

THE IMPACT OF PREECLAMPSIA ON THE CARDIOVASCULAR PHENOTYPE OF OFFSPRING IN EARLY LIFE.

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THESIS ABSTRACT

In recent times the potential impact of preeclampsia on the cardiovascular health of offspring has been identified. This thesis explores the relationship between preeclampsia and offspring cardiovascular phenotype during the first three decades of life. A systematic review and meta-analysis provided evidence that there was increased blood pressure and BMI in the offspring of preeclamptic pregnancies (n = 45,249). There was however limited data on metabolic features and inadequate characterisation of the degree of prematurity or growth restriction in existing literature. I therefore studied data on two birth cohorts with up to 28 years of detailed prospective follow up (n = 2868 and n = 926). Those born very preterm to preeclamptic pregnancies had transient perinatal reductions in insulin and cholesterol, although extreme prematurity was the only determinant of variation in cardiovascular risk in later life, with changes in both metabolism and blood pressure. In those born closer to, or at term, gestation was no longer relevant and an independent impact of preeclampsia on blood pressure was evident, so that by age 20, those born at term to preeclamptic pregnancies were four and a half times more likely to demonstrate clinically-apparent hypertension. I then investigated whether there were changes in other features of cardiovascular phenotype, independent of blood pressure, in preterm neonates born following preeclampsia (n = 46). At 3 months of age preterm infants born to hypertensive pregnancies had subclinical alterations in cardiac strain, independent of gestation or birth weight but not differences in blood pressure, or microvascular structure. These findings highlight preeclampsia and prematurity as key, independent perinatal factors, important in determining cardiovascular phenotype and risk during early life. Preeclampsia is associated with a specific lean, hypertensive phenotype, associated with cardiac functional alterations; these findings begin to define a distinct at risk population who may require targeted preventative interventions.

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DECLARATION

I declare that all of the work presented in this thesis is my own, including but not limited to

1. Study design
2. Ethical application
3. Subject recruitment
4. Study Investigations
5. Data Analysis
6. Data presentation

Unless acknowledged below

- The Very Preterm Birth Cohort and Early Vascular Study (Chapter 3, 4) was designed by multiple investigators including Professor A Singhal and Professor A Lucas (University College London). These and other investigators undertook recruitment of the birth cohort and early life investigations. Professor P Leeson and Dr A Lewandowski designed the young adult follow up study and undertook recruitment and study investigations in young adulthood. I conceptualized and undertook the analysis and presenation of this data as presented in this thesis.
- The RAINE study (Chapter 4 and 5) was designed and conducted by the RAINE study investigators, including Professor J Newnham and Professor L Beilin (University of Western Australia). Data was made available for my analysis through collaboration between the Cardiovascular Clinical Research Facility and the RAINE study investigators. I conceptualized and undertook the analysis and presentation of this data as presented in this thesis.

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ABBREVIATIONS

BMI – Body Mass Index

BP – Blood Pressure

BSA – Body Surface Area

CI – Confidence Interval

DBP – Diastolic Blood Pressure

EDV – Left Ventricular End Diastolic Volume

eNOS – Endothelial Nitric Oxide Synthase

EPOCH – Effect of Preeclampsia on Offspring Cardiovascular Health Study

HDL – High Density Lipoprotein

HDU – High Dependency Unit

HELLP syndrome – Haemolysis, Elevated Liver enzymes and Low Platelets syndrome

HOMA-IR – Homeostasis Model Assessment Insulin Resistance Score

HUVEC – Human Umbilical Vein Endothelial Cells

IDD – Left Ventricular Internal Diameter in Diastole

IDS – Left Ventricular Internal Diameter in Systole

ICU – Intensive Care Unit

IMT – Intima-Media thickness

IR – Ischeamia-Reperfusion

I.S.S.H.P. – International Society of Hypertension in Pregnancy

IVS – Interventicular Septum

LBW – Low Birth Weight

LDL – Low Density Lipoprotein

LDU – Low Dependency Unit

L-NAME – L-NG-Nitroarginine Methyl Ester

LR – Likelihood Ratio

LV – Left Ventricular

LVL – Left Ventricular Length

LVM – Left Ventricular Mass

MAP – Mean Arterial Pressure

mg – Miligram

MOOSE – Proposal for reporting Meta-Analyses of Observational Studies in Epidemiology

mmHg – Millimeters of Mercury

MMP – Matrix Metaloprotiens

NT – Normotensive

PDA – Patent Ductus Arteriosus

PET – Preeclamptic

PIH – Pregnancy Induced Hypertension / Gestational Hypertension

PlGF – Placental Growth Factor

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analysis

PW – Left Ventricular Posterior Wall

RR – Relative Risk

RUPP – Reduced Uterine Artery Perfusion Pressure

SBP – Systolic Blood Pressure

SCBU – Special Care Baby Unit

SD – Standard Deviation

SEM – Standard Error of Mean

sEng - Soluble Endoglin

sFLT-1 – Soluble Fms-like Tyrosine Kinase 1

TDI – Tissue Doppler Imaging

TG – Triglycerides

VEGF – Vascular Endothelial Growth Factor

WHO – World Health Organization

CHAPTER 1 – INTRODUCTION

1.1 PERINATAL HEALTH AND ADULT CARDIOVASCULAR DISEASE RISK

Globally cardiovascular disease is the single largest cause of mortality and is responsible for over 17 million deaths a year [1, 2]. In the United Kingdom, cardiovascular disease is also the largest cause of premature mortality [3]. Conventionally cardiovascular disease has been attributed to a combination of unhealthy lifestyle habits and genetic predisposition [4]. There is now, however, growing evidence that factors in the perinatal period may have a significant influence on cardiovascular structure and function in later life [5-8]. This 'developmental programming' hypothesis [9] suggests that an insult or deprivation during a critical period of growth and development can lead to long term alterations in the risk of chronic disease [10]. Barker and colleagues developed this hypothesis in the 1980s in response to epidemiological data associating a low birth weight to a variety of cardiovascular and non-cardiovascular outcomes. These outcomes included increases in mortality from coronary artery disease [11, 12] and stroke [13], as well as an increased incidence of hypertension [11], metabolic syndrome [14] and non-insulin dependent diabetes [15]. However being born with a low birth weight is a pregnancy related outcome with multifactorial determinates. The category of 'low birth weight infants' includes those who are growth restricted due to a variety of pregnancy related pathologies, but it also encompasses appropriate grown preterm born infants kept alive by modern intensive care practices. As a consequence using low birth weight alone to define a group at future cardiovascular risk, without reference to severity of prematurity or the underlying cause of growth restriction, may not provide an adequate understanding of an individual's risk profile.

The effect of specific pregnancy related conditions, which may or may not result in growth restriction, on the developing cardiovascular system of offspring, are therefore of considerable

interest. Understanding the impacts of these conditions on cardiovascular health will better identify populations at increased risk of adverse cardiovascular outcomes who may therefore warrant more intensive monitoring and preventative therapy. In addition, it will provide a better understanding of the mechanisms underlying cardiovascular development, and the interplay of developmental environment and genetics in determining subsequent cardiovascular risk.

1.2 PREECLAMPSIA

1.2.1 DEFINITION AND PREVALENCE OF PREECLAMPSIA

Preeclampsia affects between 2% and 5% of pregnancies and remains a major cause of maternal and perinatal morbidity and mortality [16, 17]. From a research perspective preeclampsia is defined in accordance with the current International Society for the Study of Hypertension in Pregnancy (I.S.S.H.P.) definition [18], although definitions have varied historically. The I.S.S.H.P. definition requires the de novo onset of hypertension (systolic blood pressure ≥140mmHg and/or a diastolic blood pressure ≥90mmHg) after the 20th gestational week, plus the presence of proteinuria (defined as the appearance of greater than 300mg/day of urinary protein). De novo hypertension in pregnancy in the absence of proteinuria is classified as gestational hypertension and has been argued to represent a distinct pathophysiological condition [19]. Unless otherwise stated the I.S.S.H.P. definition for preeclampsia and gestational hypertension are used in in this thesis.

In its symptomatic phase preeclampsia is characterized by systemic endothelial dysfunction and inflammation affecting multiple organs, including the cardiovascular system, central nervous system, liver, kidneys and lungs. Preeclampsia can result in serious complications including HELLP syndrome (Haemolysis, Elevated Liver enzymes and Low Platelet syndrome), and Eclampsia (tonic-clonic seizures) [17]. The condition is estimated to account for 20% of antenatal admissions and is associated with a mortality rate of 0.83 per 100 000 maternities (95% CI 0.53–1.30) in the United Kingdom [20, 21]. Preeclampsia is also associated with significant neonatal morbidity and

is an antecedent factor for 12% of infants born small for gestational age and in 20% of preterm deliveries [20, 22]. As well as these immediate implications for the perinatal health of women and infants, preeclampsia is gaining increasing public health interest as a condition with long-term health implications for both the mother and child.

1.2.2 Risk Factors for Preeclampsia

Despite an incomplete understanding of the pathogenesis, observational studies have identified several factors that are associated with an increased risk of preeclampsia. Although not exhaustive, Table 1.1 summarizes clinically assessable risk factors for preeclampsia.

Table 1.1: Risk Factors for Preeclampsia assessable in early pregnancy [23, 24]

Risk Factor	**Relative risk**	**95% Confidence Interval**
Age >40 years		
Primiparous:	1.68	1.23 - 2.29
Multiparous:	1.96	1.34 - 2.87
Nuliparity	2.91	1.28 – 6.61
Previous Preeclampsia	7.91	5.85 – 8.83
Family History of Preeclampsia	2.90	1.70 – 4.93
Multiple Pregnancy		
Twin Pregnancy:	2.93	2.04– 4.21
Triplet Pregnancy (vs twin):	2.83	1.25– 6.40
Preexisting Medical Conditions		
Insulin Dependent Diabetes Mellitus	3.56	2.54– 4.90
Antiphospholipid Syndrome	9.72	4.34– 21.75
Autoimmune Disease	6.90	1.10– 42.3
Blood Pressure		
DBP >110mmHg before 20 weeks	5.20	1.50– 17.2
DBP >100mmHg before 20 weeks	3.20	1.00– 7.80
SBP > 130mmHg at booking	2.37	1.78– 3.15
DBP > 80mmHg at booking	1.38	1.01– 1.87
Increased BMI		
Raised vs Normal BMI at booking	1.55	1.28– 1.88
Raised vs Normal BMI prepregnancy	2.47	1.66– 3.67
Interval between Pregnancies		
Each year of increase in interval	1.12	1.11– 1.13

Table 1.1: This table describes risk factors for preeclampsia, which can be clinically assessed in early pregnancy, and the odds ratio for development of preeclampsia associated with each.

In addition attempts have been made to predict those at risk of developing preeclampsia by considering serum and hemodynamic factors [24]. Of these methods, measurements of uterine artery pulsatility in the second trimester has been the best predictor of development of preeclampsia (likelihood ratio of a positive test LR 7.5, 95% CI 5.4 - 10.2; likelihood ratio of a negative test LR 0.59, 95% CI 0.47 to 0.71) [25].

1.2.3 Pathophysiology of Preeclampsia

The pathophysiology and aetiology of preeclampsia remains incompletely understood, however disturbed placentation in early pregnancy is considered the key pathological event [17, 26]. Normal placental development is characterized by invasion of maternal spiral arteries by fetal cytotrophoblasts and their transformation into high-caliber capacitance vessels, which provide low resistance placental perfusion to sustain fetal growth [27]. In preeclampsia the spiral arteries inadequately remodel so that uterine flow is reduced by 50% and there is chronic placental ischaemia or intermittent flow that induces an ischaemia-reperfusion phenomenon [28]. Reactive oxygen species and cytokines are released from the ischaemic placenta and trigger a systemic oxidative [29] and inflammatory state [28, 30]. The placenta also over-expresses anti-angiogenic factors that inhibit the normal function of pregnancy-related pro-angiogenic factors, including vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) [31]. The combination of these factors is believed to underlie the systemic endothelial dysfunction that is consistently found during symptomatic preeclampsia in the mother.

1.2.4 Treatment of Clinical Preeclampsia

Delivery of the placenta, and as a consequence the foetus, is currently the only definitive management of preeclampsia [17]. Systematic review of the current literature indicates that in women under 34 weeks gestation, expectant management is associated with an increased length of pregnancy (of between 7 and 14 days), and fewer neonatal complications, without significantly increased risk to the mother [32]. During periods of expectant management, maternal blood

pressure is controlled with a number of antihypertensive therapies (most commonly one or more of labetolol, nifedipine, hydralazine or methyldopa), as well as intensive monitoring for the complications of preeclampsia or deterioration of fetal wellbeing [17]. At or close to term, induction of labour is associated with better maternal outcomes [33]. Timing of delivery is often a balance between maternal condition, the desirability of additional length of gestation and measurements of fetal wellbeing [17]. Following delivery it is recommended that antihypertensive therapy, if it was commenced during pregnancy, be continued and that blood pressure be intensively monitored. Antihypertensive therapy can often be weaned rapidly following a preeclamptic pregnancy, although a subgroup of women will experience ongoing hypertension requiring long-term, or even permanent antihypertensive therapy [34].

1.3 Preeclampsia and Maternal Cardiovascular Risk

Preeclampsia has traditionally been viewed as a self-limited maternal condition of pregnancy that resolved completely with the delivery of the placenta [16, 17]. Significant data has now demonstrated, however, that for women a preeclamptic pregnancy is a novel independent risk factor for cardiovascular disease [35, 36]. In the 10-15 years following an episode of preeclampsia, women have an increased risk of developing hypertension (RR 3.7), coronary artery disease (RR 2.2) and stroke (RR 1.8) [35] and there is a graded relationship between the risk of cardiac disease and the severity of preeclampsia [36].

In addition to this absolute increase in adverse cardiovascular outcomes, following a preeclamptic pregnancy women also demonstrate higher levels of cardiovascular risk factors, including impaired endothelial function [37, 38], increased arterial stiffness [39], capillary rarefaction [40], impaired insulin sensitivity [41], altered lipid profile [41-43], increased BMI [42, 44] and increased incidence of type two diabetes [42, 45-47]. Women with a history of preeclampsia also have altered cardiac function, both acutely and persisting for at least a year after the affected pregnancy [48]. The severity of the preeclampsia also appears to be relevant to these aspects of

later cardiovascular phenotype. Recent work has demonstrated that women who develop early onset preeclampsia (prior to 34 weeks gestation) have unique adverse blood pressure characteristics 7-12 years following the affected pregnancy, which are not seen in women who developed the condition closer to term [49].

The mechanism of this increased risk in women is unclear. Impaired endothelial-dependent vasodilatation in conduit and resistance arteries has been demonstrated in women who have preeclampsia [50-57], and it is also a key factor in the development of atherosclerotic cardiovascular disease where it precedes clinically identifiable structural changes in the vasculature [58]. The increase in cardiovascular risk following preeclampsia may be a consequence of the pro-atherogenic impact of persistent endothelial dysfunction, a result of subclinical endothelial injury at the time of pregnancy, or related to pre-existing differences in endothelial function that predispose to both conditions (Figure 1.1).

FIGURE 1.1 THEORETICAL TIMELINES OF IMPAIRMENT OF ENDOTHELIAL FUNCTION AND DEVELOPMENT OF CARDIOVASCULAR DISEASE FOLLOWING PREECLAMPTIC PREGNANCY

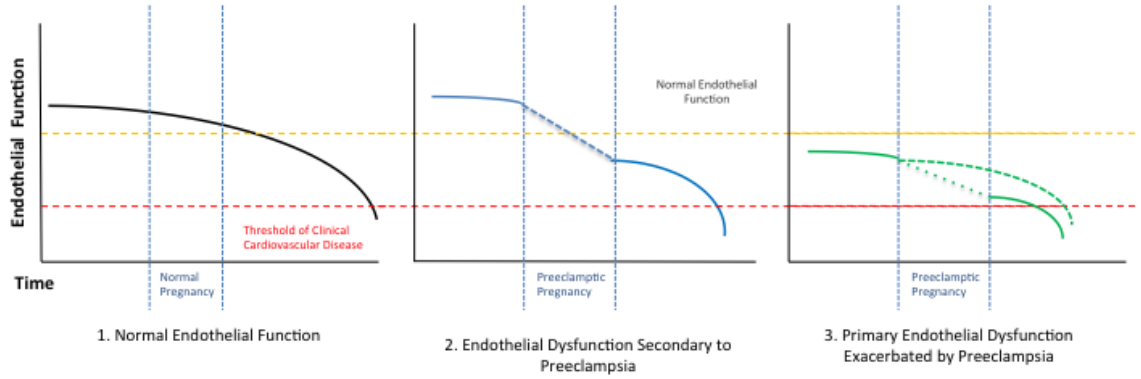


Figure Legend: (1) In normal individuals there is a gradual age related reduction in endothelial function, which can be exacerbated by the presence of cardiovascular risk factors and associates with the future risk of clinical cardiovascular disease. Women who experience preeclamptic pregnancies are known to have impaired endothelial function during pregnancy, and up to 3 years following an affected pregnancy. (2) It is possible that these women begin life with normal endothelial function, which is acutely impaired during a preeclamptic pregnancy. This followed by ongoing age related decreases in endothelial function may relate to the increased incidence of cardiovascular disease in these individuals. (3) Alternatively women who develop preeclampsia may have primary endothelial dysfunction which both puts them at risk of preeclampsia, this may then be exacerbated by the preeclamptic pregnancy (dotted then solid line), or simply persist (dashed line), in either case leading to higher incidence of cardiovascular disease.

A past history of preeclampsia is now considered an integral part of preventive cardiology assessment in women [59]. Women who have experienced preeclampsia constitute a relatively

young group, and as there is potentially a prolonged period between exposure and clinical outcome, they constitute a cohort who is likely to benefit from more intensive follow up and early intervention to reduce other cardiovascular risk factors.

1.4 Preeclampsia and Offspring Cardiovascular Risk

In addition to increased maternal risk, evidence is now emerging that being born to a preeclamptic pregnancy is associated with increased cardiovascular risk in later life.

1.4.1 Risk of Adverse Cardiovascular Events in the Offspring of Preeclamptic Pregnancies

Only one study to date has considered the long-term risk of cardiac or vascular events in the offspring of preeclamptic pregnancies [60]. This 60 year follow up study of 6140 individuals born in Helsinki between 1934 and 1944 (284 born to pregnancies complicated by preeclampsia) demonstrated that adults whose mothers had been preeclamptic had almost double the risk of stroke in adult life (RR 1.9 (95% CI 1.2 to 3.0) p = 0.01) [60]. There was also trend towards an increased incidence of coronary artery disease (RR 1.4 (95% CI 0.9-2.10 p = 0.1) although this did not reach statistical significance. Neither relationship was altered by adjustment for birth weight or gestational age suggesting that preeclampsia itself, rather than associated growth restriction or prematurity, is relevant to the mechanism of this increased risk.

1.4.2 Risk of Hypertension and Diabetes in the Offspring of Preeclamptic Pregnancies

Two studies have considered the relative risk of diagnosis of hypertension in the offspring of preeclamptic pregnancies. Kajantie et al. in their 60 year follow up of the Helsinki Birth Cohort (see section 1.4.1) found that individuals whose mothers had experienced severe preeclampsia (SBP >160 and DBP >110) also had an increased risk of hypertension in later life (RR 1.5 p = 0.03) that was not evident in those whose mothers were less severely affected. A shorter follow up study, conducted in New England, demonstrated that the offspring of preeclamptic pregnancies

were more likely to be prescribed antihypertensive medication by age 50 than those whose mothers remained normotensive during their pregnancy [61].

One study also considered whether the offspring of preeclamptic pregnancies were at increased risk of Type II diabetes [45]. Libby and colleagues undertook a 45-year follow-up of 8,648 individuals born between 1952 and 1958. This study showed a trend towards an increased risk of diabetes (1.3 (95% C.I 0.91-2.15)) but this did not reach statistical significance.

1.4.3 Alterations in Cardiovascular Phenotype in the offspring of preeclamptic Pregnancies

Alterations in the cardiovascular phenotype of the offspring of preeclamptic pregnancies have been shown to be evident from childhood. A meta-analysis of blood pressure variation, based on 10 studies undertaken in children and young adults, indicated a history of preeclampsia associated with a 2.3 mmHg higher systolic and 1.7 mmHg higher diastolic blood pressure in the offspring [62]. More recent publications have further considered the relationship between preeclampsia and offspring blood pressure in children and young adults (See Chapter 2). In addition a growing body of work is now considering the relationship between preeclampsia and other risk markers for cardiovascular disease, including endothelial function, anthropometry in childhood and adult life and lipid and glucose profiles.

In particular the offspring of preeclamptic pregnancies seem to have a distinct vascular phenotype. In a group of preterm born young adults (aged 20 to 30 years), only those born to hypertensive pregnancies had evidence of impaired flow mediated endothelial responses, although both groups had increased blood pressure compared to term born controls [8]. The offspring of preeclamptic women also have impaired endothelial function in childhood (age 5-8 years) [63] and adolescence (age 14 years) [64]. Only one study, in children aged between 9 and 10 years, has not been able to demonstrate this difference in endothelial response [65]. These offspring also show development of an early atherogenic phenotype with evidence increased

carotid intima-media thickness in their twenties [8] and aortic arterial thickening already evident at birth [66].

As improvements in neonatal intensive care has led to reduction in neonatal mortality associated with severe preeclampsia leading to premature birth, the potential long term impacts of in utero exposure on offspring health in later life has become an issue of growing importance. Furthermore as preeclampsia affects 2 to 5% of pregnancies, 1.2 to 3 million people in the United Kingdom, 6 to 15 million in the United States [16, 17] and up to 300 million individuals worldwide are the offspring of pregnancies complicated by preeclampsia and may therefore benefit from greater understanding of any cardiovascular implications.

1.5 Preeclampsia and Programming of Offspring Cardiovascular Phenotype: Insights from Mechanistic Literature.

Observational studies have demonstrated that the offspring of preeclamptic pregnancies demonstrate alterations in cardiovascular function and risk compared to the offspring of normotensive pregnancies [60, 67]. The key biological pathways underlying this difference remain undetermined. In particular the demonstrated increase in blood pressure (2-3mmHg) is insufficient to explain all of the increase in risk of cerebrovascular events that has been demonstrated in follow up studies [60, 68] .

Preeclampsia is a condition with a heritability of approximately 55% [69] which is contributed to by both maternal and fetal genes. It is plausible that some of the alterations in risk seen in the offspring of preeclamptic pregnancies relate to shared genetic factors between the mother and child. However, the observed vascular modifications in those born to preeclamptic mothers appear to be induced to some extent specifically by *in utero* exposure to preeclampsia as siblings of offspring born to preeclamptic pregnancies, who themselves were born to uncomplicated pregnancies, do not demonstrate the vascular abnormalities seen in the preeclamptic offspring

[64]. Additionally offspring of preeclamptic pregnancies are frequently also born prematurely or with low birth weight due to intra-uterine growth restriction [23]. Prematurity and intra-uterine growth restriction have themselves been associated with alterations in cardiovascular risk factors [70, 71]. Furthermore early and late onset preeclampsia are increasingly being recognized as different subclasses of disease, which may have different implications for cardiovascular risk in the offspring [72].

This complicated combination of factors means that in many currently available human studies it is challenging to separate the effects of exposure to preeclampsia from other *in utero* insults. It is known that if a baby is born at term without intrauterine growth restriction, but their mother had preeclampsia, they still have an increased risk of stroke [60] and vascular abnormalities [73] that persists after adjustment for birth weight. It therefore seems plausible that there is a unique insult within the *in utero* environment during preeclampsia that is incremental to, or acts in synergy with, any risks inferred by intrauterine growth restriction or preterm birth. This hypothesis is further supported by the observation that preterm birth largely protects offspring from the adverse associations between birth weight and later cardiovascular disease [11, 74]. In the case of preeclampsia however, which is one of the leading causes of both preterm birth and growth restriction, the largest differences in later blood pressure are seen in those offspring of preeclamptic pregnancies born preterm and growth restricted [8]. The cumulative body of work supports associations between preeclampsia exposure and offspring cardiovascular health, as well as a potential pivotal role for long-term programmed changes in vascular biology. The range of cardiovascular changes demonstrated in the human and animal offspring of preeclamptic pregnancies, and potential mechanisms underlying these changes are highlighted in Figure 1.2 and Table 1.2

FIGURE 1.2: POTENTIAL MECHANISM OF CARDIOVASCULAR PROGRAMMING IN THE OFFSPRING FOLLOWING A PREECLAMPTIC PREGNANCY.

