reduced improvement in LVMi and a trend towards reduced improvement in E/E' over time but did not correlate with absolute measures of LVMi or E/E' at six months. This could be attributable to insufficient power or inaccurate timing of preterm pre-eclampsia diagnosis.

Preterm pre-eclampsia is a heterogenous condition defined by clinical manifestations of endothelial dysfunction (proteinuria and hypertension)[28] rather than the underlying pathology. It therefore likely comprises more than one pathological process, as supported by Leavey *et al.*'s placental microarray studies[38,39] in which three subclasses of pre-eclampsia were identified. These subclasses were defined as reflecting maternal

maladaptation, placental insufficiency or immunological overactivation[38,39]. It is therefore plausible that direction of causality differs between subclasses, with a more consequential role of preterm pre-eclampsia on cardiovascular dysfunction in the maternal maladaptation subclass and a more causal role in the latter two subclasses. This potential multifactorial and multidirectional relationship is supported by the inconsistencies observed in the correlations between maternal / pregnancy characteristics and six-month echocardiography in this cohort.

The mechanism by which preterm pre-eclampsia might be a cause of cardiovascular dysfunction is not known. However, as mentioned previously, data from pre-clinical and clinical studies (both in[156,222] and outside of[173–177] pregnancy) support sFlt as a potential mediator of cardiovascular morbidity. Previous studies have reported an inverse relationship between sFlt levels and both gestation at delivery and birthweight centile[434,435]. Both of these pregnancy outcomes were associated with worse diastolic dysfunction in this cohort, supporting a potential role of sFlt in the development of cardiovascular dysfunction. However, in this cohort an association between baseline postnatal sFlt and six-month cardiovascular parameters was not demonstrated. This could represent divergent mechanistic pathways in different subclasses of preterm pre-eclampsia, absence of causality or limitations of the data (due to variable postnatal timing, inability to adjust for hours postpartum and insufficient sample size). With the increasing use of placental biomarkers in routine clinical practice, this relationship has the potential for exploration on a larger scale in the future.

4.5.5. Effect of six-month treatment with enalapril on cardiovascular function and remodelling

TVR has previously been identified as predictive of pre-eclampsia recurrence[18,26]; for this reason it was selected as the primary clinical outcome for this study given the absence of other studies which have compared baseline (at the time of birth) to six-month echocardiography changes. TVR is derived from CO and MAP. No difference was seen between the groups, despite there being a trend towards a difference in MAP. This could be due to 1) a lack of effect of enalapril on CO; 2) confounding factors affecting TVR (e.g. intrapartum events at baseline, timing of intervention/other antihypertensives); or 3)

high level biological variability of the MAP/CO measurement and therefore insufficient power in this relatively small feasibility study. Both diastolic function and left ventricular remodelling have been identified as predictors of long-term cardiovascular risk[394–396,436] and therefore these echocardiography parameters were prespecified as secondary endpoints, although it was not possible to perform formal sample size calculations for these comparisons. Despite the heterogeneity and size of the cohort, this study demonstrated an improvement in diastolic function (E/E') and remodelling (LVMi and RWT) in women treated for six months with enalapril, compared with placebo.

The difference of E/E' between groups was not reciprocated with E/A; this is likely explained by pseudonormalisation of E/A in women with stage II diastolic dysfunction and the non-linear relationship with disease severity. This study was underpowered to determine the impact of enalapril on the prevalence of cardiac pathology (diastolic or systolic dysfunction or left ventricular remodelling, as defined by predetermined criteria[281,289,318,414]). However, our results suggest that treatment with enalapril for six months could reduce the prevalence of concentric remodelling / hypertrophy. Confirmation of this finding with a larger study is required.

Diastolic, but not systolic BP, was significantly lower in the enalapril group at six weeks and six months postpartum, compared with placebo. This difference was seen despite a lower prevalence of concomitant antihypertensives (11% versus 29%) and preceded improvements in left ventricular remodelling and diastolic function. This could indicate that the cardiac improvements seen in the enalapril arm were a consequence of early improved BP control, rather than ACE inhibitor-specific actions. On the other hand, ACE inhibitors have well-established BP-independent cardioprotective mechanisms, including preservation of myocardial collagen matrix and subsequent improved myocyte relaxation[201], and elevation of bradykinin levels[193] and subsequent afterload-independent reduction in myocardial mass[199,200]. Furthermore, this was a double-blind trial in which all women's BP was treated in line with standard clinical guidelines, irrespective of treatment allocation. Cardiac remodelling has been shown to precede and predict the development of overt hypertension[5,431], indicating a cyclical relationship, in which remodelling is both a cause and a consequence of hypertension. It is therefore likely that

enalapril conferred significant cardioprotection, over and above the benefit of antihypertensive treatment alone.

