Cardiovascular Impact of Preeclampsia

on

Mother and Offspring

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ABSTRACT

Preeclampsia is one of the leading causes of maternal and fetal mortality and morbidity.[1] Furthermore, women who have had preeclampsia have an increased risk of cardiovascular events over the next 10-15 years. Indeed, preeclampsia is associated with a four-fold increase in the risk of hypertension and double the risk of fatal and non fatal ischaemic heart disease and stroke.[2-4]

In addition, offspring born to preeclampsia are more likely to have higher blood pressure from childhood[5-11] and stroke in later life.[12] The risk to mother and offspring is greatest when preeclampsia is diagnosed at an earlier gestation, suggesting a more severe form of preeclampsia.[13]

As the long term cardiovascular risk to both mother and child is known from delivery, the main interest of my research was to identify key phenotypic variations in mothers and children during the years between the episode of preeclampsia and emergence of established cardiovascular disease, which might explain the link between the two conditions. This information could then be used to devise ways to identify subjects at greatest risk of later cardiovascular disease and to establish intermediate endpoints for future preventative interventions.

Therefore, in a case control study, women diagnosed with preeclampsia between 1998 and 2003 and their offspring were recruited and underwent comprehensive cardiovascular and metabolic phenotyping. Furthermore, young adults born preterm to hypertensive pregnancy were also investigated in their twenties.

The research demonstrates that early-onset preeclampsia, diagnosed before 34 weeks gestation, is associated with blood pressure patterns in mothers 6-13 years after pregnancy that are distinct from those seen following later-onset disease. Furthermore, there is evidence of distinct differences in cardiac, vascular and metabolic profiles in these individuals with women having evidence of increased arterial stiffness, changes in cardiac function and reduced capillary density. Preterm offspring of hypertensive pregnancies similarly have higher blood pressure than seen in those born following late-onset disease and, in young adult life, have reduced endothelial function and changes in cardiac size proportional to this dysfunction.

This research demonstrates adverse cardiac and vascular remodelling after preeclampsia in mothers and offspring that are evident before the development of clinical cardiovascular disease. The identified differences in cardiac and vascular function may be useful as surrogate endpoints in future preventive trials.

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LIST OF ABBREVIATIONS

A	Late Diastolic Transmitral Filling
A'	Late Diastolic Myocardial Velocity
AA	Ascending Aorta
BP	Blood Pressure
CMR	Cardiac Magnetic Resonance
DBP	Diastolic Blood Pressure
DDA	Distal Descending Aorta
DT	Deceleration Time
E	Early Diastolic Transmitral Filling
E'	Early Diastolic Myocardial Velocity
EDd	End Diastolic Dimension
ESd	End Systolic Dimension
FBF	Forearm Blood Flow
FMD	Flow Mediated Dilatation
HDL	High Density Lipoprotein Cholesterol
IL6	Inlerleukin 6
IL8	Interleukin 8
IMT	Intima-Media Thickness
IVS	Interventricular Septum
L2AA	Abdominal Aorta at the 2nd Lumbar Spine
LA	Left Atrium
LDL	Low Density Lipoprotein Cholesterol
LVPW	Left Ventricular Posterior Wall
MAP	Mean Arterial Pressure
MBF	Maximum Blood Flux
NMD	Nitrate Mediated Dilatation
PAI-1	Plasminogen Activator Inhibitor 1
PDA	Proximal Descending Aorta
PE	Preeclampsia
PlGF	Placental Growth Factor
PORH	Post Occlusive Reactive Hyperaemia
PP	Pulse Pressure
PWV	Pulse Wave Velocity
ROS	Reactive Oxygen Species
RUPP	Reduced Uterine Perfusion Pressure

SBP	Systolic Blood Pressure
sEng	Soluble Endoglin
sFLT-1	Serum fms Like Tyrosine Kinase 1
Sm	Systolic Myocardial Velocity
SSFP	Steady State Free Recession
Tchol	Total Cholesterol
TF	Tissue Factor
TG	Triglyceride
TGFβ	Transforming Growth Factor Beta
TNFα	Tissue Necrosis Alpha
VEGF	Vascular Endothelial Growth Factor
VOP	Venous Occlusion Plethysmography
vWF	Von Willebrand Factor

Chapter 1 **: INTRODUCTION**

1.1 Preeclampsia a Novel Risk Factor for Maternal and Offspring Cardiovascular Disease

Preeclampsia, diagnosed by the new onset of hypertension and proteinuria in the second half of pregnancy, is a unique multisystem condition complicating 2-8% of all pregnancies.[3] Preeclampsia has long been a major cause of maternal and fetal mortality and morbidity.[1.14] Whilst features of preeclampsia resolve within 6 months post-partum, the affected women have a four folds increase in the risk of hypertension and double the risk of fatal and non fatal ischaemic heart disease and stroke 10-15 years postpartum.[2-4] In addition, offspring born to preeclampsia are more likely to have higher blood pressure early in life [5-11] and stroke in later life.[12] The risk to mother and child is greatest when preeclampsia is diagnosed at an earlier gestation suggesting a more severe form of preeclampsia.[13] Indeed early-onset preeclampsia, diagnosed before 34 weeks gestation, has distinctive patho-physiological features both during pregnancy and later in life.

The long term cardiovascular risk to mother and child is known from delivery. However, previous studies have failed to accurately predict preeclampsia in the general population or to identify ways to prevent it.[15-17] Hence, there is a need to better understand the patho-physiological features underlying the long term cardiovascular risk associated with preeclampsia in order to devise ways of identifying subjects at the greatest risk and to establish intermediate endpoints for future preventative interventions. Whilst, the exact cause of preeclampsia remains unknown, our understanding of its pathogenesis has greatly improved.

1.2 Pathogenesis of Preeclampsia

1.2.1 Uteroplacental Ischaemia

A large body of evidence suggests that reduced uteroplacental blood flow and placental ischaemia play crucial roles in the pathogenesis of preeclampsia.[18] During normal placental development, fetal cytotrophoblasts invade the maternal spiral arteries transforming them from small-caliber resistance vessels to high-caliber capacitance vessels providing low resistance placental perfusion adequate to sustain fetal growth. Preeclampsia, a unique condition to humans and higher apes,[19] is characterised by inadequate spiral artery remodeling leading to either chronic placental ischaemia or intermittent flow through the narrow muscular arteries thereby creating an ischaemia-reperfusion phenomenon.[20] Placental insufficiency is then thought to initiate a cascade of events culminating in vascular endothelial dysfunction, hypertension and renal endotheliosis.

Indeed, studies have demonstrated that reducing uteroplacental blood flow in animal models results in features similar to preeclampsia. Ogden and colleagues showed for the first time that clamping the aorta of anaesthetised pregnant dogs resulted in 25mmHg rise in blood pressure and a rapid return to baseline upon clamp release.[21] Woods and Brooks confirmed these observations, showing systemic hypertension consequent to 40% reduction in abdominal aortic pressure in conscious dogs. They also revealed this was independent of the renin-angiotensin system and abrogated by inhibition of cyclo-oxygenase, thromboxane A2 and prostaglandin H2.[22-23] Similar models of abdominal aortic flow manipulation in primates, rabbits and rats, or reduced uterine perfusion pressure in pregnant rats resulted in systemic hypertension, exaggerated systemic inflammation, impaired angiogenesis and endothelial dysfunction.[24-28] Impaired endothelial function and reactivity in turn caused an increase in

total peripheral resistance and renal dysfunction which then resulted in a preeclampsia-like syndrome.[29]

1.2.2 Systemic Inflammation

Normal pregnancy is characterised by an inflammatory state triggered by placentation and placental debris. In preeclampsia, the ischaemic placenta triggers oxidative stress and the release of free radicals in the systemic circulation.[14] Reactive oxygen species and cytokines, such as TNFα, derived from the placenta and neutrophils result in an exaggerated systemic inflammatory reaction and vascular endothelial injury.[20.30] Of interest, direct administration of TNFα in pregnant rats induced a preeclampsia-like state that was associated with reduced expression of renal nitric oxide synthase,[31] augmented vascular contractility and blunted nitric oxide mediated vascular reactivity.[32] In fact, the release of cytokines and acute phase proteins, such as TNFα, leptin and PAI-1, was shown to not only enhance the inflammatory response but also to induce some of the metabolic disturbances associated with preeclampsia, including insulin resistance, lipolysis and hyperlipidaemia.[14.20.33]

1.2.3 Angiogenesis

Soluble Fms-Like Tyrosine kinase (sFLT-1) and soluble endoglin, novel anti-angiogenic factors described in the last decade, are released early in pregnancy and play significant role in preeclampsia. Soluble Fms-Like Tyrosine kinase-1 (sFLT-1) is a circulating truncated form of VEGF receptor that binds to VEGF and PlGF in the maternal circulation thereby reducing the levels of angiogenic factors and inhibiting angiogenesis and vasodilatation. Soluble endoglin, a TGF-β co-receptor, enhances vascular permeability, and affects nitric oxide synthesis and vasodilatation via altered downstream signalling in the TGF-β pathway.[30]

These changes precede and predict both the occurrence and severity of preeclampsia.[34-35] It is hence plausible they underlie the defective placentation and induce endothelial dysfunction contributing therefore to the disease process. Studies have shown that administration of sFLT-1-expressing-adenovirus in rats resulted in a dose-dependent increase in blood pressure, proteinuria and the pathognomonic glomerular endotheliosis described in preeclampsia.[36] Preeclampsia-like syndrome induced by reduced uterine perfusion pressure (RUPP) in rats was in turn associated with increased release of sFLT-1,[28] reduced growth factors and increased expression of soluble endoglin.

1.2.4 Endothelial dysfunction

Endothelial dysfunction plays a pivotal role in the pathogenesis of preeclampsia. Placental ischaemia, systemic oxidative stress and inflammation as well as the release of anti-angiogenic factors[20] play a central role in mediating endothelial dysfunction in preeclampsia.[34-35.37] In addition, syncytiotrophoblasts undergoing apoptosis shed increased numbers of microparticles in the maternal circulation. These further promote the inflammatory and oxidative stress responses and have been shown to dampen endothelial mediated vasodilatation (Figure 1-1).[38] Subsequently, impaired endothelial function in association with an imbalance of vasopressors and vasodilators leads to the clinical features of preeclampsia of hypertension and renal endotheliosis causing proteinuria (Figure 1-2).

Women with pregnancies complicated by preeclampsia have been shown to have evidence of reduced endothelial-dependent vasodilatation in conduit and resistance arteries,[39-46] increased arterial stiffness,[47] increased atherosclerosis[48] and diminished capillary density[49] that may precede development of the clinical syndrome. Consistent with these findings, biochemical markers of endothelial dysfunction are also elevated in preeclampsia.[1.14] It is therefore

plausible the increase in cardiovascular risk in women following preeclampsia is a consequence of persistent alterations in vascular structure and function, either as a result of subclinical vascular injury at the time of pregnancy or due to pre-existing biological differences that predispose to both conditions.

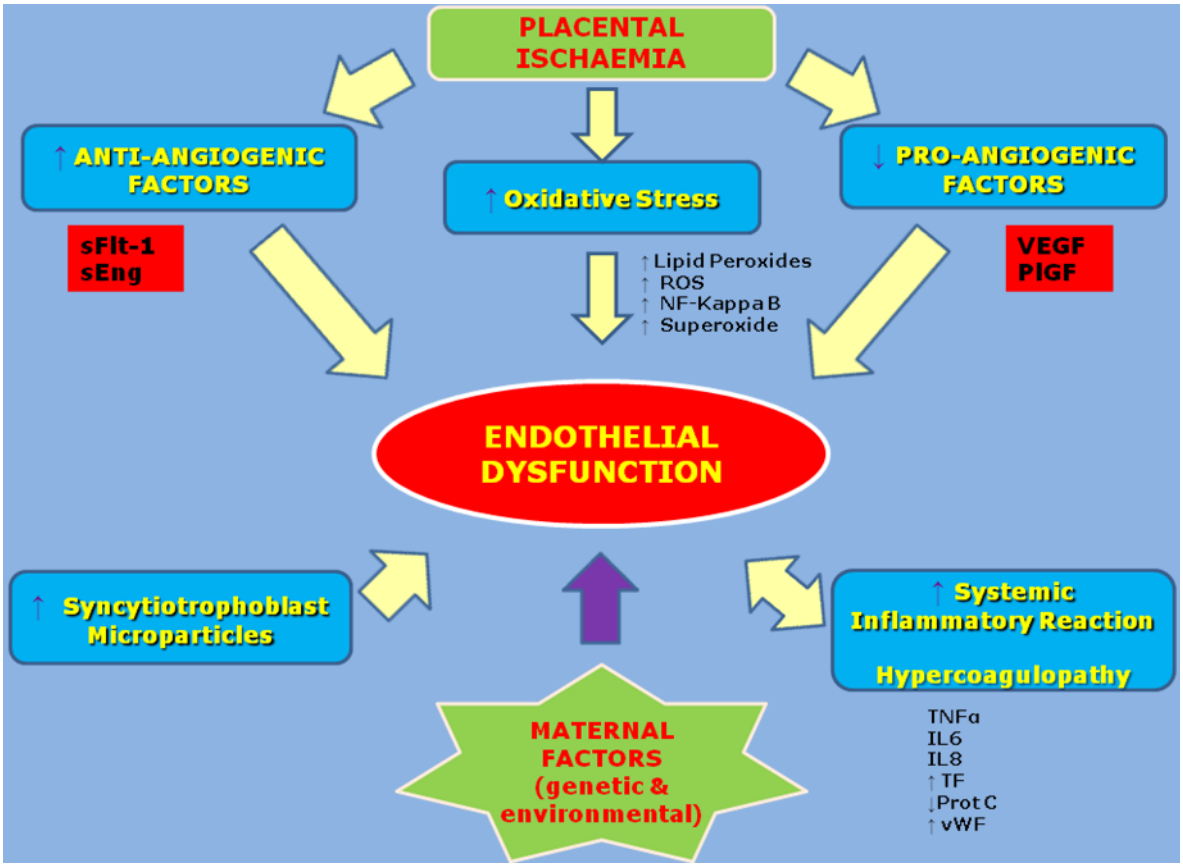


Figure 1-1 Endothelial Dysfunction Plays a Central Role in the Pathogenesis of Preeclampsia

The ischaemic placenta triggers an excessive systemic inflammatory response, oxidative stress and endothelial dysfunction via the release of a number of substances including reactive oxygen species, sFlt-1, endoglin and syncytiotrophoblast microparticles which contribute to development of maternal syndrome of hypertension and proteinuria. (Adopted from Sibai B,[1] Redman CWG & Sargent I[20] and Levine RJ & Karumanchi SA[34])

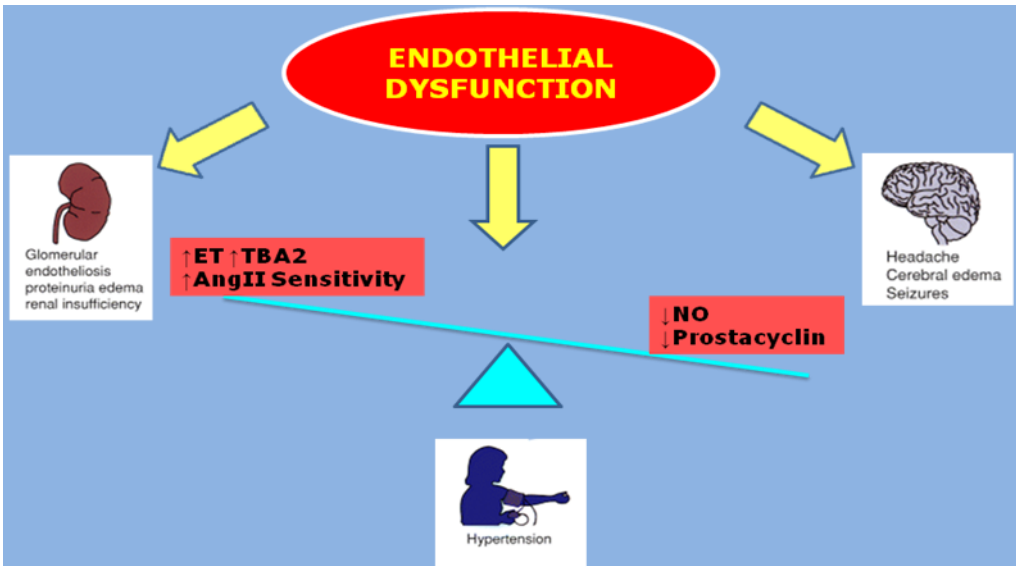


Figure 1-2 The Maternal Syndrome of Preeclampsia

Impaired endothelial function is associate renal endotheliosis causing proteinuria and an imbalance of vasopressors and vasodilators leading to hypertension. (Adopted from Karumanchi SA[50])

1.3 Preeclampsia, Vascular Function and Cardiovascular Disease

1.3.1 Maternal Vascular Function

Impaired endothelial responses and vascular function are common features of preeclampsia and cardiovascular disease. The vascular endothelium is central in regulating vascular tone, anticoagulation, antiplatelet function and fibrinolysis. Endothelial dysfunction is characterized by a pro-atherogenic and pro-inflammatory state, enhanced vasoconstriction as well as vascular remodelling.[51-52] It is a well-recognized key factor in the initiation, development and progression of cardiovascular disease[53-55] and a key feature of classical risk factors such as hypertension, insulin resistance and obesity.[56-57] Reduced endothelial responses are evident from early life in young adults even in the absence of cardiovascular risk factors[58] and in those predisposed to hypertension.[59-60] In addition, impaired endothelial function is also associated with increased carotid atherosclerosis,[55] reduced elasticity and compliance of small arteries; leading to elevated systemic vascular resistance; and large

vessel remodelling.[61] Both arterial stiffness[62] and atherosclerosis[63] are also predictors of cardiovascular risk and disease later in life.

Indeed, several relatively small-scale studies have demonstrated vascular dysfunction after pregnancy complicated by preeclampsia with evidence of endothelial dysfunction in the macrocirculation[64-70] up to a median of 3 years and the microcirculation up to 25 years later [71] as well as incrased arterial stiffness[47.67-68.72] up to almost 5 years after the index pregnancy and atherosclerosis over 3 months postpartum.[73] Furthermore, elevation of systemic biomarkers of endothelial injury and inflammation have also been described between 6 weeks[74] and 20 years[75-77] following preeclampsia (Table 1-1).

Nonetheless, others failed to show vascular abnormalities following preeclampsia.[46.78-80] This disparity may in part reflect heterogeneity and size of patient cohorts, degree of severity of preeclampsia, the timing of the studies following the affected pregnancy as well as differences in the vascular beds studied and the techniques employed (Table 1-1). Hence, there is a need for a large study to characterise the structure and function in a well-defined cohort of women in order to identify key phenotypic variations in mothers during the years between the index pregnancy and emergence of established cardiovascular disease, which might explain the link between these conditions and inform future preventative strategies.

Study	Subjects (Preeclampsia/control)	Interval after Delivery	Vascular Measures	Results in women with previous Preeclampsia
Aortic Stiffness				
Robb AO, 2009 [47]	15/22	7 weeks	AI, PWV	Increased PWV and AI.
Yinon Y, 2010 [68]	24/16	6-24 months	AI	Increased AI in women with early onset preeclampsia.
Evans CS, 2011 [81]	18/50	6-36 months	PWV	No significant differences in central arterial stiffness.
Pàez O, 2009 [67]	20/20	2 years	PWV, AI	Elevated PWV and AI.
Elvan-Tas A, 2005 [72]	44/46	4-56 months	PWV	Elevated PWV.
Lampinen KH, 2006 [70]	30/21	5-6 years	AI	No significant differences in AI.
Subclinical Atherosclerosis				
Blaauw J, 2006 [73]	22/22	≥ 3 months	Femoral and Carotid IMT	Increased IMT with early-onset preeclampsia.
Goynumer G, 2012 [82]	34/42	12-24 months	Carotid IMT	Increased IMT in severe early-onset preeclampsia.
Conduit Artery Endothelial Function				
Kuscu NK, 2003 [83]	15/11	2 and 6 weeks	FMD	Reduced FMD both during pregnancy and postpartum (no control data post partum).
Noori M, 2010 [46]	16/67	6-32 weeks	FMD	No significant differences in FMD compared to controls. FMD reduced in women with preterm delivery.
Hamad R, 2012 [84]	35/30	3-6 months	FMD	Reduced FMD in women with preeclampsia compared to controls (lower in women with early-onset preeclampsia).
Yinon Y, 2010 [68]	24/16	6-24 months	FMD	Reduced FMD in women with early-onset preeclampsia.
Hamad R, 2007 [66]	18/17	15±3 months	FMD	Reduced FMD and NMD in women with severe preeclampsia.
Germain AM, 2007 [65]	25/22	16±3.5 months	FMD	Reduced FMD.
Pàez O, 2009 [67]	20/20	2 years	FMD	Reduced FMD.
Chambers JC, 2001 [64]	113/48	3 years median	FMD	Reduced FMD particularly in women with recurrent preeclampsia that was reversed with ascorbic acid.
Goynumer G, 2012 [82]	34/42	12-24 months	FMD	Reduced FMD in severe early-onset preeclampsia.
Microvascular (Resistance Artery) Endothelial Function				
Lommerse T, 2007 [78]	32/10	0.64-1.6 years	VOP	No significant difference in FBF.
Agatisa PK, 2004 [69]	16/14	9.9±0.5 months	VOP	Reduced endothelium-mediated FBF.
Evans CS, 2011 [81]	18/50	6-36 months	VOP	Reduced FBF in response to mental stress.
Lampinen KH, 2006 [70]	30/21	5-6 years	VOP	Reduced endothelial-dependent and -independent FBF

Table 1-1 Studies of Vascular Function in Formerly Preeclamptic Women

PWV refers to pulse wave velocity, AI represents augmentation index, IMT refers to intima-media thickness, FMD refers to flow mediated dilatation, NMD refers to nitrate mediated dilatation, VOP represents venous occlusion plethysmography, FBF represents forearm blood flow.

1.3.2 Preeclampsia and Offspring Vascular Function

Offspring of preeclamptic mothers have elevated blood pressure in childhood[5-9] and an increased risk of stroke in later life.[12] This is greatest in those born to severe or early-onset preeclampsia. Although the underlying mechanisms remain unknown, there is substantial evidence of 'programming' of aspects of vascular biology during fetal development, in particular endothelial responses[85] and arterial stiffness.[86] The risk to the offspring is likely to be mediated through changes in maternal blood pressure, vascular resistance in the placenta or exposure to maternal factors (such as anti-angiogenic factors,[87] vasoactive substances[88] and reactive oxygen species) in the feto-maternal circulation. The early period of organogenesis is also susceptible to changes in fetal nutrient supply resulting in long term changes to offspring vascular function even in the absence of gross morphological changes.[89] In fact, peri-conceptional nutrient restriction leads to raised fetal blood pressure in sheep[90] as well as endothelial dysfunction in rats[91] which has been shown in animals to transmit through generations.[92] Furthermore, severe preeclampsia is commonly associated with premature delivery and intrauterine growth restriction.[93] Prematurity and growth restriction have previously been linked to hypertension and cardiovascular disease in the offspring[86.94-95] possibly through the adverse effects of the intrauterine environment on the offspring's vascular system.[96]

The cardiovascular risk to the offspring is also known from birth. Therefore, this presents an opportunity for early prevention.[97] Hence, better understanding of the long-term vascular changes in offspring of preeclampsia may allow novel primary cardiovascular prevention strategies to be targeted at key aspects of vascular function.[97]

1.3.3 Preeclampsia and Cardiac Function

Women who develop preeclampsia also undergo an exaggerated cardiac remodelling during pregnancy, with left ventricular hypertrophy, increased end-systolic volume[98] and diastolic dysfunction. These features only partially resolve within six months postpartum[99-101] and are more prominent in women with early-onset preeclampsia.[99] Indeed, left ventricular hypertrophy and diastolic dysfunction are regarded as early markers of imminent cardiovascular disease[102] and poor cardiovascular outcomes.[103] These are more prevalent in subjects at risk of hypertension[104] and relate to endothelial dysfunction.[105-106] It is possible that endothelial dysfunction in preeclampsia predisposes to cardiac and vascular remodelling later in life. Therefore, as preeclampsia is closely associated with cardiovascular disease and possible vascular dysfunction, subclinical changes in left ventricular mass and function may be early features of disease development of particular relevance to these women.

The endothelial dysfunction noted in preeclampsia may also be of relevance to the cardiac function. Not only does endothelial dysfunction play a key role in cardiovascular disease development,[107] endothelial health and nitric oxide synthase activity are crucial in modulating arterial distensibility[108-110] and carotid intima-media thickness[55.58] independent of classical risk factors[58] as well as myocardial hypertrophic responses in animals.[111-112] Left ventricular mass, a powerful independent predictor of mortality and morbidity in adults free of clinical disease,[113] has a graded relationship with vascular endothelial vasomotor responses in old hypertensive adults.[114-119] Left ventricular mass has also been related to arterial stiffness[120-121] and subclinical atherosclerosis[122] in the absence of hypertension. It is therefore of interest to establish whether the persistence of endothelial dysfunction is a better marker of adverse cardiovascular phenotype and long term risk.

THESIS OBJECTIVES

The aim of my programme of research was to investigate the long term impact of preeclampsia on the cardiovascular system of both mother and child before the development of established cardiovascular disease. This would allow me to identify key intermediate phenotypes that might link preeclampsia and later cardiovascular disease as well as identify key pregnancy characteristics that predict these changes. The research was based on recruitment of groups of women and children to undergo comprehensive and detailed cardiovascular phenotyping at a median duration of 10 years after a pregnancy complicated by preeclampsia as well as a study of young adults born to hypertensive pregnancy.

The specific objectives of my thesis were to:

1. Characterise blood pressure behaviour in women during pregnancy and after preeclampsia and determine whether this differed depending on the severity of preeclampsia (Chapter 3).
2. Establish whether there is evidence of impaired endothelial function, vascular remodelling or changes in cardiac structure and function in mothers 6-13 years after preeclampsia (Chapter 4).
3. Determine whether preeclampsia is associated with evidence of adverse vascular phenotype in offspring born to preeclampsia in childhood (Chapter 5) or early adult life (Chapter 6).
4. Determine whether persistent endothelial dysfunction is associated with changes in cardiac or vascular structure and function (Chapter 7).

Chapter 2 : RESEARCH METHODS

2.1 Study Populations and Recruitment

The research focused on characterisation of the long term cardiovascular effects on women and offspring, both in childhood and young adulthood, in observational case control studies. Each study cohort is described in detail in sections 2.1.1 to 2.1.4. The same methods were used to collect data and measure cardiovascular parameters for each cohort and these methods are described in sections 2.2 to 2.8.

2.1.1 Vascular and Cardiac Phenotyping of Mothers

2.1.1.1 Subject Selection

In collaboration with the Nuffield Department of Obstetrics, participants were identified from the John Radcliffe Hospital Oxford Maternity database (OxMat). Cases were women diagnosed with preeclampsia that delivered a live-born baby between 1998 and 2003. Controls were women with uncomplicated normotensive pregnancies matched for age, parity and year of delivery to preeclamptic mothers. Maternity records were reviewed to ascertain diagnosis of preeclampsia (PE) and verify exclusion criteria (AH, ML).

Preeclampsia was defined in accordance with the International Society for the Study of Hypertension in Pregnancy (ISSHP)[123] - *de novo* hypertension≥140/90mmHg with proteinuria≥2+ on urine dipstick measured at least on 2 separate occasions, 4-6 hrs apart, or ≥300mg in 24hr urine collection, after 20 weeks gestation. Controls with previous uncomplicated normotensive pregnancies were matched for age, parity and year of delivery. Women with pre-existing hypertension, diabetes, renal impairment, polycystic ovaries syndrome, inflammatory disease or pregnancy demise were excluded.

Controls with evidence of raised blood pressure or proteinuria of >1+ during any pregnancy or delivering a small for gestational age infant during the index pregnancy (birth weight <10th centile) were also excluded.