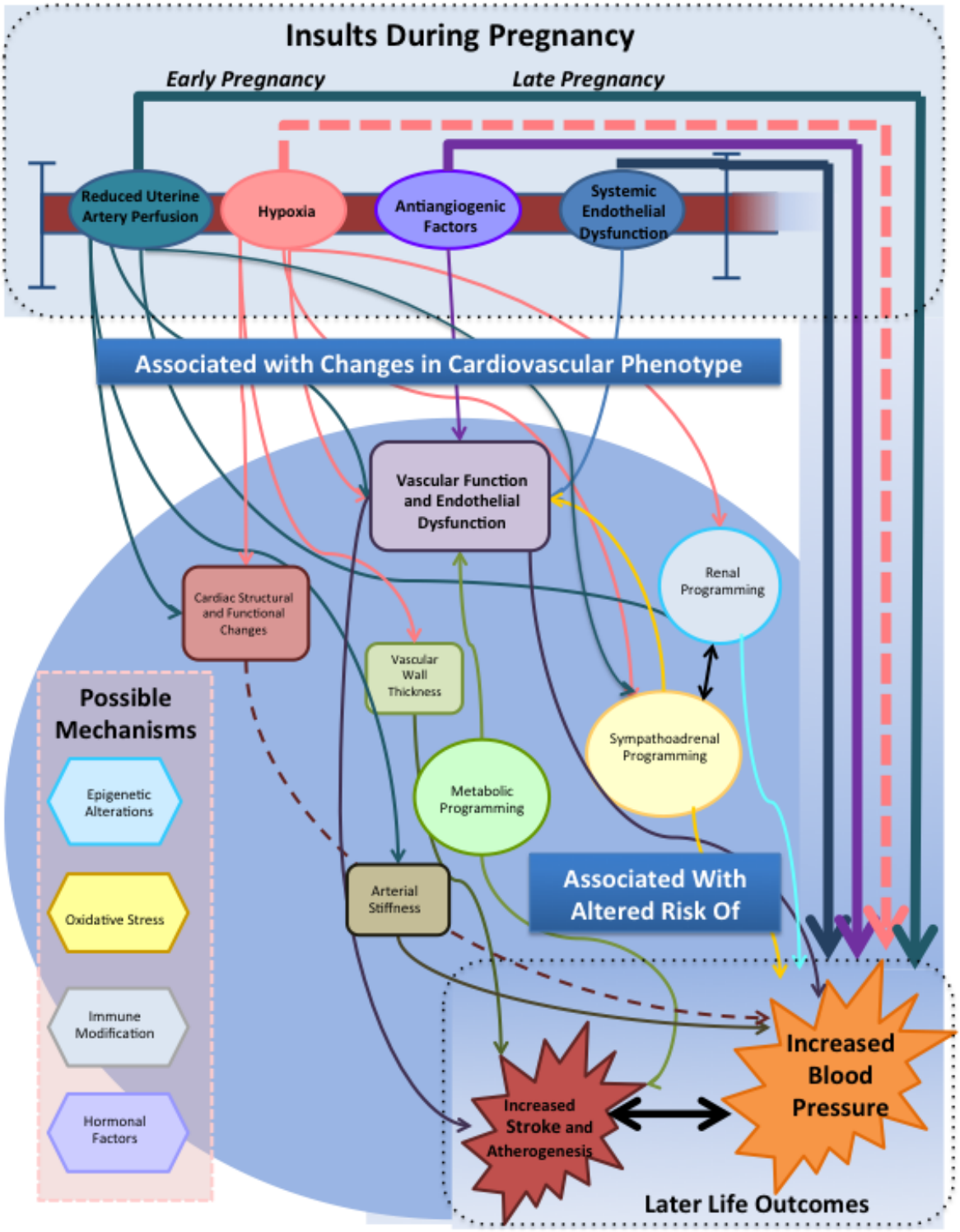


Figure 1.2: Summary of the potential and demonstrated mechanisms by which in utero exposure to preeclampsia may lead to altered cardiovascular physiology and risk in the offspring in later life. Insults during pregnancy that associate with increased blood pressure in adult offspring are indicate at the top of the figure. These insults also lead to changes in cardiovascular phenotype during life as indicated in the central panel. These alterations may relate directly or indirectly to later increases in blood pressure and/or stroke risk. Finally potential mechanistic or modifying influences – which may act both during early life and/or modifiers of adult phenotype - are indicated on the pink tinted panel on the left.

TABLE 1.2 EVIDENCE OF CARDIOVASCULAR PROGRAMMING IN THE OFFSPRING OF PREECLAMPTIC PREGNANCIES.

	Animal model offspring				Human offspring						
	RUPP	Hypoxia	sFLT1	eNOS	Placenta	Cord Blood	HUVECs	Neonate	Childhood	Adolescence	Young Adult
Blood Pressure											
↑ Blood Pressure	[75] [76] [77] [78] [79] [80] [81] [82] [83] [84] [85] [86] [87] [88] [89]	[90] [91]	[92] [93]	[94] [95]	--	--	--	[96]	[63] [97] [98] [65] [99] [100] [101]	[64] [102] [103] [104] [105]	[8]
Altered BP under stress	--	--	[90]	[94]	--	--	--	--	--	--	--
Vascular Structural Changes											
↑ IMT/ vascular wall thickness	[75] [76] [106]	[107] [108] [109]	--	--	--	--	--	[66]	--	--	[8]
↑ Arterial Stiffness	[79] [75]	[110]	--	[111] [112]	--	--	--	--	[65]	--	[8]
Vascular Functional Changes											
Endothelial Dysfunction	[75] [113] [78] [114]	[110] [115] [116]	--	[117] [112] [95] [111]	--	[118]	[119]	[73]	[65]	[64]	[8]
Non-endothelial function	[114] [113]	[116]	[120]	[112] [95]	--	--	--	--	--	[64]	[8]
Cardiac Structural and Functional Changes											
Altered Cardiac Structure	[77] [81] [106]	[90] [121] [122] [123] [124]	[93]	--	--	--	--	--	[125]	--	--
Left Ventricular Dysfunction	--	[121]	--	--	--	--	--	[126]	[125]	--	--
↑ IR injury	--	[127] [128] [91] [129] [130]	--	--	--	--	--	--	--	--	--
Renal Structural and Function Changes											
Altered Structure and Function	[131] [85] [77] [81] [82] [84] [76] [77] [106] [86] [87] [132]	--	[133]	--	--	--	--	--	--	--	--
Metabolic Changes											
↑BMI	[78] [80] [81] [82] [131] [85] [77] [106] [114] [89] [134] [135] [136] [137]	[116] [90] [108] [127] [121] [91] [138] [139]	[140] [93] [120]	[94] [112]	--	--	--	[66]	[100] [97] [65, 98, 102, 103]	[64] [105] [141]	[8] [104]
↑ serum Lipids	[80] [75] [137]	[139] [138]	[93]	--	--	[66] [142] [143] [144] [145] [146]	--	--	[65] [63]	[105]	[8]
Altered glucose metabolism	[75] [80] [136] [137] [147]	[139]	[93]	--	--	--	--	--	--	[105]	[8]
Altered Sympathoadrenal function											
Adrenal hormones	[85, 132, 137]	[90] [148] [149]	--	--	--	[150, 151]	--	--	--	[152] [105]	--
Sympathetic Changes	[153]	[90] [154]	--	--	--	--	--	--	--	--	--
Genetic/Epigenetic Changes											
Gene Expression	[81] [84] [77] [87] [132] [85] [75] [86]	[122] [123] [91] [138] [139] [130]	--	[133]	[155] [156] [157] [158] [159] [160] [161] [162] [163]	--	--	--	--	--	--
Epigenetics	--	[129]	--	--	[164, 165] [166]	--	--	--	--	--	--
Other											
↓ antioxidants	--	--	--	--	[167]	[143] [168]	--	--	--	--	--
↑ oxidative stress	[80]	--	--	--	[169]	[143] [168, 169]	--	--	--	--	--
↑ antiangiogenic factors	--	--	--	--	--	[170, 171]	--	--	[63]	--	--
↑ PIGF/ VEGF	--	--	--	--	--	[171]	--	--	[63]	--	--
Altered Inflammation	[75]	[138]	[93]	--	--	--	[172] [173] [174] [175]	[176]	[65] [63]	--	--

1.5.1 The In Utero Insult

The disturbance of placentation believed to underlie the development of preeclampsia has its origins in early pregnancy. Offspring of preeclamptic pregnancies therefore develop from the first trimester onwards within an environment of placental insufficiency and restricted oxygen supply. By the start of the second trimester there is, in addition, exposure to elevated circulating antiangiogenic factors [28-30]. These factors predate the later emergence of the clinical preeclamptic syndrome and its associated diffuse maternal inflammatory, oxidative and dysfunctional endothelial state. These factors could, individually or in concert, influence the programming of future cardiovascular risk in these individuals.

The spontaneous development of preeclampsia during pregnancy is a condition largely unique to humans [177, 178]. To mimic the human syndrome an animal model would require the development of pregnancy-specific hypertension, proteinuria and associated alterations in vascular function and biomarkers, and develop secondary to impaired trophoblastic invasion [178]. Although a variety of models based on different aspects of the pathophysiology of preeclampsia exist, [177, 178] no model has successfully mimicked all of the pathophysiological features of preeclampsia or accurately replicated the first trimester origin of the human condition. Another limitation is that the models have been primarily developed to investigate preeclampsia in the mother, not the offspring.

The adequacy of animal models developed to study preeclampsia have in part been defined by their ability to induce intrauterine growth restriction. As a result, these 'preeclampsia' models have been adopted to study how intrauterine growth restriction impacts on the offspring [179]. The findings from these offspring studies, in particular those that maintain experimental set ups that would be expected to induce preeclampsia-like syndromes in the mothers, thereby provide insights into the impact of preeclampsia-related insults on offspring cardiovasculature.

Uterine perfusion: Mechanical reduction in maternal uterine artery blood flow by unilateral or bilateral uterine artery ligation induces a pregnancy-specific increase in maternal blood pressure [180]. The model has been applied in a range of species including dogs [181], rats [178], rabbits [182], sheep [183], guinea-pigs [184] and primates [185]. In rats, reduced uterine artery perfusion pressure (RUPP) induces a full preeclamptic pregnancy phenotype including markedly elevated blood pressure and changes in renal function [186], dysfunction of endothelial-dependent vascular relaxation [187, 188] as well as increased circulating levels of soluble fms-like tyrosine kinase-1 (sFLT-1) with resultant reduction in VEGF and PIGF [189]. Variations of the model have been used to study the influence of reduced uterine perfusion on offspring blood pressure in rats. Although offspring blood pressure does not differ between cases and controls in a large number of the studies [78-85, 131], when all the studies are considered together, the timing of the reduction in uterine perfusion seems critical in determining offspring phenotype (Figure 1.3).

If perfusion is reduced at, or before 14 days gestation, resting blood pressure is consistently elevated throughout life. When uterine perfusion is reduced only in late gestation, the studies either show no increase in offspring blood pressure or modest changes in later life, despite the fact that both early and late restriction of perfusion consistently induce growth restriction in exposed offspring [75-77]. These findings suggest early exposure to uterine perfusion abnormalities are critical to the blood pressure programming in the offspring.

FIGURE 1.3 - TIMING OF REDUCTION OF UTERINE ARTERY PERFUSION AND OFFSPRING BLOOD PRESSURE

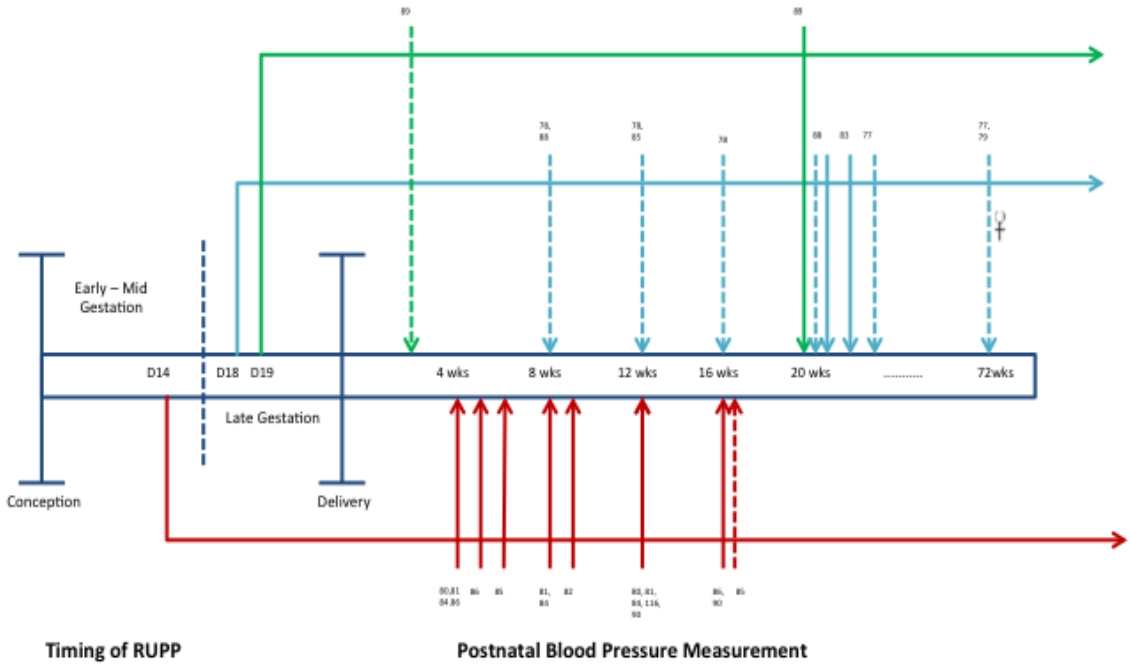


Figure 1.3: Summary of the results of studies considering the effect of RUPP at different points of gestation on offspring blood pressure. Solid lines indicate that studies have demonstrated an increase in blood pressure in RUPP animals compared to controls, while dashed lines indicate no difference in blood pressure. The colour of the lines indicates the timing of the insult; the red line showing the result of studies in which uterine artery perfusion was reduced at day 14, the blue line day 18 and the green line day 19 of gestation. These suggest that the timing of the insult may be critical in determining the effect on offspring blood pressure.

Hypoxia: In all pregnancies early placental development takes place under low oxygen conditions as this predates formation of an effective maternal-fetal circulation [155, 190]. Even in later stages of normal pregnancy, foetuses whose mothers have normal placental perfusion are still exposed to intervillous oxygen levels equivalent to only 8-11% inspired O_2. In preeclampsia abnormal placentation most likely results in lower oxygen delivery for the majority of gestation. In pregnant mice, exposure to an inappropriate level of hypoxia from early pregnancy (9.5% O_2 from day 7.5 to day 17 of gestation) induces a range of preeclampsia-like symptoms including hypertension, proteinuria, intra-uterine growth restriction, renal pathology and elevations in maternal soluble endoglin [191]. Interestingly less severe hypoxia (11% O_2) or hypoxia later in gestation (9.5% O_2 from day 10 or 15 to day 17 of gestation) does not induce these symptoms [191]. When the data from models of pregnancy hypoxia are collated, despite a range of changes

in other cardiovascular measures, there is a striking lack of any consistent reports of elevated blood pressure in the offspring. This may be because the majority of studies are based on the use of oxygen concentrations of higher than 9.5%, and/or exposed animals later in pregnancy [110, 115, 116]. Blood pressure variation in the offspring has been demonstrated in response to stress [90], but this blood pressure phenotype is distinct from that observed in human offspring of preeclamptic pregnancies, who have increased resting blood pressure in early life [67].

Antiangiogenic factors: Multiple studies have now demonstrated that a key step in the progression of preeclampsia from placental underperfusion and hypoxia to a clinical syndrome, is an increase in levels of circulating antiangiogenic factors. These include the soluble fms-like tyrosine kinase-1 (sFLT-1), the alternatively spliced truncated form of vascular endothelial growth factor receptor 1 [192], and soluble endoglin (sEng)[193-195]. sFLT-1 inhibits the normal function of pregnancy related angiogenic factors, including VEGF and PlGF [31]. A rat model based on adenovirus vector administration of sFLT-1 early in the second trimester (day 8 to 9 of pregnancy) has been developed [193] and the transfected rats develop hypertension, heavy proteinuria and glomerular endotheliosis. The same intervention in mice induces a slightly different phenotype with maternal hypertension, fetal growth restriction and impairment of maternal platelet count [140]. Male offspring of transfected animals demonstrate sustained increase in blood pressure evident from the first day of life that persists over the first 6 months [92]. These results suggest even with normal placentation and uterine blood flow in early pregnancy, exposure to an excess of antiangiogenic factors, specifically raised sFLT-1 and its biological sequelae, is sufficient to induce long term changes in blood pressure in the offspring.

Systemic endothelial dysfunction: The specific role of systemic maternal endothelial dysfunction is relatively simpler to explore. This is because targeted animal models are available that allow *in utero* development in a mother with endothelial dysfunction to be studied. These models are based either on systemic inhibition of endothelial nitric oxide synthase (eNOS) by administration

of L-NAME or by eNOS knockout [177]. One limitation is that these are not good models of preeclampsia *per se* as the hypertension in eNOS knockout mice is not pregnancy-specific [196, 197]. Nevertheless the mice consistently exhibit abnormal endothelial function, and through selective breeding of an eNOS knockout mother with a wild type male, an experimental scenario can be generated to study the impact of maternal endothelial dysfunction on the *in utero* development of an eNOS heterozygous offspring. These offspring have higher blood pressure during adulthood than the genetically similar offspring bred from a wild type mother and an eNOS knockout father, who differ only in that their *in utero* development was in a wild type mother with normal endothelial responses [94]. Such findings highlight the potential pivotal importance of maternal endothelial dysfunction during pregnancy, even in the absence of other preeclamptic features, on long-term offspring outcome.

1.5.2 Mechanisms Underlying the Long-Term Programming of the Cardiovascular System

To understand how the above insults may lead to long term alterations in blood pressure, a more in depth study of the cardiovascular phenotype that may be relevant to blood pressure in the available experimental models and human studies, is required.

Vascular Structure: Arterial stiffness is a key component of blood pressure in humans with increased arterial stiffness associated with elevation of central blood pressures [198, 199]. Therefore one possibility is that exposure to the abnormal intrauterine milieu of preeclampsia leads to vascular structural remodelling that persists into post-natal life [200]. Supporting this, stress strain studies of arteries have found increased aortic stiffness, greater vessel wall elastic fiber content in the vessel wall, and increased thickness of the aortic media in RUPP offspring [75, 106]. Changes in arterial stiffness are not found however in studies of human offspring of preeclamptic pregnancies in adolescence (age 14 years) [64] or young adulthood (age 20 – 30 years) [8], and other hypoxia and RUPP based studies suggest development of an early

atherogenic phenotype is more relevant than vascular stiffening [76, 109, 199]. Consistent with the observations in animal studies, human offspring of preeclamptic pregnancies also show an increased intima-media thickness in their twenties [8] with aortic arterial thickening already evident at birth [66]. Therefore, the features of vascular remodeling that have been observed are predominantly not associated with the arterial stiffening that tends to underlie increased blood pressure.

Vascular Function: One possibility is that the variation in blood pressure is driven by underlying induced abnormalities in vascular function rather than structural changes. In particular, systemic endothelial function is associated with traditional cardiovascular risk factors, including hypertension [201]. Endothelial dysfunction is also an early biological factor in the development of atherosclerotic vascular disease [58, 202] and predisposes to the development of left ventricular hypertrophy [203]. Consistent with vascular function being a central factor in the association between preeclampsia and long-term cardiovascular health, abnormalities in vascular responses are found in nearly all offspring models that mimic preeclampsia, as well as in the majority of human studies of offspring of preeclamptic pregnancies. In humans, endothelial dysfunction has been demonstrated in children and young adults born to preeclamptic pregnancies [63, 118, 204] and is distinct from changes in vascular function associated with prematurity [8]. Evidence of reduced endothelial function has also been demonstrated in the offspring of all major animal models of preeclampsia [75, 78, 110, 112, 114, 115, 117, 205], and data suggests that a reduction in nitric oxide induced vasodilation may underlie this change [78, 110, 115, 116, 206]. The underlying mechanism causing this reduction in nitric oxide synthesis is unclear and further studies are required to clarify these. Such changes may represent the effects of anti-angiogenic factors [207, 208], shared genetic or epigenetic factors between mother and child [164, 165, 209, 210], or alternatively the endothelial dysfunction may represent a biomarker of other underlying metabolic abnormalities related to cardiovascular disease.

Cardiac Structure and Function: An alternative possibility to explain the variation in blood pressure is that the *in utero* insult alters cardiac development. Cardiac structural and functional changes, such as increased cardiac output or left ventricular hypertrophy, then predispose to development of hypertension [211]. Animal models of hypoxia in pregnancy have demonstrated alteration in cardiac structure although these appear to be predominantly of a cardiomyopathic phenotype, more commonly linked with development of heart failure [109, 121-123, 127], and complex alterations in cardiac collagen and MMP function [122, 212]. Studies in both animals and humans have not however consistently demonstrated alterations in cardiac function that would be consistent with such changes in blood pressure [121, 125, 213].

Other factors: Animal model studies have implicated alterations in sympathoadrenal, renal, immune and hormonal function in the cardiovascular programming in the offspring of preeclamptic pregnancy [214]. Such changes have not, to date been replicated in human populations. Animal model findings suggest however that they may provide interesting avenues of investigation aimed at further understanding the phenotype of individuals exposed to preeclampsia in utero.

1.6 Conclusions and Thesis Objectives

With increasing data suggesting that individuals born following preeclamptic pregnancies are at greater risk of future cardiovascular events, further studies are needed to clarify and explore the detailed cardiovascular phenotype associated with in utero exposure to preeclampsia and whether this phenotype is present from birth, or develops during post natal life. Additionally work is needed to distinguish the phenotype associated with prematurity from that of preeclampsia. The primary objectives of my thesis are therefore:

1) To investigate the known impact of in utero exposure to preeclampsia on offspring cardiovascular physiology and phenotype (Chapter 2),

2) To investigate the impact of in utero exposure to preeclampsia on infant metabolic phenotype (Chapter 3),

3) To investigate the impact of in utero exposure to preeclampsia on cardiometabolic and cardiovascular phenotype in adolescence and young adulthood in preterm and term born offspring (Chapters 4 and 5) and

4) To investigate whether the alterations in cardiovascular phenotype associated with exposure to a preeclamptic pregnancy are identifiable in the neonatal period and in early infancy, and where possible to differentiate the effects of preeclampsia from those of prematurity (Chapters 6 to 9).

CHAPTER 2 – CARDIOVASCULAR PHENOTYPE OF CHILDREN AND YOUNG ADULTS EXPOSED IN UTERO TO PREECLAMPSIA – A SYSTEMATIC REVIEW AND META-ANALYSIS.

2.1 THESIS CONTEXT

Offspring of preeclamptic pregnancies are reported to exhibit altered cardiovascular phenotype and risk [60, 62], however the total cardiovascular risk profile of these individuals has not been established. Understanding this phenotype will guide future mechanistic and detailed phenotypic research. This chapter aims to quantify the known cardiovascular risk profile of children and young adults born to preeclamptic pregnancies by systematic review and meta-analysis.