4.5.6. Long-term implications

Given the association between diastolic dysfunction, left ventricular remodelling and mortality[426,430,437], postnatal treatment with enalapril could have significant implications for long-term cardiovascular health. Modin *et al.*[394] quantified the prognostic value of different echocardiography parameters for the prediction of ten-year risk of ischaemic heart disease or heart failure. A 1 unit increase in E/E' (the between-group difference seen in this study at six months) had a hazard ratio of 1.11 (95% C.I. 1.09-1.13) and 5g/m^2 increase in LVMi (compared with 7g/m^2 between-group difference seen in this study at six months) had a hazard ratio of 1.16 (95% C.I. 1.13-1.19)[394]. Additionally, Ladeiras-Lopes *et al.*'s meta-analysis[426] demonstrated a 3.53-fold increase in cardiovascular events or death associated with a diagnosis of diastolic dysfunction within 11 years. From the current study, however, it is not possible to determine whether these improvements in cardiac function would persist beyond cessation of the intervention, or whether longer treatment duration would be required for improvement in long-term health. A sub-study of the SOLVD trial[438] compared left ventricular volumes between enalapril and placebo groups, 15 days after treatment cessation. Importantly they demonstrated partial, but not complete, reversibility of enalapril effects, indicating the potential for short-term treatment with enalapril to slow progression of left ventricular remodelling. In order to relate the findings from the observational and interventional studies to long-term cardiovascular function, all participants have been consented to follow-up in the future. However, given the moderate overall sample size (n=99, including the enalapril, placebo and observational arms), correlation of early postnatal cardiovascular dysfunction with long-term cardiovascular events will not be possible with this cohort alone.

Given the progressive nature of cardiovascular dysfunction and risk with increasing age, it is likely that in this relatively young cohort, subclinical differences in cardiac function and morphology will increase over time[439]. The difference in remodelling seen between treatment groups could therefore be associated with a reduction in long-term cardiovascular risk, as supported by the Framingham study[395], which found that a 50g

increase in LVM was associated with a 1.57-fold increase in cardiovascular risk. Although plausible, larger studies are needed to investigate 1) the significance of postnatal cardiovascular dysfunction on long-term cardiovascular risk and 2) whether long-term cardiovascular and obstetric risks are reduced by short-term postnatal treatment with enalapril. The intention for future pregnancy needs to be considered in the design of future interventions. Cardioprotective therapies need to either be safe in pregnancy or have short-term efficacy, to allow conception following treatment cessation. Enalapril is safe in breastfeeding mothers[207,328], but contraindicated in pregnancy[405]. For this reason, further work is needed to determine whether short-term treatment with enalapril (i.e. six months) is sufficient to confer long-term reduction in cardiovascular risk.

4.6. Conclusion

Preterm pre-eclampsia is associated with persistent diastolic dysfunction, left ventricular remodelling and hypertension at six months postpartum. These have significant implications for long-term cardiovascular health. The graded severity of diastolic dysfunction with worsening prematurity and FGR suggests a dose-effect. However, the mechanism linking preterm pre-eclampsia and cardiovascular dysfunction remains uncertain and requires further investigation. Postnatal treatment with enalapril for six months, in this high risk group, was acceptable to women although in this study there was a significant non-completion rate. Treatment with enalapril was not associated with a change in TVR, but was associated with improved left ventricular diastolic function and remodelling. These findings highlight the potential to use obstetric history to target intervention to improve maternal cardiovascular health in the postnatal period. A longer-term multicentre randomised controlled trial is now required to investigate the generalisability and longevity of the cardiovascular improvements seen in PICk-UP.