2.1.1.2 Power Calculation

The maternal vascular phenotyping study was powered based on studies demonstrating vascular endothelial dysfunction. An estimate of the mean and standard deviation of the FMD was used for a population comparable to the proposed study control population and measured by ultrasound (4.7 ± 4.3%).[64] Using a two-sided significance level of 0.05 and a power of 80% against a specified difference between the groups of 2.15% (0.5SD) the sample size required was 50 subjects in each group.

The primary outcome for the follow up cardiac phenotyping study was left ventricular mass. In order to detect a difference of 10 grams mass using cardiac magnetic resonance with 90% power, a two-sided significance level of 0.05, a sample size of at least 13 subjects in each group was required.[124]

2.1.1.3 Subject Recruitment

879 potential cases were identified from the Oxford maternity database using the ICD10 coding for preeclampsia (O14.0 and O14.1). Of these, 634 maternity records were successfully retrieved and reviewed. 428 women fulfilled the ISSHP criteria,[123] and after excluding 119 subjects, 309 formerly preeclamptic women were invited to participate in the study. In order to match each formerly preeclamptic woman, 536 maternity records of potential controls were reviewed and after excluding 192 subjects, 344 women with previously uncomplicated pregnancies were invited into the study (Figure 2-1). A total of 140

women completed the study comprising of 90 formerly preeclamptic women and 50 controls. In order to study the impact of the severity of preeclampsia, women were subdivided into those with early-onset preeclampsia (45 women), diagnosed before 34 weeks gestation; and those with late-onset preeclampsia (45 women), diagnosed at or after 34 weeks gestation. The 140 women participating in the vascular phenotyping study were then offered the opportunity to attend a follow-up sub-study of cardiac phenotype and 46 of these women were enrolled. Detailed cardiac assessment was carried out using cardiac magnetic resonance and echocardiography. Studies were approved by the Oxfordshire ethics committee (08/H0604/127 and 09/H0604/126).

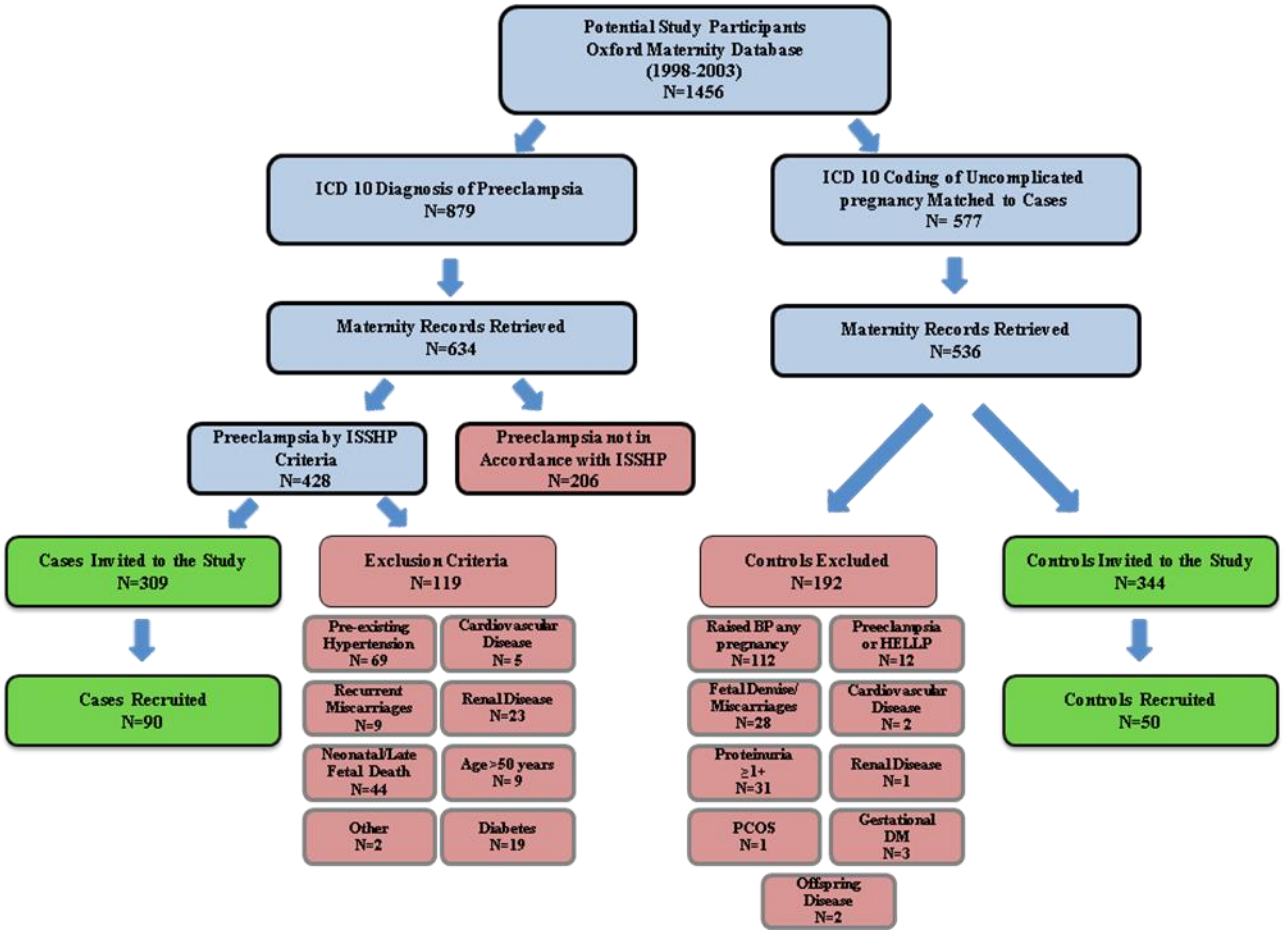


Figure 2-1 Outline of Preeclampsia Study Recruitment

2.1.2 Vascular Phenotyping of Children of Preeclampsia

2.1.2.1 Subject Selection

I then sought to determine whether offspring born to preeclampsia have evidence of adverse haemodynamic and vascular phenotype in early childhood. Therefore, mothers who initially participated in the vascular phenotyping study were offered the opportunity to enrol the offspring born to the index pregnancy into a case control study of vascular phenotype. The study was approved by the Oxfordshire ethics committee 08/H0604/128.

2.1.2.2 Power Calculation

The study was powered based on a study of similar aged subjects with an average FMD of 5.3±4.0%.[125] In order to show a difference of (0.75SD) between offspring born to preeclampsia and controls with alpha 5% and power of 80%, a sample size of 22 subjects was required in each group.

2.1.2.3 Subject Recruitment

A total of 47 offspring born between 1998 and 2003 participated in the study of whom 14 children were born to normotensive pregnancy and 33 to preeclampsia.

2.1.3 Vascular Phenotyping of Young Adults of Preeclampsia

2.1.3.1 Subject Selection

I then sought to establish whether vascular dysfunction develops later in life in offspring of preeclampsia and whether this is more likely to be evident in those born preterm to earlier onset preeclampsia. So, I studied young adults born preterm between 1982 and 1985 who were invited to participate in a 20-30 year follow-up study in Oxford (Early Vascular Study). Preterm participants were born before 37 weeks gestation, either to a hypertensive or

normotensive pregnancy and were previously enrolled from five centres in the United Kingdom and studied at age 18 months[126] and 7 years,[127] with a sub-study at 15 years.[128]

2.1.3.2 Power Calculation

The study was powered based on 20 offspring of hypertensive pregnancies and 50 preterm offspring of normotensive pregnancy to have 90% power with an alpha of 5% to identify a 0.85SD difference in vascular measures.[129-130]

2.1.3.3 Subject Recruitment

The original preterm cohort consisted of 927 subjects, of whom 140 subjects agreed to be informed about future studies and were contacted to return to Oxford for characterisation of cardiovascular risk and vascular imaging. From the 140 preterm offspring from the original cohort, 71 returned for characterisation of cardiovascular risk and vascular imaging. A control group of healthy volunteers matched for age and, born at term to uncomplicated pregnancies, were also recruited to provide normal reference ranges for the vascular measures, performed using the same techniques.

A total of 109 subjects, aged 20-30 years, participated in the study. Of these, 38 controls born at term to uncomplicated pregnancies, 19 were born preterm to hypertensive pregnancy (85% to preeclampsia), and 52 preterm to normotensive pregnancy. There were no differences in birth weight (1297.14±280.26 vs. 1329.33±330.08g, P=0.42), gestation (30.23±2.48 vs. 30.31±2.93 weeks, P=0.81), percentage of male offspring (49% vs. 55%, P=0.33) or frequency of hypertensive pregnancy (28% vs. 27%, P=0.76) between preterm offspring in the original cohort and study participants. Current and old studies were approved by the

relevant institutions research ethics committees (06/Q1604/118) and participants provided signed informed consent in accordance with the Declaration of Helsinki.

2.1.4 Vascular Endothelial Dysfunction and Cardiovascular Phenotype

2.1.4.1 Subject Selection

In a 10 year follow-up study, I explored whether persistent endothelial dysfunction or a decline over time identify subjects with more adverse cardiovascular phenotype. Research subjects who had undergone assessment of endothelial function during adolescence (age 15 years)[131] and had consented to be contacted about future studies were invited to attend the cardiovascular clinic in Oxford for reassessment of endothelial function, cardiovascular risk factors and as well as measurements of ventricular size and function and assessment of vascular phenotype at age 25 years.

2.1.4.2 Power Calculation

The study was powered based on previous reports of mean left ventricular mass and standard deviation (SD) derived using cardiac magnetic resonance in similar age groups.[132] To detect 22.5 grams (1.25SD) difference in left ventricular mass using cardiac magnetic resonance with 80% power and an alpha of 5%, a sample of 10 subjects was required in each group.

2.1.4.3 Subject Recruitment

Of the 219 individuals from the original study of adolescents,[131] 99 subjects consented to be informed of future studies and were invited to participate. A total of 55 subjects were able to attend the follow-up study in Oxford for further characterisation of cardiovascular risk, and measurements of endothelial function and ventricular mass.

2.2 Pregnancy Characteristics and Classical Cardiovascular Risk Factors

Data on pregnancy-related factors were obtained from maternity records. This comprised of data on perinatal blood pressure and weight measurements from the first trimester to delivery in controls or up to 6 weeks postpartum in preeclamptic women. The time of diagnosis of preeclampsia, gestational age at delivery, offspring birth weight, mode of delivery, and parity were also recorded. Other relevant medical and family history, drug history, and lifestyle factors (diet, exercise, smoking and alcohol) were gathered on the study day using a lifestyle questionnaire.

2.3 Demographics and Anthropometric Characteristics

Subjects attended the Cardiovascular Clinical Research Facility in the morning following a 12 hour overnight fast. On arrival, women and young adults were given a brief description of the investigations and given the opportunity to ask questions about the study prior to signing the consent form. Children were also given a brief simplified description of the study and once they expressed agreement to proceed, their parents were then able to sign an assent for the study. Subjects underwent measurements of body size using a combined digital height and weight measurement station. Body mass index was calculated as the ratio of weight to height expressed in meters and squared. Waist hip ratio was calculated after measuring hip and waist circumferences using a tape measure. Venous bloods were drawn from mothers using a vacutainer system prior to undergoing cardiovascular measurements or using a butterfly system at the end of the study in consenting children 30-45 minutes after application of local anaesthetic cream (Emla Cream 5%).

2.4 Blood Pressure Measurements

Blood pressure tracks through life and correlates with cardiovascular risk.[133] A reduction in mean blood pressure levels translates in significant clinical benefits [134] and reduced incidence of myocardial infarction and stroke.[135] In addition, Ambulatory 24 hour blood pressure levels and nocturnal levels are predictors of long term cardiovascular events and mortality.[136] Interestingly, nocturnal blood pressure was previously shown to be elevated during pregnancy in preeclampsia and correlates with perinatal outcomes[137] and measures of vascular dysfunction in these women.[138] Therefore, subjects underwent measurements of peripheral, central as well as ambulatory blood pressure in mothers.

2.4.1 Peripheral blood pressure

Two peripheral blood pressure readings were obtained following 15 minutes of supine rest using a calibrated oscillometric device (AND digital monitor, A&D Medical). The appropriate cuff sizes for arm circumference were used, ensuring the bladder of the cuff was 80% the length and at least 40% the width of the arm circumference.[139] Mean arterial blood pressure was calculated as MAP= (SBP + (2xDBP))/3 and pulse pressure as PP=SBP - DBP.

2.4.2 Central blood pressure

Applanation tonometry was used to generate the ascending aortic waveform from the radial waveform using a mathematical transfer function of the brachial blood pressure (Sphygmocor applanation tonometry, AtCor Medical).[140] Central blood pressure was then derived from the ascending aortic waveform.

2.4.3. Ambulatory blood pressure

Ambulatory blood pressure monitoring was carried out on 46 consenting mothers using an oscillometric device (TM-2430, A&D Instruments). The appropriate blood pressure cuff size was determined according to arm circumference, applied to the non-dominant arm and secured to the skin using tape. Subjects were instructed to continue with their routine daily activities, avoid strenuous exercise and to remain still during the measurements. Automated blood pressure measurements were carried out every 30 minutes at daytime and hourly between 11pm and 7am. All subjects returned a diary documenting sleep and awakening hours for accurate assessment of nocturnal BP.

2.5 Vascular Phenotyping

2.5.1 Aortic Stiffness

Arterial stiffness is a surrogate marker of cardiovascular disease. Higher aortic stiffness is associated with adverse cardiovascular outcomes including ischemic heart disease, left ventricular hypertrophy, and heart failure.[62.120.141] In fact, aortic stiffness can also precede the rise in blood pressure[142] which in turn augments the risk of coronary artery disease, stroke and cardiovascular mortality and morbidity.[143-144]

Arterial stiffness is in part determined by changes in arterial structure, vascular smooth muscle tone and correlates with measures of endothelial function.[110] The arterial wall is made up of smooth muscle cells and extracellular matrix, particularly collagen and elastin. The high elastin to collagen ratio in central arteries plays an important role in maintaining arterial elasticity whilst smooth muscle mass and tone are more important in peripheral circulation. Non-invasive measures of arterial stiffness are increasingly recognised as surrogate endpoints for cardiovascular disease and independent predictors of cardiovascular outcomes.[141.145-146]

Frederick Akbar Mohamed was the first to describe the normal radial pressure waveform and the influence of hypertension and age-related arterial degeneration on the arterial pulse waveform. A generalized transfer function, with over 90% accuracy in generating features of the ascending aortic pressure wave, was then developed by McDonald and Womersley.[147] This has made it possible to characterise properties of different vascular beds.

2.5.1.1 Carotid Femoral Pulse wave velocity (PWV)

The velocity of pulse propagation referred to as pulse wave velocity (PWV) is an important measure of arterial stiffness and a surrogate marker of cardiovascular events.[141.145] Subjects underwent recording of wave forms at the level of the carotid and femoral pulses consecutively by applanation tonometry (Sphygmocor, AtCor Medical) using ECG gating. PWV was then derived by measuring the time delay between the start of the flow waves at the femoral and carotid pulses relative to the distance between the pulse sites based on measurements from the supra-sternal notch on the surface of the body using tape measure (Figure 2-3).

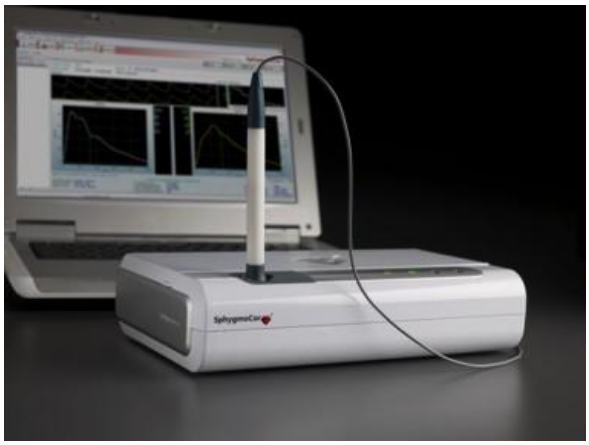


Figure 2-2 Sphygmocor Applanation Tonometry

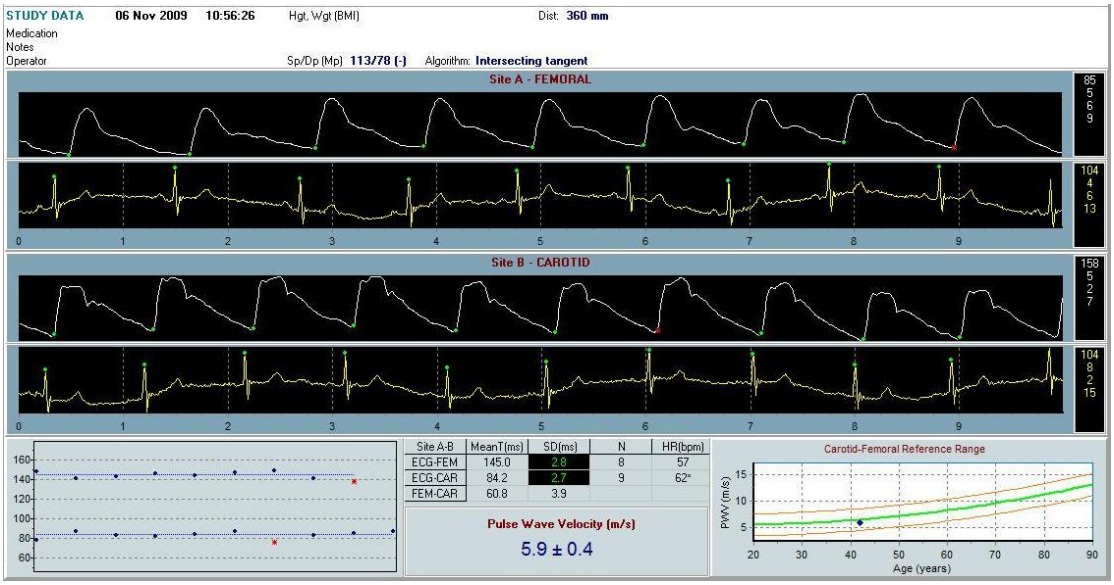
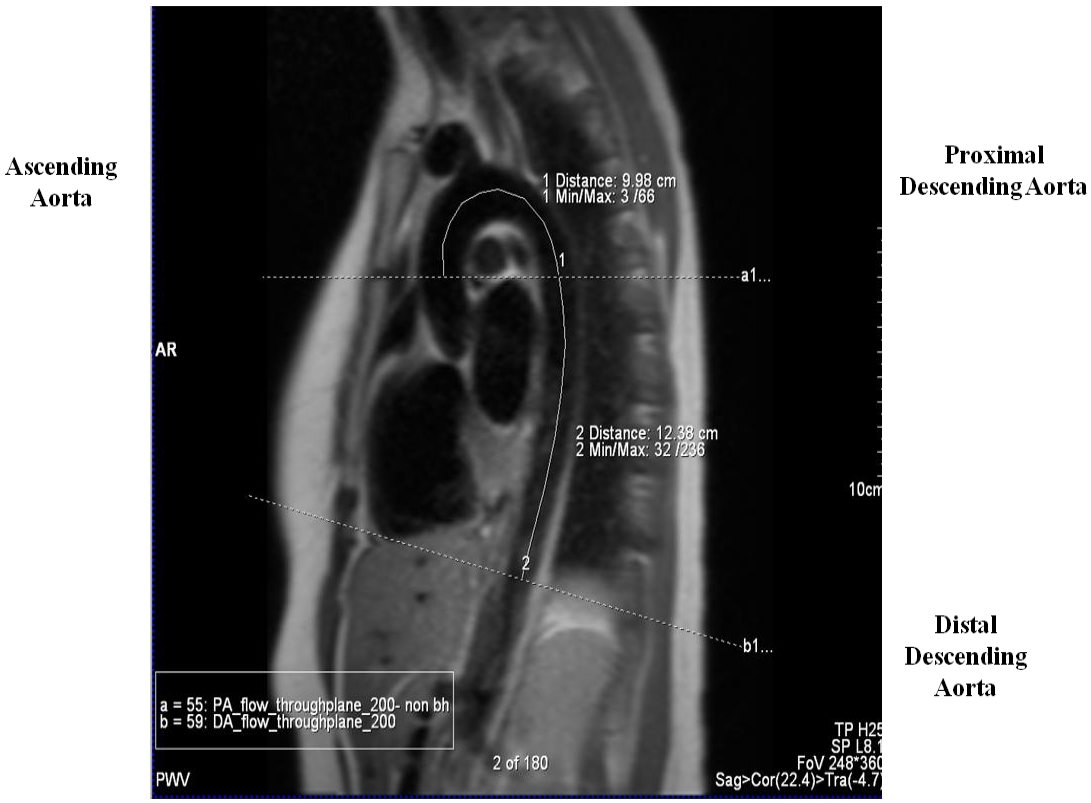


Figure 2-3 Pulse Wave Velocity (Sphygmocor)

2.5.1.2 Central Aortic Pulse wave velocity

Women taking part in the cardiac sub-study had further assessment of PWV by cardiac magnetic resonance (CMR) on a 1.5 Tesla scanner (Siemens, Sonata).[148] Images were captured using a free breathing, ECG-gated, spoiled gradient echo sequence. After acquiring an oblique saggital image of the aorta (Figure 2-4, panel a), velocity-encoding gradient for phase contrast was applied to measure through-plane flow in the ascending aorta at the level of the pulmonary artery and 12 cm distally at the level of the descending thoracic aorta. Flow images were then manually contoured (Figure 2-4, panel b) offline to generate pulse wave curves (Argus; Siemens Medical Solutions, Germany).[148] Aortic PWV was calculated from the ascending to the distal descending thoracic aorta as the distance between the two sites - measured on an aortic oblique sagittal image - divided by the time delay between the arrival of the foot of the pulse wave at each site. The arrival of the foot of the pulse wave was defined as the intersection of the tangent (determined by a least-squares method of the four flow points around the half-maximal point of flow) of the upslope of the flow curve with the x- (time) axis.

a)



b)

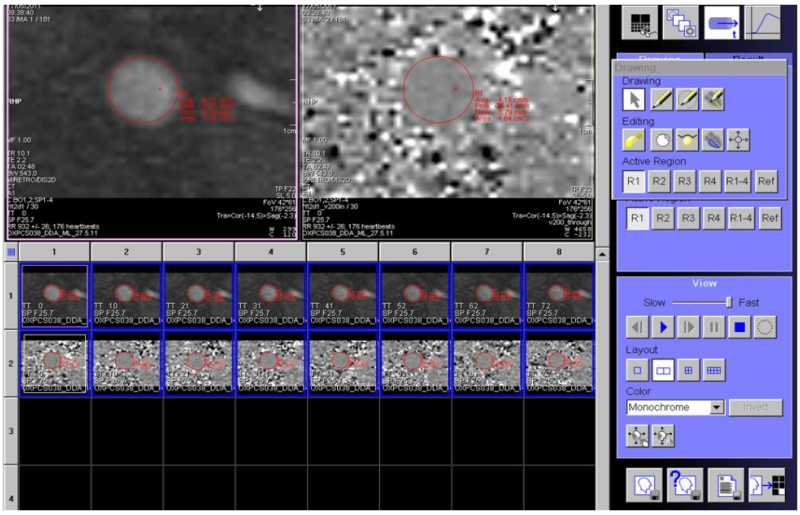


Figure 2-4 Pulse Wave Velocity (CMR)

Once the oblique saggital image of the aorta was obtained (panel a), velocity-encoding gradient for phase contrast was applied to measure through-plane flow from the ascending to the distal descending aorta. Flow images were then manually contoured using Argus to generate flow curves (panel b).

2.5.1.3 Aortic Distensibility

Aortic distensibility is also a measure of the elastic properties of the aorta. ECG-gated, steady state free precession (SSFP) cine images were acquired during breath-hold using 1.5Tesla CMR scanner (Siemens, Sonata) to assess aortic distensibility. In order to investigate regional variations in distensibility, cine images were recorded at four levels along the aorta. Images at the level of the pulmonary artery allowed cross-sectional assessment of the ascending (AA) and proximal descending aorta (PDA). Images recorded 12 cm distally provided a cross-section of the distal descending thoracic aorta (DDA) and those at the second lumbar vertebra allowed measurements of the abdominal aorta (L2AA). Both minimum and maximum aortic cross-sectional areas were determined over the cardiac cycle using semi-automated edge detection algorithms developed using Matlab software (Mathworks Inc.) as illustrated in figure 2-5. Distensibility was then calculated as the relative change in area divided by the pulse pressure.[149]

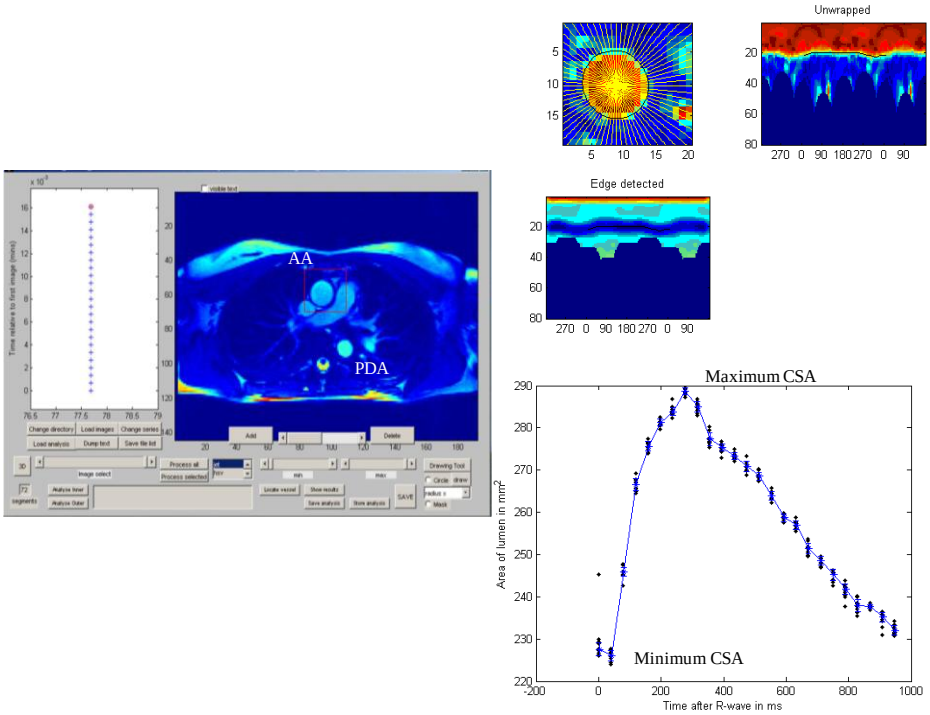


Figure 2-5 Proximal Descending Aortic Distensibility

An edge detection algorithm was applied to delineate aortic cross sectional area (CSA) through a cardiac cycle using Matlab software. Both maximum and minimum areas were determined and used to calculate aortic distensibility.

2.5.2 Subclinical Atherosclerosis

Common carotid intima-media thickness (cIMT), a well established marker of subclinical atherosclerosis and a predictor of ischaemic heart disease and stroke,[63] was measured using ultrasound.[150] High resolution ultrasound (Philips Sonos 5500 equipped with a 10MHz linear array transducer) was used to obtain longitudinal images of the right and left common carotid arteries 1cm proximal to the carotid bifurcation to obtain the clearest demarcation of the intima-media layer in the posterior wall and analysed offline by semi-automated wall tracking software. Common carotid intima-media thickness was measured as the distance between the lumen-intima and media-adventitia interface as illustrated in figure 2-6.