2.2 INTRODUCTION

As noted in Chapter 1, a growing body of evidence now suggests that individuals exposed in utero to preeclampsia have altered blood pressure in childhood [62], are twice as likely to need antihypertensive therapy [61], and are more likely to experience adverse cardiovascular events in later life [60] when compared to individuals whose mothers remained normotensive. The majority of published work to date considers offspring's blood pressure, although there is increasing research investigating other cardiovascular risk factors. Understanding the totality of the cardiovascular phenotype associated with being born to a preeclamptic pregnancy may provide insight into the reasons behind the observed increase in cardiovascular risk in this population, which is greater than would be expected from the demonstrated increase in blood pressure alone [60, 68]. Such an understanding may also allow us to characterise a population for whom primary prevention advice is particularly relevant.

I therefore undertook to investigate whether an increased cardiovascular risk profile was evident from childhood in individuals born following a preeclamptic pregnancy, and what the characteristic features of this risk profile were. I performed a systematic review and meta-analysis of the literature considering the impact of preeclampsia on cardiovascular risk factors including blood pressure, body mass index, lipid profile and glucose metabolism. In addition I have investigated whether any of these relationships varied with gender, age or in the presence of intra-uterine growth restriction.

2.3 METHODS

2.3.1 SEARCH STRATEGY

This systematic review was undertaken in accordance with the PRISMA and the MOOSE Group guidelines [215, 216]. All studies describing, or which might describe, the impact of preeclampsia on offspring cardiovascular risk factors were sought by computerized Ovid Search of Medline (1948 – August 2011) and EMBASE (1980 – August 2011). The search strategy is detailed in Appendix 1. Following the computerized search all identified studies were sifted by abstract for relevance by two investigators, independently and without reference to results, journal or author. Discrepancies between identified studies were resolved by discussion. Additionally references of identified studies were searched and potentially relevant studies identified. Data was extracted from eligible studies and cross-checked for accuracy. Where required data could not be extracted authors were contacted.

2.3.2 INCLUSION CRITERIA

Studies were selected for inclusion if they compared cardiovascular risk factors in children exposed to preeclampsia in utero to controls. Outcome measures included were: blood pressure; body mass index (BMI); lipid levels and insulin sensitivity, glucose or glucose metabolism. The primary summary measure was difference in means for continuous outcome variables. Studies reporting only relative risk of an outcome such as hypertension, obesity or diabetes, rather than

mean values in the study population were excluded. Preeclampsia was defined using the current International Society for Hypertension in Pregnancy research definition [18]. However, as diagnostic criteria have historically varied, studies where preeclampsia was diagnosed by *de novo* onset of hypertension and protienuria were also included. Where the same cohort was reported in multiple publications at similar ages the most recent publication was included. Studies where distinction between gestational hypertension and preeclampsia was impossible, and studies comparing offspring to another potential risk group such as the offspring of diabetic pregnancies, were excluded. All studies were published in peer-reviewed journals, undertaken in human subjects and published in English or French.

2.3.3 ANALYSIS

From each included study, definition of preeclampsia, age of offspring at follow up, number of cases and controls, birth weight, and gestational age of cases and controls were collected. Outcome measures were documented and for each the mean and standard deviation recorded. Funnel plots were examined for evidence of bias (Appendix 2 - Funnel Plots). Comparative review of outcome measures was undertaken and consideration given to whether results varied between genders, with age or in the presence of intrauterine growth restriction. When informative, meta-analysis was performed using Cochrane Collaborations RevMan software (Version 5.1.1) [217] based on an inverse variance method. The mean difference between outcome measures and 95% confidence interval (CI) were calculated using a fixed effect model, which reflects only the random error within each study and is less affected by publication bias [218-220]. As publication bias is a greater issue in observational studies, when compared to randomized controlled trials [218-220], this model was most appropriate. However to ensure conclusions were not unduly influenced by choice of model I also repeated the analysis with a random effects model. No differences in outcome were identified (See Appendix 3 – Fixed vs Random Effects Model Meta-Analysis Results). Statistical assessment of heterogeneity was undertaken [217].

2.4 Results

2.4.1 Study Identification

2,484 articles identified by the search yielded 83 articles for review (Figure 2.1). 18 studies were included providing data on 45,249 individuals (Table 2.1- Characteristics of Included Studies). Once studies without relevant outcome measures were excluded (30 studies), reasons for exclusion were: inability to distinguish gestational hypertension and preeclampsia (17 studies); repeat publication of data (4 studies); inability to exclude hypertension prior to pregnancy (12 studies) and studies reporting relative risk of a diagnosis rather than mean values in the study population (2 Studies).

Figure 2.1 – Search Strategy for identification of eligible trials.

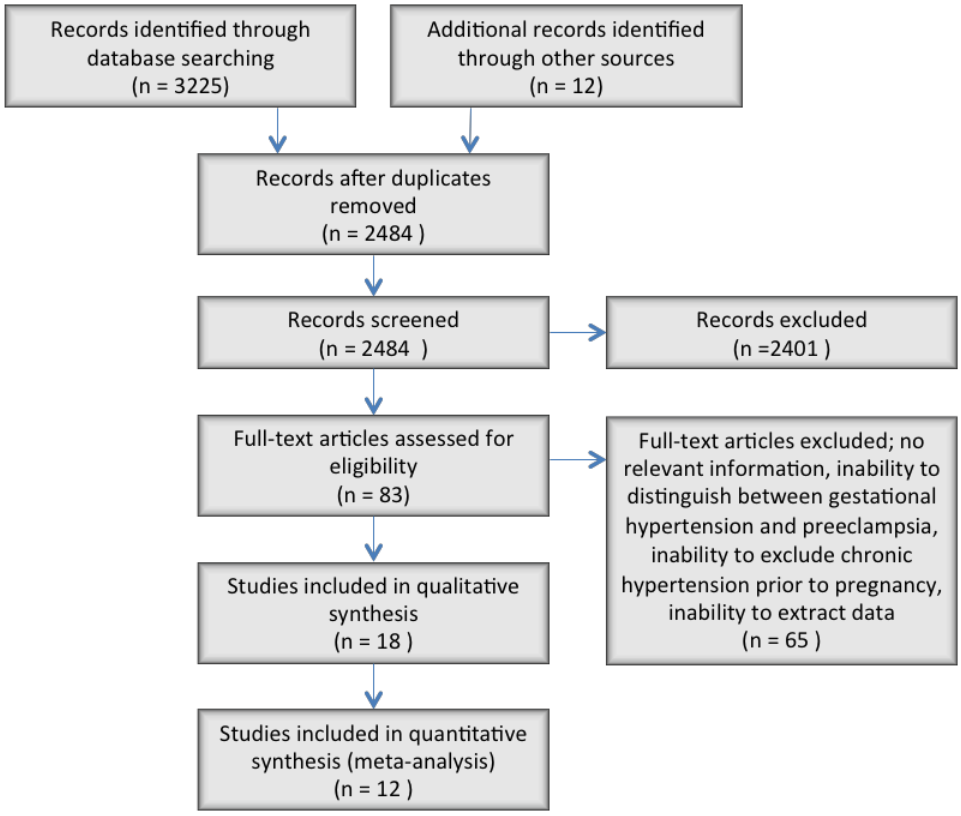


Figure 2.1: Search strategy and number of studies excluded at each stage of the search during this review.

TABLE 2.1: CHARACTERISTICS OF INCLUDED STUDIES

Study	Study Design	Definition of Preeclampsia SBP/DBP	Defin ition of Preeclampsia Protienuria	Number exposed to preeclampsia/ Controls	Birth weight Cases/Control (grams)	Gestational age Cases/Control (weeks)	Follow Up (Years)	Outcome Measure Considered
				Blood Pressure				
Hiller(2007) [101]	CS	DBP> 110mmHg or 2x DBP > 90mmHg	300mg/24 hours or 2x 2++ protein	10/78*	-	-	4-7	SBP/DBP
Jayet(2010) [64]	CC	>140/90 or rise >30/15	Consecutive dipsticks Protienuria	48/90	2843/3244	>37 and <42	14	SBP/DBP,BMI,
Kvehaugen(2010) [97]	CC	>140/90 x ≥2	≥1+ on ≥2 samples	23/17	1740/3526	32.3/38.6	5-8	SBP/DBP BMI
Lawlor(2011) [65]	CS	>139/>89	1+	143/5367	3042/3443	37.6/39.5	9-10	SBP/DBP BMI, Lipids
Lazdam(2010) [8]	CC	>140/90	2+	16/38	1323/ ?	30.93/ >37	20-30	SBP/DBP, BMI Lipids, Glucose/Insulin
Oglaend(2009) [141]	CC	>in DBP to >90	1+	181/356	~3360	37.6/40.1	10-12	SBP/ DBP
Palti(1989) [100]	CC	>140/90 or rise >30/15	Oedema and Protienuria	94/94	12.7% cases LBW/ 4.2% controls LBW	22% cases <37 / 4.3% controls <37	6	SBP/DBP
Seidman(1991) [104]	CS	1952 American Committee on Maternal Welfare Definition		428/33117	M: 3175/3338 F: 3009/3215		17	SBP/DBP BMI
Tenhola (2003) [105]	CC	>140/90 or rise >30/15	>300mg/24 hours	60/60	2622/2868	36.6/36.7	12	SBP/DBP, BMI, Lipids, Glucose/Insulin
Vatten(2003) [102]	CS	>140/90 or rise >30/15	>300mg/24 hours	220/3853	3421/3485	40.3/40.6	13-19	SBP/DBP BMI
				Other Outcome Factors				
Akcakus(2010) [221]	CC	>140/90, Severe >160/110	300mg/24 hours	30/30†/30	1817/1693†/1762	32.4/32.4†/31.5	0	Lipids
Catarino(2008) [142]	CC	ISSHP definition		46/42	2600/3400	37/38.5	0	Lipids
Howlader(2009) [143]	CC	>140/90 or rise >30/15	3000mg/24 hours	15/20	1750/2570	33.25/36.9	0	Lipids
Kvehaugen(2011) [63]	CC	>140/90 on ≥2 occasions	≥1+ on ≥2 midstream urine	26/15	-	-	6	Lipids
Ogland(2009) [103]	CC	>in DBP to >90	1+	F: 91/194 M: 92/166	F: 3010/3510 M: 2970/3600	F: 37.5/39.7 M: 37.2/39.7	10-12	BMI
Ophir(2006) [144]	CC	>140/90	>300mg/24 hours	36/35	2883/3274	37.7/38.8	0	Lipids
Rodie(2004) [145]	CC	ISSHP definition.		14/41	2510/3770	36.5/40	0	Lipids
Yavuz(2006) [146]	CC	>140/90	>300mg/24 hours	7/26	2640/3500	38/39	0	Lipids

*(*Includes only those children whose mothers did not receive calcium. † Cases whose mothers were defined as having severe preeclampsia.)*

2.4.2 BLOOD PRESSURE

Blood pressure data on 44,293 individuals, of whom 1,241 were exposed to preeclampsia, was available from ten studies (Table 2.1) [8, 64, 65, 97, 100-102, 104, 105, 141]. Subjects were aged from 4 to 30 years with all but one study reporting only on those aged less than 20 years. Five studies reported increased systolic blood pressure in those exposed to preeclampsia (range: 1.80 [141] to 6.70mmHg [101]) and 6 studies reported increased diastolic blood pressure(range:1.70 [102]- 6.0mmHg [101]) [8, 100-102, 104, 105]. No study reported significant decreases in either systolic or diastolic blood pressure in the offspring of preeclamptic pregnancies.

Quantitative summary measures obtained by meta-analysis indicated that in childhood offspring of preeclamptic women have a 2.39mmHg ($p < 0.0001$) greater systolic (Figure 2.2) and 1.35mmHg ($p < 0.00001$) greater diastolic blood pressure (Figure 2.3).

FIGURE 2.2: MEAN DIFFERENCE IN SYSTOLIC BLOOD PRESSURE FOLLOWING IN UTERO EXPOSURE TO PREECLAMPSIA

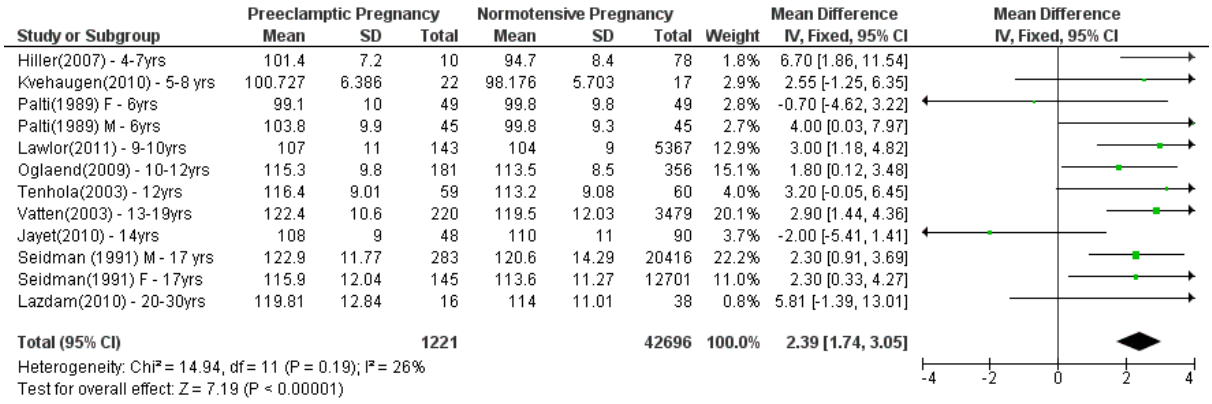


Figure 2.2: Forrest plot of the mean difference in systolic blood pressure in those exposed to preeclampsia in utero compared to those whose mothers remained normotensive. Preeclampsia exposure was associated with a 2.4mmHg increase in systolic blood pressure during young life.

FIGURE 2.3: MEAN DIFFERENCE IN DIASTOLIC BLOOD PRESSURE FOLLOWING IN UTERO EXPOSURE TO PREECLAMPSIA

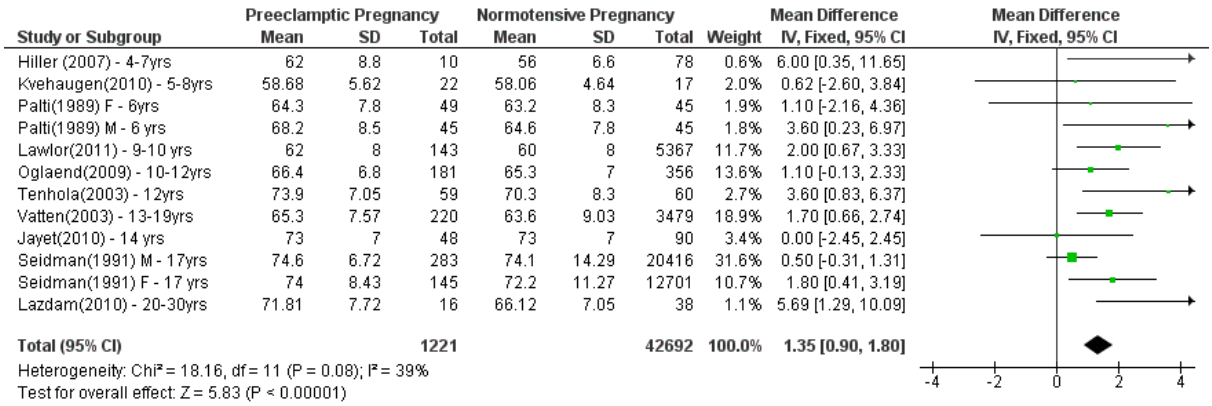


Figure 2.3: Forrest plot of the mean difference in diastolic blood pressure in those exposed to preeclampsia in utero compared to those whose mothers remained normotensive. Preeclampsia exposure was associated with a 1.3 mmHg increase in diastolic blood pressure during young life.

As some work has suggested that blood pressure differences in the offspring of hypertensive pregnancies may be sex specific [99], studies in which male and female subjects were reported separately were further investigated. Two studies reported increased systolic blood pressure in males only [100, 104]. For diastolic blood pressure two studies reported increases in females [102, 104] and one in males only [100] . Meta-analysis of studies in which data was reported in genders separately, demonstrated a 2.41mmHg ($p < 0.0001$) increase in systolic blood pressure in females, which was similar to the 2.48mmHg ($p < 0.0001$) increase in males. However for diastolic blood pressure only females had a significant 1.69mmHg (95% CI 0.89-2.50, $p < 0.0001$) increase whiles males demonstrated a non-significant 0.67mmHg (95% CI -0.312-1.45, $p = 0.1$) difference.

As blood pressure increases with increasing age I then considered whether the observed blood pressure difference varied with age of follow up. Studies of younger children, aged less than ten years at the time of follow up, had a mean increase of 2.91mmHg in systolic blood pressure (95% CI 1.55-4.27, $p < 0.00001$), similar to the 2.24mmHg (95% CI 1.49-2.98 $p < 0.00001$) in those aged ten years or over.

Finally as birth weight [71] and gestational age [70] are associated with later blood pressure, I also analysed 4 studies that reported findings in term-born offspring and in which those born

to pregnancies complicated by preeclampsia had a mean birth weight greater than 2.5kg (The WHO defines a birth weight of <2500g as low birth weight). In these studies preeclampsia was still associated with a 2.26mmHg (95% CI 1.35-3.16, p < 0.00001) higher systolic and 1.48mmHg (95% CI 0.82-2.14 P < 0.0001) higher diastolic blood pressure (Figures 2.4a and 2.4b).

FIGURE 2.4A: MEAN DIFFERENCE IN SYSTOLIC BLOOD PRESSURE IN TERM BORN INFANTS WITH BIRTH WEIGHT >2.5KG.

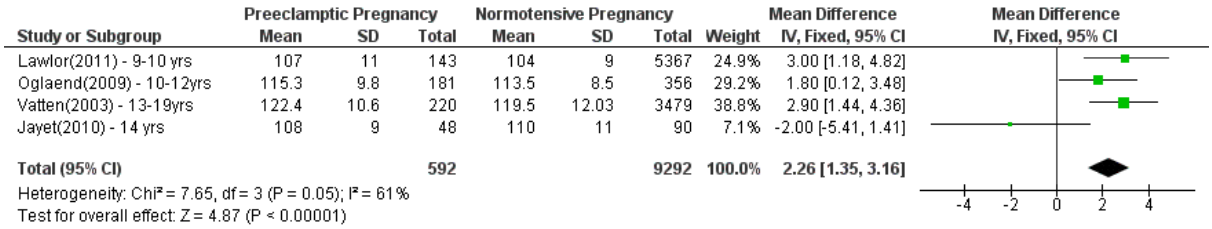


FIGURE 2.4B: MEAN DIFFERENCE IN DIASTOLIC BLOOD PRESSURE IN TERM BORN INFANTS WITH BIRTH WEIGHT >2.5KG.

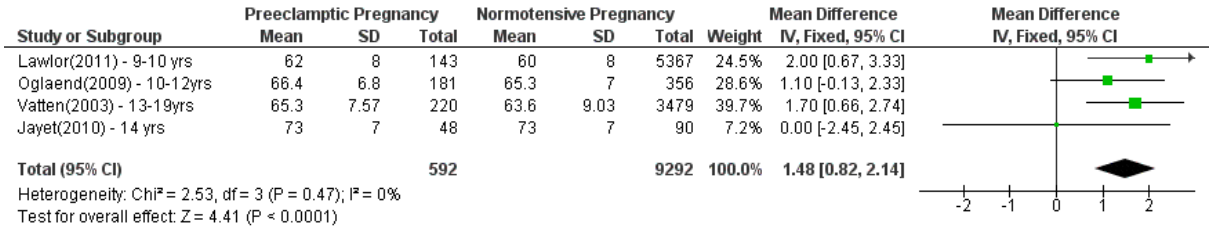


Figure 2.4 (A and B): These forest plots demonstrate the mean differences in systolic and diastolic blood pressure in those offspring of preeclamptic pregnancies born at term and with a normal birth weight, compared to those whose mothers remained normotensive. Both systolic and diastolic blood pressures were increased in term born infants whose mothers were preeclamptic during pregnancy.

2.4.3 BODY MASS INDEX

The search identified 8 eligible studies considering change in body mass index (BMI). This comprised data on 39, participants [8, 64, 97, 102-105]. In five of these studies BMI was greater in participants exposed to preeclampsia in utero, however this was significant in only 2 studies [103, 104]. In one study (undertaken in adolescents living at altitude) BMI was significantly decreased in individuals exposed to preeclampsia [64].

Meta-analysis demonstrated a significant increase of 0.57kg/m^2 (p<0.00001) in the children of preeclamptic women. Heterogeneity was significant (Heterogeneity: Chi² = 23.40, df = 9 (p = 0.005); I² = 62%). This appeared to be driven by the study of offspring living at altitude [64]. As some work has suggested that living at high altitude is associated with a lower BMI this was considered a plausible explanation for the observed heterogeneity [222]. When this study was removed an increase in BMI without significant heterogeneity was noted (0.62kg/m^2 (95% CI 0.41–0.84 p < 0.0001) Figure 2.5).

FIGURE 2.5: MEAN DIFFERENCE IN BODY MASS INDEX FOLLOWING IN UTERO EXPOSURE TO PREECLAMPSIA.

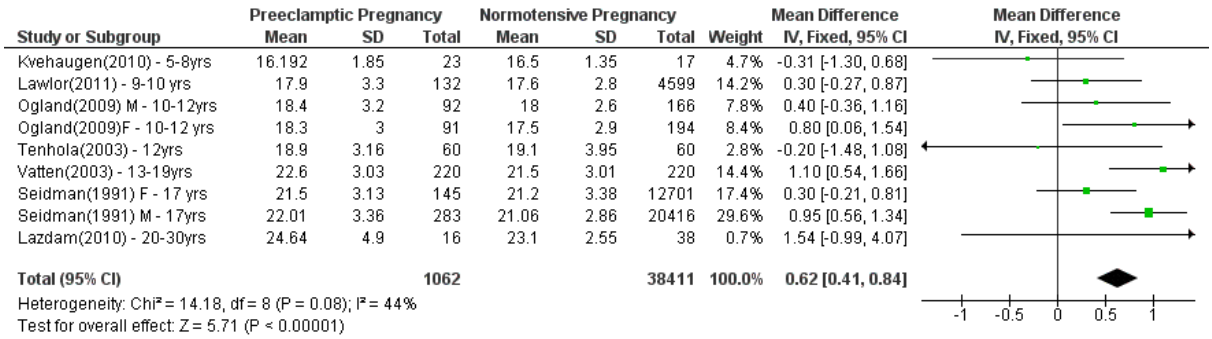


Figure 2.5 Forrest plot of mean difference in body mass index between the offspring of preeclamptic and normotensive pregnancies. In utero exposure to preeclampsia is associated with a 0.62 kg/m^{2}increase in body mass index.

Significant increases in BMI were seen both when females were considered alone (0.69kg/m^2 (95% CI 0.35–1.03, p < 0.0001)) and in studies considering males (0.83kg/m^2 (95% CI 0.48–1.18, p<0.006)) alone. Children aged less than 10 years of age at the time of follow up did not demonstrate an increased BMI (0.15kg/m^2 (95% CI -.34–0.64, p < 0.55)) whereas there was a significant increase in those ten years or older (0.69kg/m^2 (95% CI 0.35–1.03 p < 0.0001). When studies that reported findings in term-born offspring and in which those born to pregnancies complicated by preeclampsia had a mean birth weight greater than 2.5kg, a significant increase in BMI was still observed (0.67kg/m^2 (95% CI 0.35-0.99, p < 0.0001).