CHAPTER 5: DISCUSSION

5.1. Introduction

There is abundant evidence linking pre-eclampsia with postnatal cardiovascular dysfunction[5–7] and long-term cardiovascular risk[8–17]. Despite this, the mechanistic link between them is poorly understood and efforts to mitigate this risk are to-date insufficient. The aims of this thesis were to explore the relationship between pre-eclampsia and cardiovascular dysfunction and the potential reversibility of preterm pre-eclampsia-related cardiovascular dysfunction using a postnatal intervention.

In order to address these aims, I conducted a mechanistic study (chapter three), two observational studies (chapters two and four) and a randomised controlled trial (chapter four). Data from this thesis demonstrated, in the absence of targeted cardioprotective intervention, a high prevalence of persistent maternal cardiovascular morbidity six months following a pregnancy complicated by preterm pre-eclampsia. These findings have significant implications for long-term cardiovascular health and therefore highlight the need to target interventions at this high risk group. My results did not support a causal role of cardiac dysfunction in the development of pre-eclampsia, as pre-eclampsia prevalence was not increased in women with pre-existing cardiac dysfunction and severity of pre-existing left ventricular systolic or diastolic dysfunction did not correlate with pregnancy outcome. In contrast, correlations between aspects of preterm pre-eclampsia severity and postnatal maternal cardiovascular dysfunction indicated a pre-eclampsia dose-effect. This supports a causal role of pre-eclampsia in the development of cardiovascular morbidity. Unfortunately, attempts at recapitulating an sFlt-induced pre-eclampsia like rat model[135–137] were unsuccessful, meaning that it was not possible to determine the potential role of sFlt in the development of pre-eclampsia-associated cardiovascular dysfunction. Finally the interventional study demonstrated a significant improvement in left ventricular diastolic dysfunction and remodelling in women who were randomised to six months' treatment with enalapril, compared to placebo. This indicates the potential to improve maternal cardiovascular health with a postnatal medical intervention.

5.2. Cardiac dysfunction as a cause of pre-eclampsia

One would anticipate that if cardiac dysfunction contributes to impaired placental development, women with pre-existing cardiac dysfunction should have a disproportionately high prevalence of pre-eclampsia and FGR. Additionally, there should be a correlation between different measures of pre-pregnancy cardiac dysfunction and pre-eclampsia / FGR prevalence or severity.

Results from chapter two indicated that pre-eclampsia prevalence was not increased in women with pre-existing cardiac dysfunction (LVEF <55%), compared with the general population. However it is likely that term pre-eclampsia prevalence was masked by the fact that approximately one in four women underwent iatrogenic preterm delivery. To account for this, preterm pre-eclampsia prevalence was also investigated, which was again similarly comparable with the general population.

The increased prevalence of FGR and preterm delivery highlights the high risk nature of this group and further complicates interpretation of the direction of causation between placental and cardiac dysfunction. The fact that normotensive FGR, but not pre-eclampsia, predominated in this cohort suggests a different aetiology to that of early placental failure which subsequently develops into preterm pre-eclampsia. If cardiac dysfunction was a direct contributor of placental failure, it is likely that there would be a dose-effect related to the severity of pre-existing cardiac dysfunction, which was not observed in my retrospective study. Although there is obviously overlap between normotensive FGR and pre-eclampsia, a shared aetiology of abnormal spiral artery remodelling and subsequent early placental failure, as a consequence of pre-existing cardiac dysfunction is not supported by findings from this study. On the other hand, antenatal exposure to ß blockers was associated with lower birthweight Z score and earlier delivery, indicating that the high prevalence of FGR and preterm birth could in part be secondary to concomitant medication, rather than the underlying cardiac disease. This is supported by the persistence of correlation, after adjustment for the severity and cause of cardiac dysfunction. An association between ß blockers and impaired fetal growth has been demonstrated in previous clinical studies[355–359,365,366], with *in vivo*[360] and *in vitro*[364,371] studies aiming to explore potential mechanistic links. Bisoprolol, the most

commonly used ß blocker in this cohort, is a selective ß1 blocker[367]. Selective ß1 blockade, in particular, has been associated with reduced maternal CO and increased vascular resistance in the umbilico-placental circulation[368,440]. This provides a plausible mechanistic link between antenatal ß blocker exposure and FGR. A causal role of ß blockers in the development of FGR is further supported by the lack of correlation between any pre-existing echocardiography parameter and birthweight Z score, making a direct link between pre-existing cardiac dysfunction and FGR less likely.