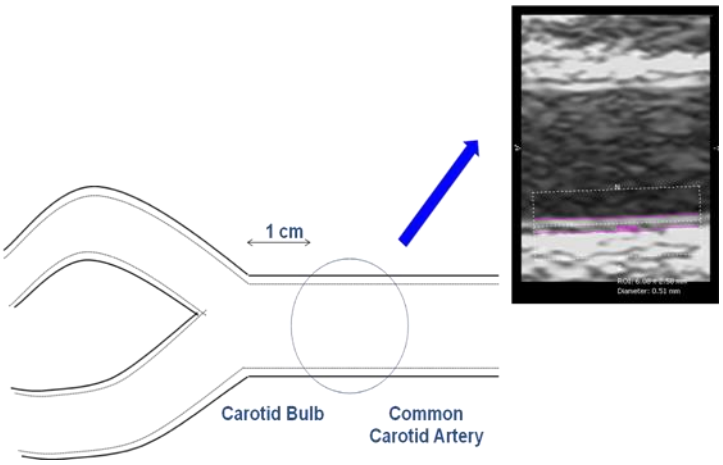


Figure 2-6 Carotid Intima-Media Thickness

2.5.3 Endothelial Dependent and Independent Macrovascular Function

A key feature of preeclampsia, shared with cardiovascular disease, is a pivotal role of the endothelium in its pathogenesis. Initiation and progression of atherosclerosis is characterised by endothelial dysfunction. Loss of vascular endothelial integrity has an important impact on vascular dilatation and tone.[51-52] Women with preeclampsia exhibit reduced endothelial-dependent vasodilatation in the conduit[39-40] and small resistance arteries.[43] Impaired endothelial function plays a key role in cardiovascular disease development[107] and is

associated with increased risk of clinical events[54] and all cause mortality.[53] Thus, study participants underwent measurements of brachial artery endothelial dependent and independent vasodilatation using ultrasound.[151-152]

Brachial artery flow-mediated dilatation (FMD), a close correlate of coronary endothelial function,[153] was measured as a quantitative measure of endothelial-dependent vasodilatation.[151-152] Whilst the subject was laying supine with an outstretched right arm, a pneumatic cuff was placed around the forearm immediately distal to the elbow. The forearm was stabilized in a pre-formed armrest and the probe fixed in position with a stereo-tactic clamp. Digital ECG-gated longitudinal 2D images of the brachial artery were obtained 5-10cm proximal to the antecubital fossa using a 7-10 MHz linear array ultrasound probe (Philips Sonos 5500) and standard vascular imaging presets at baseline and every 15 seconds after cuff deflation. As illustrated in figure 2-7, FMD was calculated as the proportional change in brachial artery diameter between baseline (B_0) and 60 seconds of hyperaemia (B_{60}) induced by 5 minute cuff occlusion at 250 mmHg.[154]

In order to assess endothelial-independent vasodilatation, a measure of vascular smooth muscle function, the blood vessel was allowed to return to resting diameter for 5 minutes before administration 25µg (mothers) or 400µg (adult offspring) of sublingual glyceryl trinitrate. The lower dose of GTN has previously been demonstrated to mimic the physiological levels of vascular dilatation observed in response to shear stress[152] whilst the higher dose induces maximal endothelial-independent vasodilatation. Children were not given GTN. Nitrate-mediated dilatation (NMD) was calculated as the proportional change in brachial artery diameter 3 minutes after subjects received sublingual glyceryl trinitrate. The inter-assay variability for FMD is around 10%[148] which is comparable to other centres.[155]

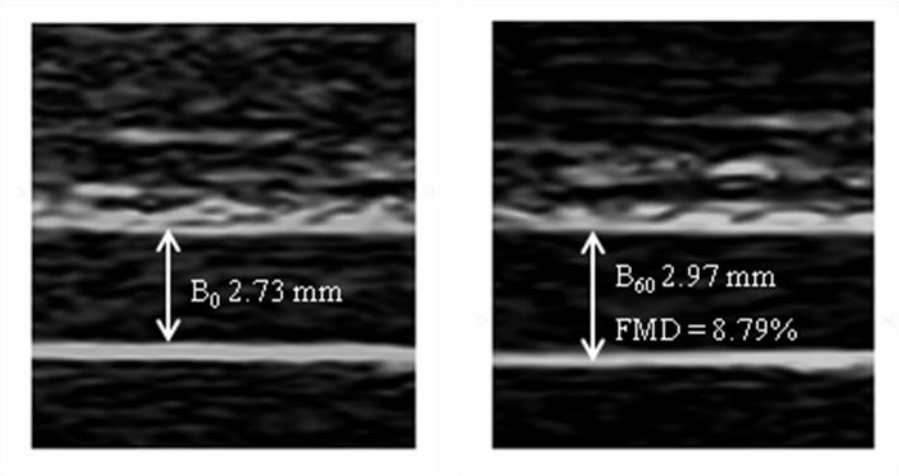


Figure 2-7 Brachial Artery Flow Mediated Dilatation

2.5.4 Microvascular Function

Impaired microvascular responses have been demonstrated during pregnancy[42] and in one study up to 25 years after preeclampsia in mothers.[71] Cutaneous microvascular dysfunction per-se is associated with elevated blood pressure and insulin resistance.[156-157] The laser Doppler applied to the skin surface allows real-time measurement of the collective physiological response (flux) in the superficial cutaneous vascular bed within at least $1mm^3$ area, of up to 10 arteriolar and venular branches. Flux is derived from the reflection of a laser beam light on encountering moving erythrocytes (Doppler shift). The frequency of the oscillation produced by the Doppler frequency shift of the red blood cells in a peripheral tissue is then translated to an intensity oscillation. Laser Doppler flowmetry was used to measure two validated markers of microvascular function, relevant to cardiovascular risk factors, namely maximum hyperaemic blood flux (MBF) and post occlusive reactive hyperaemic (PORH). Day to day inter-assay variability was assessed on ten healthy volunteers who underwent two measurements and was <10% for MBF and 29% for PORH.

2.5.4.1 Maximum hyperaemic Blood Flux

MBF in response to 30 minutes of skin heating at 42°C is primarily endothelial-mediated via nitric oxide and EDHF,[158] as presented in figure 2-8, and has previously been shown to be reduced in subjects at risk of diabetes.[159] Endothelial mediated cutaneous vasodilatation inversely correlates with coronary heart disease risk.[156]

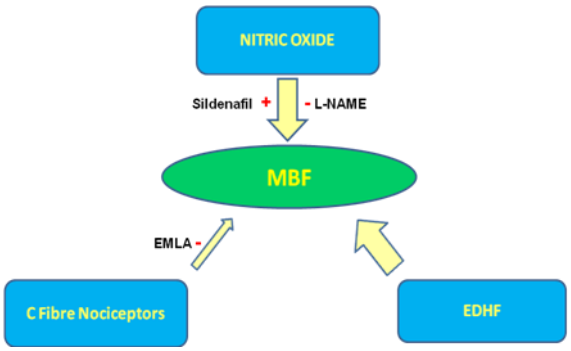


Figure 2-8 Mechanisms of Maximum Hyperaemic Blood Flux

Briefly, a small skin heater probe was placed on the dorsum of the left foot over intact skin and heated up to 42°C over a minimum of 30 minutes. A calibrated laser probe was then inserted into the heater head to measure maximum hyperaemic flux (MoorLAB unit, Moor Instruments, Axminster, UK) and recorded for 30 seconds from 8 adjacent positions using Moor Software (Moor Instruments, Axminster) with a time constant of 0.1sec (Figure 2-9).

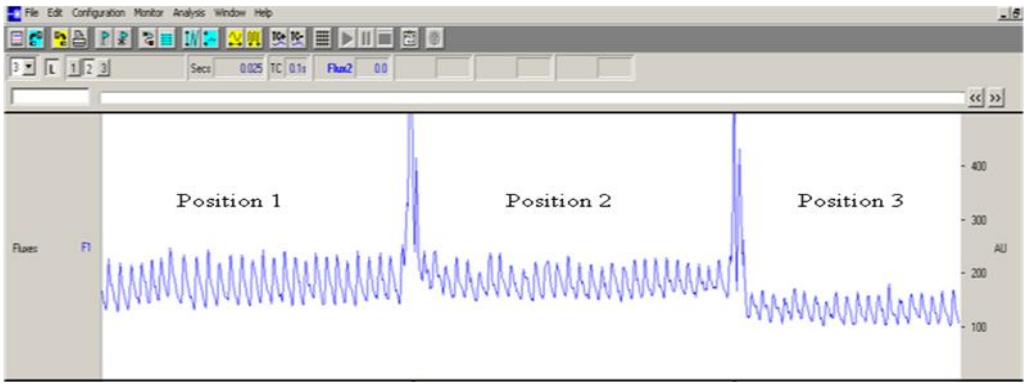


Figure 2-9 Measurement of Maximum Blood Flux

2.5.4.2 Post-occlusive Reactive Hyperaemia (PORH)

PORH is a global marker of microvascular integrity,[160] induced by short arterial occlusion (Figure 2-10).[161] Whilst subjects were laying flat with the left foot supported, a small pneumatic pressure cuff was secured around the lower 1/3 of the calf above ankle and attached to a rapid inflator deflator plethysmography unit (Hokanson, USA).

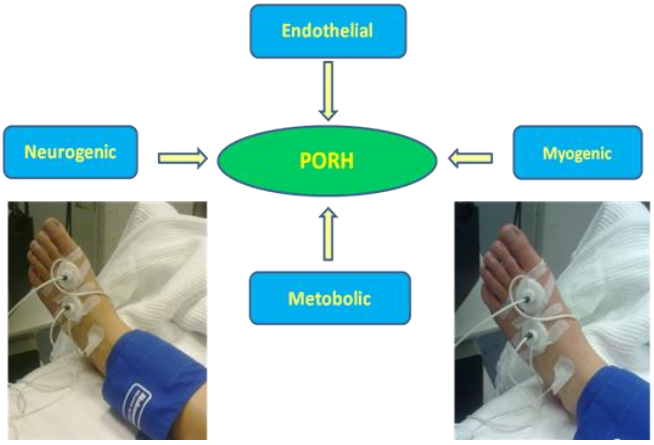


Figure 2-10Mechanisms of Post Occlusive Reactive Hyperaemia

Flux was recorded using a separate calibrated Doppler flowmetry probe attached to a MoorLAB unit (Moor Instruments, Axminster, UK) at baseline, prior to cuff inflation to 200 mmHg for 4 minutes, and during hyperaemia immediately upon cuff deflation (Figure 2-11).

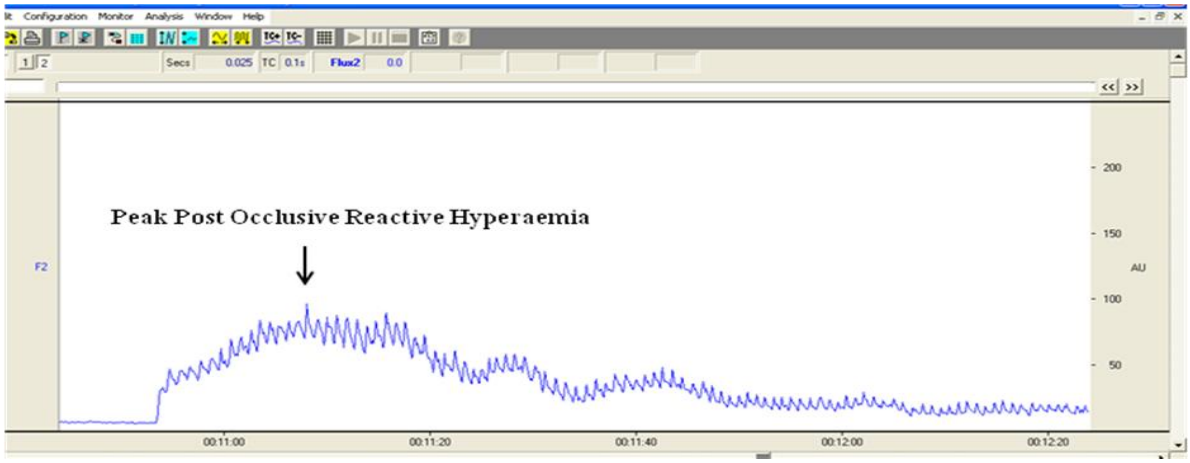


Figure 2-11 Measurement of Post Occlusive Reactive Hyperaemia

2.5.4.3 Hyperaemic flow velocity and shear stress

Reactive hyperaemia is a measure of microvascular function and acts as the stimulus for flow mediated endothelial vasodilatation.[162] Indeed, the peak brachial artery hyperaemic flow velocity after 5 minutes of cuff occlusion of the arm is inversely correlated with traditional cardiovascular risk factors.[163] Flow velocity measurements were taken using pulsed-wave Doppler ultrasound. The cursor was placed in the centre of the brachial artery and aligned to the direction of the vessel at baseline immediately before cuff inflation and the peak within 20 seconds of cuff deflation. Baseline and Hyperaemic flow volumes (ml/min) were calculated using the formula: V x CSA x 60. V refers to mean flow velocity and CSA to the cross sectional area ($CSA=(\pi \times D^2)/4$ with D being vessel diameter). Reactive Hyperaemic Flow was calculated as the proportion of hyperaemic to baseline flow volume. Flow velocity (V) was also converted to shear stress (dyne/cm2) using Poiseuille's equation: $8 \times \mu \times V/D$ where μ represents blood viscosity which was assumed to be 0.035 dyne.s.cm-2.[162]

2.5.5 Microvascular Structure – Capillary Rarefaction

Preeclampsia is associated with impaired angiogenesis[35] and capillary rarefaction in the acute phase.[49] As, capillary rarefaction appears to precede hypertension,[157.164] we hypothesised that capillary density is reduced long term. Capillary density was measured by intravital microscopy on the dorsum of the left middle phalanx using a combined Leica system and digital camera (Bosch DCC) and an external LED light source (Schott 1500) covering the absorption spectrum of haemoglobin (540-580 nm). Capillary density was defined as the total number of erythrocyte-perfused capillaries per mm^2 of skin. Capillary density was measured and averaged from 6 fields both at baseline and following 5 min of venous occlusion at 50 mmHg (Peni-Cuff, Hokanson, USA) and analysed offline.

2.6 Cardiac Phenotyping

Women who develop preeclampsia display signs of exaggerated cardiac remodelling during pregnancy with left ventricular hypertrophy, increased end-systolic volume[98] and diastolic dysfunction that only partially resolves 6 months postpartum.[99-101] These changes are more prominent in women with early-onset preeclampsia.[99] Left ventricular diastolic dysfunction is also regarded as an early marker of imminent cardiovascular disease,[102] poor cardiovascular outcomes,[103] and is more prevalent in subjects at risk of hypertension[104] and relates to endothelial dysfunction.[105-106] Therefore, I sought to determine whether women who develop preeclampsia have evidence of increased left ventricular mass and impaired cardiac function that precede cardiovascular disease development using highly sensitive cardiac magnetic resonance and echocardiographic methods for assessing myocardial mass and performance.

2.6.1 Left Ventricular Mass and Volumes

Cardiovascular Magnetic Resonance (CMR) imaging on a Siemens 1.5T Sonata scanner was used for accurate and reproducible measurement of ventricular mass and volumes. All cardiovascular images were ECG gated with a precordial three lead ECG and acquired during end-expiration breath-holding using a steady state free precession (SSFP) sequence as previously described.[165] Following acquisition of cine localisation images (HASTE - half Fourier single-shot turbo spin echo - sequences), an SSFP left ventricular short axis stack of contiguous images was acquired with a slice thickness of 7mm and an inter-slice gap of 3mm. Image analysis for left ventricular volumes and mass was performed offline based on the short axis stack using Argus Syngo MR Software (version B15, Siemens Medical Solutions, Germany). The endocardial and epicardial borders were delineated from base to apex at end-diastole to yield myocardial volume, with inclusion of papillary muscles and trabeculations (Figure 2-12). Left ventricular mass (LVM) was then calculated as the myocardial volume

multiplied by 1.05 (specific gravity of myocardium). The endocardial border was delineated both at end-diastole and end-systole to derive end-diastolic (EDV) and end-systolic volumes (ESV).

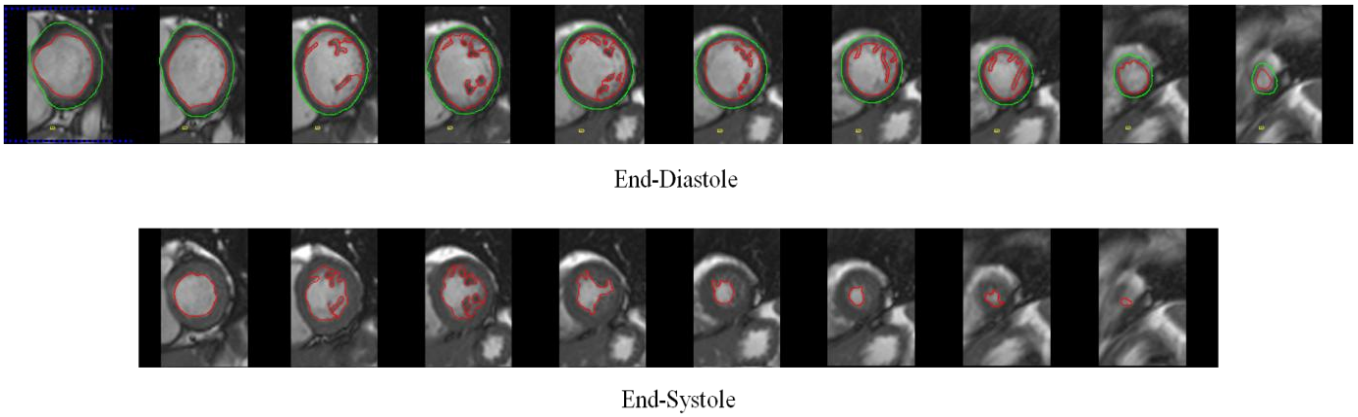


Figure 2-12 Left Ventricular Stack for Ventricular and Myocardial Volume Assessment

2.6.2 Left Ventricular Systolic Function

Global left ventricular function was assessed by measuring ejection fraction (EF) and myocardial strain. Left ventricular stroke volume was calculated as the difference between both volumes. Left ventricular ejection fraction (EF) was calculated as the proportion of stroke volume to the end-diastolic volume and expressed as a percentage. Myocardial strain is an accurate and sensitive method of assessing ventricular function and was measured by CMR using myocardial tagging. A rectangular tagging grid with a spacing of 8 mm was applied to cine images acquired at the base, mid and apical ventricular short axis slices with field of view 360mm, slice thickness 10mm, flip angle 4^{o}, and an acquisition window of 800 msec (Figure 2-13). Peak circumferential systolic strain and strain rate were measured using semi-automatic quantification with CIM (CimTag2D v.7, Auckland MRI Research Group, New Zealand).

Horizontal and vertical long axis cine images were obtained by CMR and analysed using semi-automated 2D Cardiac Performance Analyis Software (TomTec, Germany) to calculate global longitudinal strain. Once the endocardial and epicardial borders were contoured at end-diastole manually, images were analysed automatically through the cardiac cycle to derive longitudinal strain.

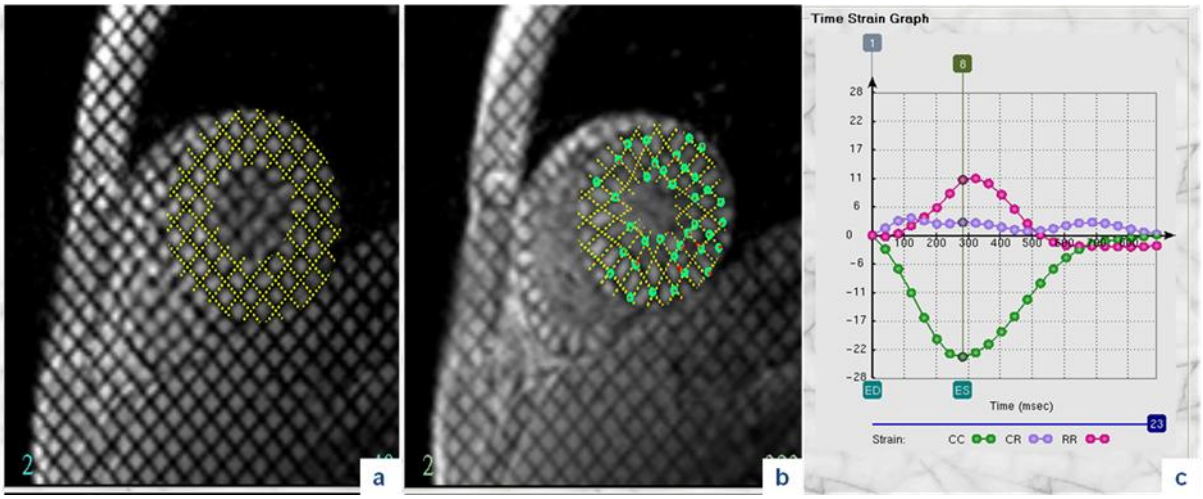


Figure 2-13 Myocardial Tagging and Circumferential Strain (CMR)

2.6.3 Left Ventricular Diastolic Function

For accurate assessment of diastolic function, subjects underwent cardiac echocardiography in the lateral decubitus position with Toshiba Artida equipped with a 2D transducer using standard views. Analysis was carried out offline with Toshiba ACP Software. Left ventricular posterior (LVPW) and inter-ventricular septal (IVS) wall thickness; end diastolic (EDd) end systolic (ESd) dimensions were measured from parasternal 2D images. Left atrial (LA) dimension and volume were measured from parasternal 2D images and apical four chamber using modified Simpson's. A pulsed wave Doppler sample volume was placed at the level of mitral valve tips in the apical 4 chamber view to record trans-mitral flow and measurements of early (E) and late (A) filling velocities, and E wave deceleration time (DT). Myocardial

velocities were recorded at the mitral annulus using tissue Doppler imaging, including peak systolic myocardial velocity (Sm), early (E') and late (A') diastolic velocities, and isovolumic relaxation time (IVRT). Measurements were averaged over three cardiac cycles.

Additional assessment of myocardial strain and diastolic function was carried out using speckle tracking technology. This is an echocardiographic software that uses the inherent acoustic 'speckles' within the myocardium to track them during the cardiac cycle and allows a more detailed assessment of myocardial strain and relaxation (Figure 2-14). Short axis 2D images were obtained at the base, mid- and apical left ventricular levels. In the peak systolic frame, the endocardial border was delineated. The myocardium was then automatically contoured throughout the cardiac cycle allowing tracking myocardial speckles and generating peak circumferential and radial strains.



Figure 2-14 Circumferential Strain and Speckle Tracking (Echocardiography)

2.7 Metabolic and Cardiovascular Biomarkers

Preeclampsia and cardiovascular disease share common metabolic derangements. Therefore, the metabolic profile of women and offspring was measured. Blood samples were drawn, centrifuged and separated within 30 minutes, then stored at -80^{o}C for later analysis. Fasting blood glucose, total cholesterol (TChol), high density lipoprotein cholesterol (HDL), and triglycerides (TG) were measured at the Oxford John Radcliffe Hospital Biochemistry Laboratory using routine validated clinical biochemistry assays. Low density lipoprotein (LDL) was calculated using the Friedewald formula. Homeostatic model assessment was calculated as a measure of insulin resistance (HOMA-IR=fasting glucose x fasting insulin/22.5). Protein: creatinine ratio was measured from a midstream urine sample after exclusion of urinary tract infection.

Levels of anti-angiogenic factors were also measured. Soluble fms-like tyrosine kinase (sFLT-1) and soluble endoglin (sEng) – as well as transforming growth factor β1 (TGF-β1) were measured using commercially available ELISA kits (R&D Systems). Circulating levels of vascular endothelial factor (VEGF), e-Selectin, tissue necrosis factor α (TNFα), interleukin 8 (IL8). ICAM and VCAM were measured by multi-analyte profiling using the Luminex platform (Millipore and R&D Systems).

2.8 Statistical Analysis

Statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences, Version 17) and GraphPad Prism Version 5. Normality of variables was assessed using both a Shapiro-Wilk test and visual assessment of normality curves. When comparing variables between groups, unpaired t-test or ANOVAs test was used for continuous variables and $\chi 2$ for categorical data. Pearson's correlations (parametric) or Spearman's correlations (non

parametric) were used to study the relationship between vascular phenotype and subject characteristics. Results are presented as Mean ± Standard Deviation for continuous data or number (frequency) for categorical data. Two tailed P-values <0.05 were considered statistically significant.

Chapter 3 : PREECLAMPSIA AND MATERNAL BLOOD PRESSURE

3.1 Introduction

Preeclampsia is a common multisystem condition complicating 2-8% of all pregnancies. Not only is it associated with increased perinatal mortality and morbidity,[1.166] it also carries 2-4 fold increase in the risk of hypertension and cardiovascular disease up to 14 years later.[2-3] Cardiovascular risk is greatest in women who develop severe preeclampsia diagnosed early or resulting in preterm delivery or intrauterine growth restriction.[13.167] Whilst the hypertension and proteinuria resolve by 6 months post-partum in most women,[168] some studies have shown that compared to those with normotensive pregnancies formerly preeclamptic mothers have higher office blood pressure up to 6 years[47.64-66.70] and ambulatory blood pressure measurements up to 1 year[66] after the index pregnancy.

Early-onset preeclampsia, diagnosed before 34 weeks gestation, has distinct patho-physiological features that are not commonly observed in late-onset disease, diagnosed after 34 weeks gestation[84.169-171] and greater long term sequelae on blood pressure and vascular function.[46.68.73.84] In addition, mothers who develop preeclampsia before 34 weeks gestation are more likely to have a family history of preeclampsia and have recurrent disease in subsequent pregnancies.[166.172] These observations raise the possibility that mothers develop early-onset disease because they have underlying physiological or biological differences that persist beyond pregnancy and transfer between generations.[173] Hence, I initially sought to determine whether women with early-onset preeclampsia have distinct blood pressure behaviour characteristics remote from pregnancy.

3.2 Hypotheses and Aims

The first stage of the research was aimed to determine whether:

a. Women who develop preeclampsia have higher blood pressure during pregnancy and at 6-13 years post-partum.
b. Women with early-onset preeclampsia have distinct blood pressure characteristics.
c. The association between preeclampsia and blood pressure is not explained by delivery preterm or birth of small for gestational age offspring.

3.3 Methods

3.3.1 Study Design and Population

Study design and subject recruitment was described in the detailed methods section 2.1. Study methods are described in sections 2.2 to 2.4. Briefly, the key measurements for this study were:

3.3.2 Demographics, Anthropometry and Pregnancy Data

Mothers attended in the morning and underwent measurements of body size. Fasting venous blood samples were drawn, centrifuged and separated within 30 minutes, then stored at -80^{o}C. Relevant data on medical, family history and lifestyle factors was gathered using a questionnaire administered by a research midwife.[60] Maternal clinical details recorded during pregnancy were obtained from maternity records.

3.3.3 Antenatal and Postnatal Blood Pressure Measurements

Antenatal blood pressure measures were extracted from the maternity records both for cases and controls. Blood pressure measurements were taken at standardised clinical antenatal visits: around 12 weeks (booking visit 12.14±2.69 weeks), 20 weeks (mid-term 21.36±1.34

weeks), and 32 weeks gestation (third trimester 32.49±1.43 weeks), as well as at the last antenatal check pre-delivery (varied between groups) and at hospital discharge as recorded in the postnatal charts. Women with preeclampsia attended the hospital at six weeks post-partum and blood pressure was also recorded. As described in section 2.4, women underwent measurements of peripheral and central blood pressure at study visit 6-13 years after the index pregnancy. Ambulatory blood pressure was measured on the 46 participants who attended at a later date.

3.3.4 Statistical Analysis

Statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences, Version 17) and GraphPad Prism Version 5 for graphs. Women were grouped according to whether preeclampsia developed before 34 weeks (early-onset) or at 34 weeks or later (late-onset).[171] 45 subjects per group provided a 90% power with an alpha of 5% to identify at least 0.75SD difference between variables, equating to a difference of 4-12mmHg between groups. Pearson's correlation was used to study the relationship between booking blood pressure and blood pressure in later life, as well as whether this association varied dependent on baseline blood pressure in early pregnancy (booking blood pressure). Additionally, the group was divided by booking diastolic blood pressure tertiles to compare the increases in blood pressure relative to booking pressure between preeclampsia-type. Two tailed P-values of <0.05 were considered statistically significant.