2.4.4 CHOLESTEROL

Serum lipid levels were considered in 6,164 individuals of whom 423 were exposed to preeclampsia [8, 63, 65, 105, 142-146, 221]. 6 of these studies, comprising 366 participants reported levels at birth in cord blood samples [142-146, 221]. As these reflect the unique post-delivery biological situation they were considered separately. Five of these cord blood studies reported changes in lipid profile; four an increase in triglycerides [142, 143, 145, 221], two an increase in total cholesterol, two an increase in LDL cholesterol [143, 144] and three a decrease in HDL cholesterol [142, 143, 221] in offspring of preeclamptic pregnancies. It is plausible that these acute changes may relate to alterations in maternal lipid levels during preeclamptic pregnancies [223-225].

In studies in later life (considering children and young adults aged 9-30 years), one was performed on non-fasted samples [65]. Two of the fasting studies found increased total cholesterol with one an increase in LDL-cholesterol [8, 63] No other differences were demonstrated. These studies included 208 subjects and in view of the small number of participants meta-analysis was not performed. The results of all studies considering serum cholesterol (both in the perinatal period and later life) are summarised in Table 2.2.

TABLE 2.2: LIPID STUDIES IN OFFSPRING OF PREECLAMPTIC PREGNANCIES.

	Total Cholesterol	HDL	LDL	Triglyceride
Studies on Cord Blood				
Akcakus et al 2010[221]	No difference	Decreased	No difference	Increased
(mg/dl)	73.3vs 80.5 p=0.72	17.3 vs.27.6 p=0.002	48.2 vs.49.9 p=0.82	39.2 vs.14.9 p=0.039
Catarino et al 2008[142]	No difference	Decreased	No difference	Increased
(mg/dl)	73.0 vs 86.0 p=0.09	32.0 vs 53.0 p<0.001	34.0 vs.32.0 p=0.68	49.0 vs.39.0 p=0.049
Howlander et al2009[143]	Increased	Decreased	Increased	Increased
(mg/dl)	70.9 vs 48.2 p<0.001	10.3 vs.13.3 p<0.001	65.6vs 28 p<0.001	52.4 vs 31.6 p<0.01
Ophir et al 2006[144]	No difference	Not reported	Increased	No difference
(mg/dl)	70.6vs 82 p=NS		42vs.36.8 p<0.01	46.5vs 45 p=NS
Rodie et al 2004[145]	Increased	No difference	Not reported	Increased
(log mmol/L)	0.36vs 0.11 p=0.003			-0.21vs -0.49 p=0.02
Yavuz et al 2006[146]	No difference	No difference	No difference	No difference
(mg/dl)	65.0vs.56.0 p=NS	28.3vs 25.2 p=NS	30.4vs 25.5 p=NS	30.6vs.32.6 p=NS
Studies in Children and Young Adults				
Kvehaugen et al 2011 [63]	Increased	No difference	No difference	No difference
(mmol/L)	5.01vs 4.43 p=0.04	1.60vs.1.41 p=0.1	1.81vs 1.53 p=0.3	0.60vs.0.58 p=0.9
Lazdam et al 2010[8]	Increased	No difference	Increased	No difference
(mmol/L)	4.7vs.3.87 p=0.001	1.6vs.1.46 p=0.42	2.65vs.2.0 p=0.0001	0.99vs 0.89 p=0.44
Lawlor et al 2011[65]	Not reported	No difference	No difference	No difference
(mmol/L)		1.37vs.1.40 p=0.21	2.87vs 2.87 p=0.76	0.99vs.1.03 p=0.78
Tenhola et al 2003[105]	No difference	No difference	No difference	No difference
(mmol/L)	4.54vs.4.50 p=0.618	1.31vs.1.36 p=0.468	2.82vs.2.75 p=0.342	0.90vs.0.86 p=0.617

Table 2.2: Summary of results of studies reporting cholesterol levels in the offspring of preeclamptic pregnancies at birth or during childhood, adolescence and young adult life.

2.4.5 GLUCOSE METABOLISM

Only two studies involving only 174 participants have considered fasting glucose in the offspring of preeclamptic pregnancies [8, 105]. At age 12 years there was no difference in serum insulin, glucose or glucose to insulin ratio [105]. In young adulthood offspring of preeclamptic mothers born preterm had increased fasting glucose (5.04 vs 4.57mmol/L p = 0.0001) [8]. In view of the limited numbers no meta-analysis was performed.

2.5 DISCUSSION

This systematic review and meta-analysis generates the first comprehensive study of multiple aspects of the cardiovascular phenotype of children and young adults whose mothers suffered from preeclampsia. The results are based on published data on multiple outcomes, and on around 45,000 individuals. In childhood and early adult life individuals born to preeclamptic pregnancies have higher blood pressure and a small increase in body mass index. Differences are consistently evident in both males and females as well as in those born at term with a normal birth-weight. Based on current reports there are insufficient studies to allow an appreciation of the long-term effects, if any, of preeclampsia on offspring glucose metabolism or lipids, except for probable acute changes in perinatal lipid profiles, which may well reflect the acute alterations in maternal lipid levels evident during preeclamptic pregnancies [223-225].

If the 2.4mmHg difference in systolic blood pressure tracks into adulthood as would be expected [226], this would be associated with around an 8% increased risk of mortality from ischaemic heart disease and 12% increased risk from stroke [68]. The only long term mortality follow up so far reported, showed a 1.9-fold increased risk of stroke in the offspring of preeclamptic pregnancies [60]. The discrepancy between the increase in risk expected given the observed increase in blood pressure, and that demonstrated by long term follow up suggest key biological pathways relevant to offspring cardiovascular health are likely to have additional effects on other features of stroke risk beyond clinic blood pressure measures alone. Regardless of the underlying mechanism, this data suggests this may be a population of children for whom intensive monitoring and early primary prevention advice may be warranted. Current data is limited almost exclusively to young cohorts and it is possible the blood pressure difference increases with age to explain the greater observed stroke risk. Despite that there was no evidence that the observed increase in blood pressure varies

between childhood and young adulthood and it is possible preeclampsia exposure is associated with other biological variations that alter risk.

Some studies found gender-specific variation in associations between preeclampsia and offspring blood pressure [99]. Work in animal models has certainly suggested that hormonal status may play some role in phenotypic modulation [214]. However, our combined analysis indicates gender is unlikely to be a further significant factor, certainly for systolic blood pressure, although there was some evidence for differences in diastolic pressure that may warrant further investigation.

Preeclampsia is a recognised cause of fetal growth restriction [20], which is itself associated with increased blood pressure in adult life. I therefore performed an analysis restricted to studies of individuals who were born at term, and where the mean birth weight of the cases was greater than 2.5kg. These offspring still had a 2.26mmHg higher systolic and 1.48mmHg higher diastolic blood pressure as well as a 0.67kg/m^2 increase in BMI. The use of 2.5kg birth weight as a cut off is a crude manner of defining growth restriction, however the currently available data does not allow for more detailed analysis of the effects of various degrees of growth restriction. Interestingly some evidence suggests that very low birth weight individuals born preterm have higher blood pressure irrespective of whether the mother had preeclampsia [17,37,38]. However the underlying mechanistic link with later blood pressure may vary depending on the blood pressure of the mother [17]. This highlights the potential highly complex interaction of growth restriction and exposure to preeclampsia in modulating offspring cardiovascular phenotype.

Rapid postnatal growth may also impact on blood pressure [71], and offspring of affected pregnancies appear to have a consistently higher BMI of around 0.6kg/m^2. It seems unlikely that this alone accounts for the variation in blood pressure as, based on previous reports, at least a 1 to 2 kg/m^2 difference in BMI would be needed to explain the greater than 2mmHg

difference [227-229]. Although some studies have shown an attenuation of the difference in blood pressure when offspring BMI was adjusted for [103], more recent data in children exposed to preeclampsia in utero suggests that the increase in blood pressure remains after adjustment for BMI [98] and childhood growth trajectory [230]. Furthermore in this analysis the increase in BMI was most evident in early adulthood, whereas the blood pressure difference was already established in childhood. Nevertheless it remains possible that raised BMI contributes in part to increased blood pressure in later life or that the impact of preeclampsia exposure on body fat distribution, and thereby blood pressure in childhood has so far been underestimated. Additionally BMI may not be the best predictor of adiposity in children, particularly in thin individuals [231]. Alternatively as raised maternal BMI is a risk for both preeclampsia and offspring obesity [23, 232], shared environmental influences may be important in this association.

There are several potential limitations to this analysis. I have only considered the effect of preeclampsia on traditional cardiovascular risk factors. Recent studies have linked preeclamptic pregnancy to alterations in other recognised cardiovascular risk factors, such as depression in the offspring [233]. This and other novel cardiovascular risk factors warrants further consideration in future studies. Furthermore the data available in the current literature does not allow for full exploration of potential confounding factors. It is possible that these findings may in part reflect such factors. For example, raised maternal BMI is a risk for both preeclampsia and offspring obesity [23, 232], and raised maternal pregnancy BMI is associated with higher offspring blood pressure, although this relationship attenuates when childhood BMI is considered [230]. Conversely some potential confounding factors may lead to underestimation of the true effect size of in utero exposure to preeclampsia. For example, maternal smoking reduces the risk of preeclampsia by up to 50% [234], but increases offspring blood pressure in adult life [235].

Interpretation of these findings also needs to take into account that observational studies are more prone to publication bias than randomised clinical trials [218]. In this analysis funnel plots for considered outcome measures were grossly symmetrical, however the possibility of publication bias cannot be excluded. Meta-analysis of continuous outcome variables can also be significantly affected by heterogeneity and without source data it can be difficult to explore subgroup variation. Although statistical analysis did not highlight heterogeneity, the included studies were published during a period when preeclampsia definitions varied. Because of this it is impossible to exclude the possibility that studies misidentified cases or included individuals who would not meet current diagnostic criteria. Finally, nearly all studies were on infants born close to term, presumably to mothers with less severe preeclampsia. An outcome study of hypertension risk found this was greater in those born to more severe preeclampsia [60] and there is some evidence that the blood pressure differences may be greater in those born preterm [8]. Therefore the impact of maternal disease severity on offspring phenotype needs further exploration.

2.6 CONCLUSIONS

Children and young adults born to pregnancies complicated by preeclampsia have adverse changes in cardiovascular risk factors present from early life. The predominant phenotype is that of increased blood pressure and body mass index. Greater mechanistic understanding of these differences in blood pressure may provide useful insights into why some people are predisposed to hypertension and preeclampsia. From a public health perspective, children born to a pregnancy complicated by preeclampsia appear to have a unique, lifetime cardiovascular risk profile that is present from early life. They may therefore constitute a population that would benefit from risk profile monitoring and early implementation of primary prevention strategies.

CHAPTER 3 – GLUCOSE AND LIPID METABOLISM IN PRETERM BORN OFFSPRING OF PREECLAMPTIC PREGNANCIES

3.1 THESIS CONTEXT

The literature exploring cardiovascular phenotype in the offspring of preeclamptic pregnancies deals predominately with blood pressure in children and young adults aged between 4 and 30 years. Meta-analysis (Chapter 2) has identified a paucity of data considering lipid and glucose metabolism in the offspring of early onset preeclamptic pregnancies. In addition to this no current data considers these parameters separately from the acute situation of delivery where levels may be influenced by maternal oxidative stress and inflammatory state. This chapter investigates the effect of in utero exposure to preeclampsia on glucose and lipid metabolism in a group of preterm born infants during neonatal period, and whether these varied with gestation at delivery and degree of fetal growth restriction.

3.2 INTRODUCTION

As demonstrated in Chapter 2, systematic review has identified a paucity of data considering lipid and glucose metabolism in the offspring of early onset preeclamptic pregnancies [67]. Current studies suggest that the offspring of preeclamptic pregnancies have acute alterations in cord blood lipids [142-146, 221], but there is insufficient data to make conclusions about metabolic profile separate from the acute effects of delivery. These levels may well reflect acute changes during the process of delivery particularly in the offspring of preeclamptic pregnancies where the mother experiences systemic endothelial dysfunction and oxidative

stress [214]. As deregulation of glucose or lipid metabolism are established risk factors for cardiovascular disease [236, 237], it is possible that changes in these factors may underlie some aspect of the alteration in long-term cardiovascular risk observed in individuals exposed to preeclampsia in utero. Moreover insulin resistance has its origins in childhood, and early life cholesterol has been demonstrated to track into adult life so it is possible that alterations in these factors may be present from early life [238, 239].

Preeclampsia is estimated to be an antecedent factor in 12% of small for gestational age infants and 20% of premature deliveries [20, 22]. In utero growth restriction has been linked to a variety of cardiovascular and non-cardiovascular outcomes including changes in glucose and lipid metabolism [11, 12, 14, 15]. More recently prematurity, independent of fetal growth restriction, has been linked to adverse changes in cardiovascular phenotype [7]. To date little work has dissected the impact of in utero exposure to preeclampsia from that of prematurity or growth restriction. In view of established phenotypic changes associated with prematurity and growth restriction, separating these perinatal influences is likely to be particularly relevant when considering glucose and lipid metabolism. Furthermore these factors are of particular interest as it is plausible that if preeclampsia does significantly impact on lipid and glucose metabolism then the effects will be more marked in those infants born prematurely, as these infants have likely been exposed to a more severe preeclampsia phenotype. This is evidenced by the fact that early onset preeclampsia appears to have a more severe impact on offspring blood pressure, with offspring of such pregnancies demonstrating a 6-10mmHg increase in blood pressure during childhood [240], which is greater than the 2-3mmHg associated with later onset preeclampsia [67].

Using prospectively collected data on preterm born offspring of preeclamptic pregnancies I have undertaken to determine the effect of in-utero exposure to preeclampsia on

metabolism of glucose and lipids in the first weeks of life taking into account degree of prematurity and fetal growth restriction.

3.3 METHOD

3.3.1 STUDY POPULATION AND MEASUREMENTS

Nine hundred and twenty six infants, with birth weights of less than 1850g and free of major congenital abnormalities, were recruited into a randomized trial of infant feeding regimes at five centers in the UK between 1982 and 1985. These infants were studied in the postnatal period until discharge from the neonatal unit, or until they reached a weight of 2000g with detailed data collection on clinical course, obstetric and perinatal events, regular anthropometry, and repeated biochemical studies [241]. There was no difference between preeclamptic and normotensive infants in dietary assignment, and there was no association between different dietary subgroups and mean metabolic parameter in infancy, therefore dietary subclass assignment was not considered in this analysis.

Weight was measured daily to 1-gram accuracy using an electronic balance (Sartorius MP models), and repeated biochemical studies (every 2-3 days) were undertaken during the inpatient course including repeated measurements of Total Cholesterol, Triglycerides, Glucose and Insulin levels [241].

3.3.2 STATISTICS

Statistical analysis was carried out with SPSS version 20. Normality testing was undertaken by visual assessment of normality curves and the Shapiro-Wilk test. Repeated measure ANOVA was undertaken for each metabolic factor over time to look for evidence of a between subject variable effect (with maternal hypertension as the between subject variable). The number of infants with blood results at all time points between 0 and 5 weeks was small in comparison to the group as a whole (less than 30 infants for some variables). To minimize the

impact of this initial repeated measure testing was undertaken on 3 time points comprised of 1) average values in the first week of life, 2) average values in weeks 2 and 3 and finally 3) average values in weeks 4 and 5. Following this detailed comparison between groups were performed with a 2-sided, independent-samples, Student t tests on mean levels across the whole neonatal period, and at each individual time point. Triglyceride, Insulin, Glucose and Total Cholesterol values were log transformed prior to analysis. Linear regression models were undertaken using a Forced Entry Model. Pearson correlations (r) were used for bivariate-associated models, and unstandardized regression coefficients (β) were used for bivariate and multivariate regression models. Comparisons between groups were adjusted for gestational age at delivery, birth weight z score and sex. P values less than 0.05 were considered statistically significant.

3.4 RESULTS

3.4.1 STUDY POPULATIONS

Of the total 926 infants enrolled in the trial, 860 were classified as being born to normotensive (n = 657) or preeclamptic pregnancies (n = 203). In the remainder, information regarding maternal hypertension status during pregnancy was not available. The perinatal characteristics of these infants and their mothers are presented in Table 3.1.

Infants born to preeclamptic pregnancies were born slightly later than those whose mothers remained normotensive (31.71 ± 2.35 vs 30.47 ± 2.86 weeks p <0.001) and there was a small, but significant difference in birth-weight between the groups (p = 0.001) after adjustment for gestation and sex. The preterm offspring of preeclamptic pregnancies also had significantly lower birth weight z scores, indicating a greater degree of intra-uterine growth restriction (-.53 ± 1.29 vs -1.55 ± 1.09 p < 0.001). These infants were more likely to be female (59.6% vs 47.4% p = 0.009), had a higher rate of operative delivery (91% vs 34% p = 0.001) and were less likely to have been treated with antenatal steroids for lung maturation (5.9% vs 18.8% p <

0.001). There were no significant differences between the groups in other factors describing the perinatal or postnatal course. As would be expected mothers who experienced preeclampsia were more often primparous (51.2 % vs 38.1% p = 0.035) and had slightly higher BMI's than those who experienced normotensive pregnancies (23.47 vs 22.28 p <0.001)

TABLE 3. 1 - PERINATAL CHARACTERISTICS OF INFANTS BORN TO PREECLAMPTIC AND NORMOTENSIVE PREGNANCIES.

	Normotensive (n = 656)	**Preeclamptic (n = 203)**	**p-Value**
Birth Weight (g)	1386 (313)	1336 (315)	0.046
Gestation (weeks)	30.47 (2.86)	31.71 (2.35)	<0.001
% Small for Dates	24.7.3%	58.4%	<0.001
Birth Weight Z score	-.528 (1.29)	-1.55 (1.09)	<0.001
% Male	52.6 %	40.4%	0.009
Antenatal Steroids (%)	18.8 %	5.9%	<0.001
C Section Delivery	34.0%	92.1%	<0.001
1 min APGAR	5.6 (2.45)	5.81 (2.33)	0.17
Breast Fed at Discharge	65.3%	71.8%	0.14
Multiple Pregnancy (%)	25%	10.4%	<0.001
Days Supplemental O2			
Total	9.42 (16.31)	8.46 (15.42)	0.42
Ventilated	4.90 (10.24)	3.96 (8.84)	0.14
Positive blood cultures (%)	8.1%	3.0%	0.24
Days admitted (days)	32.82 (21.41)	33.69 (20.85)	0.47
Mortality (%)	11.1%	8.1%	0.22
Primiparous Mother (%)	38.1%	51.2%	0.035
Maternal BMI (kg/m^2)	22.26 (3.57)	23.48 (3.72)	<0.001
Maternal Smoking in pregnancy	44.6%	18.1%	0.012

Table 3.1: Perinatal characteristics of the infants born to preeclamptic and normotensive pregnancies. Data presented is mean+/- standard deviation, p-values from T-test, or χ^2 for difference across groups.

3.4.2 NEONATAL GLUCOSE AND INSULIN

Glucose was measured during the perinatal period in 597 preterm born infants (152 born to preeclamptic pregnancies). Repeated measure ANOVA showed no evidence of an impact of maternal preeclampsia (p= 0.26). There was no difference in mean glucose (4.69 ± 2.71 vs

5.20 ± 1.93 mmol/L p = 0.455) levels between those born to preeclamptic and normotensive pregnancies after adjustment for sex, gestation and birth weight z score. Gestation (β = -0.301 p < 0.001) was the only perinatal factor that independently predicted mean serum glucose.

Insulin levels were measured at one or more points during postnatal period in 264 infants (207 normotensive, 57 preeclamptic). Repeated measures ANOVA (undertaken on 84 infants, 20 preeclamptic) suggested a significant impact of maternal preeclampsia on serum insulin (p=0.021). Detailed analysis indicated that offspring of preeclamptic pregnancies had lower mean (15.36 ± 11.61 vs 18.86 ± 11.67 pmol/L p = 0.027) and maximum (22.91 ± 15.82 vs 33.99 ± 31.62 pmol/L p = 0.014) insulin levels during the neonatal period independent of degree of prematurity and birth weight z score. There was no independent relationship between mean insulin and gestational age (β = 0.343, p = 0.573) or birth weight z score (β = 0.537, p = 0.264). There was no significant difference in mean glucose to insulin ratio between groups, although the offspring of preeclamptic pregnancies tended to have lower values (4.71 ± 5.32 vs 3.25 ± 2.43 p = .118). Differences in insulin were predominately evident in the first four weeks of life with significant differences present at week 2 (p=0.016) and week 3 (p=0.009) of life (Figure 3.1). By 5 weeks of post-natal age there was no longer a significant difference in insulin between those born to preeclamptic and normotensive pregnancies.

FIGURE 3.1 – MEAN INSULIN LEVELS IN THE NEONATAL PERIOD IN THE OFFSPRING OF PREECLAMPTIC AND NORMOTENSIVE PREGNANCIES

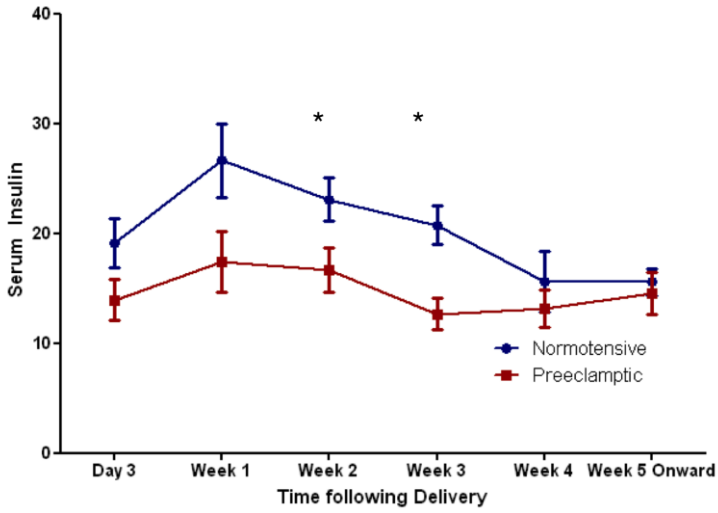


*Figure 3.1: Mean Insulin levels (pmol/L+/- SEM) at different time points following delivery in premature born offspring of preeclamptic and normotensive pregnancies. Significantly different results at individual time points are indicated by *.*

3.4.3 NEONATAL CHOLESTEROL

Total cholesterol levels were measured in 659 individuals (159 preeclamptic) who did not receive intravenous lipid therapy during the neonatal period. Repeated measures ANOVA suggested a significant impact of maternal preeclampsia on cholesterol (p=0.026). Offspring of preeclamptic pregnancies had lower mean (2.83 ± 0.82 vs 2.59 ± 0.82 mmol/L p = 0.006) total cholesterol levels after adjustment for gestation, birth weight z score and sex. Gestation (β = 0.074 p <0.0001), and birth weight z score (β = 0.14 p < 0.001) also independently related to neonatal total cholesterol levels. Differences in mean total cholesterol were predominately present in the first week of life with significant differences present at day 3 and 7. From week 2 of neonatal life there was no difference in cholesterol levels (Figure 3.2). Triglyceride levels were measured during the postnatal period in 651 infants (492 normotensive, 159 preeclamptic). Repeated measures testing showed no between subject effect of maternal hypertension (p=0.12) and there was no difference in mean triglycerides (1.36 ± 0.57 vs 1.28

± 0.75 mmol/L p = 0.53) between groups. Triglyceride levels were related to birth weight z score (β = -0.12, p < 0.001) and gestation at delivery (β = 0.066, p < 0.001).

FIGURE 3.2 TOTAL CHOLESTEROL LEVELS IN THE NEONATAL PERIOD IN THE OFFSPRING OF PREECLAMPTIC AND NORMOTENSIVE PREGNANCIES.