Another consideration is that pre-existing left ventricular remodelling (LVMi), valvular stenosis and right ventricular systolic dysfunction were associated with increased pre-eclampsia prevalence. Given the small numbers included in these analyses, these findings should be interpreted with caution, however they support a potential multidirectional multifactorial relationship between pre-eclampsia and cardiac dysfunction.

Multiple large retrospective registry studies[146–151,153–155] have investigated general obstetric outcomes in women with known cardiac disease. However pre-eclampsia prevalence was not the primary outcome and results have been conflicting, with some demonstrating increased prevalence[146,148–151] and others not[147,153–155]. Due to the lack of primary focus on the relationship between cardiac disease and pre-eclampsia, these studies demonstrate inconsistencies in cardiac and obstetric phenotyping, timing of cardiac impairment relative to pregnancy and adjustment for pre-eclampsia risk factors. Additionally, studies specifically looking at pregnancy outcomes in women with cardiomyopathy are less common. In Koutrolou-Sotiopoulou *et al.*'s retrospective cohort study[147], composite maternal and perinatal outcomes were investigated and therefore it is not possible to compare absolute pre-eclampsia rates. Lima *et al.*[148] and Owens *et al.*'s[150] larger retrospective cohort studies demonstrated an increased prevalence of pre-eclampsia in those with cardiomyopathy (26.6% and 25.3%, respectively), however registry-derived data were not verified and timing of cardiac diagnosis relative to pregnancy could not be confirmed. It is therefore not possible to infer temporal associations between cardiac dysfunction and pre-eclampsia, limiting direct comparison with results from chapter two.

Given the observational nature of this study, causality cannot be confirmed or refuted, however results suggest that pre-eclampsia-associated postnatal cardiovascular dysfunction is not solely attributed to pre-pregnancy cardiac status. In this way, it is likely that postnatal cardiovascular dysfunction is at least in part a consequence rather than cause of pre-eclampsia. Since this relationship is not definitively unidirectional, mechanistic links need to be considered in both directions. In cases where cardiac dysfunction might have a causal role in the development of pre-eclampsia, it could be due to an insufficient antenatal rise in CO and subsequent uteroplacental hypoperfusion. Consequently, pre-existing cardiac dysfunction could contribute to the pathogenesis of pre-eclampsia in some cases. This theory is supported by the association between valvular stenosis (which can cause reduced CO[340]) and pre-eclampsia, albeit with small numbers, but is limited by the lack of CO data in this study. The absence of relationship between the degree of left ventricular systolic function (which also leads to reduced CO[337]) adds further uncertainty to this theory. The high prevalence of FGR in this cohort could be attributed to reduced uteroplacental supply as a result of reduced CO, however birthweight Z score did not correlate with any pre-existing cardiac measures and therefore this may instead be attributed to confounding factors, like concomitant ß blocker use (as discussed above).

Hypertensive cardiomyopathy was associated with an increased prevalence of pre-eclampsia, however this is not surprising as pre-existing hypertension is the second most predictive risk factor for pre-eclampsia (relative risk: 5.1 [95% C.I. 4.0-6.5])[441]. Hypertensive cardiomyopathy also represents a vascular rather than purely cardiac disease. Ischaemic heart disease was associated with lower birthweight Z score and gestation at delivery. It was not associated with pre-eclampsia, however all women (n=12) with ischaemic heart disease took aspirin antenatally, thereby potentially reducing their risk. Correlations between these two vascular-originating cardiac diagnoses and pregnancy outcome indicate that in cases where pre-existing cardiovascular impairment contributes to the development of pre-eclampsia, the mechanistic link is more likely vascular than purely cardiac.

5.3. Pre-eclampsia as a cause of cardiovascular dysfunction

Within this thesis, two methodologies were used to investigate pre-eclampsia as a cause of postnatal cardiovascular dysfunction: 1) evaluation of the cardiovascular phenotype in a sFlt-induced pre-eclampsia-like rodent model in chapter three, and 2) exploration of a preterm pre-eclampsia dose-effect on postnatal cardiovascular dysfunction and remodelling in chapter four. Unfortunately, recapitulation of the rodent model was unsuccessful and therefore answering this question relies on interpretation of clinical observational data alone, which has its limitations.