3.4 Results

3.4.1 Study Groups and Cardiovascular Risk Factors

140 women participated in the study, of whom 45 had early-onset preeclampsia, 45 late-onset preeclampsia, and 50 had uncomplicated pregnancies. Subjects were aged 28-50 years at enrolment with a mean follow-up of 9.75 years (6-13 years) after the index pregnancy. 80%

of women were primiparous. There were no differences in booking blood pressure between responders and non-responders (Table 3-1). There were also no differences in birth weight or proportion of small-for-gestational-age offspring between participants and non-participants (21.3% vs. 22.6%, P=0.84).

Blood Pressure	Preeclampsia		P Value	Controls		P Value
	Responders	Non-Responders		Responders	Non-Responders	
N	90	219	-	50	259	-
Booking SBP, mmHg	115 (14)	116 (13)	0.55	108 (10)	108 (10)	0.99
Booking DBP, mmHg	68 (9)	70 (10)	0.10	64 (8)	64 (8)	0.99

Table 3-1 Comparison of responders and non-responders

Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported. SBP refers to systolic blood pressure, and DBP to diastolic blood pressure.

As presented in table 3-2, Mothers with early-onset preeclampsia were more likely to have a preterm delivery (77.8% vs. 20%, P<0.001) and give birth to a small for gestational age offspring (51.1% vs. 17.8%, P<0.001) compared to mothers with late-onset preeclampsia. Both groups of preeclamptic women were 2cm shorter (P=0.005) than controls, tended to have higher BMI and had higher insulin, HOMA-IR and lipid abnormalities.

As expected, preeclamptic women were more likely to have a recurrence of the condition at a later pregnancy and to be born to preeclampsia particularly those women who developed preeclampsia before 34 weeks gestation. Furthermore, whilst all subjects were normotensive at antenatal booking, six women with early-onset preeclampsia had diagnosed hypertension and one was on oral hypoglycaemic agents for diabetes at the time of the study (Table 3-2).

Parameters	Early PE (n 45)	Late PE (n 45)	Controls (n 50)	P Value		
				All PE vs. Controls	Early PE vs. Controls	Late PE vs. Controls
During Index Pregnancy						
Maternal Age at Delivery, years	30.33 (5.34)	30.69 (4.60)	30.12 (3.59)	0.60	0.82	0.51
Primiparous, n (%)	36 (80)	37 (82)	40 (80)	0.52	1.00	0.78
Antenatal Booking BMI, Kg/m^2	26.07 (4.61)	25.70 (6.24)	23.95 (4.33)	0.13	0.11	0.27
Gestational Age at diagnosis, weeks	29.67 (3.44)	37.07 (1.78)†	–	–	–	–
Gestational Age at Delivery, weeks	34.09 (2.70)	37.87 (1.53)	40.16 (1.26)	**<0.001**	**<0.001**	**<0.001**
Offspring Birth Weight, Kg	2.01 (0.78)	2.97 (0.54)	3.65 (0.40)	**<0.001**	**<0.001**	**<0.001**
Premature Delivery, n (%)	35 (77.8)	9 (20) †	0 (0)	**<0.001**	**<0.001**	**<0.001**
SGA Offspring, n (%)	23 (51.1)	8 (17.8) †	0 (0)	**<0.001**	**<0.001**	**<0.001**
Subject Characteristics at Study Enrolment						
Age*, years	39.78 (5.30)	40.04 (5.09)	40.51 (3.16)	0.40	0.42	0.60
Height, m	1.63 (0.05)	1.63 (0.05)	1.65 (0.06)	**0.005**	**0.02**	**0.02**
Weight, Kg	74.22 (17.78)	71.77 (16.04)	69.39 (14.40)	0.21	0.15	0.45
Body Mass Index, Kg/m^2	28.07 (6.78)	27.22 (6.27)	25.42 (5.45)	**0.05**	**0.05**	0.14
Waist Hip Ratio	0.81 (0.07)	0.82 (0.09)	0.80 (0.07)	0.15	0.70	0.34
Current Smoking, n (%)	5 (12.5)	1 (2.3)	2 (4.2)	0.16	0.20	0.84
Total Cholesterol, mmol/l	4.88 (0.90)	4.99 (1.15)	4.65 (0.82)	0.10	0.21	0.11
HDL Cholesterol, mmol/l	1.45 (0.36)	1.55 (0.36)	1.63 (0.33)	**0.05**	**0.01**	0.30
LDL Cholesterol, mmol/l	2.89 (0.80)	2.96 (0.96)	2.61 (0.75)	**0.04**	0.09	0.06

Total:HDL Cholesterol Ratio	3.53 (0.86)	3.30 (0.84)	2.95 (0.74)	**0.002**	**0.001**	**0.04**
Triglycerides, mmol/l	1.19 (0.80)	1.02 (0.41)	0.90 (0.46)	**0.05**	**0.04**	0.17
Insulin, mU/l	9.34 (5.08)	9.03 (5.67)	6.76 (3.62)	**0.002**	**0.006**	**0.03**
Glucose, mmol/l	5.06 (0.77)	4.97 (0.43)	4.92 (0.36)	0.51	0.53	0.58
HOMA-IR	2.08 (1.14)	2.01 (1.29)	1.52 (0.85)	**0.01**	**0.01**	**0.04**
Albumin:Creatinine Ratio, mg/mmol	0.50 (0.57)	0.62 (0.63)	1.03 (2.19)	0.16	0.12	0.23
Recurrent Preeclampsia, n (%)	7 (15.6)	10 (22.2)	_	_	_	_
Current Hypertension, n (%)	6 (13.3)	2 (4.4)	0	**0.03**	**0.01**	0.14
Current Diabetes, n (%)	1 (2.2)	1 (2.2)	0	0.29	0.29	0.29
Born to Preeclampsia, n (%)	8 (17.8)	4 (8.9)	1 (2)	**0.002**	**0.02**	0.18
Maternal Hypertension, n (%)	23 (52.3)	18 (40)	13 (26.5)	**0.02**	**0.01**	0.17
Paternal Hypertension, n (%)	12 (27.9)	15 (33.3)	15 (31.3)	0.95	0.73	0.56

Table 3-2 Subject Characteristics during Pregnancy and Follow-up

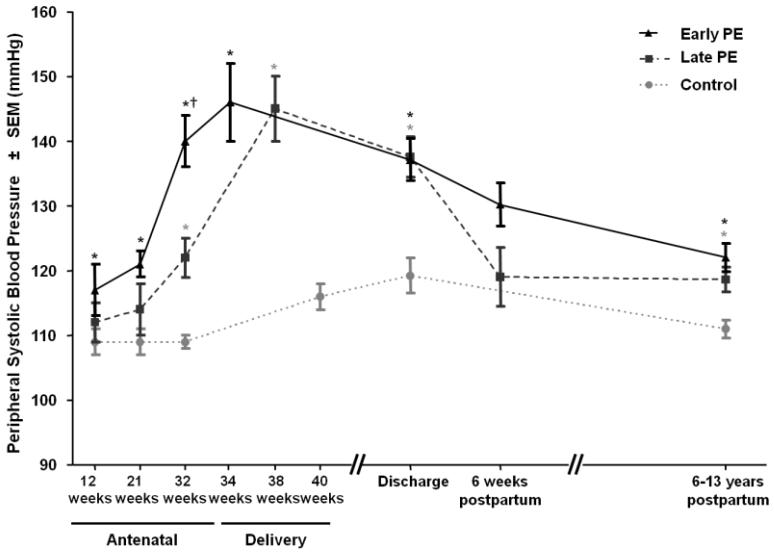
Data are presented as Mean (SD). Two tailed unpaired student t Test or χ^2 P values reported. SGA refers to small for gestational age ($<10^{th}$ centile on the birth charts), HDL refers to high density lipoprotein cholesterol, LDL to low density lipoprotein, and HOMA-IR to the homeostatic model assessment insulin resistance factor. †P<0.05 early-onset compared to late-onset preeclampsia.

3.4.2 Maternal Antenatal and Postnatal Blood Pressure

All participants had normal blood pressure at the time of their antenatal booking appointment however when the group was divided according to time of onset of preeclampsia women who developed early-onset disease had significantly higher systolic and diastolic booking blood pressures compared to controls (115.70±14.09 vs. 108.41±9.84mmHg, P=0.03 and 70.61±8.73 vs. 63.83±8.02mmHg, P=0.005). There were non significant differences in blood pressure between those who developed late-onset disease and controls (114.80±14.34 vs. 108.41±9.84mmHg, P=0.09 and 66.15±10.48 vs. 63.83±8.02mmHg, P=0.38, Table 3-3).

Systolic and diastolic blood pressure levels rose rapidly in those with early-onset disease, and were significantly higher than controls and those with late-onset disease by the middle trimester (Figure 3-1). In contrast, blood pressure in women who developed late-onset preeclampsia only reached the levels seen in the early-onset group in the third trimester. In addition, despite having similar peak blood pressure levels during pregnancy and at discharge from hospital, a striking divergence between preeclamptic groups was evident at the six weeks post-partum (Figure 3-1). Of interest, the early-onset preeclampsia group had higher systolic and diastolic blood pressures at 6 weeks postpartum. Nonetheless, diastolic blood pressures were unchanged in these women compared to levels measured at discharge from hospital, and remained significantly higher than the late-onset group (86.25±13.46 vs. 75.00±5.00mmHg, P=0.04). In contrast, the late-onset preeclampsia group had similar systolic and diastolic blood pressures at six weeks to levels seen at hospital discharge in women who had a normotensive pregnancy (SBP 119.00±10.25 vs. 119.25±13.27mmHg, P=0.97; DBP 75.00±5.50 vs. 74.70±7.74mmHg, P=0.93) .

a)



b)

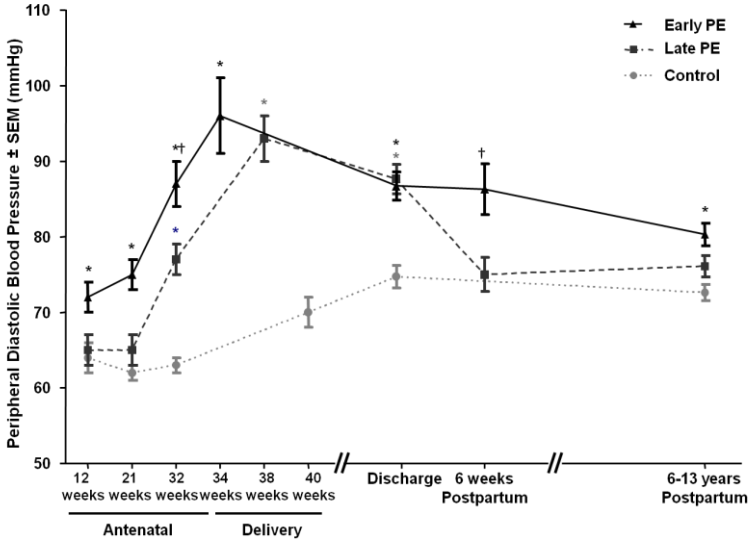


Figure 3-1 Trajectory of Perinatal and Postnatal Blood Pressure in Mothers

As illustrated in panel (a) women with early-onset preeclampsia had significantly higher SBP throughout pregnancy and 6-13 years postpartum compared to uncomplicated pregnancies (*). Women with late-onset preeclampsia had a slower increase in SBP during pregnancy compared to controls (*) but similar peak blood pressure compared to those with early-onset disease. By six weeks postnatal, SBP returned to levels seen following normotensive pregnancy at hospital discharge. Panel (b) shows women with early-onset preeclampsia had significantly higher DBP during pregnancy compared to controls (*) and at six weeks post-partum compared to those with late-onset disease (†). Women with late-onset preeclampsia had comparable DBP to controls at booking but higher levels 6-13 years later (grey asterisks). (Error bars represent Mean and SE).

Parameters	Early PE	Late PE	Controls	*P Value*		
				All PE vs. Controls	**Early PE vs. Controls**	**Late PE vs. Controls**
Booking Antenatal Blood Pressure						
Number	45	45	50			
Peripheral SBP, mmHg	115.70 (14.09)	114.80 (14.34)	108.41 (9.84)	**0.02**	**0.03**	0.09
Peripheral DBP, mmHg	70.61 (8.73)	66.15 (10.48)	63.83 (8.02)	**0.03**	**0.005**	0.38
Blood Pressure at study follow up						
Number	45	45	50			
Peripheral SBP, mmHg	122.04 (14.75)	118.57 (12.95)	110.96 (10.03)	**<0.001**	**<0.001**	**0.001**
Peripheral DBP, mmHg	80.31 (9.98)	76.11 (9.48)	72.63 (7.76)	**0.001**	**<0.001**	**0.05**
Central SBP, mmHg	115.86 (15.51)	112.13 (13.45)	104.39 (11.57)	**<0.001**	**<0.001**	**0.003**
Central DBP, mmHg	81.82 (10.11)	78.22 (9.17)	74.39 (8.54)	**0.001**	**<0.001**	**0.02**
Ambulatory Blood Pressure						
Number	15	16	15			
24 hour SBP, mmHg	119.86 (11.99)	116.07 (6.32)	109.27 (8.92)	**0.006**	**0.01**	**0.02**
24 hour DBP, mmHg	74.57 (6.78)	71.73 (6.05)	67.13 (6.11)	**0.005**	**0.004**	0.05
Daytime SBP, mmHg	122.36 (12.07)	120.13 (6.55)	111.73 (9.70)	**0.003**	**0.01**	**0.01**
Daytime DBP, mmHg	77.00 (6.94)	75.33 (7.05)	70.27 (6.58)	**0.01**	**0.01**	**0.05**
Nocturnal SBP, mmHg	111.07 (13.18)	101.13 (11.49)	100.27 (9.41)	0.15	**0.02**	0.83
Nocturnal DBP, mmHg	67.00 (7.25)	58.60 (5.79)	58.00 (7.29)	0.06	**0.003**	0.81

Table 3-3 Maternal Blood Pressure

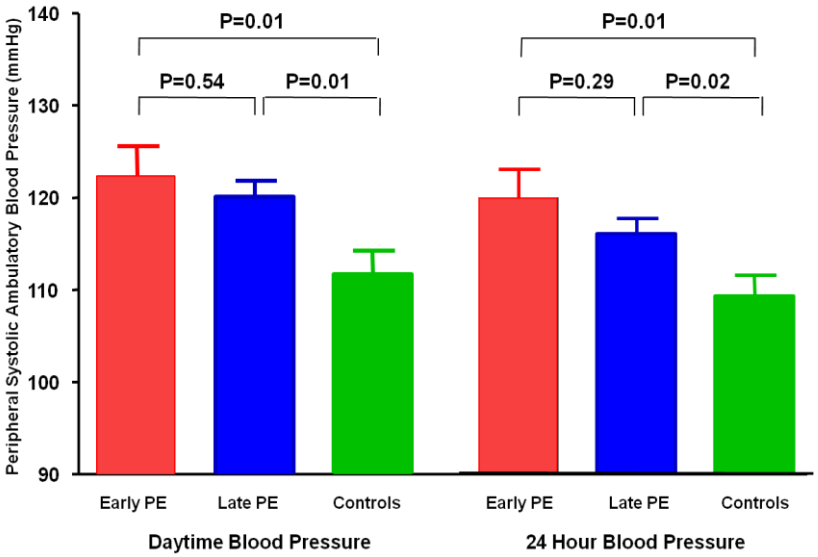
Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported. SBP refers to systolic blood pressure and DBP to diastolic blood pressure.

3.4.3 Maternal peripheral, central and ambulatory blood pressure in later life

At study enrolment, 6 to 13 years later, women from both preeclampsia groups had higher peripheral and central blood pressure measured during the study visit (Table 3-3). These differences remained significant after excluding women with a recent diagnosis of hypertension ($P \leq 0.05$).

46 women underwent twenty four hour ambulatory blood pressure monitoring. This demonstrated a graded difference in blood pressure dependent on the time of onset of preeclampsia (Figure 3-2, panel A). Furthermore, whilst both groups of preeclamptic women displayed higher levels of daytime systolic and diastolic blood pressures compared to controls (Figure 3-2, panel A), only women with early-onset preeclampsia had approximately 10mmHg higher nocturnal blood pressure compared to both controls and those with late-onset disease, having similar nocturnal levels (Figure 3-2, panel B).

a)



b)

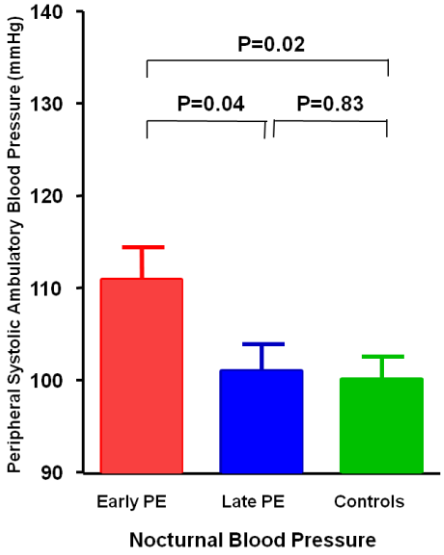


Figure 3-2 Maternal Ambulatory Blood Pressure 6-13 after Pregnancy

Panel (a) shows that women with early (red bars) and late-onset preeclampsia (blue bars) had significantly greater 24 hour and daytime systolic blood pressure compared to controls (green bars). Panel (b) demonstrates that only women with early-onset preeclampsia had significantly higher nocturnal systolic blood pressure compared to both women with uncomplicated pregnancies and late-onset preeclampsia. (The bar graph represents mean SBP and SE).

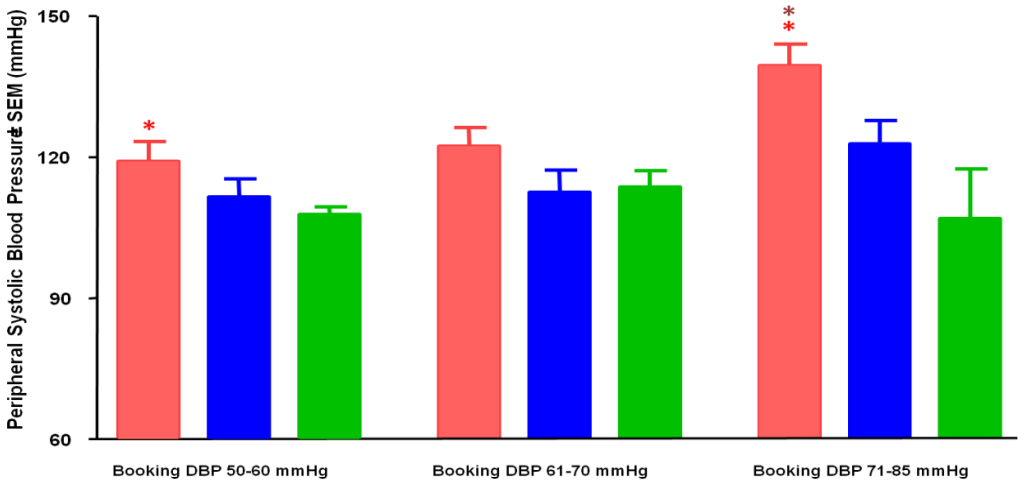
3.4.4 Associations between antenatal and long term blood pressures

I next studied the relation between blood pressure measured in early pregnancy and later in life at study enrolment. When the cohort was studied as a whole, there was a positive correlation between blood pressures at booking and 6-13 years postpartum both for systolic (r0.512, P<0.001) and diastolic (r0.404, P<0.001) blood pressures.

I then divided groups by tertiles of booking DBP to investigate patterns of blood pressure changes at follow-up as a function of baseline booking blood pressure during pregnancy. I found a distinct pattern of blood pressure behaviour depending on the time of onset of preeclampsia. Interestingly, the early-onset preeclampsia group had higher levels of blood pressure in later life for any level of booking diastolic blood pressure compared to the normotensive pregnancy group and to late-onset subjects in the highest tertile (Figure 3-3, panel A).

I then assessed the mean change in blood pressure between the 1st antenatal booking and that measured 6-13 years later. I found women developing early-onset preeclampsia had between 12 and 18mmHg greater increase in blood pressure over this period, irrespective of the level of booking pressure (Figure 3-3, panel B). Nonetheless, in the control and late-onset preeclampsia groups the mean rise in blood pressure was smaller and, in fact, in those with high booking blood pressure, there was a relative drop in later blood pressure.

a)



b)

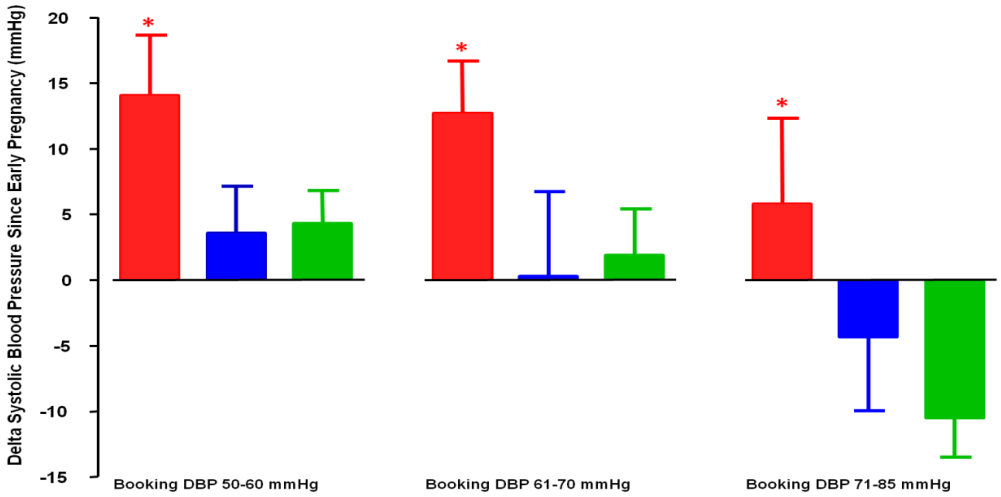


Figure 3-3 Patterns of Maternal Long-term Blood Pressure

Groups were divided by tertiles of booking diastolic blood pressure to further assess patterns of blood pressure long term. Panel (a) demonstrates that blood pressure was persistently higher in the early-onset preeclampsia group (red bars) compared to the normotensive pregnancy (green bars) irrespective of booking blood pressure (*), and to the late-onset preeclampsia group (blue bars) at the highest tertile (*). Panel (b) shows that mothers with early-onset preeclampsia had significantly greater mean rise in systolic blood pressure between early pregnancy and later life irrespective of booking blood pressure. Women with uncomplicated pregnancies (green bars) and late-onset preeclampsia (blue bars) had a smaller blood pressure rise or fall. (The bar graphs represent Mean and SE).

3.4.5 Associations between small for gestational age and long term blood pressures

Delivery of small for gestational age offspring per se is associated with increased cardiovascular risk in mother and child. A large proportion of women with preeclampsia in our cohort delivered an SGA offspring (34%). Therefore, I sought to determine whether preeclamptic mothers who delivered an SGA infant had distinct metabolic or blood pressure profile. So, I divided the group of formerly preeclamptic women according to whether they delivered a small for gestational age infant (SGA) or an appropriate for gestational age offspring (AGA). I found no differences in body size, fasting lipids, insulin, or glucose 6-13 years after preeclampsia (Table 3-4). Moreover, whilst women who went on to develop preeclampsia had higher blood pressure during pregnancy and many years later, there were no differences in blood pressure between SGA and AGA groups. Nonetheless, higher blood pressure was noted in the 3rd trimester in women delivering an SGA offspring suggesting a possible interaction between placental pathology and pregnancy blood pressure and fetal growth.

Parameters	**SGA (n 31)**	**AGA (n 59)**	**P Value**
BMI, kg/m^2	27.16 (7.12)	27.89 (6.20)	0.20
Total Cholesterol, mmol/L	4.91 (1.07)	4.95 (1.01)	0.87
HDL Cholesterol, mmol/L	1.46 (0.36)	1.52 (0.37)	0.45
LDL Cholesterol, mmol/L	2.93 (0.90)	2.92 (0.88)	0.96
Total : HDL Cholesterol	3.47 (0.77)	3.39 (0.90)	0.67
Triglycerides, mmol/L	1.12 (0.89)	1.09 (0.44)	0.83
Insulin, mU/L	8.35 (4.07)	9.63 (5.91)	0.29
Glucose, mmol/L	4.81 (0.61)	5.06 (0.45)	0.32

Table 3-4 The Impact of Small for Gestational Age Delivery on Preeclamptic Mothers

Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported.
SGA refers to small for gestational age (<10th centile on birth charts), AGA to appropriate for gestational age, and HDL high density lipoprotein.

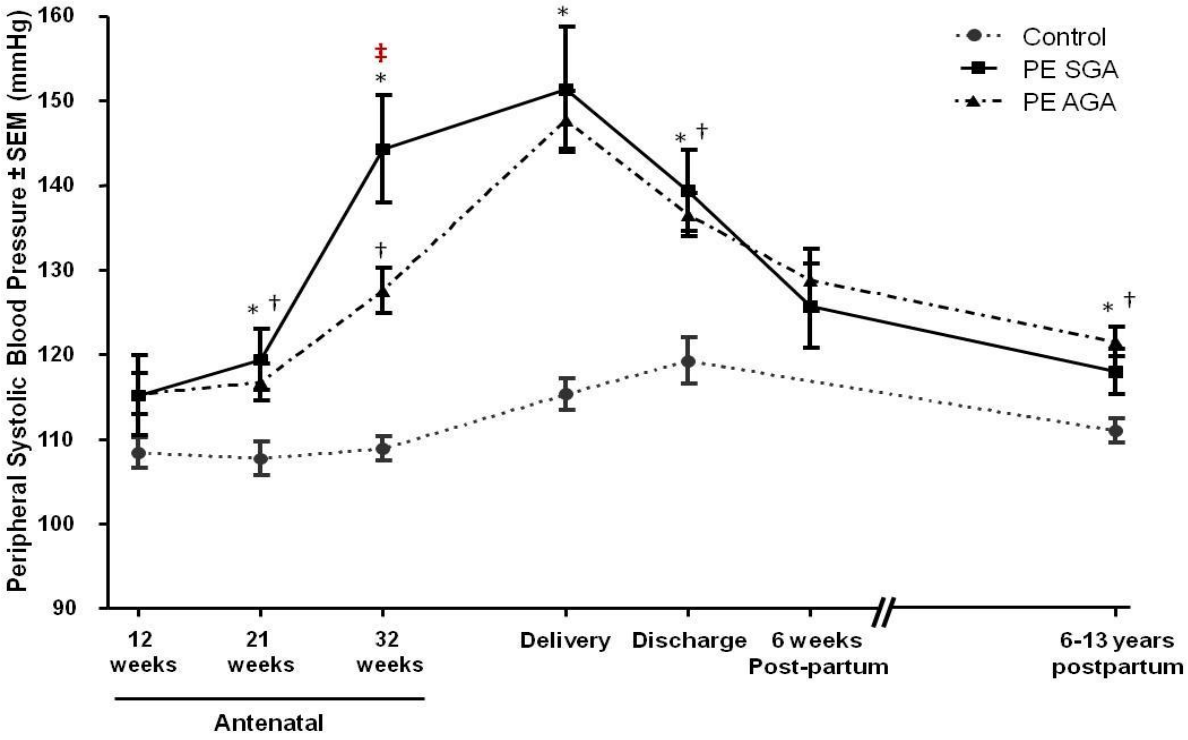


Figure 3-4 Small for Gestational Age Delivery and Maternal Blood Pressure

Blood pressure characteristics in women who had preeclampsia (PE) with a small for gestational age (SGA), compared to appropriate for gestational age (AGA), infant. There were no differences in booking or post-partum blood pressures, in contrast to the differences identified when time of onset of preeclampsia is used to define groups. A difference in blood pressure is noted in the third trimester (‡) which may suggest intra-pregnancy blood pressure differs in the presence of a small for gestational age infant.
(* represents a significant difference between preeclamptic women delivering SGA with controls whereas † represents a significant difference between preeclamptic women delivering AGA compared to controls.)