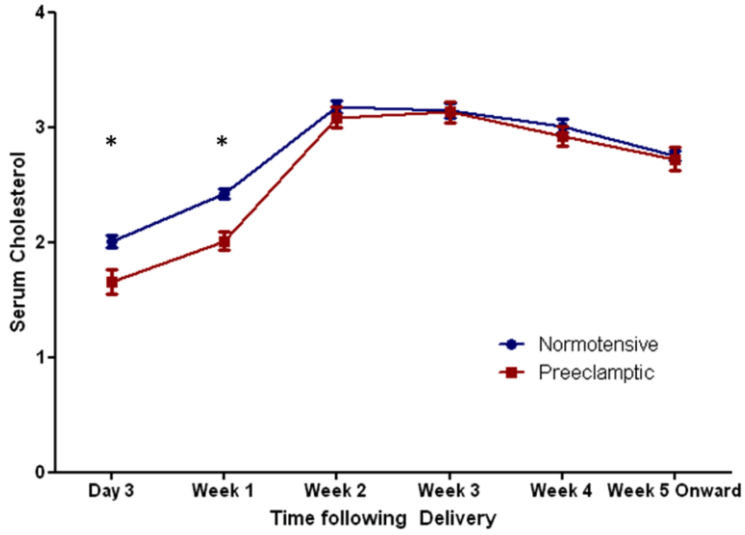


*Figure 3.2: Mean Cholesterol levels (pmol/L+/- SEM) at different time points following delivery in premature born offspring of preeclamptic and normotensive pregnancies. Significantly different results at individual time points are indicated by *.*

3.5 DISCUSSION

This work has examined glucose and lipid handling in preterm infants born to preeclamptic pregnancies, taking into account degree of prematurity and severity of fetal growth restriction. In preterm born infants in utero exposure to preeclampsia is associated with short-term alterations in lipid and glucose metabolism. In particular they demonstrate lower total cholesterol in the first week of life, and lower insulin during the first month, independent of gestation at delivery and birth weight z score. By the fifth week of post-natal age these changes had resolved and there was no difference between those whose mothers developed preeclampsia and those whose mothers remained normotensive.

Previous studies of glucose and lipids in the offspring of preeclamptic pregnancies have been largely restricted to cord blood [142-146, 221] and have reported increased lipid levels in

exposed infants [67]. These results must, however, be interpreted with caution as they likely reflect acute conditions at delivery in a mother with increased circulating oxidative and inflammatory stimuli. In later life only two studies have reported fasting lipid and glucose levels and these did not demonstrate consistent effects [8, 105].

Additionally to date much of the literature concerning the effect of preeclampsia on offspring health has been limited by an inability to distinguish the effects of in utero exposure to preeclampsia from those of fetal growth restriction and prematurity. Preeclampsia is a recognised cause of prematurity and fetal growth restriction [20, 22]. Consistent with this just over 21% of this preterm cohort were born to preeclamptic pregnancies and these infants were more commonly small for gestational age with significantly lower birth weight z scores.

Growth restriction itself has been associated later insulin resistance and type 2 diabetes in adulthood [15] but the impact on lipid and glucose metabolism in neonatal life is less well understood. In some studies preterm born small for gestational age infants have lower serum insulin and glucose levels immediately after birth [242, 243]. Conversely others have reported higher insulin levels and HOMA-IR scores in both preterm and term born small for gestational age infants [244, 245]. Cord blood cholesterol also varies with prematurity [246] and small for gestational age infants have been reported to have higher or lower serum cholesterol dependent on whether infants are growth restricted or constitutionally small [247]. In view of the potentially complex array of perinatal factors it has been difficult to distinguish the effects of the in utero environment associated with preeclampsia, from the prematurity and growth restriction that often accompany it.

An advantage of this study is the large number of infants who underwent multiple biochemical tests during the perinatal period. This has allowed the separation of the impact of maternal preeclampsia from that of prematurity and growth restriction. The finding that preeclampsia is associated with reduced insulin and cholesterol levels in infancy, independent

of other these other perinatal factors, has not previously been reported. However animal models looking at the offspring of preeclamptic pregnancies suggest that metabolic differences may be a relevant to later cardiovascular phenotype. In particular animal models incorporating aspects of the in utero insult of preeclampsia result in offspring with alterations in BMI, altered serum insulin and altered glucose metabolism [214]. Of more relevance to these findings, Stryud and colleagues demonstrated in a rat model of preeclampsia that newborn offspring had reduced pancreatic β cell mass and insulin content and lower serum insulin when compared to control animals [147]. It is interesting to note that in later life these animals became insulin resistant. In human studies reduced pancreatic β cell mass has been associated with birth weights of less than 1500 grams but not with less severe growth restriction [248, 249]. The findings of this analysis, initially lower insulin levels with normalization in later postnatal life, may indicate that this mechanism operates in human offspring of preeclamptic pregnancies, although further and more detailed mechanistic studies of infant glucose handling will be needed to understand these differences. In addition longer term follow up will be required to demonstrate if in later life insulin resistance and hypercholesterolaemia are aspects of the metabolic profile of those exposed to preeclampsia in utero.

There are several limitations to this study. Firstly samples obtained in the neonatal cohort were understandably not collected in the fasting state. Such a collection would be neither feasible nor ethical in newborn infants. I have therefore used mean levels across the period of collection to gain an understanding of the predominate metabolic state of these infants. It is important to note, however, that long term implications of mean metabolic markers in infancy are unknown, and may have no, or limited, relationship to fasting samples later in life. Where differences were established I then considered mean values at each week of post-natal age. As the number of infants having regular blood tests reduced with increasing postnatal age it is possible that ongoing subtle changes in insulin or cholesterol levels would

be detectable in a larger population. In addition the above factors, and the fact that not every infant had blood tests each week even where the remained an inpatient, made repeated measure testing difficult and required consideration of average values at 3 time points rather than allowing all measurement points to be independently considered. To my knowledge, however, at present there are no other studies which have undertaken such intensive biochemical sampling in a neonatal population and therefore I feel these results remain informative. Additionally the prospective nature of this study and the detailed data collection in the perinatal period make it uniquely able to examine the cardiometabolic phenotype of newborns following preeclamptic pregnancies.

3.6 CONCLUSIONS

This work has for the first time considered glucose and lipid metabolism in the newborn offspring of preeclamptic pregnancies. Infants born to preeclamptic mothers have transient alterations in their metabolic phenotype characterised by lower insulin and cholesterol levels. These changes have largely resolved by the end of the first month of post-natal life. Although the long-term implications of these brief changes are unclear, further detailed investigation of metabolism and other aspects of cardiovascular phenotype during this period may be informative in leading to greater understanding of the impact of preeclampsia exposure on later cardiovascular risk in these offspring.

CHAPTER 4 – CARDIOVASCULAR AND CARDIOMETABOLIC PHENOTYPE OF PRETERM BORN OFFSPRING OF HYPERTENSIVE PREGNANCIES.

4.1 THESIS CONTEXT

For women early onset preeclampsia leading to premature delivery is associated with greater increases in cardiovascular risk compared to those who developed preeclampsia at term [36]. Preterm born offspring of preeclamptic pregnancies also appear to have a particularly adverse cardiovascular phenotype, showing up to 10mmHg higher blood pressure during childhood [240], altered vascular function [8] and altered neonatal insulin and lipid metabolism (Chapter 3). In addition some studies indicate adverse metabolic risk factors in later life [8]. Whilst both prematurity and growth restriction impact on cardiovascular phenotype, little work has been done to differentiate the different impact of these factors from maternal hypertension. This chapter seeks to distinguish the relevance of early-onset pregnancy related hypertension from that of prematurity and growth restriction, on offspring cardiovascular risk factors in two large birth cohorts, each with more than 20 years of prospective follow up.

4.2 INTRODUCTION

As outlined in Chapters 1 and 2 preeclampsia affects between 2% and 5% of all pregnancies [16, 17]. It is associated with long-term changes in cardiovascular phenotype, and almost doubles the risk of strokes in adulthood [7, 60, 67, 203, 240]. Of those exposed in utero, it is

individuals born preterm to women with early onset preeclampsia who demonstrate the most severe alterations in cardiovascular phenotype, with a 6-10mmHg increase in blood pressure during childhood [240], and alterations of endothelial function in later life [8]. These changes are greater than those seen in the offspring of preeclamptic pregnancies delivered at term in whom meta-analysis has demonstrated a 2-3mmHg increase in blood pressure, and small increase in BMI in childhood [67].

Early onset hypertensive disorders of pregnancy commonly lead to premature delivery of growth-restricted infants [20, 22]. In utero growth restriction has itself been linked to a variety of cardiovascular outcomes [11, 12, 14, 15], and those infants who are born severely prematurely go on to exhibit elevated blood pressure and alterations in cardiac structure and function as adults [7, 250]. The ability of studies to distinguish the impacts of preeclampsia from those of prematurity has to date been limited by the lack of large preterm comparison groups spanning the severe, moderate and late preterm born. In view of the fact that preeclampsia is an antecedent factor in up to 20% of preterm deliveries it is of considerable interest to understand the phenotype of those born to affected pregnancies.

I have therefore studied subgroups of two large cohorts of preterm infants, with detailed pregnancy data collected at birth, as well as prospective cardiovascular measures over the subsequent 20 to 28 years [7, 203, 251]. These studies include infants born to hypertensive pregnancies with differing degrees of prematurity, including both those very and more moderately preterm born, as well as infants born at similar gestation to normotensive pregnancies. In these cohorts I have considered the cardiovascular phenotype at multiple time points from infancy to young adulthood. In addition, these results have been compared to those of term born infants with the aim of distinguishing whether a history of early-onset pregnancy induced hypertension in the mother was associated with distinct differences in the

cardiovascular risk profile, and whether any association varied between those born very preterm compared to moderately preterm.

4.3 METHODS

4.3.1 STUDY POPULATIONS

This chapter uses data from two birth cohorts with 20 to 28 years of prospective follow up: namely the UK based '*Early Vascular Study - Very Preterm Cohort'* and the Australian based '*Western Australian Pregnancy Cohort (Raine) Study'.*

Early Vascular Study (EVS) - Very Preterm Cohort:

Nine hundred and twenty six infants, with birth weights of less than 1850g were recruited in the UK between 1982 and 1985 into a randomised trial of feeding regimes. 203 were born following an early-onset hypertensive disorder of pregnancy. All infants were studied in the postnatal period with detailed data collection, regular anthropometry, and repeated biochemical studies [241]. Growth and development were measured at 9 and 18 months, and 7 years [252-255]. A subgroup was followed up with cardiovascular phenotyping at age 15, and between 23 and 28 years [5, 6, 8]. This was done in combination with data collected on a group of young adults born term to uncomplicated pregnancies, with similar age and sex distribution to the preterm-born young adults, who were specifically recruited to undergo identical cardiovascular investigations [7]. This study was reviewed and approved by relevant institutional ethics committees at each stage. Timing of visits and investigations are outlined in Figure 4.1

FIGURE 4.1 TIMINGS OF FOLLOW UP OF INFANTS IN THE 'EARLY VASCULAR STUDY' COHORT

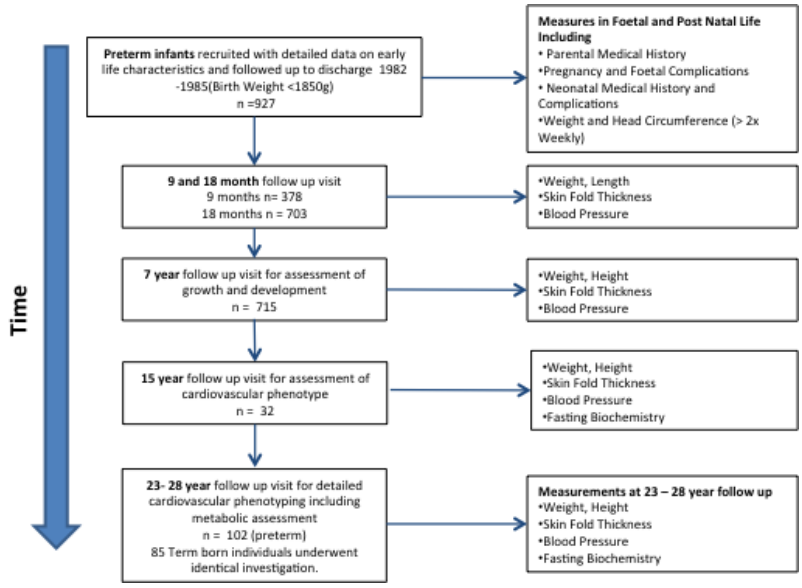


Figure 4.1: This figure highlights the timing of follow up visits for individuals enrolled in the early vascular study cohort. At 23-28 a group of term born controls was specifically recruited from the local population to undergo identical cardiovascular investigations.

Raine Study - Moderate Preterm Cohort

The Western Australian Pregnancy Cohort (Raine) Study consists of 2,900 women enrolled in a controlled trial of ultrasound frequency and pregnancy outcome in Perth, Western Australia [251]. 2,868 children born to 2,804 mothers consented to take part in a follow up cohort. Follow up visits, including assessment of growth, development, metabolic and cardiovascular health, have been undertaken at multiple time points (including 1, 2, 8, 17 and 20 years of age). Within the cohort, 225 infants were born prematurely (<37 weeks of completed gestation), of whom 83 were born to pregnancies complicated by early onset hypertensive disorders (de novo onset hypertension (>140/90) after the 20th week of pregnancy resulting in premature delivery). This group of early onset hypertensive disorders was not further subdivided into pregnancy induced hypertension and proteinuric preeclampsia because it was considered that all hypertensive disorders of pregnancy severe enough to lead to premature delivery fell on the preeclampsia spectrum, even if proteinuria had not emerged by the time of delivery. This study was reviewed and approved by the ethics committees of Princess

Margaret Hospital for Children, the King Edward Memorial Hospital For Women and The University of Western Australia. Timing of visits and investigations are outlined in Figure 4.2

FIGURE 4.2 TIMINGS OF FOLLOW UP OF INFANTS IN THE 'RAINE STUDY' GROUP

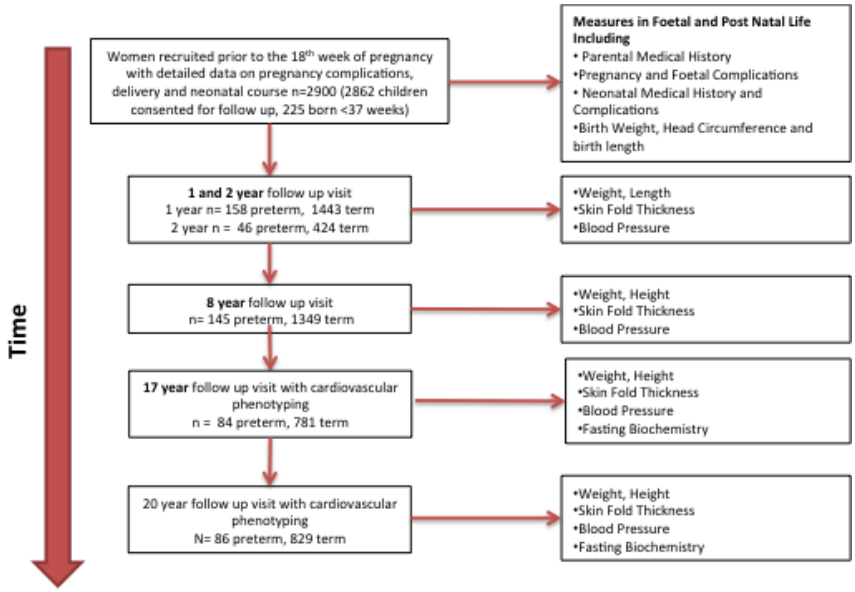


Figure 4.2: This figure highlights the timing of follow up visits for preterm individuals enrolled in the Raine study cohort. Term born individuals were also followed up from birth.

4.3.2 STUDY MEASUREMENTS

Blood pressure: In both cohorts blood pressure was measured using validated automatic sphygmomanometers with appropriately sized blood pressure cuff and the subject in the resting state. The average of two or more readings is reported.

Growth: In the Early Vascular Study group weight was measured using an electronic balance accurate to 1g in neonates (Sartorius MP models), or 0.2kg in older participants (Seca). Head circumferences were measured using paper non-stretchable tape. At follow up, height was measured on a horizontal infant/or free-standing stadiometer [6, 256, 257]. Skinfold were measured using Harpenden callipers [241, 255]. In the Raine Study, weight was measured using an electronic balance at birth. At subsequent follow up visits weight was recorded to the nearest 100g using a Wedderburn digital chair scales, length to 0.1cm on Harpenden Infantometer/ Holtain Stadiometer and head circumference was measured to the nearest

0.1cm using flexible plastic or fibreglass tape. Skinfolds were measured to the nearest 0.1mm using Slimguide Callipers.

Fasting Glucose, Insulin and Lipids: In the Early Vascular Study blood was collected after an overnight fast for metabolic parameters both during adolescence and young adulthood and analysed using routine validated assays, with clinical-level quality controls [6]. Insulin resistance scores were calculated using the HOMA2 model [258]. In the Raine Study, fasting blood samples were drawn from participants at the 17 and 20 year follow up visits and analysed in the PathWest laboratory at Royal Perth Hospital for serum insulin, glucose, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol using routine validated assays [259].

4.3.3 STATISTICS

Statistical analysis was carried out with SPSS version 20. Normality testing was undertaken and comparison between groups for continuous variables was performed with a 2-sided, independent-samples Student t-test or Mann-Whitney tests for skewed data. One-way ANOVA was used for comparison across multiple groups. Where multiple groups were compared one-way ANOVA for difference between groups was undertaken prior to T-testing. For categorical variables, comparison used a $\chi 2$ test. Triglycerides, insulin, glucose and other non-normal values were log transformed prior to analysis. Linear regression models were undertaken using a Forced Entry Model. Pearson correlations (r) were used for bivariate-associated models, and unstandardized regression coefficients (β) were used for bivariate and multivariate regression models. Comparisons between groups for cardiometabolic, anthropometric variables and blood pressure were adjusted for gestational age at delivery, birth weight z score and sex and reported p values are adjusted. P values less than 0.05 were considered statistically significant.

4.4 POPULATION CHARACTERISTICS

The perinatal characteristics of the subgroups followed into adult life are presented in Table 4.1. It has previously been shown that there was no significant differences in demographics or perinatal characteristics between those who took part in the Early Vascular Study 23-28 year follow up, and the full birth cohort [7]. In the Raine Study group, children from socially disadvantaged families were less likely to remain in the study beyond the third year, with those remaining in the study having higher birth weights and gestational ages at 17 [260, 261]. As expected there was a significant difference in mean gestational age between two studies (EVS 30.27 ± 2.47 vs Raine 34.66 ± 2.49 weeks $p < 0.001$), with the average gestational age of the Early Vascular Study falling in the WHO very preterm category, compared with the moderate to late preterm gestation of the Preterm Raine Study group [262]. The Early Vascular Study group also had significantly lower birth weights (EVS 1303 ± 286 vs Raine 2359 ± 633 g, $p < 0.001$) and birth weight z scores (EVS -0.79 ± 1.18 vs Raine 0.043 ± 1.04 $p<0.001$).

Of the 98 very preterm infants followed into adult life, 26 were born following early-onset pregnancy hypertension, and of the 88 moderate preterm infants in the Raine Study followed up in adulthood, 32 had mothers with early onset pregnancy hypertension. In both cohorts infants of hypertensive pregnancies had slightly longer gestations, with a significant one-week difference in the Early Vascular Study group. There were no differences within the study groups in other perinatal or postnatal clinical factors except for a higher prevalence of Patent Ductus Arteriosus in the Raine normotensive preterm group, and a higher rate of operative delivery in the Early Vascular Study hypertensive group. In keeping with their greater levels of prematurity the Early Vascular Study very preterm group infants had lower APGAR scores ($p=0.001$), longer post-natal admissions ($p<0.001$) and were more likely to need ventilation during the neonatal period in comparison to the more moderately preterm born Raine Study group.

TABLE 4.1: PERINATAL CHARACTERISTICS OF PRETERM BORN PARTICIPANTS FOLLOWED UP TO YOUNG ADULTHOOD

	Raine Study					**Very Preterm Born – Early Vascular Study Cohort**					**p-value differences between preterm cohorts**
	Preterm NT n = 56	Preterm HTN n = 32	p-value (Preterm)	Term Normotensive N = 1759	p-value (vs preterm)	Preterm NT n= 72	Preterm HNT n= 26	p-value	Term Born (n=85)	p-value (vs preterm)	
Sex (% Male)	37.5% (21)	46.9% (15)	0.39	49.6% (873)	0.476	51.4% (37)	29.2% (7)	0.058	47.1% (40)	0.66	0.50
Gestation (weeks)	34.61 (2.71)	34.75 (2.10)	0.79	39.83 (1.27)	<0.001	29.72 (2.59)	31.11 (1.96)	0.044	39.54 (0.78)	<0.001	<0.001
Birth Weight (g)	2374 (639)	2331 (630)	0.76	3418 (461)	<0.001	1295 (287)	1324 (286)	0.69	3428 (340)	<0.001	<0.001
Birth Length (cm)	45.02 (4.09)	44.94 (3.89)	0.93	49.38 (2.14)	<0.001	39.93 (2.98)	39.51 (3.13)	0.65	-	-	<0.001
Ponderal Index Birth	25.31 (3.11)	25.07 (2.98)	0.73	28.34 (2.86)	<0.001	21.05 (2.66)	20.10 (1.69)	0.22	-	-	<0.001
% C-Section Delivery	42.9% (24)	50.0% (16)	0.52	17% (299)	<0.001	40.3% (29)	95.8% (23)	<0.001	-	-	0.300
% Multiple Pregnancy	28.6% (16)	31.2 (10)	0.74	1.8% (32)	<0.001	22.2% (16)	8.4% (2)	0.49	-	-	0.97
Antenatal Steroids (% Received)	16.1% (9)	25.0% (8)	0.31	1.0% (17)	<0.001	20.8% (15)	12.5% (3)	0.36	-	-	0.87
APGAR 5 minutes	8.46 (1.25)	8.19 (1.51)	0.36	9.10 (2.25)	<0.001	7.86 (1.91)	7.21 (2.64)	0.27	-	-	0.001
Length of Neonatal Admission	17.16 (19.15)	16.75 (15.96)	0.92	4.93 (2.03)	<0.001	39.89 (20.34)	40.45 (23.01)	0.91	-	-	<0.001

TABLE 4.1: CONTINUED

SCBU Admission	71.4 %(40)	81.3% (26)	0.31	12.9% (227)	<0.001	-	-	-	-	-	-
ICU Admission	39.3 %(22)	37.5% (12)	0.87	1.9% (33)	<0.001	-	-	-	-	-	-
% Ventilated	19.6% (11)	18.8% (6)	0.92	0.3% (5)	<0.001	50% (36)	66.7% (16)	0.16	-	-	<0.001
Neonatal Sepsis	26.8% (15)	15.6% (5)	0.23	5.4% (95)	<0.001	75% (54)	70.8% (17)	0.41	-	-	<0.001
PDA	8.9% (5)	0% (0)	0.08	0% (0)	<0.001	-	-	-	-	-	-
Breastfed Discharge	87.5% (49)	78.1% (25)	0.41	86.7% (1525)	<0.001	30.8% (12/39)	42.9% (6/14)	0.41	-	-	0.74
Maternal Age (years)	29.91 (5.30)	30.03 (5.81)	0.93	29.38 (5.76)	0.67	-	-	-	-	-	-
Maternal BMI (kg/m^2)	21.31 (2.58)	23.37 (3.91)	0.011	21.85 (3.95)	<0.001	22.28 (3.77)	23.09 (3.79)	0.38	-	-	0.29
Maternal birth weight (g)	3183 (704)	3206 (494)	0.88	3212 (614)	0.12	-	-	-	-	-	-
Maternal smoking late pregnancy (%)	19.6% (11)	9.4% (3)	0.59	38.1% (495)	0.016	15.5% (11/71)	12.5% (3/24)	0.72	-	-	0.89
Young adult follow up age (years)	20.10 (0.42)	19.98 (0.34)	0.17	20.10 (0.44)	0.31	25.06 (1.40)	25.38 (1.36)	0.30	25.68 (2.14)	0.093	<0.001

Table 4.1: This table shows the perinatal characteristics of participants in the two cohorts who were followed up into young adulthood. P-values for comparisons between preterm normotensive and hypertensive groups, and between preterm groups and term born controls are also presented. The final column shows p-values for differences between the two cohorts in terms of perinatal characteristics.