There was no correlation between the standard metrics defining maternal disease severity[328] and six-month postnatal cardiovascular phenotype. Severe features, as defined by NICE[328], include maximum BP >160/110mmHg, and deteriorating alanine aminotransferase, creatinine or platelets. These are heavily reliant on timing and frequency of measurements and therefore could be considered subjective measures of severity. Additionally, they may not reflect the pathways that contribute to cardiovascular dysfunction and therefore may not align with cardiovascular phenotype. For this reason, it is important to look at each of these severity measures in turn. In a healthy pregnancy, glomerular filtration rate increases due to increased renal perfusion and reduced glomerular oncotic pressure, resulting in lower serum creatinine concentrations[442]. A rise in creatinine, in the context of severe pre-eclampsia, is likely attributed to glomerular endothelial dysfunction and thrombotic microangiopathy[442]. The cause of thrombocytopenia in pre-eclamptic women is largely unknown. Mechanistic theories include platelet destruction secondary to increased vascular tone or immune processes, or microangiopathy-induced platelet aggregation[443,444]. Similarly, endothelial dysfunction and vasoconstriction are thought to induce hepatic hypoxia, thereby causing a rise in alanine aminotransferase[445]. It is therefore possible that these processes could be linked with cardiac microvascular disease[173] and increased afterload, thereby contributing to abnormal cardiovascular function and remodelling. Maximum BP is often an unreliable measure of severity as it is heavily influenced by clinician response in terms of timing, dose and route of antihypertensive treatment and timing of delivery. In this way, a lack of relationship between the above severity metrics and cardiovascular phenotype could be attributable to inconsistencies of the measurements themselves, absence of causality,

confounding factors, or insufficient power (particularly as each variable is dichotomised by potentially arbitrary thresholds).

Other ways to grade pre-eclampsia exposure include duration of pre-eclampsia and severity of placental disease reflected by gestation at diagnosis and delivery and lower birthweight centile. In the PICk-UP cohort, the most prevalent cardiovascular abnormalities six months after preterm pre-eclampsia were diastolic dysfunction and raised TVR. Both of these measures correlated with the degree of FGR and gestation at diagnosis and delivery. Although crude, these measures likely reflect placental disease more than the aforementioned pre-eclampsia severity metrics which are more reflective of maternal disease. These findings support a potential causal role of the placenta in the development of postnatal cardiovascular dysfunction.

The measure of pre-eclampsia duration relies on accurate timing of pre-eclampsia diagnosis. Women categorised as low-risk antenatally (47% of the cohort) may not have seen a healthcare professional for six weeks. Given the frequent asymptomatic nature of pre-eclampsia onset, this could result in significant delay in diagnosis[446]. For this reason, the absence of correlation between preterm pre-eclampsia duration and six-month cardiovascular parameters, beyond TVR, is not surprising.

Left ventricular remodelling only correlated with pre-pregnancy factors, not pregnancy outcome. LaPlace's law states that left ventricular wall stress (afterload) is proportional to left ventricular radius and pressure and inversely proportional to left ventricular wall thickness[53]. Left ventricular hypertrophy is therefore considered a compensatory response to increased afterload (i.e. hypertension), serving to reduce the tension in individual sarcomere units and stabilise myocardial oxygen demand[447]. E/E', a measure of diastolic dysfunction, precedes and independently predicts left ventricular remodelling following myocardial infarction[428,429], indicating increased diastolic pressure as a sensitive functional measure of left ventricular injury and a precursor to remodelling. Therefore, the lack of relationship between preterm pre-eclampsia phenotype and left ventricular remodelling could be due to insufficient power, alternative causal pathways (including pre-existing maternal factors) or insufficient time postpartum. Since diastolic dysfunction

precedes and predicts left ventricular remodelling, it is likely that the prevalence of remodelling will increase over time and therefore a relationship may be unveiled following longer-term follow-up.