3.5 Discussion

This is the first study to demonstrate that, compared to women with late-onset preeclampsia or normotensive pregnancies, women who develop early-onset preeclampsia not only have higher blood pressure at antenatal booking and in later life, but also persistently higher blood pressure six weeks after delivery, a greater increase in blood pressure during the subsequent decade relative to booking blood pressure and significantly higher nocturnal blood pressures

in later life. Therefore, early-onset preeclampsia associates with distinct maternal blood pressure patterns.

Although the exact mechanisms linking preeclampsia and cardiovascular disease development remain unknown, both conditions share common biological features.[174] It has been suggested that the long term cardiovascular risk is predominantly determined by risk factors present before pregnancy.[175] Therefore, in order to better understand the association between preeclampsia and cardiovascular disease in the absence of pre-existing risk, all women with prior history of hypertension, diabetes or other classical risk factors were excluded.[1] I show that despite having comparable body size at the first antenatal visit to those with uncomplicated pregnancies, women who went on to develop preeclampsia had a tendency towards higher body mass index, higher fasting lipids and increased insulin resistance measured by homeostatic model later in life, features that may contribute to the future risk of atherosclerosis.

In addition, as early-onset preeclampsia is associated with increased future cardiovascular risk,[13.167] I sought to establish whether these women had a distinct risk profile and studied levels of blood pressure during the perinatal period and at 6-13 years after pregnancy. Not only did women with early-onset preeclampsia have higher blood pressure during pregnancy and at follow-up 6-13 years later, they also had 12-18 mmHg greater increase in blood pressure over this period compared both to mothers who remained normotensive during pregnancy or those with late-onset preeclampsia. Interestingly, those with late-onset disease or normotensive pregnancy had on average smaller changes or even a relative drop in blood pressure later in life. Indeed, a 10 mmHg difference in the middle-aged blood pressure equates to a 20% increase in age-adjusted risk of cardiovascular events[176] and even high

normal blood pressure is associated with increased cardiovascular risk.[133] Therefore the time of onset and severity of preeclampsia appear to predict additional patterns to subsequent postpartum rises in blood pressure than could have been determined by booking blood pressure alone.[175]

Furthermore, by combining blood pressure measures during the perinatal period and later life, it was possible to distinguish between any short-term persistent effects of pregnancy on blood pressure behaviour and longer term variation remote from pregnancy. It has previously been shown that blood pressure levels can take between six months and two years to normalise after delivery in some women.[168] Studies have also demonstrated persistent differences in cardiac and vascular responses during this early post-partum period.[173] Some specifically investigated differences related to early-onset preeclampsia and found increased blood pressure in women with early-onset preeclampsia up to 18 months post-partum.[68.84] This is in keeping with the finding in this study of persistent elevations in blood pressure up to six weeks following delivery in mothers with early-onset preeclampsia.

Furthermore, although formerly preeclamptic women had significantly higher daytime ambulatory blood pressure later in life, only mothers who were diagnosed with preeclampsia before 34 weeks gestation had significantly higher nocturnal blood pressure compared to controls or those with late-onset preeclampsia. Elevated nocturnal blood pressure is closely associated with cardiovascular risk both in normotensive and hypertensive subjects.[177] Previous studies have demonstrated that women with preeclampsia have a reduced drop in nocturnal blood pressure evident as early as the second trimester of pregnancy[137.178] and in some high risk pregnancies the circadian pattern can even reverse.[179]

This study sample may appear small compared to larger population-based studies of mother-offspring pairs[180] but this is primarily due to the meticulous review of maternity records. This was of prime importance to ensure only women who fulfilled appropriate criteria for preeclampsia and had no pre-existing disease relevant to long term outcomes were recruited. The study sample of 90 preeclamptic subjects is of a similar size to other reports of the long term impact of preeclampsia based on population recruitment.[180] In addition, this study succeeded in recruiting a greater proportion of cases with early-onset preeclampsia.[64.68] Furthermore, because of the study design, and the inherent difficulty in recruiting controls, the control group was substantially smaller, but powered to identify clinically relevant blood pressure variation. Although, blood pressure remains within the normal limits in the majority of subjects, it would be of interest to determine whether differences in vascular responses and cardiac hemodynamics can explain the observed unique blood pressure differences.

In summary, whilst women who develop preeclampsia have a greater risk of hypertension and cardiovascular disease later in life, those who develop early-onset preeclampsia had several unique adverse blood pressure characteristics following pregnancy. They were more likely to have elevated blood pressure at six weeks postpartum, a greater increase in blood pressure relative to their booking blood pressure over the subsequent decade and significantly higher nocturnal blood pressure in later life. Furthermore, they were more likely to have been born to a preeclamptic pregnancy themselves. A better understanding of the biological differences that underlie these characteristics may help identify why those with early-onset preeclampsia develop more severe later disease and identify targeted approaches to disease prevention.

Chapter 4 : PREECLAMPSIA AND MATERNAL VASCULAR STRUCTURE AND FUNCTION

4.1 Introduction

In the next part of the study, I investigated the potential vascular biological differences to better understand the mechanisms for future cardiovascular risk and determine whether differences in vascular responses and cardiac hemodynamics may explain the unique blood pressure differences in early-onset preeclampsia. Cardiovascular disease undergoes a gradual progression from subclinical alterations in vascular structure and function to overt disease and organ dysfunction. Impaired vascular responses are a common feature of both preeclampsia and cardiovascular disease[14.30] and several studies have demonstrated increased arterial stiffness,[47] atherosclerosis,[48] endothelial dysfunction[39-40.43] and impaired diastolic function[101.181] in these women during pregnancy. It is plausible that women with preeclampsia have evidence of vascular dysfunction predisposing them to cardiovascular disease development and by targeting these changes one may reduce their future cardiovascular risk. Indeed, there is growing evidence from relatively small studies showing vascular dysfunction.[173] Hence, I sought to establish whether preeclampsia is associated with impaired vascular structure and function remote from the affected pregnancy even in the absence of clinical hypertension.

4.2 Hypotheses and Aims

This stage of the research was aimed to determine whether 6-13 years post-partum formerly preeclamptic women have evidence of:

a. Adverse vascular phenotype.
b. Increased ventricular mass and impaired diastolic function.

c. An imbalance in the circulating angiogenic and anti-angiogenic factors (VEGF, sFLT-1, sEng, TGFβ) and cytokines and adhesion molecules.

4.3 Methods

4.3.1 Study Design and Population

Study design and subject recruitment was described in the detailed methods section 2.1 and represented the same study cohort as reported in Chapter 3. The key measures reported in this chapter are described in detail in sections 2.5, 2.6 and 2.7. Briefly these comprised of:

4.3.2 Vascular Structure

Subjects underwent assessment of aortic stiffness by measuring carotid-femoral pulse wave velocity (SphygmoCor, AtCor Medical, Australia),[182] regional variations in aortic distensibility using cardiac magnetic resonance imaging (1.5 Tesla, Siemens, Sonata),[149] common carotid intima-media thickness using high resolution ultrasound (Philips Sonos 5500),[150] and capillary density by intravital microscopy (Leica).[183]

4.3.3 Vascular Function

Endothelial-mediated vasodilatation of brachial artery was measured by flow mediated dilatation using high resolution ultrasound (Philips Sonos 5500).[154] Endothelial-independent vasodilatation was assessed following administration of 25μg of sublingual glyceryl trinitrate. Subjects then underwent an assessment of cutaneous microvascular function by measuring maximum blood flux, in response to heating,[158] and post-occlusive reactive hyperaemic (PORH)[161] using laser Doppler flowmetry (Moor Instruments).

4.3.4 Cardiac Structure and Function

A 1.5 Tesla cardiovascular magnetic resonance scanner (Siemens, Sonata) was used for accurate and reproducible assessment of ventricular mass and volumes as described in section 2.6. Peak circumferential systolic strain and strain rate were measured using semi-automated quantification with CIM (CimTag2D v.7, Auckland MRI Research Group, New Zealand). Horizontal and vertical long axis cine images were obtained by CMR and analysed using 2D Cardiac Performance Analyis Software (TomTec, Germany) to calculate global longitudinal strain. Diastolic function was assessed using Pulse Wave and Tissue Doppler.

4.3.5 Angiogenesis and Inflammation

Levels of soluble fms-like tyrosine kinase (sFLT-1), endoglin (sEng) and transforming growth factor β1 (TGF-β1) were measured using commercially available ELISA kits (R&D Systems). Circulating levels of vascular endothelial factor (VEGF), e-Selectin, tissue necrosis factor α (TNFα), interleukin 8 (IL8), ICAM and VCAM were measured by multi-analyte profiling using the Luminex platform (Millipore and R&D Systems).

4.3.5 Statistical Analysis

Statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences, Version 17) and GraphPad Prism Version 5 for graphs as described earlier.

4.4 Results

4.4.1 Subject Characteristics

As described in Chapter 3, a total of 140 women, aged 28-50 years, completed the study comprising of 90 formerly preeclamptic women (PE) and 50 controls. The mean time from delivery to follow up was 9.75 years (6-13 years). In order to study the impact of the severity

of preeclampsia on cardiovascular phenotype, women were subdivided into 45 women with early-onset preeclampsia, diagnosed before 34 weeks gestation, and 45 with late-onset preeclampsia, diagnosed after 34 weeks gestation. The baseline characteristics were presented earlier in table 3-2.

4.4.2 Vascular Structure and Function

As women with early-onset preeclampsia had distinct blood pressure both during pregnancy and in later life, I initially sought to determine whether this could be explained by differences in vascular structure and function. Of interest, they had higher PWV (6.99±1.31 vs. 6.50±0.77m/s, P=0.04) and a modest increase in cIMT (0.54±0.07 vs. 0.52±0.05mm, P=0.05) compared to those with uncomplicated pregnancies (Figure 4-1). These differences in aortic stiffness were also evident in the group undergoing cardiovascular magnetic resonance with regional variation in central aortic distensibility, so that women with early-onset preeclampsia had significantly reduced proximal descending aortic distensibility (Table 4-1). In addition, women with early-onset preeclampsia had significant functional (108.28±16.03 vs. 122.87±16.42 mm^{-2}, P=0.005) and structural (114.39±16.72 vs. 126.75±16.35 mm^{-2}, P=0.02) capillary rarefaction.

On the other hand, women with late-onset preeclampsia had significantly higher cIMT compared to controls (0.57±0.09 vs. 0.52±0.05 mm, P=0.002) but no differences in PWV (6.60±0.91 vs. 6.50±0.77 m/s, P=0.59) or functional capillary density (119.74±17.94 vs. 122.87±16.32, P=0.51) (Figure 4-1). However, endothelial function, measured by conduit artery endothelial-dependent vasodilatation and cutaneous microvascular function, was comparable between women exposed to preeclampsia and those with uncomplicated pregnancies (Table 4-1).

Parameters	Early PE	Late PE	Controls	*P Value*		
	(n 45)	(n 45)	(n 50)	All PE vs. Controls	Early PE vs. Controls	Late PE vs. Controls
Peripheral Vascular Function						
Brachial Artery Diameter, mm	3.00 (0.34)	2.90 (0.30)	3.02 (0.33)	0.23	0.73	0.08
Flow Mediated Dilatation, %	7.15 (5.49)	6.73 (3.41)	6.00 (3.32)	0.21	0.23	0.30
Baseline Microvascular Flux, FU	9.64 (6.03)	9.83 (4.31)	9.81 (3.01)	0.14	0.11	0.40
Maximum Blood Flux, FU	173.81 (56.31)	161.76 (44.05)	173.90 (62.15)	0.54	0.99	0.59
Peak PORH, FU	55.98 (33.18)	54.51 (24.75)	55.87 (25.67)	0.90	0.39	0.55
Time to Peak PORH, sec	23.60 (14.77)	24.28 (17.80)	26.98 (15.86)	0.32	0.34	0.29
Central Aortic Distensibility **(x10^{-3})**	n 15	n 16	n 15			
Ascending Aorta	4.79 (1.98)	5.01 (1.21)	5.66 (2.25)	0.24	0.28	0.49
Proximal Descending Aorta	4.72 (1.60)	5.11 (0.89)	6.04 (1.58)	**0.02**	**0.04**	0.11
Distal Descending Aorta	7.53 (2.26)	8.02 (1.75)	8.63 (2.57)	0.28	0.29	0.61
Abdominal Aorta L2	5.41 (2.18)	5.58 (2.36)	6.89 (3.11)	0.13	0.15	0.18

Table 4-1 Maternal Vascular Phenotype

Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported.

FU refers to flux units, PORH to peak post occlusive reactive hyperaemia, and L2 refers to the level of the 2nd lumbar vertebra.

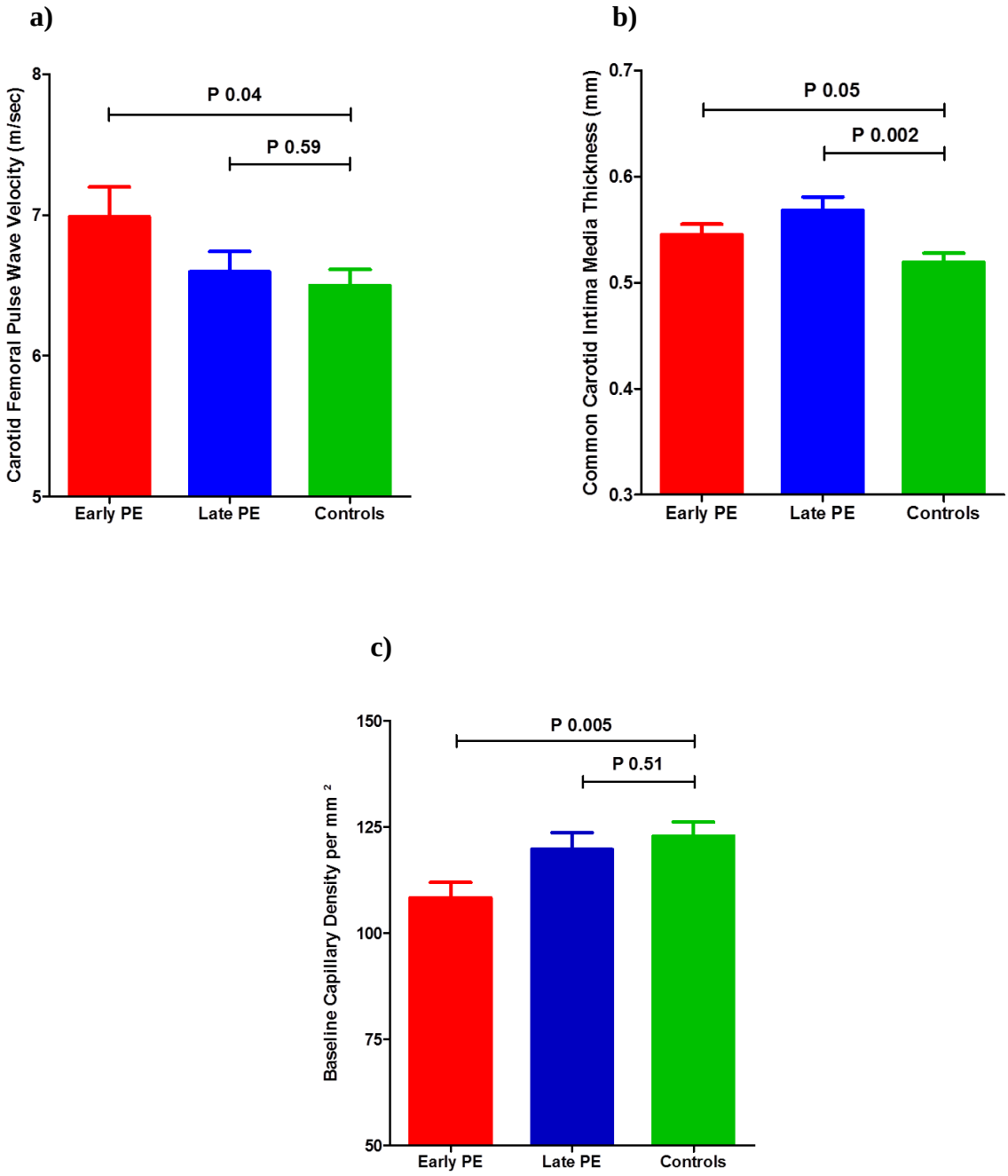


Figure 4-1Maternal Vascular Structure

Changes in arterial structure were proportional to the severity of preeclampsia. Women with early-onset preeclampsia (early PE) had increased arterial stiffness, carotid atherosclerosis and reduced capillary density. In contrast, late-onset preeclamptic women (late PE) had increased carotid intima-media thickness and a tendency to increased aortic stiffness.

4.4.3 Cardiac Structure and Function

After showing distinct blood pressure profile and vascular phenotype in women with early-onset preeclampsia, I investigated the long-term impact on cardiac structure and function on 46 women using echocardiography and CMR. Of these women, 31 were diagnosed with preeclampsia and 15 had uncomplicated pregnancies. There were no differences in blood pressure or metabolic profile between controls who responded and those who did not. However, preeclamptic women participating in the study had higher systolic blood pressure compared to non-responders (P=0.02). Age and body size were comparable between women with preeclampsia and those with uncomplicated pregnancies.

As presented in table 4-2, there were no differences in left ventricular volumes and mass or left atrial volumes between the groups. Nonetheless, women with early-onset preeclampsia had 4% greater ejection fraction compared to controls (69.79±4.65 vs. 65.97±4.81%, P=0.04) and an increase in basal (-17.06±1.67 vs. -14.24±2.98%, P=0.02) and apical (-15.57±2.45 vs. -13.25±2.19%, P=0.02) circumferential strain measured by echocardiography. Lateral E/E' ratio, a measure of LV filling pressure, was significantly elevated when comparing all women diagnosed with preeclampsia to controls (5.21±1.12 vs. 4.40±0.85, P=0.02). This appeared to be most significant in those with late-onset preeclampsia compared to controls (5.92±0.98 vs. 4.40±0.85, P=0.01). Lateral E/E' was positively associated with peripheral (r 0.37, P=0.02) and central (r 0.44, P=0.008) systolic blood pressure.

I then studied the relationship between myocardial function and the observed vascular alterations and their relevance to blood pressure variability. Ejection fraction correlated positively with peripheral and central pulse pressure (r 0.32, P=0.03 and r 0.35, P=0.02). Furthermore, left ventricular filling pressures, measured by average E/E', was directly

correlated with peripheral and central SBP (r 0.39, P=0.01 and r 0.43, P=0.01) and LVMI (r 0.37, P=0.02), and inversely correlated with longitudinal strain (r -0.40, P=0.01). Average E/E' was also positively correlated with cIMT (r0.39, P=0.01). This relationship remained significant even after correction for blood pressure (P<0.02).

Parameters	Early PE (n 15)	Late PE (n 16)	Controls (n 15)	*P Value*		
				All PE vs. Controls	Early PE vs. Controls	Late PE vs. Controls
Cardiac Structure						
Left Ventricular Mass, gram	71.24 (15.52)	68.72 (10.87)	69.36 (14.35)	0.90	0.74	0.89
IVS thickness, mm	0.68 (0.11)	0.73 (0.09)	0.73 (0.09)	0.63	0.28	0.86
LVPW thickness, mm	0.86 (0.19)	0.79 (0.11)	0.81 (0.12)	0.78	0.43	0.72
End Diastolic Volume, ml	130.82 (18.47)	135.28 (18.12)	134.09 (22.66)	0.89	0.68	0.88
End Systolic Volume, ml	39.73 (8.76)	42.68 (9.64)	45.69 (9.83)	0.16	0.10	0.41
LA Diameter, cm	30.20 (5.46)	30.99 (4.17)	30.29 (4.51)	0.68	0.96	0.66
LA volume index, ml/m^2	24.00 (6.06)	23.57 (9.37)	25.04 (8.76)	0.65	0.74	0.68
Cardiac Systolic and Diastolic Function						
Ejection Fraction, %	69.79 (4.65)	68.33 (6.24)	65.97 (4.81)	0.08	**0.04**	0.26
Apical Circumferential Strain	-15.57 (2.45)	-14.03 (4.42)	-13.25 (2.19)	0.14	**0.02**	0.57
Mid LV Circumferential Strain	-14.16 (4.04)	-16.07 (3.71)	-16.79 (2.91)	0.18	0.07	0.59
Basal Circumferential Strain	-17.06 (1.67)	-16.24 (3.51)	-14.24 (2.98)	**0.02**	**0.02**	0.12
E velocity, cm/s	73.56 (7.39)	77.20 (13.78)	70.20 (11.76)	0.15	0.37	0.14
A velocity, cm/sec	55.58 (12.66)	60.58 (11.69)	52.99 (9.80)	0.14	0.55	0.06
E/A Ratio	1.39 (0.29)	1.31 (0.33)	1.34 (0.19)	0.64	0.60	0.76
Deceleration time, msec	224.67 (26.95)	236.88 (25.57)	236.07(34.60)	0.97	0.36	0.94
Septal E/E' Ratio	7.09 (2.61)	6.73 (1.16)	6.28 (1.49)	0.92	0.33	0.36
Lateral E/E', cm/sec	4.95 (1.20)	5.37 (1.07)	4.40 (0.85)	**0.02**	0.19	**0.01**

Table 4-2 Maternal Cardiac Phenotype

IVS refers to inter-ventricular septum, LVPW to left ventricular posterior wall, LA to left atrium, E to peak early filling velocity, A to late diastolic filling velocity, and E' to early left ventricular diastolic velocity. (Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported).

4.4.4 Angiogenesis and Inflammation

Although preeclampsia is characterised by an exaggerated inflammatory reaction and increased anti-angiogenic factors evident from early pregnancy, levels of anti-angiogenic factors were comparable between study groups up to 13 years post-partum. However, women exposed to preeclampsia had significantly higher levels of eSelectin and TNFα compared to controls (Table 4-3).

Parameters	**Early PE**	**Late PE**	**Controls**	**P Value**		
				All PE vs. Controls	**Early PE vs. Controls**	**Late PE vs. Controls**
	(n 45)	**(n 45)**	**(n 50)**			
VEGF, pg/ml	58.06 (37.34)	73.09 (53.79)	54.31 (30.27)	0.10	0.60	**0.05**
Soluble FLT-1, pg/ml	72.65 (13.51)	81.11 (20.87)	79.43 (17.85)	0.43	**0.05**	0.68
Soluble Endoglin, ng/ml	3.72 (0.55)	3.87 (0.89)	3.76 (0.62)	0.78	0.76	0.48
TGF-β1, ng/ml	6.20 (5.82)	8.01 (6.45)	5.51 (4.20)	0.10	0.73	0.06
eSelectin, ng/ml	41.13 (27.81)	31.23 (13.07)	25.52 (8.71)	**<0.001**	**0.001**	**0.02**
TNFα, pg/ml	2.96 (1.94)	2.83 (1.76)	1.81 (1.36)	**0.001**	**0.005**	**0.008**
IL8, pg/ml	8.39 (4.59)	7.48 (3.44)	7.06 (3.52)	0.21	0.12	0.58
ICAM, ng/ml	124.05 (55.42)	112.94 (52.68)	107.57 (29.87)	0.21	0.09	0.56
VCAM, ng/ml	939.30 (315.9)	951.48 (355.86)	872.22 (200.67)	0.12	0.71	0.20

Table 4-3 Inflammatory and Angiogenic Markers 6-13 years after Preeclampsia

Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported.

4.5 Discussion

In this chapter, women who developed early-onset preeclampsia not only had an adverse metabolic profile and higher peripheral and central blood pressures, but also exhibited increased central aortic stiffness, hyperdynamic systolic function and reduced capillary

density whereas mothers with late-onset preeclampsia displayed more generalised changes in carotid intima-media thickness and diastolic function.

These results are consistent with previous studies showing increased arterial stiffness from 7 weeks to 6 years following preeclampsia.[47.67-68.72] The unique differences in arterial stiffness shown in this study may explain the distinct blood pressure features in mothers with early-onset preeclampsia. Arterial stiffness is determined by alterations in vascular structure and function leading to increased wave reflection in the aorta and augmentation of systolic pressure consequently driving further aortic remodelling. Indeed, arterial stiffness predates the development of hypertension and is an independent predictor of cardiovascular risk per-se.[142] Whilst the clinical relevance of 0.5 m/s increase in pulse wave velocity in this age group remains unknown, studies on middle aged adults show that 1 m/s increase in pulse wave velocity is associated with 14-15% higher risk of cardiac events and mortality.

Women developing early-onset preeclampsia also displayed significant changes in the microcirculation with reduced capillary density evident up to 13 years after the affected pregnancy. Whilst Hasan et al showed reduced functional and structural capillary density during pregnancy,[49] this is the first study to demonstrate capillary rarefaction many years after preeclampsia. The mechanism of microvascular rarefaction is unknown. In fact, preeclampsia is characterised by increased circulating levels of soluble FLT-1 and endoglin weeks or months before the onset of preeclampsia.[34-35] Soluble fms-like tyrosine kinase-1 binds to the vascular and placental endothelial growth factors inhibiting eNOS-mediated endothelial proliferation, migration and neovascularization.[184] Soluble endoglin, a co-factor for transformin β factor, increases vascular permeability by inhibiting its function on the vasculature.[35] Therefore, it is plausible excess of these anti-angiogenic factors in pregnancy markedly affects microvascular neovascularisation leading to reduced capillary density long

term. Capillary rarefaction may in turn lead to a gradual increase in peripheral vascular resistance and arterial stiffness.[61] Indeed capillary rarefaction predates clinical hypertension and is associated with increased cardiovascular risk and microvascular angina.[156.185] Microvascular rarefaction also relates to changes in glucose and insulin homeostasis.[186]

Previous studies have demonstrated that women who develop preeclampsia exhibit exaggerated cardiac remodelling during pregnancy, with increased left ventricular mass and diastolic dysfunction, that can persist up to two years postpartum.[98-101.181] Women with early-onset preeclampsia had significantly higher ejection fraction and circumferential strain, as well as increased left ventricular filling pressures in the absence of myocardial hypertrophy. These characteristics suggest early subclinical reduction in ventricular relaxation[187] and hyperdynamic systolic function. These are probably compensatory mechanisms for the increased arterial stiffness and microvascular rarefaction, of particular relevance to premature cardiovascular disease development. Impaired left ventricular diastolic function per-se is also regarded as an early marker of imminent cardiovascular disease,[102] poor cardiovascular outcomes,[103] and is more prevalent in subjects at risk of hypertension.[104]

Pregnancy is well known to be a considerable stress test to the maternal cardiovascular physiology. Women who develop preeclampsia are exposed to an exaggerated inflammatory, metabolic and anti-angiogenic milieu culminating in endothelial injury and activation. Endothelial dysfunction plays a central role in pathogenesis of preeclampsia and has been thought to persist postpartum hence augmenting cardiovascular disease risk. Previous studies have reported impaired conduit artery[64-65.67-68.83.188] and microvascular endothelial function after preeclampsia.[71] Only few studies investigated vascular function in mothers with early-onset preeclampsia and showed impaired endothelial mediated vasodilatation 3-24 months

after preeclampsia.[68.84] However, in the present study, there were no differences in flow mediated dilatation or cutaneous microvascular function up to 13 years postpartum between women with preeclampsia or normotensive pregnancy. This is in fact consistent with results at 6 weeks,[79] 12 weeks[46] and 2 years after delivery from other studies.[78] It is hence plausible the activated endothelium during pregnancy drives cardiac and vascular remodelling that either persist or progress postpartum linking preeclampsia to cardiovascular disease. Indeed, aortic distensibility in part is mediated by endothelial vasoactive substances.[109] It is very probable the endothelium recovers its normal vasoactive function upon resolution of the physiological changes that accompany preeclampsia which can take up to two years after delivery.[168]

As demonstrated in this study, levels of circulating soluble-FLT 1 and endoglin are comparable to controls after delivery in accordance with studies showing return to baseline postpartum.[35.46] Moreover, women who developed preeclampsia had significantly higher levels of TNFα, eSelectin, lipids and insulin compared to women with normotensive pregnancies suggesting an ongoing process of sub-acute inflammation and metabolic derangement remote from the index pregnancy contributing therefore to cardiovascular disease development.