4.5 Blood Pressure

In early adulthood those born very preterm had significantly higher blood pressure than age matched individuals born at term to uncomplicated pregnancies (Preterm NT 122.28 ± 10.23, PET 120.58 ± 11.93, Term 112.83 ± 10.67 mmHg $p < 0.001$ (ANOVA)) (Figure 5.3). The increase in blood pressure however was similar in adults born following both normotensive and hypertensive pregnancies (122.28 ± 10.23 vs 120.58 ± 11.93 mmHg $p = 0.92$). Review of earlier follow up data showed there had not been any difference between these preterm born groups at any earlier time in life, including at 18 months $p = 0.46$, 7 years $p = 0.43$ and 15 years $p = 0.86$ of age. Blood pressure differences were also evident between groups in those born moderately preterm (p (ANOVA) = 0.026). However in contrast to those born extremely preterm levels of blood pressure in the offspring of normotensive pregnancies were similar to the term control group (Term 116.49 ± 13.08 vs Preterm Normotensive 114.29 ± 14.04 mmHg $p = 0.36$). Differences were only evident in those adults born following a hypertensive pregnancy, who had significantly higher systolic blood pressure compared to both preterm offspring whose mothers had a normotensive pregnancy (Preterm Hypertensive 122.00 ± 13.75 vs Preterm Normotensive 114.29 ± 14.04 mmHg $p=0.008$) and term born adults ($p = 0.005$, Figure 1). Review of earlier follow up data showed this difference was not present during infancy (1 year) and childhood (8 years), but had emerged by age 17 years (119.5 ± 9.71 vs 113.12 ± 9.31 mmHg $p=0.001$).

FIGURE 4.3 YOUNG ADULT SYSTOLIC BLOOD PRESSURES IN MODERATE AND VERY PRETERM BORN INDIVIDUALS BY MATERNAL HYPERTENSIVE STATUS.

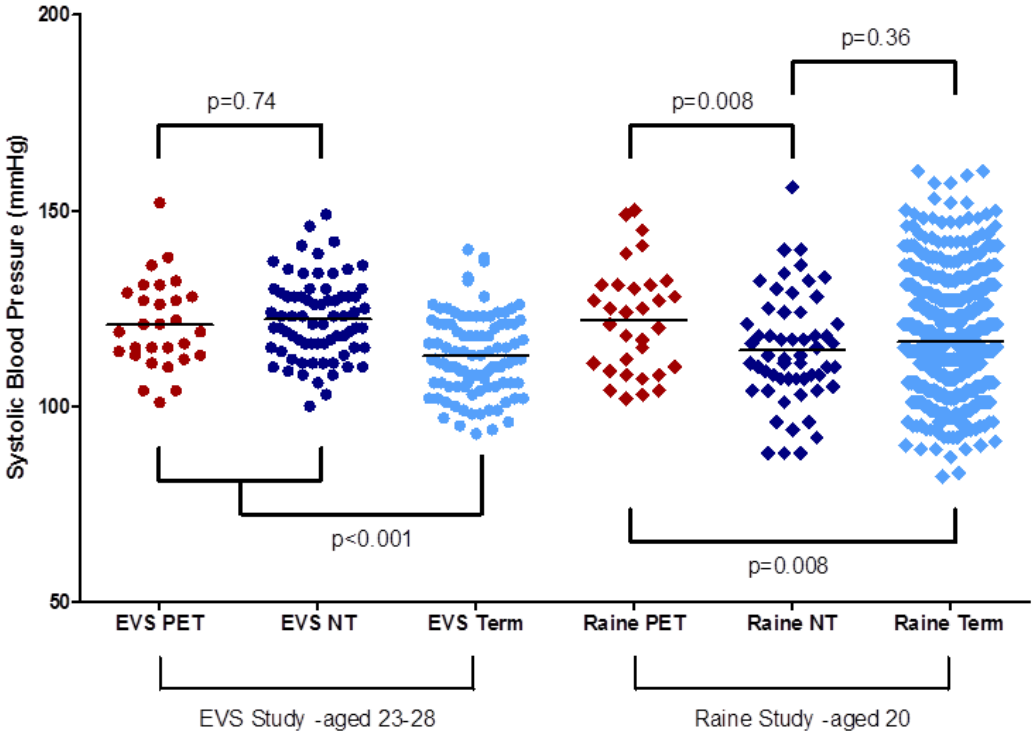


Figure 4.3: This figure shows systolic blood pressure levels in young adulthood in moderate and very preterm offspring of hypertensive and normotensive pregnancies.

Diastolic blood pressure was not consistently elevated in those born preterm to hypertensive pregnancies when compared to those born preterm to normotensive pregnancies, however the very preterm born had elevated diastolic blood pressure compared to term born controls (p=0.001). Data for both cohorts is presented in Table 4.2.

TABLE 4.2 DIASTOLIC BLOOD PRESSURES IN RAINE AND EARLY VASCULAR STUDY GROUPS

Age	Preterm Normotensive	Preterm Hypertensive	p-value	Term Born	p-value (Term vs Preterm NT)	p-value (Preterm vs Preterm HTN)
			Raine Study			
1 year	59.72 (15.63) n = 36	54.45 (6.82) n = 22	0.13	58.75 (12.93) n= 565	0.41	0.064
2 Years	-	-	-	-	-	-
8 Years	54.84 (8.16) n = 44	56.21 (9.21) n = 24	0.45	54.38 (8.16) n = 694	0.96	0.28
17 Years	59.88 (5.86) n = 41	61.11 (7.99) n=27	0.56	58.82 (6.28) n = 660	0.65	0.62
20 Years	65.24 (9.71) n = 54	66.00 (8.64) n = 32	0.45	64.92 (7.88) n = 826	0.87	0.47
			Very Preterm Group – Early Vascular Study			
15 Years	62.50 (7.14) n = 26	64.06 (6.23) n = 8	0.78	-	-	-
23-28 Years	73.19 (7.40) n = 72	73.15 (6.73) n = 26	0.85	68.37 (6.71) n = 85	0.001	0.13

Table 4.2: This table reports the diastolic blood pressure levels (mmHg) in the moderate and very preterm born individuals in the Raine Study and Early Vascular Study cohorts. p-values are adjusted for sex, gestation and birth weigh z score, in the Early Vascular Study young adult cohort p-values are additionally adjusted for age at follow-up.

4.5.1 PERINATAL DETERMINATES OF BLOOD PRESSURE IN VERY AND MODERATELY PRETERM BORN INDIVIDUALS

In a regression model that included sex, gestation at delivery, birth weight z score, and maternal hypertension status, only sex (age 17 $\beta = -9.91$ $p < 0.001$, age 20 $\beta = -11.42$ $p < 0.001$) and early onset hypertensive disorders (age 17 $\beta = 7.24$ $p = 0.001$, age 20 $\beta = 7.13$ $p = 0.008$) were independent predictors of systolic blood pressure in the Raine Study cohort. In this group there was no independent effect of prematurity (age 17 $p = 0.240$, age 20 $p = 0.575$) or birth weight z score (age 17 $p = 0.675$, age 20 $p = 0.930$). When the same model,

with the addition of age at follow up was undertaken in the Early Vascular Study cohort sex (β = -8.61 p <0.001), and gestation at delivery (β = -1.02 p = 0.002) were independent predictors of systolic blood pressure without an independent effect of preeclampsia exposure (p = 0.74).

4.6 Fasting Glucose, Insulin and Lipids

Fasting glucose, insulin and lipids were measured at age 15 and 23-28 in the Early Vascular Study – Very Preterm study group, and at age 17 and 20 in the moderately preterm Raine Study group. At age 25 very preterm born individuals had increased fasting glucose (p < 0.001), fasting insulin (p<0.001), higher insulin resistance scores (p < 0.001) and elevated fasting total cholesterol (p<0.001) and triglycerides (p= 0.015) compared to age matched term born young adults (Table 4.3). The elevation in these measures was similar in adults born following both hypertensive and normotensive pregnancy. In contrast, in the moderate preterm Raine Study group at age 20 (Table 4.3) there were no differences in metabolic factors either between the preterm groups when divided according to the presence or absence of maternal hypertensive disorders, or between those born preterm and those born at term to normotensive mothers. The moderately preterm born offspring the Raine study did show increased LDL cholesterol at age 17 (p ANOVA = 0.021 p (adjusted) = 0.019), when compared to those born at term, however this was no longer evident at young adulthood.

In the very preterm born group age (β = 0.110 p < 0.001), sex (β = 0.459 p = 0.010), birth weight z score (β = -0.26 p = 0.003) and gestation (β = -0.072 p = 0.001) predicted insulin resistance with no independent effect of hypertensive disorders of pregnancy (p = 0.39). Gestation (p = 0.005), age (p < 0.001) and sex (p = 0.038) predicted total cholesterol in young adulthood in this group, again without an independent impact of preeclampsia. As metabolic factors did not vary at age 20 in the moderate preterm group regression analysis was not performed.

4.3 FASTING GLUCOSE INSULIN AND LIPID LEVELS AT ADOLESCENT AND YOUNG ADULT FOLLOW UP.

	Raine Study (17 year follow up)					**Early Vascular Study (15 year Follow up)**				
	Preterm NT n = 52	**Preterm HTN** n = 30	p- value	**Term n = 783**	p-value	**Preterm NT** n = 26	**Preterm HTN** n = 8	p- value	**Term**	p-value
Glucose mmol/L	4.71 (.395)	4.79 (.562)	.511	4.76 (0.44)	0.41	4.69 (.42)	4.38 (.45)	.377	-	-
Insulin pmol/L	99.6 (90.1)	98.9 (67.8)	.948	92.12 (108.49)	0.89	66.65 (85.4)	34.87 (16.89)	.063	-	-
Total Cholesterol mmol/L	4.27 (.755)	4.46 (.871)	.359	4.11 (0.76)	0.069	3.81 (.579)	3.97 (.312)	.850	-	-
HDL-C mmol/L	1.32 (.285)	1.29 (.282)	.464	1.30 (0.29)	0.59	1.16 (.377)	1.06 (.270)	.192	-	-
LDL-C mmol/L	2.48 (.600)	2.65 (.833)	.392	2.32 (0.67)	0.019	-	-	-	-	-
Triglycerides mmol/L	.998 (.448)	1.13 (.594)	.299	1.07 (0.57)	0.14	1.07(.290)	1.07 (.686)	.533	-	-
	(20 Year follow up)					**(23-28 year follow up)**				
	Preterm NT n = 49	**Preterm HN** n = 24	p- value	**Term** N=735	p-value	**Preterm NT** n = 70	**Preterm HTN** n = 20	p- value	Term n = 82	p-value
Glucose mmol/L	4.91 (.329)	5.14 (.814)	.200	4.95 (0.41)	0.18	5.01 (.450)	4.99 (.394)	.764	4.62 (0.30)	<0.001
Insulin pmol/L	61.4 (55.1)	69.9 (53.4)	.455	69.6 (63.7)	0.69	65.2 (45.6)	61.9 (35.2)	.738	35.64 (16.39)	<0.001
Insulin Resistance	-	-	-	-	-	1.20 (0.81)	1.21 (0.65)	.772	0.67 (0.30)	<0.001
Total Cholesterol mmol/L	4.43 (.823)	4.39 (.861)	.960	4.33 (0.80)	0.18	4.82 (.978)	5.19 (1.31)	.425	4.24 (0.84)	<0.001
HDL-C mmol/L	1.35 (.260)	1.26 (.327)	.297	1.33 (0.32)	0.25	1.54 (.381)	1.54 (.473)	.411	1.48 (0.41)	0.63
LDL-C mmol/L	2.61 (.710)	2.58 (.631)	.921	2.49 (0.67)	0.84	2.75 (.876)	3.06 (1.14)	.562	2.37 (0.65)	0.33
Triglycerides mmol/L	1.02 (.424)	1.17(.761)	.624	1.09 (0.82)	0.29	1.08 (.776)	1.31 (.872)	.365	0.87 (0.41)	0.015

Table 4.3: Fasting serum glucose, insulin and lipids at young adult follow up. Values are means ± standard deviation.

4.7 Growth and Anthropometry

In early adult life there was no difference in anthropometric measures between those born very preterm and term controls despite their greater blood pressure and increased metabolic risk factors. Those born preterm to hypertensive pregnancies had lower birth weight z scores than those whose mothers remained normotensive (-0.65 ± 1.22 vs -1.22 ± 0.94 p=0.039). Consistent with this observation of growth restriction in those born following early onset hypertension, were smaller sub-scapular and triceps skinfold thicknesses at 9 months of age (9 months subscapular p (adjusted) = 0.008, triceps p (adjusted) = 0.017). However, differences in athropometric measures had disappeared by later childhood. In the moderately preterm Raine Study group again there was no significant differences between the preterm born groups in anthropometric measures (Table 4.4). Furthermore earlier data showed similar birth weight, ponderal index and birth length in those born preterm following hypertensive pregnancy disorders and those born preterm to normotensive pregnancies (See Table 4.1).

TABLE 4.4 MEASUREMENTS OF GROWTH AND ADIPOSITY IN THE RAINE AND EARLY VASCULAR STUDY COHORTS.

	Early Vascular Study					Raine Study				
	Normotensive	**Hypertensive**	**p-value**	**Term**	p-value	**Preterm**	**Preterm**	**p-value**	**Term**	**p-value**
Weight (kg)										
1 Year /9 months	8 (1) n = 41	8 (1) n = 14	0.69	-	-	10 (1) n=48	10 (1) n=28	0.16	10 (1) n=784	0.19
8 Years/7 Years	23 (4) n= 72	24 (3) n = 23	0.52	-	-	27 (5) n=50	29 (7) n=25	0.07	28 (5) n=747	0.16
17 Years/15 Years	57 (12) n = 41	57 (10) n = 14	0.89	-	-	66 (11) n=42	67(13) n=27	0.58	67 (13) n=650	0.82
20 Years/25 Years	69 (16) n = 55	74 (19) n = 32	0.08	72 (16) n = 85	0.88	74 (21) n =73	68 (19) n = 26	0.21	70 (13) n = 85	0.77
BMI kg/m^2										
1 Year/9 months	17 (1) n = 41	16 (1) n=14	0.53	-	-	17 (2) n=48	18 (1) n=27	0.05	17 (1) n=780	0.46
8 Years/7 Years	15 (2) n = 72	15 (1) n =23	0.64	-	-	16 (2) n=50	17(3) n=25	0.09	17 (2) n=747	0.21
17 Years/15 Years	21 (4) n = 41	22 (3) n = 14	0.79	-	-	23 (4) n=42	23 (4) n=27	0.18	23 (4) n=650	0.98
20 Years/25 Years	24 (4) n = 55	25 (5) n = 32	0.19	24 (5) n = 82	0.59	25 (5) n=73	26 (7) n = 26	0.48	22 (2) n = 85	0.27

TABLE 4.4 CONTINUED

Triceps Skinfold (mm)										
1 Year /9 months	8 (2) n = 35	6 (2) n = 13	0.02	-	-	8 (1) n=39	9 (2) n=25	0.07	8 (2) n=682	0.10
17 Years/15 Years	14 (6) n = 41	15 (7) n =14	0.50	-	-	16 (6) n=39	16 (7) n=27	0.66	15 (7) n=621	0.70
20 Years/25 Years	14 (6) n = 53	13 (5) n = 16	0.72	-	-	18 (8) n = 55	19 (9) n = 32	0.61	17 (8) n = 836	0.35
Subscapular Skinfold (mm)										
1Year /9 months	6 (1) n = 35	5 (1) n = 13	0.01	-	-	7 (2) n=45	7 (1) n=26	0.55	7 (1) n=744	0.43
17Years/15 Years	12 (5) n = 41	12 (5) n = 14	0.71	-	-	14 (5) n=39	15 (8) n=27	0.48	14 (7) n = 617	0.84
20 Years/25 Years	19 (9) n = 52	18 (7) n = 16	0.24	-	-	16 (7) n = 55	18 (9) n = 32	0.70	15 (8) n = 832	0.17

4.8 DISCUSSION

This study has, for the first time, compared the cardiovascular phenotype of individuals born at differing degrees of prematurity following early onset hypertensive disorders of pregnancy with prospective follow up from infancy into young adult life. Very preterm born individuals have increased blood pressure, fasting lipids and insulin resistance in young adult life. Although early onset hypertension was associated with greater growth restriction in these very preterm individuals there was no additional impact of maternal hypertension during pregnancy or growth restriction on these later metabolic risk factors. Rather, the levels of risk factors were independently predicted by degree of prematurity. This observation is consistent with the finding that a more moderately born preterm group did not have significant changes in cardiovascular risk factors compared to term infants. Interestingly however those born moderately preterm still had higher blood pressure in adulthood if the pregnancy had been complicated by an early onset hypertensive pregnancy disorder. This elevation in blood pressure was independent of degree of prematurity or growth restriction.

To date much of the literature on the effect of preeclampsia on offspring has been limited by the inability to distinguish the effects of in utero exposure to preeclampsia from those of growth restriction and prematurity. Preeclampsia is a recognised cause of prematurity and fetal growth restriction [20, 22]. Consistent with this just over 21% of the Early Vascular Study cohort, and 33% of the preterm born Raine Study were born to hypertensive pregnancies and these offspring were more commonly small for gestational age with lower birth weight z scores. Prematurity itself comprises a spectrum ranging from those born on the edge of viability at 24 weeks gestation, to those born near term at 36 weeks [262]. Preterm born individuals may, therefore, vary widely in their perinatal treatment needs,

prognosis and plausibly in the manner in which differing perinatal factors influence their cardiovascular phenotype.

This analysis has identified that greater prematurity is associated with adverse changes in both metabolic and blood pressure characteristics in later life independent of degree of growth restriction or history of maternal hypertension. The changes in metabolic factors in the very preterm group are of interest. Prematurity has been associated with increased cord blood cholesterol [246], and small for gestational age infants have been reported to have higher or lower serum cholesterol dependent on whether infants are growth restricted or constitutionally small [247]. In later life prematurity has been associated with altered lipoprotein metabolism [263] and as previously demonstrated with higher cholesterol in this preterm born population [203].

This data suggests however that in the absence of extreme, or very preterm birth, the key factor that determines later cardiovascular risk profile is a history of early-onset hypertensive pregnancy disorder. Moderately preterm born offspring of hypertensive pregnancies demonstrate a specific adult hypertensive phenotype, without evidence of alteration in metabolic risk factors or anthropometry. This phenotype is consistent with several other recent studies that have shown only small or no metabolic differences in the offspring of preeclamptic pregnancies [8, 63, 105]. The increase in blood pressure related to maternal pregnancy hypertension in the moderately preterm group is around 8mmHg, similar to that observed in a previous study of children born following early-onset preeclampsia [240], and equivalent to that associated with severe prematurity in the very preterm study group. As 80% of preterm births occur between 32 and 37 weeks (moderate to late prematurity) [262] this finding is potentially of significant public health importance in defining a subgroup of those born preterm who may experience increased cardiovascular risk.

Additionally this work has demonstrated in two cohorts that in utero exposure to hypertensive disorders is not associated with alterations in glucose or lipid metabolism separate from those associated with prematurity. This is despite the fact that those born to hypertensive pregnancies are more growth restricted at birth than those whose mothers remained normotensive. Being born small for gestational age has been associated with an adverse metabolic profile from childhood which has been demonstrated in other studies of comparable sample sizes [15, 264] Although sample sizes in these studies in later life are relatively small, replication in two long-term follow up cohorts at two time points allows for some confidence in these results. Furthermore these results are consistent with other studies, which have shown small or no metabolic differences in the offspring of preeclamptic pregnancies [8, 63, 105]. The lack of differences in the offspring of preeclamptic pregnancies is unexpected and better understanding of the reasons underlying this may provide additional insight into the long-term implications of preeclampsia. Animal studies have reported metabolic alterations associated with models mimicking preeclampsia [147, 214, 265], and it is possible that these will become apparent in large populations or with longer term follow up.

There are several limitations to this study. Primarily not all individuals recruited into either cohort were followed up to young adulthood. In addition the smaller sample size in adolescent and young adult follow up means it is impossible to exclude the possibility of subtle changes in metabolism or anthropometry, unseen in this study group, that may be detectable in a larger population. Additionally in the UK based early feeding cohort, detailed perinatal data was not available on the term born individuals recruited in young adulthood. Accurate gestation and birth weight data is available in this population, however given the long period between delivery and recruitment the possibility cannot be excluded of conditions of pregnancy which were not reported to investigators. Finally the two cohorts considered in this work were recruited in different countries, and therefore from differing

baseline populations. There is no reason to suspect major underlying demographic differences in the recruited populations in the United Kingdom and Western Australia, however it is possible that some population based difference may underlie some aspects of the observed differences.

4.9 CONCLUSION

This work has demonstrated for the first time that those infants born at moderate to late preterm gestation to mothers with hypertensive disorders have a significant increase in systolic blood pressure in later life independent of gestation. This is not seen in extremely preterm born individuals where gestation at delivery remains the most important perinatal predictor of adult blood pressure. Furthermore severe prematurity, but not preeclampsia, impacts on lipid and glucose metabolism in later life. These data suggest that metabolic alterations are not the key factor underlying the increased risk of cardiovascular events in those born to preeclamptic pregnancies, although future larger scale follow up studies will allow more precise determination of whether subtle alterations in glucose or lipid metabolism are present in these individuals. In view of the fact that the vast majority of preterm births are after 32 weeks, and at least 20% of these are due to hypertensive disorders of pregnancy, these findings are of significant interest in defining a population that may warrant more intensive cardiovascular risk surveillance into from young adult life.

CHAPTER 5 – CARDIOVASCULAR PHENOTYPE AND CARDIOVASCULAR RISK IN TERM BORN OFFSPRING OF PREECLAMPTIC AND HYPERTENSIVE PREGNANCIES.

5.1 THESIS CONTEXT

Early onset hypertensive disorders of pregnancy leading to premature delivery appear to have particularly adverse impacts on offsprings' cardiovascular phenotype. It should be noted however that the vast majority of women with hypertensive disorders of pregnancy deliver at term. This chapter considers the cardiovascular phenotype of term born offspring of preeclamptic pregnancies and whether this differs from that associated with non-protienuric hypertensive disorders of pregnancy. It also considers whether exposure to either condition is already associated with increases in markers of clinical risk in young adulthood.

5.2 INTRODUCTION

Offspring exposed to preeclampsia in utero demonstrate persistent changes in cardiovascular phenotype, with almost double the risk of strokes in adulthood [7, 8, 60, 67, 240]. Gestational hypertension, a more common and less severe pregnancy related complication than protienuric preeclampsia [266], is also associated with altered cardiovascular phenotype in childhood and adolescence [98, 267, 268]. Individuals born preterm because their mother developed hypertension in early pregnancy, have particularly marked differences in cardiovascular phenotype, with up to 10mmHg higher blood pressure during childhood [8, 240]. As demonstrated in Chapter 4, adverse offspring metabolic risk factors after early-onset pregnancy hypertension relate to degree of prematurity. However an independent impact of

maternal hypertension during pregnancy on offspring blood pressure is also evident in those born at a moderately preterm gestation. These findings highlight that the moderately preterm born offspring of hypertensive pregnancies, may as a group warrant early and intensive primary prevention to reduce later cardiovascular risk.

While the preterm offspring of hypertensive pregnancies are clearly a group at increased risk, the vast majority of women with hypertensive disorders of pregnancy go on to deliver at term. A Norwegian study considering data from close to 1.7 million deliveries, over 50 years, showed that of 44,220 cases of preeclampsia, 83.8% (37,044) were delivered at more than 37 weeks completed gestation [269]. Because of this, even if the offspring phenotype associated with maternal preeclampsia at term were less severe, the number of potentially affected individuals would make it of considerable public health importance.