Due to the three-day postnatal window of visit one in the PICk-UP study and the rapid decline of placental biomarkers in the first 48 hours postpartum[248], it was not possible to correlate peak sFlt levels with postnatal cardiovascular phenotype. Number of days (but not hours) postpartum were included as a covariate in the linear regression analyses, however no correlation between baseline sFlt or PlGF and six-month cardiovascular parameters was demonstrated. This differs from previous studies[156,178], in which maternal cardiovascular health has correlated negatively with antenatal sFlt levels and positively with antenatal PlGF levels. Shahul *et al.*[156] explored the relationship during pregnancy and revealed an association between increased sFlt levels and worse left ventricular systolic function and remodelling. Garrido-Gimenez *et al.*[178] correlated antenatal angiogenic factors with cardiovascular risk ~12 years postpartum. They demonstrated correlations between 1) increased sFlt levels and worse lipid profile and carotid atherosclerosis and 2) increased PlGF levels and improved lipid profile, left ventricular systolic function, carotid atherosclerosis and MAP at the same timepoint[178].

The observational findings from PICk-UP suggest that, at least in part, there is a causal link between the placenta and postnatal cardiovascular dysfunction. Given the inconsistencies, the relationship is most likely multifactorial and influenced by underlying maternal factors, which may predispose some women to cardiovascular susceptibility. Due to the number of correlations assessed in a relatively small cohort, these results are subject to type I error. On the other hand, in support of their accuracy, the findings linking birthweight and gestation with worse cardiovascular parameters, are in keeping with several much larger epidemiological studies investigating pre-eclampsia and long-term cardiovascular disease[10,11,13–15,184].

Another consideration is the heterogenous definition of preterm pre-eclampsia, which likely comprises more than one pathological process. In Leavey *et al.*'s microarray studies[38,39], three subclasses of pre-eclampsia were identified, including maternal

maladaptation, placental insufficiency and immunological overactivation. The first subclass is thought to arise, despite a healthy placenta, from pre-existing maternal cardiovascular risk factors and typically results in term pre-eclampsia in the absence of FGR[39]. The placental insufficiency subclass encompasses the traditional two-stage model of pre-eclampsia[40,314], in which placental malperfusion leads to hypoxia and subsequent release of antiangiogenic factors into maternal circulation, thereby inducing maternal endothelial dysfunction. Finally the third subclass is a result of maternal-fetal incompatibility, subsequent perivillous fibrin deposition, placental insufficiency and severe FGR[39]. It is probable that the majority of preterm pre-eclampsia cases comprise the last two subclasses. Both sFlt[156,174–178] and immunological processes[448] have been linked to cardiovascular disease within and outside of pregnancy. It is therefore plausible that different mechanisms link preterm pre-eclampsia and cardiovascular abnormalities in each of these subclasses, further complicating the quest for causality, when a broad preterm pre-eclampsia definition is applied.

Alternatively, it is plausible that endothelial and subsequent microvascular dysfunction directly link pre-eclampsia and cardiovascular dysfunction in both directions. Endothelial dysfunction is associated with altered vascular integrity and haemodynamics[449]. This could contribute to defective spiral artery remodelling in early pregnancy, which is thought to underpin placental dysfunction and subsequent preterm pre-eclampsia and FGR[450]. This is supported by evidence of spiral artery remodelling before cytotrophoblast invasion[451] as well as the predictive nature of anatomically remote microvascular remodelling for pre-eclampsia and placental dysfunction[330]. It might also explain the increased prevalence of pre-eclampsia in women with established vascular disease[335] rather than non-vascular cardiac disease (as seen in chapter two). In the other direction, antiangiogenic factors, including sFlt, are known to induce endothelial dysfunction in the context of pre-eclampsia[40,164]. Endothelial dysfunction subsequently impacts on microvascular tone and integrity, potentiating myocardial malperfusion and resultant aberrant cardiac function and remodelling[452]. This potentially explains the predictive nature of sFlt[174–178] and endothelial function[453] on cardiovascular function, remodelling and long-term risk. Unfortunately, using data from this doctoral thesis, it is not possible to

confirm or refute the mechanistic role of sFlt in the context of pre-eclampsia-associated cardiovascular dysfunction.