In summary, young women who develop early preeclampsia have a more adverse metabolic, biochemical and cardiovascular phenotype evident up to 13 years following preeclampsia and may need targeted for surveillance and primary preventative intervention. These vascular changes may provide useful intermediate endpoints in future prevention studies.

Chapter 5 : BLOOD PRESSURE AND VASCULAR FUNCTION IN CHILDREN BORN TO EARLY-ONSET PREECLAMPSIA

5.1 Introduction

Offspring born to hypertensive pregnancy have higher blood pressure in childhood[5-9] and increased risk of stroke in later life.[12] The risk to the offspring is greatest in those whose mothers had severe preeclampsia or gestational hypertension.[12] It is suggested the risk to the offspring is mediated through familial genetic variation that determines the long term cardiovascular development of the fetus or intrauterine conditions adversely affecting the development of their vascular system.[96] Indeed, there is extensive evidence of 'vascular programming' during fetal development.[95] Intra-uterine growth restriction and prematurity, two common complications of preeclampsia,[93] have previously been independently linked with hypertension and cardiovascular complications in the offspring[86.94-95] and are characterised by underlying vascular dysfunction per se.[60.85.95.189] Levels of circulating anti-angiogenic factors and asymmetrical dimethylarginine have previously been show to be elevated in the fetal circulation of those born to preeclampsia.[87-88] These are likely to adversely affect fetal vascular development that may predispose to offspring cardiovascular disease.

Therefore, I hypothesised that offspring born to mothers with preeclampsia have evidence of vascular dysfunction and that it is most likely to be apparent in young children of mothers with severe preeclampsia diagnosed before 34 weeks gestation.

5.2 Hypotheses and Aims

The first stage of the research was aimed to determine whether young children born to preeclampsia have:

a. Increased blood pressure
b. Impaired macrovascular and microvascular function

And whether early-onset preeclampsia is associated with more adverse phenotype

5.3 Methods

Study design and subject recruitment was described in the detailed methods section 2.1.2. Study methods are described in sections 2.2 to 2.5. The key measurements were:

5.3.1 Anthropometric and Blood Pressure Measurements

Offspring attended the research clinic in the morning following an overnight fast and underwent measurements of body size, peripheral blood pressure using appropriate cuff size for arm circumference and central blood pressure using applanation tonometry. Fasting blood samples were drawn from all willing participants following the application of topical local anaesthetic cream (Emla 5%) for later analysis of metabolic and angiogenic factors. Perinatal clinical details were obtained from maternity records.

5.3.3 Vascular Measurements

In a temperature controlled room, children underwent measurements of brachial artery flow-mediated dilatation (FMD) and common carotid intima-media thickness using high resolution ultrasound. Aortic stiffness was measured by carotid femoral pulse wave velocity (PWV) by (applanation tonometry), microvascular function by laser Doppler flowmetry and capillary density using intravital microscopy.

5.3.4 Statistical Analysis

The study was powered based on study of similar aged subjects with an average FMD of 5.3±4.0%.[125] In order to show a difference of 0.75SD between offspring born to preeclampsia and controls with 5% alpha and 80% power, a sample size of 16 subjects was required in each group. Results are presented as Mean (Standard Deviation). Two tailed P-values of <0.05 were considered statistically significant.

5.4 Results

5.4.1 Subject Characteristics

A total of 47 offspring with a mean age of 9.38±1.58 years took part in the study, of whom 14 were born to a normotensive pregnancy and 33 were born to preeclampsia (15 born to early-onset preeclampsia and 18 to late-onset disease). There were no differences in birth weight (2822.32±974.63 vs. 2968.80±842.60g, P=0.36), overall gestational age at delivery (37.02±3.52 vs. 37.66±2.97 weeks, P=0.26) or proportion of small-for-gestational-age offspring (21.3% vs. 22.6%, P=0.84) between participants and non-participants.

As expected, offspring of early-onset preeclampsia were more likely to be born preterm, before 37 weeks gestation, (86.67% vs. 22.22%, P<0.001) and small for gestational age (46.67% vs. 16.67%, P=0.06) compared to late-onset preeclampsia. Compared to children born to normotensive pregnancy, offspring of preeclampsia had comparable age and anthropometric measures including height, head circumference and BMI at study visit with no differences in metabolic or inflammatory markers between groups (Table 5-1). Of interest, those born to late-onset preeclampsia appeared to have smaller waist and left arm circumference compared to controls.

Parameters of Offspring Born to	Early PET (n 15)	Late PET (n 18)	Controls (n 14)	*P Value*		
				All PE vs. Controls	Early PE vs. Controls	Late PE vs. Controls
Birth Characteristics						
Birth Gestation, weeks	33.07 (2.89)	37.67 (1.41)	40.43 (1.02)	**<0.001**	**<0.001**	**<0.001**
Birth Weight, g	1819.20 (817.69)	2978.39 (542.01)	3696.43 (489.23)	**<0.001**	**<0.001**	**<0.001**
Born SGA n (%)	7 (46.7)	3 (16.7)	0	**0.02**	**0.003**	0.11
Characteristics at Enrolment						
Age, years	9.13 (1.77)	9.17 (1.65)	9.93 (1.21)	0.13	0.17	0.16
Height, m	1.39 (0.11)	1.40 (0.13)	1.44 (0.11)	0.23	0.23	0.35
Weight, Kg	35.64 (11.30)	33.79 (8.68)	39.70 (11.59)	0.13	0.35	0.11
BMI, kg/m^2	18.01 (3.26)	16.96 (2.10)	18.73 (3.16)	0.16	0.55	0.07
Waist circumference, cm	65.36 (11.45)	59.00 (8.06)	66.07 (10.75)	0.20	0.87	**0.04**
Hip Circumference, cm	79.93 (12.52)	74.28 (9.16)	78.19 (9.74)	0.67	0.69	0.25
Waist/Hip ratio	0.82 (0.07)	0.79 (0.07)	0.84 (0.06)	0.07	0.31	**0.04**
Arm circumference, cm	21.07 (3.41)	20.14 (2.47)	22.42 (3.17)	0.06	0.30	**0.03**
Head circumference, cm	53.72 (2.20)	53.88 (1.89)	53.68 (1.79)	0.88	0.96	0.82
Heart Rate, bpm	70.67 (7.94)	72.25 (9.12)	70.57 (8.51)	0.73	0.98	0.60
Peripheral SBP, mmHg	108.40 (7.90)†	99.58 (7.15)	102.64 (8.14)	0.73	**0.06**	0.27
Peripheral DBP, mmHg	67.30 (7.28)	62.31 (6.87)	62.82 (6.42)	0.44	0.09	0.83
Peripheral MAP, mmHg	81.00 (6.52)	74.73 (5.87)	76.10 (6.34)	0.49	**0.05**	0.53
Peripheral PP, mmHg	41.10 (7.82)†	37.28 (7.95)	39.82 (6.49)	0.74	0.64	0.34
Central SBP, mmHg	96.27 (7.30)†	88.39 (7.57)	89.14 (7.08)	0.27	**0.01**	0.78
Central DBP, mmHg	69.93 (7.91)	65.17 (7.41)	64.64 (6.11)	0.26	0.06	0.83
Central MAP, mmHg	78.71 (7.14)†	72.91 (7.02)	72.81 (5.58)	0.23	**0.02**	0.97

Central PP, mmHg	26.33 (6.18)	23.22 (5.39)	24.50 (6.85)	0.95	0.46	0.56
Biochemical Profile	**(n 9)**	**(n 13)**	**(n 11)**			
Total Cholesterol, mmol/L	4.29 (0.59)	4.24 (0.81)	4.37 (0.43)	0.63	0.72	0.61
HDL Cholesterol, mmol/L	1.46 (0.42)	1.51 (0.32)	1.58 (0.24)	0.43	0.41	0.54
Triglycerides, mmol/L	0.85 (0.51)	0.58 (0.22)	0.87 (0.46)	0.25	0.94	0.06
LDL Cholesterol, mmol/L	2.44 (0.47)	2.46 (0.55)	2.39 (0.36)	0.72	0.80	0.72
Total:HDL Ratio, mmol/L	3.17 (1.05)	2.85 (0.39)	2.82 (0.50)	0.51	0.34	0.86
Glucose, mmol/L	4.57 (0.31)	4.68 (0.37)	4.61 (0.23)	0.85	0.73	0.60
Insulin, mU/L	5.66 (1.94)	4.67 (1.91)	6.66 (4.70)	0.31	0.56	0.18
HOMA_IR	1.15 (0.40)	0.99 (0.44)	1.40 (1.07)	0.32	0.52	0.22
eSelectin(ng/ml)	39.69 (19.30)	46.34 (13.97)	49.70 (20.61)	0.42	0.34	0.67

Table 5-1 Characteristics of Offspring born at Index Pregnancy

Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported.

SGA refers to small for gestational age, BMI refers to body mass index, SBP refers to systolic blood pressure, DBP refers to diastolic blood pressure, MAP refers to mean arterial pressure, PP refers to pulse pressure.

(† Represents a significant difference between offspring born to women developing early-onset preeclampsia compared to those born to late-onset preeclampsia)

5.4.2 Offspring Blood Pressure

Both peripheral and central systolic blood pressures were significantly higher in offspring of early-onset preeclampsia compared to those born following late-onset disease (108.40±7.90 vs. 99.58 ±7.15mmHg, P=0.002 and 96.27±7.30 vs. 88.39±7.57mmHg, P=0.005, Figure 5-1). The association between peripheral and central blood pressure and the severity of preeclampsia remains significant even after correcting for delivery of small for gestational age offspring (r 0.303, P=0.04 and r 0.34, P=0.02).

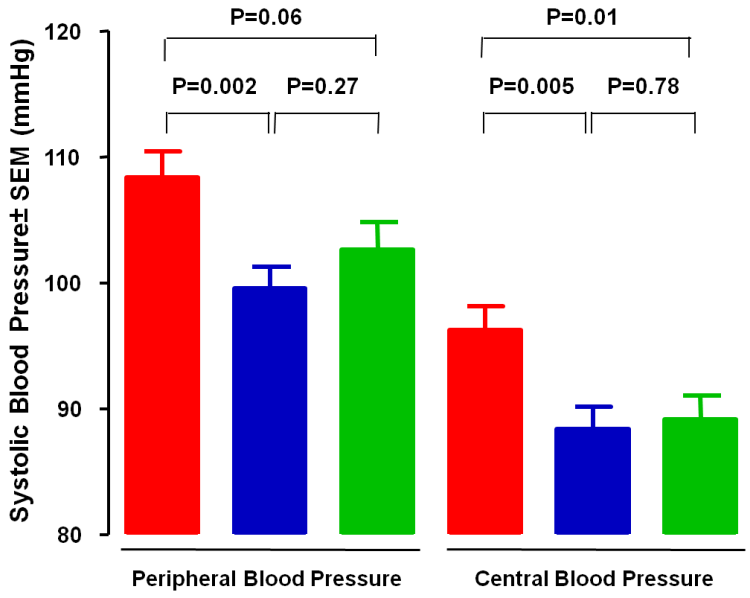


Figure 5-1 Offspring Blood Pressure

Offspring born to mothers diagnosed with early-onset preeclampsia (red bars) have around 6mmHg higher peripheral and central blood pressure compared both to those born late-onset preeclampsia (blue bars) or normotensive pregnancy (green bars). (The bar graph represents mean SBP±SE).

5.4.3 Vascular Measurements

Offspring born to preeclampsia had comparable levels of arterial stiffness, carotid intima-media thickness, brachial artery diameter at rest and endothelial mediated dilatation in response to shear stress, as well as microvascular function (Table 5-2). Unfortunately, it was difficult to assess capillary density in children as intravital microscopy was not well tolerated and movement artifacts significantly affected the measurements.

Parameters of Offspring Born to	**Early PE**	**Late PE**	**Controls**	***P Value***		
	(n 15)	**(n 18)**	**(n 14)**	**All PE vs. Controls**	**Early PE vs. Controls**	**Late PE vs. Controls**
Pulse Wave Velocity, m/sec	4.68 (0.34)	4.75 (0.56)	4.65 (0.48)	0.67	0.87	0.62
Common Carotid Artery Intima-media Thickness, mm	0.45 (0.02)	0.46 (0.05)	0.46 (0.04)	0.81	0.57	0.99
Brachial Diameter, mm	2.59 (0.39)	2.66 (0.21)	2.72 (0.20)	0.34	0.30	0.47
Flow Mediated Dilatation, %	4.59 (4.83)	5.14 (4.40)	5.14 (4.54)	0.87	0.76	1.00
Maximum Blood Flux, FU	141.93 (39.42)	163.30 (38.20)	161.77 (51.76)	0.59	0.28	0.93
Mean Baseline Flux, FU	10.68 (4.14)	10.40 (4.72)	10.19 (4.67)	0.82	0.78	0.90
Peak PORH, FU	52.15 (24.09)	49.81 (21.15)	51.62 (31.17)	0.92	0.96	0.85
Fold Increase in PORH, FU	3.88 (1.00)	4.16 (1.82)	4.04 (1.45)	0.99	0.74	0.85
Time to PORH, FU	25.44 (15.43)	30.52 (18.39)	27.79 (13.38)	0.92	0.68	0.65

Table 5-2 Offspring Cardiovascular Phenotype

Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported.
FU refers to flux units and PORH refers to post occlusive reactive hyperaemia.

In order to also study the impact of birth weight on cardiovascular phenotype, the group of children born to preeclampsia were divided into those delivered small for gestational age (SGA) or appropriate for gestation (AGA). There were no significant differences in blood pressure, vascular structure or function between SGA and AGA offspring or with controls. There were also no significant differences between subjects when offspring of preeclampsia were divided into those delivered at term or preterm.

5.5 Discussion

Offspring whose mothers developed preeclampsia early in pregnancy had 6mmHg higher peripheral and central systolic blood pressure at mean age of 9.38±1.58 years in the absence of significant differences in body size or vascular phenotype. In contrast, blood pressure measures in offspring born to late-onset preeclampsia did not differ from controls. Hence, early-onset preeclampsia appears to also be associated with distinct blood pressure patterns in the offspring.

Previous studies have reported increased blood pressure in children and young adults born to a pregnancy complicated by preeclampsia.[10.190] Nonetheless, the majority of studies have not necessarily grouped children according to the time of onset of preeclampsia. Since long term cardiovascular risk associated with preeclampsia in mothers and offspring is greater with more severe forms of preeclampsia; I tested the hypothesis that early-onset disease, often associated with preterm delivery or birth of a small infant, may lead to greater blood pressure differences and an adverse vascular phenotype in offspring. Of interest, in this study, the only differences in blood pressure during childhood were present in those born to early-onset disease with a 6mmHg higher peripheral and central blood pressure. Many in this group were also born preterm and, some, small for gestational age, factors which are linked with higher blood pressure during childhood. However, further analysis showed no relationship between blood pressure, gestational age at delivery or birth weight and no differences between controls and those either born small for gestational age or preterm.

There is paucity of studies that investigated vascular function in children after preeclampsia. In this study, there were no differences in vascular structure or function between controls and children of preeclamptic mothers. Indeed, These findings are consistent with a recent large

population study in children of comparable age which suggests that vascular dysfunction may not be evident at such an early age.[191] In a different study, endothelial dysfunction was only evident in mothers and children when the offspring was born small for gestational age.[192] This observation suggested that severity of placental pathology may be relevant to later outcomes. Nonetheless, this combination did not determine post-partum blood pressure and metabolic parameters in the children in this study. Fetal growth restriction may be a better identifier of offspring exposed to severe placental pathology than small-for-gestation. However, it was not possible from the maternal records to accurately identify fetal growth restricted infants. Furthermore, offspring delivered at high altitude to mothers with preeclampsia were also found to have evidence of impaired endothelial function and pulmonary hypertension compared to their siblings or to other subjects born to a normotensive pregnancy in the absence of increased peripheral blood pressure.[193] The authors speculated that epigenetic alterations *in-utero* induced by preeclampsia accounted for the observed vascular dysfunction.

Of interest, mothers who experienced early-onset disease were more likely to have been born to early-onset preeclampsia themselves. This observation raises the possibility that blood pressure variation, programmed genetically or in-utero, may be relevant to familial predisposition to preeclampsia.

In summary, young children born to early-onset preeclampsia have 6mmHg higher blood pressure which predates changes in vascular phenotype and in the absence of significant differences in body size, metabolic or inflammatory markers.

Chapter 6 : BLOOD PRESSURE AND VASCULAR FUNCTION IN PRETERM OFFSPRING BORN TO PREECLAMPSIA

6.1 Introduction

Offspring born to hypertensive pregnancy have higher blood pressure in childhood[5-9] and increased risk of stroke in later life.[12] The risk to the offspring is greatest in those whose mothers had severe preeclampsia or gestational hypertension.[12] It is postulated the risk to the offspring is mediated through intrauterine conditions adversely affecting the development of their vascular system.[88.96] Indeed, prematurity and growth restriction common complications of preeclampsia[93] have been independently linked with hypertension and cardiovascular complications in the offspring[86.94-95] and are characterised by underlying vascular dysfunction [60.85.95.189].

In the children study, young offspring born to mothers with early-onset preeclampsia had significantly higher blood pressure at a mean age of 9.38 years. This seemed to predate changes in vascular structure or function. Indeed, atherosclerosis has a long pre-clinical phase that starts earlier in life and is characterised by changes in vascular structure and function that progress into advanced lesions in adult life.[194] Therefore, I hypothesised that vascular dysfunction is most likely to be apparent later in life, in young adulthood, as a result of earlier onset of preeclampsia or gestational hypertension resulting in preterm delivery.

In a twenty year prospective follow up study of a cohort born preterm, I initially compared the vascular phenotype to that of young adults born at term to uncomplicated pregnancies. I then sought to determine whether the vascular phenotype of young adults born preterm to

pregnancies complicated by maternal hypertension was distinct from that associated with prematurity when the mother did not develop hypertension.

6.2 Hypotheses and Aims

The aim of this study was to determine whether young adults born preterm to preeclampsia or gestational hypertension have:

c. Greater blood pressure in adulthood.
d. Impaired endothelial function and a distinct vascular phenotype to offspring born preterm to normotensive pregnancy.

6.3 Methods

Study design and subject recruitment was described in the detailed methods section 2.1.3. Study methods are described in sections 2.2 to 2.5. Briefly, the key measurements for this study were:

6.3.1 Anthropometric Measurements and Blood pressure

The young adults attended the department in the morning following an overnight fast and underwent measurements of body size. Three measurements of peripheral blood pressure were recorded after 10 minutes of supine rest for each participant with an OMRON automatic digital monitor (HEM-705CP, OMRON, Japan). Central aortic blood pressures were derived from applanation tonometry (SphygmoCor System-Atcor Medical, Sydney, Australia).

6.3.3 Vascular Measurements

Subjects underwent measurement of brachial artery flow-mediated dilatation (FMD) and nitrate-mediated dilatation (NMD) in response to administration of 400µg of glyceryl trinitrate as well

as common carotid intima-media thickness using high resolution ultrasound. Aortic stiffness was measured by carotid femoral pulse wave velocity (PWV) using applanation tonometry.

6.3.4 Statistical Analysis

The study was powered based on 20 offspring of hypertensive pregnancies and 50 preterm offspring of normotensive pregnancy to have 90% power at P=0.05 to identify a 0.85SD difference in vascular measures.[129-130] Post hoc analysis demonstrated that in the final cohort differences of 0.8SD in carotid intima-media thickness, 1.9SD in central systolic blood pressure, 0.57SD in flow mediated dilatation and 0.58SD in pulse wave velocity were identified. As smoking has acute effects on functional vascular measures,[59] active smokers were excluded from analysis of associations with pulse wave velocity and flow-mediated dilatation. Spearman bivariate correlations were performed to identify relations between blood pressure, cardiovascular measures and other characteristics. Results are presented as Mean (Standard Deviation). Two tailed P-values of <0.05 were considered statistically significant.

6.4 Results

6.4.1 Subject Characteristics

Preeclampsia accounted for 85% of cases born to a hypertensive pregnancy. Preterm offspring born to a hypertensive or normotensive pregnancy did not differ in the length of gestation (31.05±2.34 vs. 29.96±2.5weeks, P=0.1), birth weight (1293.9±315.38 vs. 1306.9±267.44g, P=0.86) or the proportion of those born small for gestational age (37 vs. 23%, P=0.25). Maternal smoking was reported in 18% of the preterm hypertensive pregnancies and 46% of the preterm normotensive pregnancies (P=0.06). Antenatal steroids were administered in 16% of the preterm hypertensive pregnancies and 19% of the preterm

normotensive pregnancies (P=0.74). Offspring born preterm to a pregnancy complicated by hypertension were shorter than those delivered at term but body mass index and waist-hip ratio were comparable between the three groups (Table 6-1). Subjects born preterm, whether to hypertensive or normotensive pregnancy, had significantly higher fasting plasma glucose, total cholesterol and low-density lipoprotein cholesterol than those born at term. However, fasting insulin and high density lipoprotein levels were comparable between groups (Table 6-1). There were no differences in body size, lipid profile or glucose levels between subjects born preterm to a noromotensive or a hypertensive pregnancy.

Variables	Preterm Offspring of		Term Controls	P Value (All Preterm vs. Term Offspring)	P Value Preterm HP vs. Controls	P Value Preterm NP vs. Controls
	Hypertensive Pregnancy n 19	Normotensive Pregnancy n 52	n 38			
Male Gender, n (%)	8 (42)	25 (48)	20 (53)	0.54	0.45	0.67
Age in years (range)	24.71 (23-26)	24.48 (22-26)	24.46 (20-30)	0.87	0.79	0.97
Height, m	1.70 (0.11)	1.69 (0.09)	1.75 (0.11)	**0.004**	0.09	**0.004**
Weight, kg	72.0 (18.56)	69.48 (12.92)	71.31 (12.0)	0.68	0.89	0.50
BMI, kg/m^2	24.64 (4.64)	24.3 (4.2)	23.1 (2.55)	0.10	0.19	0.10
Waist to hip ratio	0.79 (0.09)	0.82 (0.08)	0.76 (0.13)	**0.04**	0.33	**0.03**
Number of smokers (%)	4 (21)	10 (19)	5 (13)	0.39	0.44	0.45
Total cholesterol, mmol/l	4.7 (0.62)	4.87 (1.06)	3.87 (0.75)	**0.001**	**0.004**	**0.001**
HDL cholesterol, mmol/l	1.6 (0.43)	1.58 (0.41)	1.46 (0.33)	0.42	0.34	0.31
LDL cholesterol, mmol/l	2.65 (0.46)	2.78 (0.9)	2.0 (0.54)	**<0.001**	**0.002**	**0.002**
Triglycerides, mmol/l	0.99 (0.5)	1.12 (0.88)	0.89 (0.35)	0.44	0.51	0.31
Glucose, mmol/l	5.04 (0.39)	5.08 (0.46)	4.57 (0.3)	**<0.001**	**0.001**	**<0.001**
Insulin, pmol/l	64.89 (37.63)	69.32 (47.87)	53.27 (32.82)	0.17	0.36	0.23

Table 6-1 Baseline Characteristics of Term and Preterm Offspring

Data are presented as Mean (SD). Two tailed unpaired student t Test or χ^2 P values reported.

HP refers to hypertensive pregnancy, NP refers to normotensive pregnancy, BMI refers to body mass index, HDL refers to high density lipoprotein, LDL refers to low density lipoprotein.

6.4.2 Peripheral and Central Blood Pressure

Preterm subjects from both preterm normotensive and hypertensive pregnancies had significantly higher peripheral and central blood pressures than term controls (Table 6-2). There was a significant difference in peripheral diastolic and mean arterial blood pressures (P=0.0001 for both groups) with a similar pattern for systolic pressure (P=0.06 for hypertensive group and P=0.006 for normotensive group). Based on estimates of central blood pressure there were consistent differences in systolic, mean arterial pressure and pulse pressure for both groups (P=0.0001) compared to term controls. The absolute difference in central systolic blood pressure by birth history was 9mmHg.

I then sought to determine whether the observed differences were merely a reflection of the effects of being born small for gestational age. So, I compared blood pressure between young fadults born preterm small for gestational age and those born preterm appropriate for gestation and found them to be similar (P=0.65). Furthermore, there was no correlation between blood pressure in early adulthood and birth weight (for SBP r=-0.26, P=0.83).

Variables	Preterm Offspring of		Term Controls	P Value (All Preterm vs. Term Offspring)	P Value Preterm HP vs. Controls	P Value Preterm NP vs. Controls
	Hypertensive Pregnancy n 19	Normotensive Pregnancy n 52	n 38			
Peripheral SBP, mmHg	120.21 (11.84)	120.66 (10.35)	114.0 (11.01)	**0.005**	0.06	**0.006**
Peripheral DBP, mmHg	71.79 (7.37)	71.61 (6.52)	66.12 (7.05)	**<0.001**	**0.008**	**<0.001**
Peripheral MAP, mmHg	87.93 (7.61)	88.07 (6.90)	82.07 (7.66)	**<0.001**	**0.01**	**<0.001**
Peripheral PP, mmHg	48.42 (10.62)	48.88 (8.49)	47.88 (8.23)	0.64	0.84	0.59
Central SBP, mmHg	115.96 (10.86)	115.78 (10.55)	96.71 (9.86)	**<0.001**	**<0.001**	**<0.001**
Central DBP, mmHg	69.4 (6.28)	68.48 (9.79)	66 (7.36)	0.20	0.13	0.30
Central MAP, mmHg	84.92 (7.00)	84.13 (8.90)	76.24 (7.96)	**<0.001**	**0.001**	**0.001**
Central PP, mmHg	46.56 (8.63)	46.94 (10.27)	30.71 (4.82)	**<0.001**	**<0.001**	**<0.001**

Table 6-2 Haemodynamic Characteristics of the Young Offspring

Data are presented as Mean (SD). Two tailed unpaired student t Test P values reported.

SBP refers to systolic blood pressure, DBP refers to diastolic blood pressure, MAP refers to mean arterial pressure, PP refers to pulse pressure.

6.4.3 Vascular endothelial function

I then investigated the vascular phenotype of term and preterm offspring and found that offspring born to hypertensive pregnancies had 30% lower endothelial mediated vasodilatation compared to preterm offspring of normotensive pregnancy (4.25±4.02 vs. 6.79±4.38%, P=0.05) (Figure 6-1, panel a) who in turn had similar levels of flow-mediated dilatation to term controls (6.79±4.38 vs. 6.87±4.88%, P=0.94).

6.4.4 Subclinical Atherosclerosis

Carotid intima-media thickness, a robust measure of subclinical atherosclerosis, also differed between the groups. As presented in figure 6-1, panel b; offspring born to hypertensive pregnancy had significantly higher common carotid intima-media thickness than those born preterm to a normotensive gestation (0.52±0.04 vs. 0.48±0.06mm, P=0.01). The difference was even greater when offspring of hypertensive pregnancy were compared to term born offspring to an uncomplicated pregnancy (0.52±0.04 vs. 0.36±0.06mm, P=0.002).

6.4.5 Arterial stiffness

I then sought to determine whether arterial stiffness differed between subjects based on their maternal pathology during pregnancy. I found that overall preterm offspring born to normotensive pregnancies had significantly higher aortic stiffness compared to term controls (5.92±0.84 vs. 5.56±1.09m/sec, P=0.03). However, pulse wave velocity was similar in preterm subjects born to hypertensive pregnancies compared to term controls (5.42±0.73 vs. 5.56±1.09m/sec, P=0.97) but was significantly lower than those born preterm to normotensive pregnancies (5.42±0.73 vs. 5.92±0.84m/sec, P=0.04) (Figure 6-1, panel c). This suggests the increased blood pressure in offspring of hypertensive pregnancy is not related to aortic stiffness.