Hypertensive disorders of pregnancy requiring premature delivery are generally considered to fall on the preeclampsia spectrum, whether or not overt proteinuria has developed by the time of delivery. At term, however, there are potential differences in both the pathophysiology and consequences of preeclampsia and gestational hypertension [19, 269-271]. In particular, gestational hypertension at term may occur in the absence of the placental dysfunction that is characteristic of protienuric preeclampsia, and infants born to these pregnancies do not necessarily show evidence of exposure to an adverse in utero environment [19, 269-271]. It is therefore unclear whether the long-term cardiovascular phenotype associated with in utero exposure to preeclampsia and non-proteinuric hypertensive disorders of pregnancy will be similar. Differences, if they exist, in the cardiovascular phenotype associated with these conditions may be informative in understanding the mechanisms by which hypertensive disorders of pregnancy impact on offspring cardiovascular risk.

Additionally while the association between in utero exposure to hypertension and small increases in blood pressure is relatively well established [67], it remains unclear whether these alterations translate into increases in clinically relevant risk markers, such as increase in global risk scores, or increased likelihood of clinically evident hypertension or obesity in early life. Such changes, if demonstrated, may allow the definition of a subgroup at particularly increased risk who would therefore benefit from risk factor modification.

Using data from the Western Australian Pregnancy Cohort Study (The RAINE Study) [251], I have undertaken to investigate the separate effects of preeclampsia and gestational hypertension on offspring cardiovascular phenotype. In addition I have investigated whether these changes in cardiovascular phenotype translate into apparent and relevant alterations in clinical risk in young adulthood.

5.3 METHODS

5.3.1 STUDY POPULATION

The Western Australian Pregnancy Cohort Study (The RAINE Study) is described in detail in Chapter 4. In brief, 2,900 women were enrolled at or before, the 18th week of gestation at a tertiary level obstetric hospital in Perth, Western Australia [272]. Only subjects born at, or greater than 37 weeks gestation without congenital abnormalities were included in this analysis. Follow up visits including assessment of growth, development, metabolic and cardiovascular health have been undertaken at multiple time points (including 1, 3, 5, 8, 10, 14, 17 and 20 years of age) [273]. Timing of visits included in this analysis and relevant investigations at each time point are outlined in Figure 5.1.

FIGURE 5.1 FOLLOW UP VISIT SCHEDULE AND INVESTIGATIONS IN THE RAINE STUDY COHORT

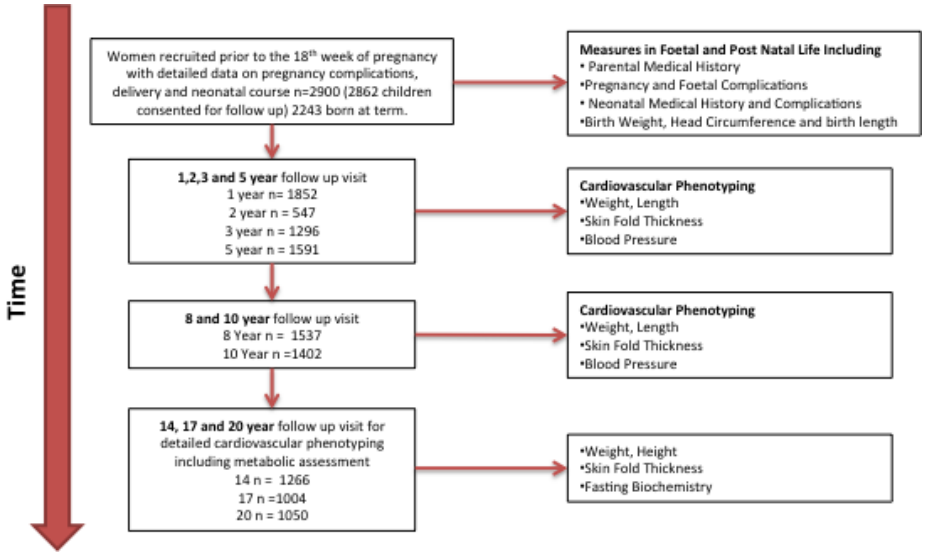


Figure 5.1: this figure shows the timing and investigations in the term-born Raine Study group used in this analysis.

During pregnancy lifestyle information was collected by Questionnaire at 18 and 34 weeks gestation. Information on antenatal course, delivery and neonatal course was obtained from review of maternity, and where relevant, neonatal medical records. Later lifestyle factors were recorded with questionnaires completed by the primary care giver and/or the subject. Preeclampsia (PET) was defined as de novo onset hypertension (>140/90) and proteinuria after the 20th week of pregnancy. Pregnancy induced hypertension (PIH) was defined as de novo onset hypertension (>140/90) after the 20th week of pregnancy in the absence of proteinuria. Offspring of mothers with neither of these conditions were classed as normotensive (NT).

5.3.2 CARDIOVASCULAR PHENOTYPING

At birth, weight was measured using an electronic balance. Length was measured using a Harpeden Neonatometer and head circumference was measured using flexible plastic or fibreglass tape to the nearest 0.1cm. At subsequent follow up visits, weight was recorded to the nearest 100g using a Wedderburn digital chair scales, length to 0.1cm on Harpenden Infantometer/Holtain Stadiometer. Blood pressure was measured using a clinically validated electronic blood pressure recorder. The average of two or more readings was reported.

Fasting blood samples were drawn at the 14, 17 and 20 year follow up visits. These samples were analysed in the PathWest Laboratory at Royal Perth Hospital for serum insulin, glucose, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol using routine clinically validated assays [259].

Clinical risk - Lifetime QRisk scores (to age 95) were calculated for each individual for whom a minimum dataset (weight, height, blood pressure, serum lipids and smoking status) was available[21]. These variables for each individual were entered into an online automated QRisk lifetime calculator (http://www.qrisk.org/lifetime/) and a % lifetime risk score to age 95 was recorded for each individual. Subjects were classed as falling in the top 25% of lifetime risk if their QRISK lifetime score was 39% or more [22]) Clinically apparent hypertension at age 20 was defined as an examination systolic blood pressure of more than 140mmHg and/or a diastolic blood pressure of more than 90mmHg. Subjects were defined as overweight at age 20 if the calculated BMI was between 25kg/m^2 and 29.99 kg/m^2, and defined as obese if BMI was 30kg/m^2 or more.

5.3.3 STATISTICS:

Statistical analysis was carried out with SPSS version 20. Normality testing was undertaken by visual assessment of normality curves and the Shapiro-Wilk test. Subjects were divided into those born to normotensive pregnancies (NT), those born to hypertensive pregnancies (PIH) and those born to preeclamptic (PET) pregnancies. Comparisons were undertaken between the hypertensive groups (PET and PIH), between combined hypertensive groups (PET +PIH) and the NT offspring and between individual hypertensive groups (PET or PIH) and the NT group. Comparison between groups for continuous variables was performed with a 2-sided, independent-samples Student t test for comparison of two groups and one-way ANOVA for comparison across multiple groups for normally distributed data and Mann-Whitney and Kruskal-Wallis tests for skewed data. Linear regression models were undertaken using a

Forced Entry Model. Pearson correlations (r) were used for bivariate-associated models, and unstandardized regression coefficients (β) were used for bivariate and multivariate regression models. Triglyceride, Insulin, Glucose and other non-normal values were log transformed prior to analysis. Comparisons between groups were adjusted for gestational age at delivery, birth weight and sex. For categorical variables, or binary categories of clinical risk, comparison was done with a $\chi 2$ test and where informative addition odds ratios were calculated for the odds of clinical outcome in hypertensive groups (PET or PIH) compared to normotensive offspring. P values less than 0.05 were considered statistically significant.

5.4 Population Characteristics

2,243 infants were born at term and without major congenital abnormalities. 445 of these were born to pregnancies complicated by gestational hypertension (PIH), and 39 to pregnancies complicated by protienuric preeclampsia (PET). The remaining 1,759 infants were born to mothers who remained normotensive (NT). The perinatal characteristics of these infants and their mothers are presented in Table 5.1, and the perinatal characteristics of the 1,050 individuals who were followed up to age 20 are presented in Table 5.2.

TABLE 5.1: PERINATAL CHARACTERISTICS OF BIRTH COHORT

	Normotensive	PIH	Preeclamptic	p-value
	N= 1759	N= 445	N=39	
Infant Characteristics				
Sex (% male)	49.6% (873)	54.2% (241)	59% (23)	0.14
Birth weight (g)	3418 (461)	3410 (481)	3355 (455)	0.67
Birth weight z score	-0.057 (0.91)	-0.028 (0.97)	0.12 (0.91)	0.44
Gestational Age (weeks)	39.83 (1.27)	39.70 (1.33)	39.00 (1.25)	<0.001
Head Circumference (cm)	34.61 (1.46)	34.67 (1.52)	34.68 (1.23)	0.72
Placental Weight (g)	607 (127)	614 (124)	622 (111)	0.48
	n= 1651	n = 428		
Perinatal Characteristics				
C-Section Delivery (%)	17% (299)	18.4% (82)	23.1% (9)	0.50
Antenatal Steroids (% Received)	1.0% (17)	0.7% (3)	2.6% (1)	0.48
APGAR 5	9.10 (2.25)	8.95 (0.70)	9.28 (0.65)	0.30
Neonatal Hospital Care				
ICU admission (%)	1.9% (33)	2.0% (9)	0.0% (0)	0.67
SCBU admission (%)	12.9% (227)	22.5% (100)	38.5% (15)	<0.001
Ventilation (%)	0.3% (5)	0.7% (3)	0.0% (0)	0.44
Neonatal Sepsis (%)	5.4% (95)	8.5% (38)	5.1% (2)	0.040
Length of Neonatal Stay (days)	4.93 (2.03)	5.40 (2.19)	6.33 (3.19)	<0.001
Breast Fed at Discharge (%)	86.7% (1525)	86.3% (384)	92.3% (36)	0.030
Maternal Characteristics				
Maternal Age (years)	29.38 (5.76)	28.98 (5.78)	29.35 (6.30)	0.66
Maternal BMI (kg/m^2)	21.85 (3.95)	23.36 (4.47)	23.32 (5.43)	<0.001
Smoking in Late Pregnancy (%)	28.1% (495)	22.2% (99)	17.9% (7)	0.28
Multiple Pregnancies (%)	1.8% (32)	0.8% (4)	5.2% (2)	0.32
Gestational Diabetes (%)	1.6% (29)	1.8% (8)	2.6% (1)	0.89

Table 5.1: Perinatal characteristics of infants and mothers in the Raine Study by group. Data presented is mean+/- standard deviation, p-values from ANOVA, or χ^2 for difference across groups. ICU = Intensive Care Unit, SCBU = Special Care Baby Unit, BMI = Body Mass index.

TABLE 5.2: PERINATAL CHARACTERISTICS OF THOSE INDIVIDUALS ATTENDING 20-YEAR FOLLOWUP.

	Normotensive n= 829	PIH n= 207	Preeclamptic n=14	p-value
Infant Characteristics				
Sex (% male)	50.8 (421)	58.9 (122)	57.1 (8)	0.10
Birth weight (g)	3429 (449)	3420 (479)	3405 (438)	0.96
Birth weight z score	-0.049 (0.91)	-0.031 (0.97)	0.17 (0.99)	0.67
Gestational Age (weeks)	39.86 (1.24)	39.72 (1.36)	39.04 (1.41)	0.025
Head Circumference (cm)	34.69 (1.48)	34.77 (1.55)	34.61 (1.72)	0.76
Placental Weight (g)	606 (124)	615 (130)	647 (123)	0.35
Perinatal Characteristics				
C-Section Delivery (%)	17.9% (148)	18.8% (39)	28.6% (4)	0.57
Antenatal Steroids (% Received)	0.7% (6)	0.5 (1)	0% (0)	0.89
APGAR 5	9.08 (0.65)	8.96 (0.66)	9.50 (0.65)	0.004
Neonatal Hospital Care				
ICU admission (%)	1.8% (15)	0.5% (1)	0% (0)	0.34
SCBU admission (%)	12.8% (106)	23.2% (48)	21.4% (3)	0.005
Ventilation (%)	0.1% (1)	0% (0)	0% (0)	0.88
Neonatal Sepsis (%)	4.7% (41)	8.2% (17)	0% (0)	0.12
Length of Neonatal Stay (days)	5.03 (2.04)	5.42 (2.24)	6.93 (4.05)	<0.001
Breast Fed at Discharge (%)	90.6% (751)	90.8% (188)	100% (14)	0.41
Maternal Characteristics				
Maternal Age (years)	29.41 (5.76)	28.96 (5.79)	29.53 (6.30)	0.64
Maternal BMI (kg/m^2)	21.80 (3.93)	23.06 (4.15)	22.49 (3.91)	<0.001
Smoking in Late Pregnancy (%)	23.2% (192)	20.8% (43)	14.2 (2)	0.87
Multiple Pregnancies (%)	2.2% (18)	1% (2)	14.2% (2)	0.06
Gestational Diabetes (%)	1.6% (13)	2.9% (6)	7.1% (1)	0.16

Table 5.2: Perinatal characteristics those individuals attending 20 year follow up by group. Data presented is mean+/- standard deviation, p-values from ANOVA, or χ^2 for difference across groups. ICU = Intensive Care Unit, SCBU = Special Care Baby Unit, BMI = Body Mass index.

In both the birth cohort, and those returning for 20 year follow up, infants whose mothers had experienced protienuric preeclampsia were born slightly earlier than other infants ($p < 0.001$). As expected, mothers who experienced gestational hypertension and preeclampsia had slightly higher body mass indexes (BMI) than those mothers who remained normotensive ($p < 0.001$), although the average BMI in all groups fell within the normal range. The offspring of hypertensive and preeclamptic pregnancies more commonly required special care and had a longer length of hospital stay than those whose mothers remained normotensive ($p < 0.001$). In the birth cohort, those born to preeclamptic pregnancies were more likely to be

breastfed at discharge than other groups (92.3 vs 86.3% and 86.7% p = 0.03). Finally in those who attended the 20-year follow up, the offspring of preeclamptic pregnancies had higher 5-minute APGAR scores (p = 0.004) although this was attenuated by adjustment for gestation (p = 0.65).

5.5 ADIPOSITY

BMI measurements were calculated at multiple time points. From 1 year of age (p = 0.042) to young adulthood (p = 0.002), the offspring of mothers with gestational hypertension showed significant increases in BMI compared to those whose mothers remained normotensive. This increase in BMI was not seen in the offspring of preeclamptic pregnancies who did not demonstrate a significant increase compared to the normotensive group at any time point (Figure 5.2A). By young adulthood offspring of mothers with gestational hypertension were, on average, 1.18kg/m^2 heavier than the normotensive offspring (p=0.002), and 1.24 kg/m^2 heavier than preeclamptic offspring, although this relationship did not reach statistical significance. There was no significant difference between the preeclamptic and normotensive offspring at this, or any other, age (PET 24.08 ± 5.53 vs NT 24.16 ± 4.78 kg/m^2 p = 0.90).

FIGURE 5.2: TRAJECTORIES OF BMI AND SYSTOLIC BLOOD THROUGH CHILDHOOD AND YOUNG ADULT LIFE

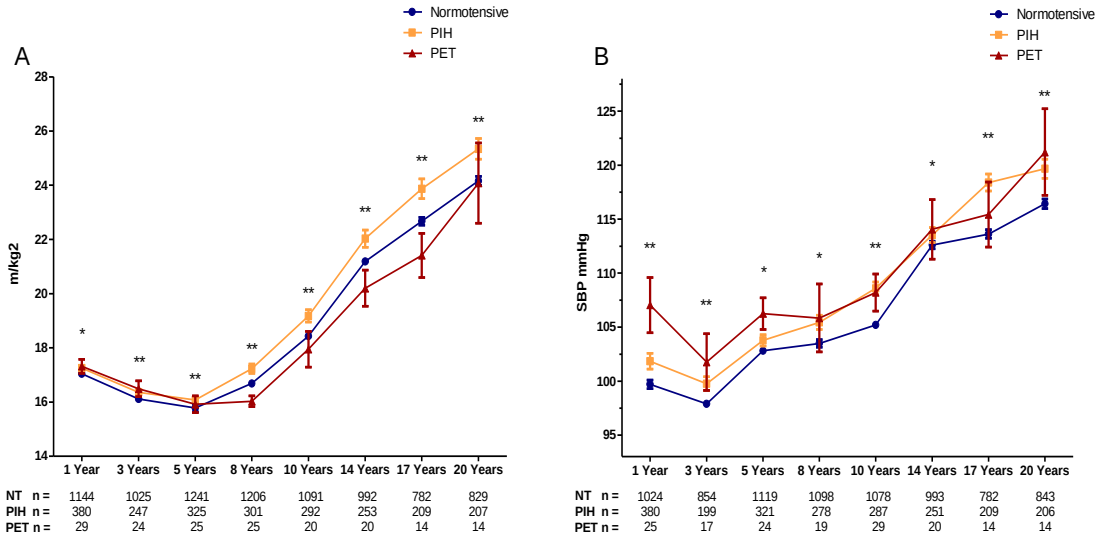


*Figure 5.2: This figure shows the trajectories of BMI and systolic blood pressure throughout life in the: offspring of NT (blue), PIH (orange) and PET (red) pregnancies. Data presented is mean ± SEM, * indicates p <0.05 and ** indicates p <0.01.* ***Panel A*** *shows BMI kg/m^2 from 1 to 20 years of age. The offspring of PIH pregnancies have greater BMIs at all stages, p-values are for the difference between the PIH and NT group adjusted for sex, gestation at delivery and birth weight z score. There was no difference between the PET and NT group (p-values not reported).* ***Panel B*** *shows systolic blood pressure (mmHg) from 1 to 20 years of age. At all stages PIH and PET offspring have increased blood pressure compared to those whose mothers remain normotensive, the magnitude of change was similar in both hypertensive groups. P-Values for difference of offspring of hypertensive pregnancies compared to the normotensive group adjusted for sex, gestation at delivery and birth weight z score.*

5.6 BLOOD PRESSURE

Blood pressure was measured at multiple time points within the cohort. Consistent with previous work, the offspring of hypertensive pregnancies (P.I.H. and PET) demonstrated increased resting systolic blood pressure at each time point compared to the offspring of normotensive pregnancies (Figure 5.2B). This increase in blood systolic blood pressure persisted after adjustment for gestation at delivery, birth weight z score and sex. The magnitude of the increase in systolic blood pressure was similar in both PIH and PET offspring (Figure 5.2B). An elevation in diastolic pressure was not as consistently associated with in utero exposure to hypertension. In the gestational hypertensive group only significant increases in diastolic blood pressure were evident at age 8 (PIH 56.3 ± 8.40 vs NT 54.8 ± 8.50

mmHg p = 0.006) and age 20 (PIH 64.9 ± 7.9 vs ± NT 66.5 ± 8.4 mmHg p = 0.011), while both gestational hypertensive and preeclamptic groups demonstrated elevations at ages 10 (NT 56.2 ± 6.6, PIH 58.5 ± 6.8, PET 57.7 ± 6.0 mmHg p <0.001) and 16 (NT 58.9 ± 6.2, PIH 61.0 ± 7.0, PET 60.1 ± 6.4 mmHg p <0.001). At other time points there was no significant difference in diastolic blood pressure between groups.

Finally in view of the distinct differences in BMI demonstrated by the offspring of hypertensive (PIH) pregnancies, the impact of BMI on this group's blood pressure was considered at age 20. The relationship between gestational hypertension and systolic blood pressure was attenuated by adjustment for BMI from an association with a 3.69mmHg increase (p < 0.001) to a more modest 1.77mmHg increase (p =0.039).

5.7 Glucose and Lipid Metabolism

Fasting glucose, insulin and cholesterol levels were measured in 1081 (221 PIH and 17 PET) subjects at 14 years of age, 986 subjects (206 PIH and 16 PET) at 17 years of age and 927 subjects (185 PIH and 13 PET) at 20 years of age. Glucose, insulin and cholesterol results at each age point are presented in Table 5.3. The offspring of hypertensive (PIH) pregnancies appeared to have a slightly higher serum insulin level at age 14 (p=0.040), although this was no longer present at age 17 or 20. There were no other differences between the NT and PIH groups. Additionally there were no differences in blood glucose, insulin or cholesterol between the offspring of normotensive pregnancies and the offspring of preeclamptic pregnancies at any point during adolescence or young adulthood.

TABLE 5.3: SERUM GLUCOSE, INSULIN AND CHOLESTEROL AT DIFFERENT AGES

	Normotensive (NT)	**P.I.H**	**p-value P.I.H. vs NT**	**PET**	**p-value PET vs NT**	**Across all groups**
14 Years	*n = 842*	*n = 221*		*n = 17*		
Glucose (mmo/L)	4.80 (.559)	4.80 (0.40)	0.76	4.72 (0.32)	0.37	0.04
Insulin (pmol/L)	11.92 (8.87)	12.97 (8.90)	0.040	11.52 (4.67)	0.68	0.74
Total cholesterol (mmol/L)	4.17 (0.70)	4.10 (0.71)	0.15	4.14 (0.55)	0.94	0.77
Triglycerides (mmol/L)	1.00 (0.57)	0.99 (0.45)	0.87	1.03 (0.45)	0.54	0.35
HDL – Cholesterol (mmol/L)	1.41 (0.32)	1.37 (0.30)	0.30	1.39 (.274)	0.87	0.93
LDL – Cholesterol (mmol/L)	2.30 (0.62)	2.27 (0.66)	0.27	2.28 (0.50)	0.85	0.36
17 Years	*n = 764*	*n = 206*		*n = 16*		
Glucose (mmo/L)	4.76 (0.44)	4.48 (0.53)	0.62	4.80 (.32)	0.76	0.32
Insulin (pmol/L)	9.21 (10.85)	9.20 (8.61)	0.95	7.41 (5.78)	0.24	0.32
Total cholesterol (mmol/L)	4.11 (0.76)	4.12 (0.77)	0.19	4.27 (0.83)	0.30	0.26
Triglycerides (mmol/L)	1.07 (0.57)	1.02 (0.48)	0.16	0.98 (0.37)	0.70	0.94
HDL – Cholesterol (mmol/L)	1.30 (0.29)	1.26 (0.29)	0.22	1.32 (0.20)	0.47	0.72
LDL – Cholesterol (mmol/L)	2.32 (0.68)	2.29 (0.63)	0.99	2.50 (0.65)	0.32	0.37
20 Years	*n = 729*	*n = 185*		*n = 13*		
Glucose (mmo/L)	4.95 (0.41)	4.97 (0.47)	0.82	4.90 (0.35)	0.58	0.52
Insulin (pmol/L)	6.96 (6.37) n = 375	6.13 (4.35) n = 105	0.28	4.54 (2.61) n = 6	0.21	0.24
Total cholesterol (mmol/L)	4.33 (0.80)	4.20 (0.74)	0.13	4.30 (0.79)	0.79	0.054
Triglycerides (mmol/L)	1.09 (0.82)	1.08 (0.59)	0.71	1.05 (0.39)	0.96	0.25
HDL – Cholesterol (mmol/L)	1.34 (0.33)	1.29 (0.32)	0.26	1.31 (0.32)	0.82	0.16
LDL – Cholesterol (mmol/L)	2.49 (0.68)	2.41 (0.62)	0.21	2.51 (0.53)	0.97	0.33

Table 5.3: Results of fasting biochemical tests for glucose, insulin, and lipids at age 14, 17 and 20 years. Values reported are mean± standard deviation. Reported p-values for differences between groups are adjusted for sex, gestational age at delivery and birth weight z score.