5.4. Postnatal cardiovascular dysfunction following preterm pre-eclampsia

The observational findings from PICk-UP (chapter four) revealed a high prevalence of cardiovascular abnormalities at six months postpartum following pregnancies complicated by preterm pre-eclampsia. In particular, diastolic dysfunction and raised TVR affected 61% and 75% of women, respectively, in the absence of postnatal cardioprotection. The cardiovascular phenotype in the first few days after birth did not relate to six-month postnatal cardiovascular phenotype. This could highlight the multitude of peripartum confounding factors or the variation in rate and degree of cardiovascular resolution postpartum. Although the numbers are small, it potentially indicates a lack of clinical utility for early postnatal echocardiography and biomarkers to screen for lasting cardiovascular morbidity.

A six-month postnatal endpoint was chosen due to the expectation that cardiovascular parameters normalise by six months postpartum following a healthy pregnancy[84,92]. It was therefore anticipated that six-month cardiovascular parameters would be representative of longer term cardiovascular status. Interestingly, there was little change in cardiovascular status from six weeks to six months postpartum in the observational and placebo arms, indicating six weeks as a potential alternative timepoint for cardiovascular assessment in future observational studies.

At one year postpartum, Melchiorre *et al.*[5] demonstrated comparable rates of altered geometry (41%) with our six-month data but lower rates of diastolic dysfunction (52% versus 61% in PICk-UP, using comparable definitions). At two years, they similarly demonstrated lower rates of hypertension (40% hypertension at two years versus 57% at six months in PICk-UP). This is consistent with Benschop *et al*.'s[454] study which assessed hypertension prevalence (41%) one year following a pregnancy complicated by severe pre-eclampsia. The elevated prevalence in PICk-UP could be due to a more severe phenotype (in particular earlier gestation and lower birthweight), or partial resolution of diastolic dysfunction and hypertension beyond six months postpartum. Diastolic

dysfunction prevalence varies depending on the definition used. In PICk-UP, diastolic dysfunction was defined using the BSE age-related 95% confidence intervals for Doppler derived diastolic measurements[289], similar to Melchiorre *et al.*'s study[5]. Importantly, only two women had a completely normal echocardiogram postpartum, highlighting the need to target counselling and intervention in this high risk group.

5.5. Reversibility of preterm pre-eclampsia-related postnatal cardiovascular dysfunction

To our knowledge, PICk-UP was the first interventional study exploring reversibility of cardiovascular dysfunction and remodelling following preterm pre-eclampsia. The cohort represented a severe but diverse range of preterm pre-eclampsia phenotypes and, in the absence of cardioprotective intervention, cardiovascular abnormalities remained prevalent at six months postpartum. In the interventional arm, six months postnatal treatment with enalapril was associated with improved measures of diastolic function (E/E') and left ventricular remodelling (LVMi and RWT). Postnatal hypertension was treated as per routine clinical practice, independent of treatment allocation. Despite this, dBP was lower in the enalapril arm, as was prevalence of concomitant antihypertensives (11% versus 29%). Left ventricular remodelling and hypertension are thought to have a cyclical relationship in which both share causal and consequential roles[5,431]. In this way, it is likely that postnatal enalapril confers significant cardioprotection after pregnancy in women with preterm pre-eclampsia, over and above the benefit of BP treatment alone.

Importantly, the magnitude of treatment effect on diastolic dysfunction and remodelling observed in PICk-UP was in line with previous work investigating echocardiography parameters as predictors of ten-year risk of ischaemic heart disease or heart failure[394]. A 1 unit increase in E/E' (the between-group difference seen in PICk-UP at six months) had a hazard ratio of 1.11 (95% C.I. 1.09-1.13) and 5g/m^2 increase in LVMi (compared with 7g/m^2 between-group difference seen in PICk-UP at six months) had a hazard ratio of 1.16 (95% C.I. 1.13-1.19)[394]. Cardiovascular dysfunction and remodelling are progressive and increase with age[455]. It is therefore plausible that the differences seen between treatment groups in PICk-UP will increase over time[439]. Globally, hypertension is the leading

modifiable risk factor for all-cause mortality[432] and is a well-established measure of cardiovascular risk. Women with pre-eclampsia have a sixfold increased risk of developing hypertension within six years of the index pregnancy (OR 6.6, 95% C.I. 4.6-9.5)[456]. Given the cyclical relationship between left ventricular remodelling and hypertension, early correction of abnormal left ventricular remodelling could protect against later development of hypertension.