I then studied the relationship between blood pressure and vascular parameters. Peripheral systolic blood pressure was significantly correlated with pulse wave velocity (r 0.308, P=0.008) and flow-mediated dilatation (r -0.198, P=0.04).

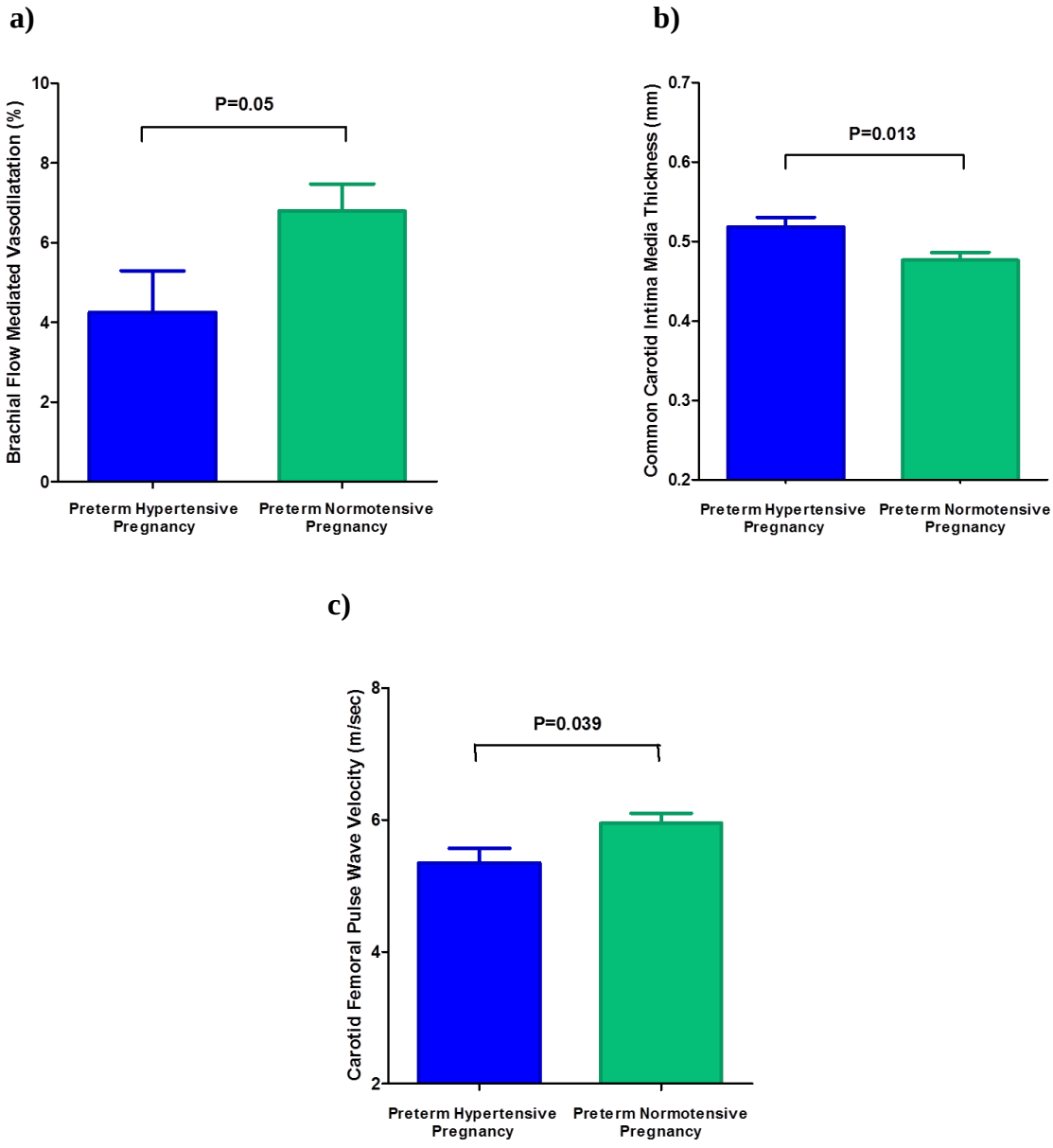


Figure 6-1 Vascular Phenotype following Hypertensive Pregnancy

Offspring born preterm to preeclampsia or gestational hypertension have impaired endothelial function and increased carotid intima-media thickness whereas preterm offspring of normotensive gestation have increased arterial stiffness.

6.5 Discussion

Young adults born to severe hypertensive pregnancy not only displayed 5-10mmHg higher blood pressure but also exhibited an adverse vascular phenotype in their twenties. However, the vascular phenotype also differed at this age and was dependent upon the underlying pregnancy-related pathology. Preterm offspring of hypertensive pregnancies had evidence of reduced endothelial function and greater subclinical atherosclerosis. Whereas, offspring born preterm to normotensive pregnancies had a predominant finding of increased aortic stiffness but reduced carotid intima-media thickness and normal endothelial responses.

The finding of 9mmHg higher systolic blood pressure in these offspring is higher than the observed difference in children reported in the previous chapter which may partly be explained by the premature birth and age related increase in blood pressure in this cohort. Previously published studies have shown similar results in offspring born to preeclampsia at different ages.[5-9.195] Although all the subjects were normotensive, the differences in blood pressure represent a substantial variation of potential clinical relevance particularly as blood pressure tracks through life and relates directly to increased age-adjusted risk of cardiovascular events.[176]

A defining feature of preeclampsia is endothelial dysfunction in the mother and endothelial dysfunction has also been reported in mothers with gestational hypertension. The degree of maternal endothelial dysfunction is proportional to the severity of the hypertensive condition being most severe in pregnancies that result in premature delivery.[39-40.43] There is substantial evidence of 'vascular programming' during fetal development,[95] in particular endothelial responses,[85] and arterial stiffness. [86]

Although younger children born to preeclampsia did not appear to exhibit significant differences in vascular function in their first decade of life, adults born preterm to a hypertensive pregnancy displayed a 30% reduction in endothelial function in their twenties. This seems to relate primarily to exposure to hypertensive pregnancy rather than premature delivery as offspring born preterm to normotensive pregnancy had comparable endothelial responses to controls. In addition, premature infants have been shown to have preserved endothelial function despite being small for gestational age.[196-197] This finding may relate to a heritable predisposition to endothelial dysfunction in both mother and child or occurs in the offspring in response to the same placenta-related factors that influence the mother. However, it appears that endothelial dysfunction develops gradually and only becomes evident over many years. Indeed, impaired endothelial function is an important feature of established hypertension[198] and may predate the development of clinical disease. Studies have shown that reduced nitric oxide availability[199] and increased endothelial derived constrictor factors[198] secondary to endothelial dysfunction or raised endogenous inhibitors of nitric oxide synthase[200] play a role in the pathogenesis of hypertension even in children. There is great interest in better understanding the mechanisms by which endothelial dysfunction predisposes to or promotes early cardiovascular remodelling and disease development.[194]

Carotid intima-media thickness is also a well-established, independent predictor of future cardiovascular risk in asymptomatic adults. In a meta-analysis, Lorenz and colleagues reported that an absolute difference in intima-media thickness of 0.1mm was associated with a relative risk of myocardial infarction and stroke of 1.15 and 1.18 respectively in adults over the age of 40 years.[63] Carotid wall thickening has previously been demonstrated in children and adults with cardiovascular risk factors such as smoking[201-202] and hypercholesterolaemia.[203-204] This is the first study to my knowledge to show that preterm

offspring of hypertensive pregnancies have on average a 0.15mm greater carotid wall thickness compared to term controls. The levels of thickness in the term controls were equivalent to those previously reported in healthy young adults[129] and the differences may in part be determined by the prematurity and higher levels of cardiovascular risk factors such as blood pressure and lipids, which are well known to relate to carotid intima-media thickness in young adult life. However, there was also around 0.05mm difference between normotensive and hypertensive preterm offspring in whom levels of risk factors were equivalent. This observation is novel and the increase in carotid wall thickness is equivalent to that reported in young adults with a family history of cardiovascular disease[129-130] and four times that observed in young adult smokers.[202] Furthermore, the difference may also be accounted for by the independent association between endothelial dysfunction and progression of carotid wall thickness.[55] Alternatively, the findings may be a manifestation of the *in-utero* developmental vascular changes or the vascular phenotype associated with a family history of hypertensive pregnancy.

In the present study, there was a similar level of flow mediated dilatation in preterm infants of normotensive pregnancies compared to full term controls despite differences in blood pressure and other cardiovascular risk factors. However, the predominant finding in preterm offspring of normotensive pregnancies is of increased arterial stiffness. Arterial stiffness is a composite measure of vascular tone, in part determined by endothelial function, and vascular structure. As endothelial function did not differ, the increased arterial stiffness may be accounted for by inadequate structural vascular development and differentiation of the central arteries in premature infants with insufficient elastin deposition in central arteries predisposing to abnormalities in aortic elasticity.[205]

Of interest, there was a significantly higher proportion of reported smoking in women who had a premature delivery with a normotensive pregnancy than those with a hypertensive pregnancy. This difference is consistent with increased prematurity in smoking mothers and generally lower rates of smoking in mothers with preeclampsia. However, maternal smoking was not associated with vascular parameters in this cohort and did not account for the differences in measures between groups.

In summary, offspring born preterm to hypertensive pregnancy had significantly higher blood pressure in young adult life and displayed evidence of endothelial dysfunction and increased intima-media thickness in their twenties. Therefore, preeclampsia and gestational hypertension represent clinical scenarios where cardiovascular risk for the offspring is known from birth and there may be opportunities to start prevention much earlier in life.[97]

Chapter 7 : IMPAIRED ENDOTHELIAL RESPONSES AND SUBCINICAL VARIATION IN CARDIOVASCULAR PHENOTYPE

7.1 Introduction

Preterm offspring of hypertensive pregnancies have reduced endothelial function and greater subclinical atherosclerosis in their twenties as demonstrated earlier in this thesis.[11] Reduced endothelial responses have previously been described in young people free of overt cardiovascular disease.[58.180] This has also been reported in subjects with novel risk factors for hypertension such as family history of hypertension[206-207] and low birth weight[60.97.208] or exposure to a hypertensive pregnancy.[11.193] It raises the possibility that reduced endothelial responses in a young people may not only be a sensitive early marker of underlying 'subclinical' disease development but also a plays a crucial role in cardiovascular remodelling and disease progression.

It is plausible impaired endothelial responses reflect endothelial injury as a consequence of 'subclinical' risk exposure in the first decades of life and that the reduction in nitric oxide bioavailability contributes to early disease development through its impact on cardiac and vascular remodelling.[109.112] Indeed, endothelial dysfunction is associated with progression of carotid intima-media thickness, independent of risk factors, in older adults.[55.58] Furthermore, endothelial responses inversely correlate with left ventricular mass in pre-hypertensive people[122] suggesting that changes in cardiac structure precede clinical hypertension.[111-112.209-211] Although some studies have shown that cardiovascular structural changes precede the onset [122.212] and predict those at risk [209-211.213-214] of hypertension in younger people, no studies to date have investigated the relationship between endothelial function and ventricular mass in young people

In order to determine whether persistent vascular endothelial dysfunction over a ten year period reflects adverse cardiovascular remodelling independent of classic cardiovascular risk factors, I studied a subgroup of the Early Vascular Study who had had endothelial function measures during adolescence.

7.2 Hypotheses and Aims

The aim of this study was to determine whether the presence of persistently reduced endothelial responses:

1. Identifies a group of individuals with subclinical elevation in risk factors.
2. Is associated with variation in cardiac and vascular structure relevant to later disease development.

The study then explored whether a decline in endothelial responses over this time period identified a group with a less severe cardiovascular phenotype than that associated with persistently reduced endothelial function.

7.3 ***Methods***

Study design and subject recruitment was described in the detailed methods section 2.1.4. Study methods are described in sections 2.2 to 2.5. Briefly, a subgroup of adults from the Early Vascular Study who had also attended a follow up study ten years earlier at age 15 years were investigated. The key measurements for this study were:

7.3.2 Demographic, Anthropometric and Metabolic Characteristics

Subjects attended in the morning following an overnight fast and underwent routine measurements of body size. Two peripheral blood pressure (BP) measurements using

appropriate cuff size. Data on medical history, smoking, and parental medical history were obtained by questionnaire.[60]

7.3.3 Endothelial Vasomotor Function and Vascular Structure

Subjects underwent measurement of brachial artery flow-mediated dilatation (FMD) using the same standardised methodology at ages 15 and 25 years by ultrasound(Philips Sonos 5500, 10MHz linear array transducer).[215] Brachial artery hyperaemic flow and shear stress were measured at baseline and after cuff deflation.[162] As previously described, carotid-femoral pulse wave velocity (PWV)[145] and carotid intima-media thickness (cIMT) were also measured.[150]

7.3.4 Left Ventricular Mass and Function

As previously described, cardiovascular magnetic resonance (CMR) was used for measurement of ventricular volumes and mass (Siemens 1.5T Sonata scanner).

7.3.5 Statistical Analysis

SPSS 17 was used for analysis and GraphPad Prism 5 for graphs. A sample of 10 subjects in each group was required to detect a 22.5gram (1.25SD) difference in LVM using CMR with 80% power and an alpha of 0.05.[132] Post hoc analysis demonstrated differences of 2.23SD in left ventricular mass between those in the top and bottom quarters for average FMD, 1.1SD in PWV and 1.2SD in cIMT with a power of 99.9%, 82% and 82% respectively (P=0.05). Data are presented as Mean (SD) for continuous variables and frequency for categorical parameters. Comparisons between measurements at both time points for subjects were carried out using paired t-Test. Differences between groups characterised by average FMD, or change in FMD, over 10 years were assessed using unpaired t-Test or ANOVA for

continuous variables and χ^2 for categorical data. Univariate correlations were performed to identify relations between average FMD, LVMI and classical cardiovascular risk factors. Variables associated with FMD or LVMI with significance P<0.1, were entered in a linear regression model of LVMI to adjust for its relationship with endothelial vasomotor function.

7.4 Results

7.4.1 Subject characteristics

Of the 99 individuals who participated in the follow up study at age 15, 47 were able to attend for reassessment at age 25 years. All subjects were normotensive subjects based on age and gender specific centiles for blood pressure at ages 15 and 25 years [216]. There were no differences in demographics or cardiovascular risk profile at age 15 between responders and the original cohort (Male gender: 45.7% vs. 45%; SBP 113.6±7.2 vs. 115.8±8.4mmHg; DBP 61.0±4.8 vs. 63.7±7.4 mmHg; fasting total cholesterol 3.8±0.6 vs. 4.0±0.7mmol/l; HDL cholesterol 1.1±0.3 vs. 1.2±0.3mmol/l; and glucose 4.7±0.4 vs. 4.7±0.5mmol/l). As described in table 7-1, despite increases in body size, blood pressure and lipids, overall levels of cardiovascular risk factors were within normal population ranges between the age of 15 and 25 years. There were no differences between mean levels of FMD measured at age 15 (5.68±3.59%) and 25 years (5.93±4.81%, P=0.13).

7.4.2 Low Average Endothelial Responses over Ten Years

To identify a group of subjects with, on average, the lowest endothelial function over the ten year period, subjects were stratified according to average FMD at the two time points and divided by quartiles into four groups (FMD ranges 1.32-3.25%, 3.40-4.81%, 4.90-7.54%, and 7.84-16.13%). Differences in cardiovascular risk factors between groups were then studied (Table 7-2).

Subject Characteristics	Age 15 years (n=47)	Age 25 years (n=47)
Age, years	15.12 (0.88)	25.27 (1.35)
BMI, Kg/m^2	21.13 (3.42)	24.63 (5.96)
Waist Hip Ratio	0.79 (0.07)	0.79 (0.09)
Systolic Blood Pressure, mmHg	113.61 (7.15)	117.04 (11.50)
Diastolic Blood Pressure, mmHg	60.99 (4.76)	69.30 (7.76)
Mean Arterial Blood Pressure, mmHg	82.37 (5.42)	85.23 (8.18)
Pulse Pressure, mmHg	52.74 (9.54)	47.63 (8.75)
Number of Smokers (%)	4 (8.51)	9 (19.15)
Total Cholesterol, mmol/l	3.82 (0.55)	4.76 (0.91)
High Density Lipoprotein, mmol/l	1.15 (0.33)	1.49 (0.26)
Low Density Lipoprotein, mmol/l	2.29 (0.64)	2.78 (0.74)
Triglycerides, mmol/l	0.96 (0.32)	1.08 (0.70)
Fasting Glucose, mmol/l	4.64 (0.44)	4.99 (0.46)
Fasting Insulin, pmol/l	58.50 (70.86)	58.00 (32.79)
Baseline Brachial Artery Diameter, mm	2.92 (0.41)	3.45 (0.64)
Flow Mediated Dilatation, %	5.68 (3.59)	5.93 (4.81)
Mean Baseline Shear Stress, dyne/cm^2	4.15 (2.27)	12.73 (7.58)
Mean Hyperaemic Shear Stress, dyne/cm^2	21.90 (6.22)	68.79 (20.47)
Reactive Hyperaemia, %	781.83 (357.67)	755.63 (375.92)

Table 7-1 Baseline Subject Characteristics at Age 15 and 25 years

Values are reported as Mean (Standard Deviation) for continuous variables and Number (Percentages) for categorical data.

7.4.2.1 Low Average Endothelial Responses and Cardiovascular Risk Factors

FMD measured at age 15 or age 25 was not related to blood pressure at either time point (Table 7-2). However, subjects whose endothelial responses were consistently reduced at both time points (low average FMD) had 5-8mmHg greater systolic (117.59±7.76 vs. 110.79±5.80mmHg, P=0.03, Figure 7-1), mean arterial (85.32±4.60 vs. 80.67±5.56mmHg, P=0.04) and pulse pressure (57.00±9.45 vs. 48.92±9.49mmHg, P=0.05) at age 15 years and 10mmHg greater systolic BP at age 25 years (124.18±8.69 vs. 113.54±11.06mmHg, P=0.02).

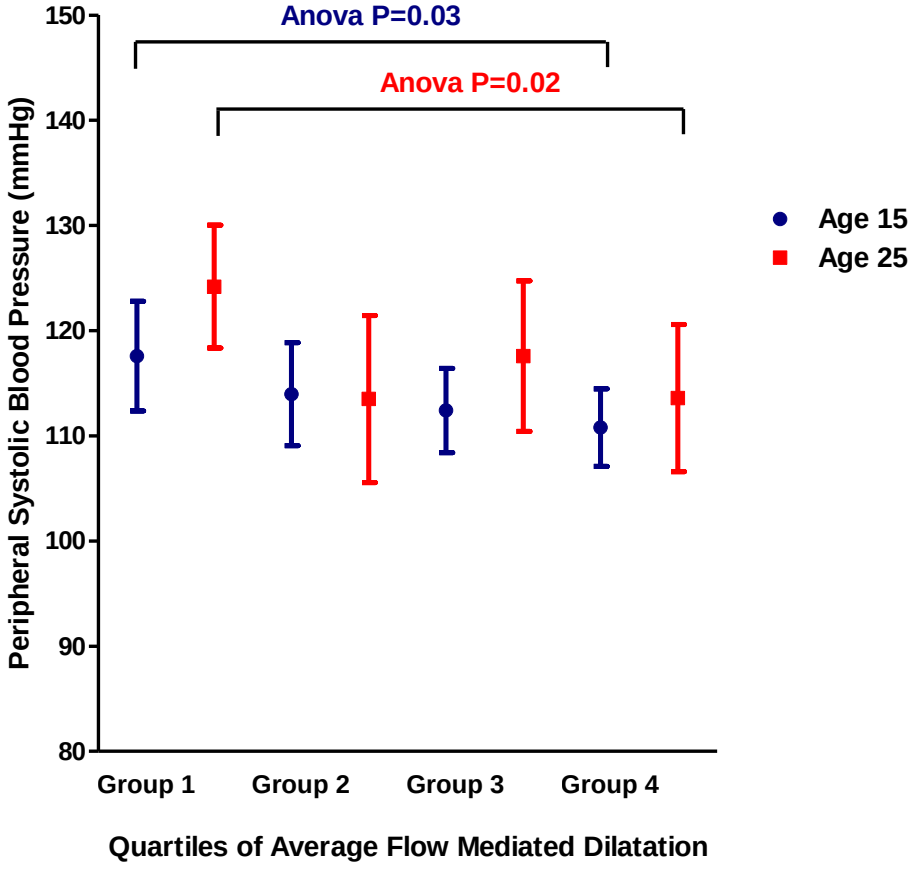


Figure 7-1 Impaired Endothelial Function and Variation in Blood Pressure

Systolic blood pressure was measured at age 15 and 25 years. There was a graded relationship between systolic blood pressure and average levels of endothelial responses over ten years. (Error bars represent the mean and 95% Confidence Interval)

Parameters	Quartiles of Average Flow Mediated Dilatation				P Value for Trend*	P Value†
	Group 1 (n 11)	Group 2 (n 12)	Group 3 (n 12)	Group 4 (n 12)		
Group Characteristics at Age 15 Years						
BMI, Kg/m^2	21.03 (4.23)	21.15 (3.76)	21.28 (3.12)	21.06 (2.96)	0.99	0.98
Waist Hip Ratio	0.82 (0.05)	0.80 (0.07)	0.76 (0.06)	0.77 (0.09)	0.19	0.16
Systolic Blood Pressure, mmHg	117.59 (7.76)	113.96 (7.69)	112.42 (6.30)	110.79 (5.80)	0.13	**0.03**
Diastolic Blood Pressure, mmHg	68.82 (3.00)	60.54 (4.17)	59.71 (4.75)	61.04 (6.42)	0.47	0.41
Total Cholesterol, mmol/l	3.99 (0.51)	3.65 (0.52)	3.69 (0.58)	3.97 (0.60)	0.30	0.94
High Density Lipoprotein Cholesterol, mmol/l	1.07 (0.15)	1.23 (0.49)	1.11 (0.29)	1.15 (0.22)	0.66	0.31
Low Density Lipoprotein Cholesterol, mmol/l	2.64 (0.42)	1.97 (0.76)	2.25 (0.60)	2.45 (0.53)	0.06	0.36
Triglycerides, mmol/l	0.97 (0.37)	1.06 (0.27)	0.94 (0.37)	0.89 (0.36)	0.67	0.59
Glucose, mmol/l	4.72 (0.48)	4.91 (0.35)	4.59 (0.39)	4.49 (0.40)	0.09	0.22
Insulin, pmol/l	60.09 (29.44)	50.33 (19.98)	37.42 (12.78)	81.75 (124.16)	0.41	0.58
Number of Smokers (%)	1 (9)	4 (33)	3 (25)	6 (50)	0.29	**0.03**
Maternal Pregnancy Induced Hypertension (%)	3 (27.3)	1 (8.3)	5 (41.7)	3 (25)	0.63	0.90
Group Characteristics at Age 25 Years						
BMI, Kg/m^2	24.63 (6.36)	23.22 (3.27)	26.10 (8.57)	24.58 (4.72)	0.72	0.98
Waist Hip Ratio	0.82 (0.10)	0.79 (0.07)	0.78 (0.08)	0.78 (0.12)	0.69	0.36
Systolic Blood Pressure, mmHg	124.18 (8.69)	113.47 (12.54)	117.58 (11.25)	113.54 (11.06)	0.08	**0.02**
Diastolic Blood Pressure, mmHg	70.75 (5.90)	66.96 (7.43)	72.74 (7.54)	66.88 (8.98)	0.17	0.24
Central Systolic Blood Pressure, mmHg	114.18 (10.41)	103.92 (8.38)	104.58 (11.31)	104.30 (7.35)	**0.04**	**0.02**
Central Diastolic Blood Pressure, mmHg	75.91 (6.99)	66.96 (7.43)	72.74 (7.54)	71.70 (4.57)	0.34	0.12
Total Cholesterol, mmol/l	4.72 (1.21)	4.20 (0.41)	4.76 (0.57)	5.37 (0.83)	0.21	0.17

High Density Lipoprotein Cholesterol, mmol/l	1.37 (0.34)	1.51 (0.19)	1.44 (0.26)	1.61 (0.23)	0.21	0.07
Low Density Lipoprotein Cholesterol, mmol/l	2.79 (1.07)	2.34 (0.29)	2.78 (0.60)	3.21 (0.59)	0.05	0.27
Triglycerides, mmol/l	1.22 (0.97)	0.80 (0.26)	1.17 (0.45)	1.17 (0.85)	0.49	0.90
Glucose, mmol/l	5.05 (0.36)	5.12 (0.47)	4.84 (0.46)	4.89 (0.53)	0.53	0.44
Insulin, pmol/l	56.04 (27.51)	41.32 (11.35)	52.69 (26.44)	78.02 (44.24)	0.05	0.19
Number of Smokers, %	2 (16.7)	3 (25)	2 (16.7)	2 (16.7)	0.94	0.92
Maternal Essential Hypertension, %	4 (36.4)	0	4 (33.3)	1 (8.3)	0.37	0.10
Paternal Essential Hypertension, %	3 (27.3)	3 (25)	1 (8.3)	3 (25)	0.75	0.90

Table 7-2 Subject Characteristics and Classical Cardiovascular Risk Factors at Age 15 and 25 Years

Values are reported as Mean (SD) for continuous variables and Number (Percentages) for nominal data. *Group comparison using Anova or Chi Square. †Comparison between subjects with the lowest and highest average FMD (groups 1 and 4).

Cardiovascular Phenotype	Quartiles of Average Flow Mediated Dilatation				ANOVA P Value*	P Value†
	Group 1 (n 11)	Group 2 (n 12)	Group 3 (n 12)	Group 4 (n 12)		
Age 15 Years						
Baseline Brachial Artery Diameter, mm	2.93 (0.37)	3.21 (0.42)	2.89 (0.32)	2.64 (0.32)	**0.005**	**0.05**
Flow Mediated Dilatation, %	3.49 (1.77)	4.10 (1.71)	4.77 (2.91)	10.17 (3.07)	-	-
Reactive Hyperaemia	626.65 (213.76)	776.21 (379.89)	714.03 (326.06)	650.94 (308.15)	0.66	0.83
Brachial Distensibility, x 10^{-3} $mmHg^{-1}$	0.12 (0.07)	0.13 (0.05)	0.12 (0.07)	0.12 (0.06)	0.99	0.90
Age 25 Years						
Baseline Brachial Artery Diameter, mm	3.74 (0.67)	3.74 (0.55)	3.43 (0.62)	2.91 (0.37)	**0.002**	**0.001**
Flow Mediated Dilatation, %	2.34 (0.56)	4.19 (1.79)	7.16 (3.64)	10.81 (2.24)	-	-
Brachial Artery Distensibility, x 10^{-3} $mmHg^{-1}$	0.57 (0.22)	0.80 (0.39)	0.80 (0.25)	0.83 (0.27)	0.15	**0.02**
Hyperaemic Flow Ratio	6.44 (2.91)	8.27 (4.79)	8.15 (4.13)	6.84 (2.56)	0.66	0.77
Baseline Systolic Shear Stress	79.01 (22.86)	72.73 (18.66)	86.34 (38.75)	103.51 (20.94)	0.05	**0.02**
Baseline Diastolic Shear Stress	2.90 (2.74)	3.01 (4.12)	1.25 (1.68)	1.55 (2.30)	0.47	0.63
Hyperaemic Systolic Shear Stress	110.76 (33.57)	108.18 (19.29)	113.22 (23.86)	134.39 (17.41)	0.06	0.07
Hyperaemic Diastolic Shear Stress	36.12 (8.48)	39.19 (12.29)	47.21 (19.68)	54.86 (11.73)	**0.02**	**0.001**
Left Ventricular Mass Indexed, gram/m^2	73.48 (7.73)	66.39 (6.23)	62.18 (11.37)	56.25 (9.54)	**0.0004**	**0.0001**
Left Ventricular Ejection Fraction, %	66.88 (7.36)	63.30 (5.20)	62.46 (7.40)	65.38 (8.59)	0.49	0.68
Pulse Wave Velocity, m/s	5.97 (0.63)	5.84 (0.74)	5.57 (0.69)	5.29 (0.59)	0.11	**0.02**
Common Carotid Intima-Media Thickness, mm	0.53 (0.06)	0.46 (0.05)	0.50 (0.04)	0.47 (0.04)	**0.02**	**0.04**

Table 7-3 Cardiovascular Phenotype at Age 15 and 25 Years

Values are reported as Mean (Standard Deviation) for continuous variables and Number (Percentages) for nominal data. *Group comparison using Anova or Chi Square. †Comparison between subjects with the lowest and highest average FMD (groups 1 and 4).