Since women with preterm pre-eclampsia may wish to conceive within a few years of their index pregnancy, cardioprotective interventions need to either be short-term or compatible with pregnancy. Enalapril is safe with breastfeeding[406,457] but contraindicated in pregnancy[405] and therefore the longevity of effect beyond treatment cessation requires further investigation.

5.6. Future work

5.6.1. Mechanistic next steps

Chapter three aimed to test the impact of sFlt in pregnancy on the cardiovascular system. In order to definitively answer this question it is likely that an alternative animal model will need to be used. Two candidate models are the STOX1 mouse model[214] and the adenoviral sFlt vector model[164]. The STOX1 model is associated with elevated sFlt levels[214] and persistent postnatal cardiovascular dysfunction[225], however the relationship between the two is yet to be explored. This model would be interesting to use to test the impact of different postnatal interventions on the cardiovascular phenotype, however it would be less feasible to explore the specific role of sFlt. Postnatal investigation of the adenoviral sFlt vector model demonstrated no difference in BP or vascular function six to eight months postpartum[224], but has demonstrated maternal proteomic alterations favouring cardiovascular disease[222]. The early or late-postnatal cardiac phenotype has not been explored in this model. Recapitulation of the adenoviral sFlt model in our unit will allow assessment of the impact of sFlt on the cardiovascular system using a similar protocol to that described in chapter three. Co-administration of VEGF[399] will determine direct causality of sFlt in the development of cardiovascular dysfunction and remodelling. Given the significant variability in BP measured via tail-cuff and carotid cannulation techniques,

going forward I plan to use a more reliable continuous BP monitoring method, like telemetry[297]. Use of this technique is established in our institution and therefore should be a feasible method for BP measurement for future studies.

5.4.2. Clinical next steps

I am in the process of seeking ethical approval for follow-up of the PICk-UP participants four to five years postpartum. At this visit women will undergo echocardiography, BP measurement, cardiovascular risk scores and consent to data linkage. This will provide information on cardiovascular status and risk four to five years following preterm pre-eclampsia. It will also enable correlation between pre-conception, pregnancy and early postnatal parameters with cardiovascular events up to ten years postpartum. Furthermore, blood samples (including, serum and lithium heparin-anticoagulated plasma and ethylenediaminetetraacetic acid [EDTA]-anticoagulated plasma) have been stored from the PICk-UP study for future molecular phenotyping studies. I plan to perform proteomic screening of the plasma samples in order to identify key modulators in the development of cardiovascular disease following preterm pre-eclampsia and explore their association with pre-existing and pregnancy phenotypes.

Finally, PICk-UP has confirmed the feasibility of using enalapril as a targeted intervention in the postnatal period for women with preterm pre-eclampsia. The next step is to carry out a multicentre study to 1) confirm the findings seen in PICk-UP, 2) determine if they persist beyond cessation of the intervention and 3) determine if these improvements confer lasting reduction in hypertension and therefore cardiovascular risk. Data from PICk-UP have informed dosing regimens, refinement of outcome selection and sample size calculations for the proposed multicentre study. I am a co-applicant on the grant, which has been submitted to Efficacy and Mechanism Evaluation programme (EME) for funding consideration. If successful, this study will be due to commence in March 2022. Within PICk-UP 2, I aim to apply for additional funding to enable sub-studies including cardiac magnetic resonance phenotyping, providing a sensitive reproducible adjunct to echocardiography and potentially further proteomic work.

5.7. Conclusion

Following preterm pre-eclampsia there is a high prevalence of cardiovascular abnormalities, including diastolic dysfunction, left ventricular remodelling, raised TVR and hypertension. In the absence of cardioprotective measures, diastolic dysfunction, raised TVR and hypertension persist in a high proportion of women six months after a pregnancy complicated by preterm pre-eclampsia. The relationship between pre-eclampsia and cardiovascular dysfunction is likely multifactorial, however data from this thesis support the hypothesis that pre-eclampsia poses a direct insult on the cardiovascular system. In this way, long-term cardiovascular risk is likely a consequence of both mutual pre-disposing risk factors and pre-eclampsia itself. This body of work has demonstrated potential for postnatal treatment with enalapril to improve cardiovascular function and remodelling in the early postnatal period. Further work is needed to determine the longevity of improvement following treatment cessation and its long-term impact on cardiovascular risk.

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