There were no significant differences between quartile groups in level of physical activity, or the incidence of paternal or maternal hypertension (P=0.78 and P=0.61), nor in body size, or other metabolic parameters (Table 7-2). Average FMD was also correlated with total cholesterol (r=0.353, P=0.03), LDL-cholesterol (r=0.362, P=0.02) and insulin (r=0.441, P=0.004) at age 25 but did not relate to changes in cardiovascular risk factor levels over the 10 years. There were no differences between groups in brachial artery distensibility or baseline shear stress. There was a graded relationship between diastolic shear stress and levels of average FMD between time points (Table 7-3).

7.4.2.2 Low average endothelial function and left ventricular size and function - Left ventricular parameters were within normal ranges for all study subjects (LVM 115.65±26.56gram, LVMI 64.38±10.69gram/m^2, LVEDV 132.15±30.98ml, EF 64.49±7.17%, septal wall thickness 7.87±1.03mm and lateral wall thickness 7.64±1.42mm). There was a graded association between LVMI and FMD at age 15 (r=-0.303, P=0.04) and 25 years (r=-0.632, P=0.000002). Greater average FMD responses over 10 years were associated with lower ventricular mass. Subjects with an average FMD below the lowest quartile had LVMI of 73.48±7.73gram/m^2 compared to 56.25±9.54gram/m^2 for those with average FMD greater than the highest quartile (P=0.0001) (Figure 7-2, Panel a). The association remained significant after adjusting for vessel diameter and hyperaemic mean shear stress (r=-0.492, P=0.001). Average FMD was associated with LVEDV (r=-0.496, P=0.009) as well as lateral (r=-0.397, P=0.006) and septal (r=-0.367, P=0.01) wall thickness (Figure 7-2, Panels b and c). There were no associations between endothelial responses and EF (r=0.022, P=0.89). LVMI was significantly higher in males compared to females (70.21±7.17 vs. 59.26±10.76gram/m^2, P=0.002). There was no relationship between LVMI and age, body

size, smoking habit or BP at age 25 but a significant association with waist hip ratio (r=0.312, P=0.03), systolic BP (r=0.316, P=0.03) and pulse pressure (r=0.34, P=0.02) at age 15 years.

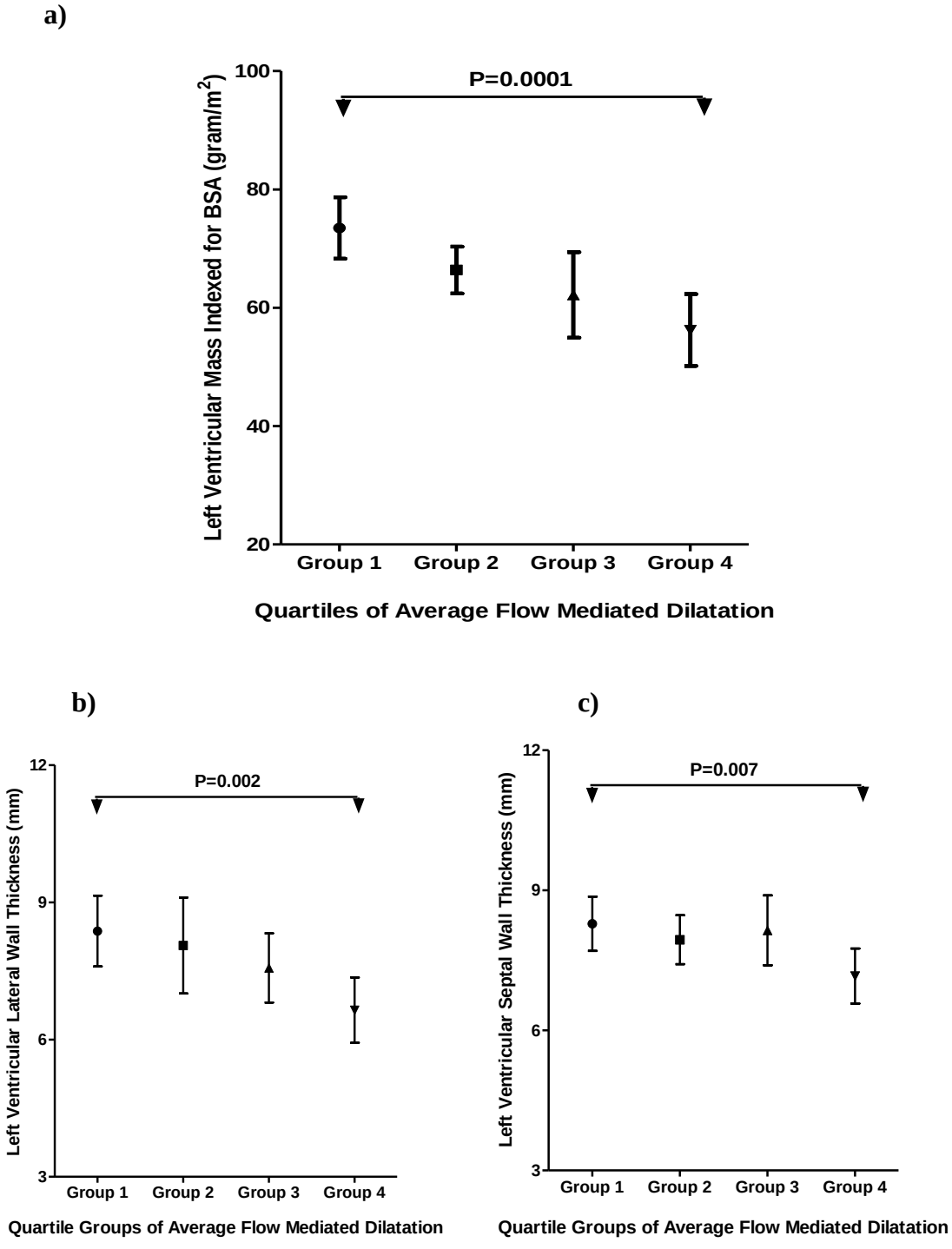


Figure 7-2 Association between Endothelial Mediated Vasodilatation and Left Ventricular Mass

There was a graded relationship between LVMI and levels of average endothelial responses over 10 years (Panel a). A similar graded relationship was observed for left ventricular lateral (Panels b) and septal (Panel c) wall thickness. Error bars represent the mean and 95% Confidence Interval.

7.4.2.3 Low average endothelial function and vascular phenotype

As presented in table 7-3, vascular phenotype also differed between groups so that subjects with low endothelial responses had significantly higher PWV (5.97±0.63 vs. 5.29±0.59m/s, P=0.02) and greater cIMT (0.53±0.06 vs. 0.47±0.04mm, P=0.04) compared to those with an average FMD above the highest quartile (Figure 3, panels a and b).

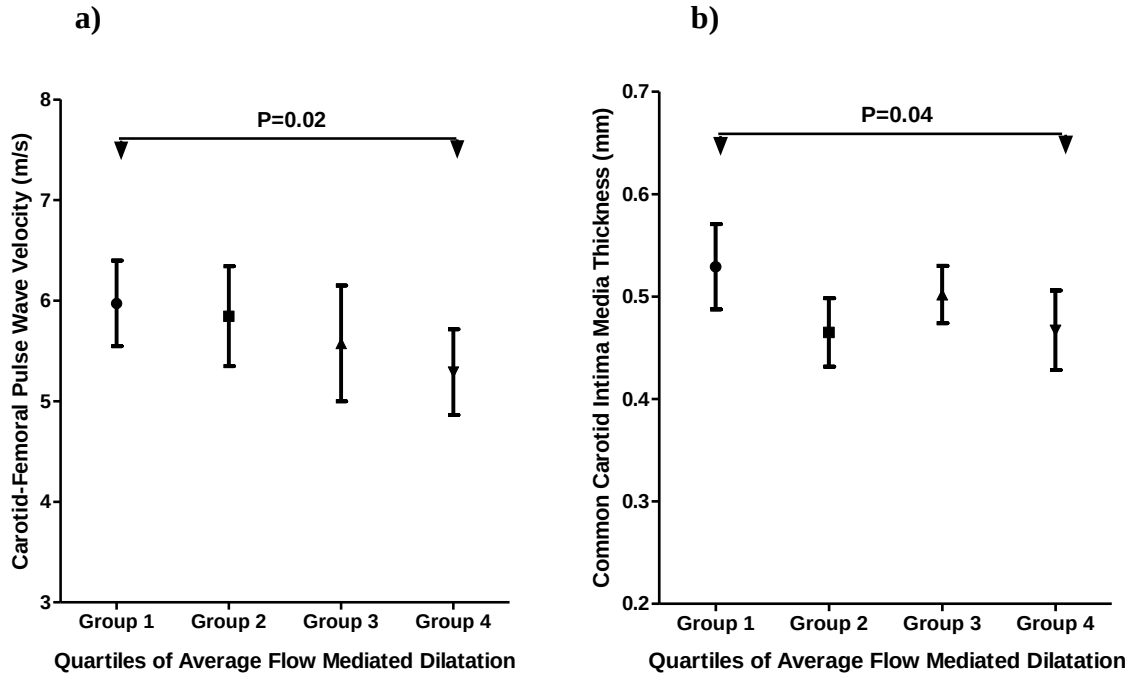


Figure 7-3 Low Endothelial Responses and Vascular Phenotype

Subjects with persistently *low* endothelial responses had significantly greater pulse wave velocity (5.97±0.63 vs. 5.29±0.59, P=0.02, Panel a) and greater common carotid intima-media thickness (0.53±0.06 vs. 0.47±0.04, P=0.04, Panel b) compared to individuals with consistently *high* endothelial responses. (Error bars represent the mean and 95% Confidence Interval).

7.4.2.3 Low Average Endothelial Function, Left Ventricular Mass and other Cardiovascular Risk Factors

As the most striking association was between average FMD and LVMI, a multivariate regression analysis was carried out to determine the role of risk factors and vascular parameters in this association. Variables associated with either FMD or LVMI with $P<0.1$ were included in multiple linear regression analysis using stepwise selection method. The analysis demonstrated that average FMD was the main determinant of LVMI (Beta=-0.625, $r^2=0.39$ and $P=0.00005$) independent of gender, body size, brachial artery diameter, blood pressure, shear stress, endothelial-independent vasodilatation, pulse wave velocity and carotid intima-media thickness and the above parameters at age 15 or 25 years (Table 7-4).

Variables	*Beta*	Confidence Interval		*P Value*
		Lower Bound	Upper Bound	
Average FMD	-0.625	-2.78	-1.26	<0.0001
Average FMD adjusted for gender	-0.625	-2.78	-1.26	<0.0001
Average FMD adjusted for gender and brachial diameter age 25	-0.625	-2.78	-1.26	<0.0001
Average FMD adjusted for gender and brachial diameter age 25 and 15	-0.625	-2.78	-1.26	<0.0001
Average FMD adjusted for gender, brachial diameter age 25 and 15 and PP25	-0.625	-2.78	-1.26	<0.0001
Average FMD adjusted for gender, brachial diameter age 25 and 15, PP25 and HSS25	-0.625	-2.85	-1.19	<0.0001
Average FMD adjusted for gender, brachial diameter age 25 and 15, PP25, HSS 25, and PP15	-0.625	-2.85	-1.19	<0.0001
Average FMD adjusted for gender, brachial diameter age 25 and 15, PP25, HSS25, PP15 and WHR15	-0.625	-2.85	-1.19	<0.0001
Average FMD adjusted for gender, brachial diameter age 25 and 15, PP25, HSS25, PP15 and WHR15, PWV	-0.625	-2.90	-1.14	<0.0001

Table 7-4 Stepwise Linear Regression Model for Determinants of Left Ventricular Mass

FMD refers to Flow Mediated Vasodilatation, PP represents Pulse Pressure (number refers to age), HSS 25: Hyperaemic Mean Shear Stress age 25 years, WHR: Waist Hip Ratio, PWV: Pulse Wave Velocity.

7.4.3 Decline in endothelial responses over ten years

In order to study the relevance of a decline in endothelial responses over the ten years, subjects were divided into new quarters based on their change in FMD between the two time points (ΔFMD) to identify the group with the largest decline in endothelial function. The change in levels of cardiovascular risk factors was compared between subjects with the greatest decline (ΔFMD -13.73 to -3.52%) and the rest of the cohort (ΔFMD -3.41 to 10.64%). Subjects with the greatest reduction in FMD over 10 years had significantly greater increase in mean arterial blood pressure (7.04±8.83 vs. 1.57±6.57mmHg, P=0.03) which appeared to be explained by a greater increase in systolic (6.86±10.86 vs. 2.39±9.05mmHg, P=0.18) than diastolic BP (10.63±10.07 vs. 7.61±7.87mmHg, P=0.30). There were no significant differences in other cardiovascular risk factors (Table 7-5). In contrast to subjects with persistently low average FMD during this time period, there were no significant differences at age 25 years in PWV (5.98±0.62 vs. 5.59±0.70m/s, P=0.13), cIMT (0.51±0.05 vs. 0.49±0.05mm, P=0.39), LVM (118.00±12.36 vs. 114.93±28.78 gram, P=0.74), LVMI (66.96±7.59 vs. 63.60±11.45gram/m^2, P=0.37) or EF (66.43±5.14 vs. 63.78±7.73%, P=0.30) in those in whom FMD had deteriorated (Table 7-5).

Change in Parameters over 10 Years	Largest FMD Decline (n=11)	Rest Cohort (n=36)	P Value
Subject Characteristics and Cardiovascular Risk Factor			
BMI	2.63 (3.66)	3.77 (4.89)	0.48
Waist Hip Ratio	0.002 (0.13)	0.006 (0.09)	0.90
Systolic Blood Pressure, mmHg	6.86 (10.86)	2.39 (9.05)	0.18
Diastolic Blood Pressure, mmHg	10.63 (10.07)	7.61 (7.87)	0.30
Mean Arterial Blood Pressure, mmHg	7.04 (8.83)	1.57 (6.57)	**0.03**
Pulse Pressure, mmHg	-2.00 (9.62)	-6.07 (10.69)	0.26
Total Cholesterol, mmol/l	0.75 (0.63)	1.00 (0.75)	0.37
High Density Lipoprotein, mmol/l	0.45 (0.29)	0.30 (0.37)	0.28
Low Density Lipoprotein, mmol/l	0.31 (0.60)	0.55 (0.71)	0.36
Triglycerides, mmol/l	-0.19 (0.17)	0.21 (0.72)	0.11
Glucose, mmol/l	0.36 (0.57)	0.34 (0.49)	0.90
Insulin, pmol/l	-29.32 (116.52)	7.87 (35.38)	0.12
Vascular Function			
∆FMD, %	-6.38 (3.41)	2.28 (3.98)	**<0.0001**
Baseline Brachial Diameter, mm	0.54 (0.35)	0.53 (0.48)	0.95
Baseline Mean Shear Stress, dyne/cm^2	6.65 (4.50)	9.22 (8.41)	0.34
Hyperaemic Mean Shear Stress, dyne/cm^2	47.52 (17.86)	46.70 (18.75)	0.90
Reactive Hyperaemia, %	-17.68 (628.16)	-29.04 (470.00)	0.95

Table 7-5 Cardiovascular Phenotype in Subjects with the Greatest Decline in Endothelial Function

Values are reported as Mean (SD) for continuous variables and Number (Percentages) for nominal data. Two tailed unpaired student t Test P values reported.

7.5 Discussion

Subjects who were born preterm and exhibited persistently reduced endothelial responses over ten years had evidence of subclinical elevation in blood pressure, increased left ventricular mass and features of an adverse cardiovascular phenotype with increased arterial stiffness and carotid intima-media thickness in early adult life compared to preterm individuals with greater endothelial responses. Moreover, subjects who had a decline in endothelial responses also had a significant increase in mean arterial pressure over this period of life. Whilst the subclinical variation in blood pressure would not normally be considered of clinical significance, the degree of endothelial impairment associated with this variation was similar to that seen in patients with severe coronary artery disease.[217-218] In addition, it identified individuals with over two standard deviations variation in left ventricular mass. These findings therefore highlight a potential link between vascular health, blood pressure and cardiovascular remodelling, during the early stages of life, before onset of clinical levels of risk factors.

An association has previously been demonstrated between endothelial function and ventricular mass in older cohorts.[119] A major limitation of these earlier observations is that cohorts included in these studies had established cardiovascular risk factors including hypertension, obesity and hyperlipidaemia[114.117-119] which also relate to endothelial dysfunction and left ventricular hypertrophy.[214] In this study, even young people who were clinically normotensive there was an association between endothelial vasomotor responses and left ventricular mass. Why endothelial function is related to ventricular mass in the absence of hypertension or other risk factors is of interest. Persistently impaired peripheral endothelial function could indirectly influence cardiac remodelling through functional and structural changes in the central and peripheral circulation. Endothelial dysfunction may be

associated with reduced elasticity and compliance of the small arteries and large vessel tone leading to elevated peripheral resistance and reduced central aortic elasticity.[61] However, the relationship between average levels of endothelial function and aortic stiffness in early adulthood did not explain the association between endothelial responses and ventricular mass. Alternatively, the observed association may reflect an endothelial-nitric oxide mediated alteration in myocyte response to hypertrophic stimuli [111-112.219-220]. Indeed, animal studies have shown an association between impaired myocardial nitric oxide synthase function and increased hypertrophic responses that were reversed by enhanced eNOS activity.[111-112] Of interest, statins, angiotensin receptor blockade and smoking cessation are known to have potent influences on endothelial function and, in studies on older normotensive adults, have also been shown to reduce ventricular mass.

It is possible that the trend for higher blood pressure in the group with low endothelial responses explained the association between endothelial function and left ventricular mass. However, in multivariate regression the association was independent of blood pressure levels. In addition, single measurements may not have fully characterised blood pressure in these subjects and those with higher left ventricular mass had more labile blood pressure. Data on ambulatory blood pressure or measures of blood pressure variability were not available in these subjects. Nonetheless, even if this were the case, findings would suggest endothelial vasomotor function is a sensitive physiological marker of vascular damage associated with this phenomenon. Consistent with this, persistent endothelial dysfunction appeared to be a marker of adverse vascular structural changes, with increased carotid intima-media thickness and aortic stiffness, in apparently healthy individuals.

Moreover, there was a parallel relationship between the decline in endothelial function over a decade and a significant increase in mean arterial pressure in young normotensive cohort. As there was an inverse correlation between endothelial function and blood pressure levels in hypertensive populations,[221] further work is needed to establish whether the subclinical variation in blood pressure is sufficient to induce the degree of endothelial dysfunction observed in these normotensive young subjects. If so then, this decline in function is likely to have a significant pro-atherogenic impact. Alternatively, as vascular tone is closely related to the balance between endothelial vasodilatation and vasoconstriction[109] the endothelial dysfunction, and reduced nitric oxide bioavailability, may have a more causal link with the increase in blood pressure.[61] No measures of left ventricular mass or carotid intima-media thickness were carried out at age 15 years and changes may already have developed by adolescence. Therefore, future follow up of young children with vascular measures, to assess the relationship between cardiovascular structure and endothelial function, as well as progression at different time points will be relevant. Finally, whether variation in ventricular mass within the normal range in young adults has long term clinical relevance is currently unexplored but in older individuals there is a linear relationship between left ventricular mass and adverse cardiovascular events even when values fall within the normal range. Future prospective follow up of this cohort to study development of hypertension will be of interest.

In summary, this study demonstrates a close relationship between endothelial responses, subclinical variation in blood pressure and cardiovascular remodelling in young individuals. The association with left ventricular mass is particularly striking. Persistently reduced, or declining, endothelial responses may be sensitive early markers of high risk groups. Furthermore, left ventricular mass may be valuable additional endpoint in future interventional studies.

Chapter 8 **: GENERAL DISCUSSION**

8.1 Key Findings of the Research and Conclusions

Preeclamptic mothers and children have increased risk of hypertension and cardiovascular disease long term.[2-3]

The research described in this thesis demonstrates that mothers with early-onset preeclampsia, diagnosed before 34 weeks gestation, exhibited distinct blood pressure patterns both during pregnancy and many years later, vascular remodelling with increased central arterial stiffness and capillary rarefaction as well as changes in cardiac haemodynamics 6-13 years after pregnancy. In contrast, women with late-onset preeclampsia displayed more non-specific cardiovascular changes including increased carotid intima-media thickness and diastolic dysfunction.

In addition, young children whose mothers developed early- rather than late-onset preeclampsia also exhibited higher peripheral and central systolic blood pressure. At this age they did not have evidence of vascular dysfunction but by age 20 years those born preterm to hypertensive pregnancy had evidence of impaired vascular endothelial responses and increased subclinical atherosclerosis. In addition, preterm offspring who had the lowest endothelial vasomotor responses both at age twenty and earlier in life also exhibited alterations in cardiac structure with increased myocardial mass and vascular remodelling with increased intima-media thickness and aortic stiffness in early adulthood.

In conclusion, severe preeclampsia diagnosed before 34 weeks gestation or resulting in premature delivery was associated with increased blood pressure and distinct cardiovascular phenotypes in both mother and offspring of relevance to future cardiovascular disease.

8.2 Future Directions

Preeclampsia is associated with increased risk of hypertension and cardiovascular disease in mother and child. The results presented in this research demonstrate subclinical increases in blood pressure in mother and offspring in association with adverse alterations in cardiovascular structure and function following preeclampsia that precedes the development of clinical cardiovascular disease.

Accordingly, it would be of interest to focus future research on whether modifications of these specific vascular phenotypes or control of blood pressure within these groups improve cardiovascular outcomes or reduce cardiovascular complications following preeclampsia. Regular aerobic exercise [222] and pharmacological interventions, including statins and renin-angiotensin system inhibitors, are known to improve endothelial function, reduce arterial stiffness and alter capillary density.[51.223-224] Statins are potent inhibitors of cholesterol biosynthesis and in clinical trials they have also been shown to be beneficial both in primary and secondary prevention of cardiovascular disease through their pleiotropic effects.[51.225] Angiotensin-converting enzyme inhibitors also increase nitric oxide bio-availability as well as reduce oxidative stress and arterial stiffness.[51.223] Therefore, it would be of interest to establish the effectiveness of these physical or pharmacological measures in improving vascular phenotype in the offspring and mother and determine whether these alterations translate into long term risk reduction.

Chapter 9 **: APPENDICES**

9.1 Sources of Funding

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9.2 Attributions

I am extremely grateful for the contributions made to this research by colleagues and collaborators. Apart from the exceptions described below, the author is directly responsible for the planning, execution and analysis of all the experiments and the preparation of this thesis.

The initial protocol for the PVS study was written and submitted for ethical approval by Dr Paul Leeson, Dr Nicholas Alp, Dr Raj Kharbanda and Dr Brenda Kelly and for the EVS study by Dr Paul Leeson. Measurement of brachial FMD by ultrasound was performed by Mr Jonathan Diesch.

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9.4 Publications Arising from Work in This Thesis

Lazdam M, de la Horra A, Diesch J, Kenworthy Y, Davis EF, Lewandowski AJ, Szmieglski C, Shore A, Mackillop L, Kharbanda R, Alp N, Redman C, Kelly B, Leeson P. Unique Blood Pressure Characteristics in Mother and Offspring following Early-Onset Preeclampsia, *Hypertension.* 2012; 60:1338-1345

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Lazdam M and Leeson, P. Response to Essential Hypertension, Hypertension in Pregnancy, And Subsequent Blood Pressure. *Hypertension*, 2010; 56(3):e33

Lazdam M, Leeson P. Letter by Lazdam and Leeson regarding article, "Pulmonary and Systemic Vascular Dysfunction in Young Offspring of Mothers with Preeclampsia". *Circulation.* 2011; 123:e386

9.2 Other Research Publications during DPhil Study

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Davis EF, **Lazdam M**, Lewandowski AJ, Worton SA, Kelly B, Kenworthy Y, Adwani S, Wilkinson AR, McCormick K, Sargent I, Redman C, Leeson P. Cardiovascular Risk Factors in Children and Young Adults Born to Preeclamptic Pregnancies: A Systematic Review. *Pediatrics*. 2012; 129(6):e1552-61

Kelly BA, Lewandowski AJ, Worton SA, Davis EF, **Lazdam M**, Francis J, Neubauer S, Lucas A, Singhal A, Leeson P. Antenatal Glucocorticoid Exposure and Long-Term Alterations in Aortic Function and Glucose Metabolism. Pediatrics. 2012; 129(5):e1282-90

Davis EF, Newton L, Lewandowski AJ, **Lazdam M**, Kelly BA, Kyriakou T, Leeson P. Pre-eclampsia and Offspring Cardiovascular Health: Mechanistic Insights from Experimental Studies. Clin Sci (Lond). 2012; 123(2):53-72

Lewandowski AJ, **Lazdam M**, Davis E, Kylintireas I, Diesch J, Francis J, Neubauer S, Singhal A, Lucas A, Kelly B, Leeson P. Short-Term Exposure to Exogenous Lipids in Premature Infants and Long-Term Changes in Aortic and Cardiac Function. Arterioscler Thromb Vasc Biol. 2011; 31(9):2125-35

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Lazdam M, de la Horra A, Diesch J, Kenworthy Y, Davis EF, Lewandowski AJ, Szmieglski C, Shore A, Mackillop L, Kharbanda R, Alp N, Redman C, Kelly B, Leeson P. Long-term Cardiac and Vascular Phenotype of Young Women with Pregnancies Complicated by Preeclampsia. *Heart*. 2012; 98: Suppl 1 A80. **(*Poster Presentation BCS Conference 2012 and ISSHP Congress Jul 2012*)**

Lazdam M, de la Horra A, Diesch J, Szmigielski C, Kharbanda RK, Alp N, Kelly B, Leeson P. Structural Vascular Changes in Young Women with Previous Preeclampsia: Link to Cardiovascular Disease Development. *EHJ*. 2011; 32 (Suppl 1): 1-312. ***(Oral Presentation ESC Congress 2011)***

Lazdam M, Pitcher A, de la Horra A, Kylintireas I, Mannie Z, Diesch J, Trevitt C, Singhal A, Lucas A, Neubauer S, Alp A, Kelly B, Leeson P. Elevated blood pressure in offspring born to hypertensive pregnancy: is endothelial dysfunction the underlying vascular mechanism? *Heart* 2010; 96: A33-A34. ***(Poster Presentation BCS Conference 2010)***

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Lazdam M, Pitcher A, de la Horra A, Kylintireas I, Mannie Z, Diesch J, Trevitt C, Singhal A, Lucas A, Neubauer S, Alp N, Kelly B, Leeson P. Hypertension in Offspring of Pregnancies Complicated by Pre-eclampsia: Underlying Vascular Mechanisms? *J Am Coll Cardiol*, 2010; 55. ***(Poster Presentation ACC Scientific Session* 2010)**

Lazdam M, Kylintireas I, Cunnington C, Diesch J, Trevitt C, Channon K, Neubauer S, Leeson P. Abstract 3279: Endothelial function during adolescence predicts left ventricular mass in early adult life. *Circulation,* 2009;120:S778b. ***(Poster Presentation AHA Annual Scientific Session* 2009)**

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