5.8 Clinically Relevant Risk Markers

To determine whether the offspring of hypertensive pregnancies already show increased levels of clinically relevant risk in young adulthood I investigated the proportion of individuals already showing blood pressures consistent with clinical hypertension or obesity. At 20 years of age 5.6% of the offspring of normotensive mothers, and 6.7% of the offspring of mothers with pregnancy induced hypertension, had systolic blood pressures consistent with clinical hypertension (> 140mmHg as per NICE Guidelines for diagnosis of hypertension[68]). Conversely 21.4% of those born to mothers with protienuric preeclampsia had hypertensive level systolic blood pressures (p= 0.012 vs controls and 0.045 vs PIH). The odds ratio for meeting diagnostic criteria for systolic hypertension was, in those born to preeclamptic pregnancies, 4.64 (95% CI 1.25 – 17.18 p = 0.022) compared to those whose mothers remained normotensive and 3.78 (95% CI 0.94-15.13 p = 0.060) compared to those who mothers had non-protienuric hypertension during pregnancy. The offspring of PET pregnancies were also 4.53 times (95% CI 1.22 – 16.79 p = 0.024 Figure 5.3) more likely to exhibit either systolic or diastolic hypertension at age 20 years of age. There was no increase in risk for PIH offspring (OR 1.28 95% CI 0.70 – 2.1 p =0.422 Figure 5.3).

Of those born to normotensive pregnancies, 72% had a body mass index of less than 25kg/m^2 and 9.8% were clinically obese at follow up (BMI > 30kg/m^2). A similar pattern was seen in the offspring of preeclamptic pregnancies with 63.6% having healthy range BMI's and 9.1% falling into the category of obesity (p = 0.74). The offspring of pregnancies complicated by gestational hypertension were however significantly more likely to be overweight (28.6%) or obese (14.1%) at 20 years of age (p < 0.001), with an odds ratio of 1.92 (95% CI 1.38 -2.66 p < 0.001) compared to the offspring of normotensive pregnancies. There was no evidence of increased prevalence of obesity in the PET offspring (p = 0.54 Figure 5.3).

Finally to investigate the global risk in this group I calculated lifetime QRISK scores up to 95 years of age for participants attending the 20-year follow up visit [274] . Although global risk levels were low, lifetime QRISK scores were higher in those born to hypertensive (27.71 ± 6.04% lifetime risk) and preeclamptic (27.78 ± 7.38%) pregnancies when compared to those whose mothers remained normotensive (26.52 ± 5.58% p=0.048). This relationship persisted after adjustment for gestational age and birth-weight z score (p= 0.017). 15.4% of those born to preeclamptic pregnancies and 7.9% of those born to hypertensive pregnancies had scores which would place them above the 75th Centile for QRISK score (>39% lifetime risk [274]), compared to 3.8% of the offspring of normotensive pregnancies (p=0.015). The odds ratios for a QRISK score greater than the 75th centile are presented in Figure 5.3.

FIGURE 5.3: ODDS RATIOS FOR RISK OF CLINICAL RISK FACTORS AT AGE 20

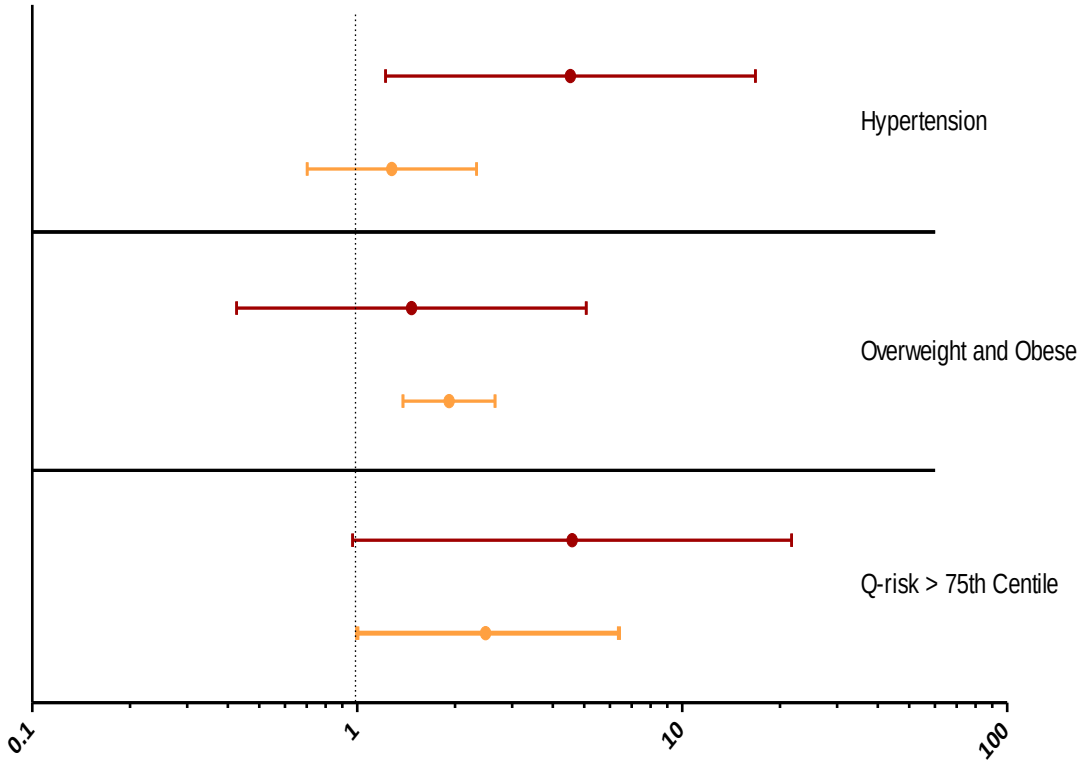


Figure 5.3: This figure shows the odds ratios of clinically relevant risk factors in the offspring of P.I.H (orange) and PET (red) pregnancies compared to those born to normotensive pregnancies at age 20. At this age the offspring of preeclamptic pregnancies are 4.5 times more likely to have clinically apparent hypertension. The offspring of P.I.H. pregnancies are 1.9 times more likely to be overweight or obese, without a significant increase in hypertension. Both hypertensive groups were more likely to have QRISK scores greater than the 75th centile in adult life

5.9 DISCUSSION

This chapter has presented a detailed investigation and differentiation of the cardiovascular and cardiometabolic phenotype of offspring of hypertensive and preeclamptic pregnancies at multiple time points throughout infancy, childhood and adolescence, with follow up into young adult life. Uniquely I have been able to consider whether the offspring of preeclamptic or hypertensive pregnancies already demonstrate alteration in clinically relevant risk as young adults. In term born individuals, the offspring of hypertensive pregnancies (both preeclamptic and non-protienuric hypertension) have increased systolic blood pressure present from infancy with some elevation of diastolic blood pressure. The offspring of non-protienuric hypertensive pregnancies additionally have increased BMI throughout life, with the mean BMI values falling into the overweight range by young adulthood. These individuals are 1.9 times more likely to be obese at 20 years of age than those whose mother remained normotensive. Interestingly the offspring of preeclamptic pregnancies do not demonstrate this increase in adiposity, but do have increases in systolic blood pressure commensurate with those of the gestational hypertension group. Furthermore those born to preeclamptic pregnancies are more likely to have blood pressure levels consistent with clinical hypertension (>140/90mmHg) compared to those born to normotensive, or non-protienuric hypertensive pregnancies. These findings suggest the possibility of two distinct phenotypes with individuals born to non-protienuric hypertensive pregnancies demonstrating a phenotype characterised by increased adiposity and associated small increase in systolic blood pressure without an increased risk of clinically apparent hypertension. Conversely those born to preeclamptic pregnancies remain lean but hypertensive. As there are potential differences in pathophysiology of protienuric preeclampsia and gestational hypertension at term [19, 269] it is plausible that they are associated with different cardiovascular phenotypes, and these differences may be relevant to the understanding of natural history of these conditions.

Of particular clinical relevance is the fact that this work has considered clinical markers of risk in young adulthood in a group with detailed perinatal information. The QRISK lifetime risk score was developed to provide risk assessments for younger patients with adverse risk profiles who despite a low 10-year risk are likely to have a high lifetime risk burden and benefit from early intervention [274]. In this cohort the offspring of hypertensive pregnancies already showed increases in their lifetime QRISK score at 20 years of age. It is necessary to interpret this data with caution as the QRISK score has not been validated for populations under the age of thirty [274]. However many of the factors from which the score is derived including blood pressure, BMI and serum lipids are known to track from childhood into adult life [226, 236, 237, 275]. It is therefore possible to cautiously speculate that these differences, already present in early in life, are likely to persist and if anything increase as the population ages. Furthermore the offspring of both preeclamptic and hypertensive pregnancies were more likely to fall into the top 25% of lifetime risk. As this cut off for the 75th centile was derived from a population aged between 30 and 84 years of age falling into this category at the young age of 20 may actually indicate a relatively greater increase in risk [274]. Further study will allow this estimate to be refined and to establish the implications of this increase in risk at such a young age.

Separate to this increase in risk score, the offspring of preeclamptic pregnancies already demonstrated a greater than four-fold increase in the likelihood of clinically apparent hypertension compared to those whose mothers remained normotensive. They were also more than 3 times more likely to be hypertensive than young adults born to mothers with non-poteinuric hypertension during pregnancy. Although it is impossible to diagnose hypertension on a single clinic blood pressure reading, if these readings are indicative of the long-term blood pressure state in these individuals, then the offspring of preeclamptic pregnancies may benefit from more intensive risk monitoring and early intervention of risk reduction strategies. In particular early attention to those individuals who do fall into the hypertensive category may go

some way to reducing the excess risk of cerebrovascular events associated with being born to a preeclamptic pregnancy [60].

Conversely young adults born following pregnancies complicated by gestational hypertension do not demonstrate an increased risk of hypertension at 20 years of age, despite having a small increase in mean systolic blood pressure. They are however significantly more likely to be overweight or obese at age 20. These clinical classifications further highlight the differences in the phenotype associated with PIH and preeclampsia. Those born to hypertensive pregnancies showed an obesity based phenotype without increased risk of overt hypertension, whilst those born to preeclamptic pregnancies showed a increased risk of a hypertension without increased incidence of obesity.

This analysis was limited by the fact that not all individuals recruited at birth were followed up into adult life. This, given the relatively small number of term born offspring of preeclamptic pregnancies in this cohort, has led to a relatively small group being followed into young adulthood. However strict criteria has been used for defining PIH and preeclamptic populations in this cohort, and this allows reasonable confidence that there is no, or limited misdiagnosis leading to confusion of the phenotype between the two categories. Furthermore the detailed and prospective nature of the data collection means that even with small numbers, clear divergences between groups can be identified over time. Finally, caution must be taken when applying single point measurements to clinical classifications or clinical risk score calculators, particularly in a young population. Notwithstanding this, given the firm trends this analysis demonstrated over a lifetime in these individuals, and the established evidence of tracking of parameters utilized in the clinical classifications [226, 236, 237, 275], I believe these estimates remain informative. In addition the prospective nature of this study and the detailed data collection in the perinatal period make it powerful in its ability to consider the cardiovascular phenotype of those born to hypertensive and preeclamptic pregnancies

5.10 CONCLUSIONS

Term born offspring of preeclamptic and hypertensive pregnancies have higher blood pressure throughout early life. In those born to non-protienuric hypertensive pregnancies this is associated with higher BMI's and increased risk of obesity. Conversely the offspring of preeclamptic pregnancies manifest a lean hypertensive phenotype. At 20 years of age the offspring of preeclamptic pregnancies are between 3.5 and 4.5 times more likely to have clinically apparent hypertension. As more than 80% of pregnancies complicated by hypertension go on to deliver at term, these findings are potentially relevant to a large number of individuals worldwide. Greater understanding in the variation in clinical risk in young adult life may explain some aspect of the increased risk of stroke in those born to preeclamptic pregnancies.

CHAPTER 6 – EPOCH PILOT STUDY POPULATION

6.1 THESIS CONTEXT

This chapter describes the study populations, study design and ethical issues of the primary study used in the remainder of this thesis. The 'Effect of Preeclampsia on Offspring Cardiovascular Health Pilot Study (EPOCH Study)', is a prospective case-control study investigating the impact of preeclampsia on infant cardiovascular phenotype.

6.2 THE EFFECT OF PREECLAMPSIA ON OFFSPRING CARDIOVASCULAR HEALTH PILOT STUDY (EPOCH STUDY)

6.2.1 OVERVIEW AND STUDY AIMS

The EPOCH Pilot Study was designed to assess the cardiovascular phenotype in neonates born to women who have experienced a preeclamptic pregnancy. The aims of the EPOCH Pilot Study are;

1) To establish the feasibility of undertaking non-invasive measurements of cardiovascular structure and function in newborn infants, including those being cared for in a high dependency setting and,
2) To investigate in detail the cardiac and vascular phenotype of infants born to women who have experienced a preeclamptic pregnancy and to compare this to a gestation matched control group.

6.2.2 STUDY DESIGN

The EPOCH Pilot study is designed as a case control study. Cases are infants born to mothers with preeclampsia or gestational hypertension, and controls are infants born to mothers who did not suffer from preeclampsia or gestational hypertension.

The target population for this study was infant-mother pairs recruited from a regional tertiary obstetric centre, the John Radcliffe Hospital, Oxfordshire. Both preterm and term born infants (and their mothers) were eligible for inclusion in the study. Women were approached about the study prior to delivery on the antenatal wards, or following delivery when infants were admitted to the Special Care Baby Unit (SCBU). The John Radcliffe Hospital SCBU is a tertiary neonatal care unit comprising of a Neonatal Intensive Care Unit, a Neonatal High Dependency Care Unit and a Neonatal Special Care Unit.

6.2.3 Inclusion Criteria

Infants: Both case and control infants were required to meet the following inclusion criteria:

- Being born at, or admitted after birth to the John Radcliffe Hospital Women's Centre,
- Available for assessment within the neonatal (< 28 days of age) or infancy (3 months post-natal age) period,
- The physical condition of the infant being suitable to allow non-invasive vascular testing, and
- The infant's mother meeting the criteria for inclusion in the study and being willing to participate in the study.

Cases only:

- Infant born to a mother diagnosed during pregnancy with preeclampsia or gestational hypertension (as per I.S.S.H.P. research definition).

Controls only:

- Infant born to mothers without a history of gestational hypertension or preeclampsia during this, or any previous pregnancy.

Mothers: In addition to the above criteria mothers participating in the study were required to be aged >/= 16 years.

6.2.4 Exclusion Criteria

Infants (and therefore mothers) were excluded from the study if:

- The infant displayed evidence of congenital cardiovascular disease (with the exception of Persistent Ductus Arteriosus (PDA) and Atrial Septal Defect (ASD)),
- The infant displayed severe cardiorespiratory instability or active infection (culture proven) at time of proposed measures or,
- The mother of infant was excluded from the study.

Mothers (and their infants) were also excluded from the study if they were unable or unwilling to give informed consent, were aged less than 16 years at the time of delivery or, if their physical condition post delivery was such that it would preclude participation in the study.

6.2.5 Recruitment Strategy and approach to participants

The recruitment strategy and approach to participants is described in Figure 6.1

FIGURE 6.1 RECRUITMENT STRATEGY AND APPROACH TO PARTICIPANTS IN THE EPOCH PILOT STUDY

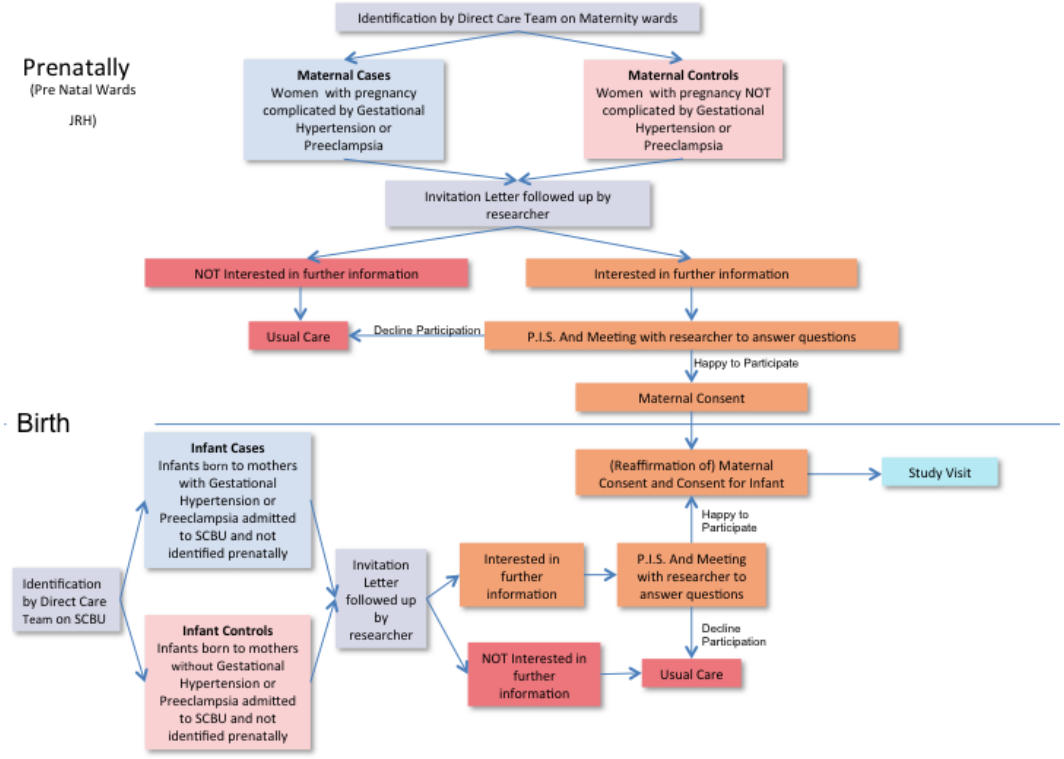


Figure 6.1: Summary of approach to participants in the EPOCH Pilot Study. Recruitment is undertaken prenatally on the antenatal wards at the John Radcliffe Hospital, or during the postnatal period on the SCBU at the John Radcliffe Hospital. Identification of participants is made by the direct care team who provide an invitation letter. This letter is followed up by the research team who provide detailed study information and a patient information sheet. If mothers are happy to participate consent for the mother and infant is undertaken and arrangements are made for study investigations.

6.2.6 CONSENT

Parents of potential participants were provided with detailed study information, a description of the study timeline and protocol information at least 6 hours before consent was taken. Written informed consent was obtained from all adult participants, and separately on behalf of all infant participants, prior to the commencement of any study measures. Where women were recruited prior to delivery, consent for infant participation was delayed until after birth. Participants were free to withdraw from further participation in the study at any time.

6.2.7 ETHICAL CONSIDERATION AND PRINCIPLES

The EPOCH Pilot Study was reviewed and approved by the Berskshire Regional Ethics Committee in application 11/SC/0006. All testing was undertaken according to principles of ethical research practice in compliance with the declaration of Helsinki [276].

The EPOCH Pilot Study raised several unique ethical issues including the following.

6.2.7.1 Research Involving Children

Research involving children, especially those who are unable to consent for themselves, requires special ethical consideration. This is particularly the case in research involving the neonatal population. Despite this medical research in children is necessary to provide information to advance the health and wellbeing both of children and the adults they will become. With reference to the MRC guidelines for research in children the following reasons underpin the decision to undertake this study in a paediatric population [277];

1. Cardiovascular disease is one of the major causes of mortality and morbidity in the world and insight into its origins is important in developing effective primary prevention techniques,
2. Information on the early development of cardiovascular disease risk can only be obtained by studying children early in life,
3. Findings on the early development of the cardiovascular system following a preeclamptic pregnancy may contribute to the development of guidelines for the clinical management of such children to reduce their risk of the development of cardiovascular disease in later life and,
4. Undertaking this study in a neonatal population allows for improved understanding of normal cardiac and vascular development of children.

6.2.7.2 Research in the Neonatal High Dependency and Intensive Care Setting

A large proportion of the EPOCH pilot study was undertaken in the John Radcliffe Hospital special care baby unit ICU and HDU. Admission of an infant to a neonatal high dependency unit has been associated with parental stress and feelings of powerlessness [278]. Furthermore infants in a high dependency setting are often medically unstable and may undergo rapid changes in clinical condition. The decision to undertake research in these infants therefore requires ethical

justification. The following reasons underpin the decision to undertake the EPOCH pilot study in the neonatal intensive care setting;

1. Infants admitted to the neonatal unit after preeclamptic pregnancies are generally born to pregnancies with early onset preeclampsia (onset of preeclampsia <34 weeks). Existing work has demonstrated women who experience early onset preeclampsia have more severe disease and are at greater risk of cardiovascular health issues in the future [36]. Follow up studies of children born to preeclamptic pregnancies have demonstrated that those born to early onset preeclampsia have the most marked changes in vascular phenotype [8, 240]. The intensive care setting therefore provides access to infants who are most likely to exhibit marked early changes in cardiovascular phenotype, and are most likely to benefit from the future development of early intervention strategies. Preeclampsia is an antecedent factor in up to 20% of preterm births and therefore a significant number of preterm admissions to the neonatal high dependency/intensive care unit are due to preeclampsia.
2. Historically many studies have compared a group of individuals born preterm to preeclamptic pregnancies to a group born at term to a normotensive pregnancy. Prematurity alone is associated with significant changes in cardiovascular phenotype in childhood and young adulthood [7]. It is therefore challenging to distinguish alterations in vascular phenotype due to preeclampsia from those caused by prematurity. Recruitment in a neonatal high dependency setting allows for the simultaneous recruitment of a group of infants born preterm to normotensive mothers, and therefore a better understanding of any additional changes to the cardiovascular phenotype associated with preeclampsia alone.

Conducting research in such a setting is also associated with further unique ethical issues.

1. Consent in Neonatal Units:

   The stresses associated with the admission of an infant to a neonatal intensive care unit have led to questions about the validity of informed consent given by parents in this context. While some studies suggest poor long term recall of consent to participate in trials [279], a study assessing the competence of consent for neonatal research in infants with critical heart disease found that parents exhibited understanding comparable to control population, and higher than that of other venerable groups [280]. This suggests that despite the stresses associated with having a critically ill child, parents are able to understand study-specific information and make informed choices about their child's participation in research.

   In order, however, to minimize stress associated with the approach to parents in the EPOCH study the following steps were undertaken:

   - Assessment of eligibility for the study was initially assessed by the direct care team who provided the parents with an abbreviated patient information sheet. Following this the parents were approached by a member of the study team, normally a minimum of 24 hours later. This ensured the parent had sufficient time to consider whether or not they were interested in the study, prior to approach by researchers.
   - After the initial approach parents were given a minimum of 6 hours, and commonly more than 24 hours, to consider detailed study information before being approached for consent. While the minimum time period was chosen to allow for measures to be taken within the first 24 hours of life, the needs of the parents were considered on an individual case by case basis with more time being allowed as required.

2. Potential for rapid change in the clinical condition of patients.

Infants admitted to SCBU are frequently clinically unstable and rapid changes to clinical condition may occur. In order to avoid causing any additional stress or distress to the parents by approaching them at a time of clinical instability, I made contact with the direct care team prior to any approach to the family to ascertain the medical status of the participant and therefore the appropriateness of approach.

6.2.7.3 Consent on behalf of unborn child:

A proportion of the recruitment for the EPOCH Pilot study was undertaken during the antenatal period. This had the advantage of allowing the approach to parents to be made at a less stressful time. It also allowed longer for them to consider their participation in the study. Prenatal recruitment did however raise issues related to consent for the child when it was not yet born. It is not generally appropriate to take consent for the purpose of research on behalf of an unborn child, and therefore in individuals approached prior to delivery, consent was taken only from the mother for her participation in the study. Following delivery the mother's consent was reaffirmed and consent was obtained for the infant's participation. This allowed the parents the opportunity to change their mind following the delivery and allowed a sensitive approach towards any medical issues at or around the time of delivery.

6.2.8 Outline of Study Visits

The outline of the EPOCH Pilot Study Visits is summarized in Figure 6.2.

FIGURE 6.2 OUTLINE OF EPOCH PILOT STUDY VISITS

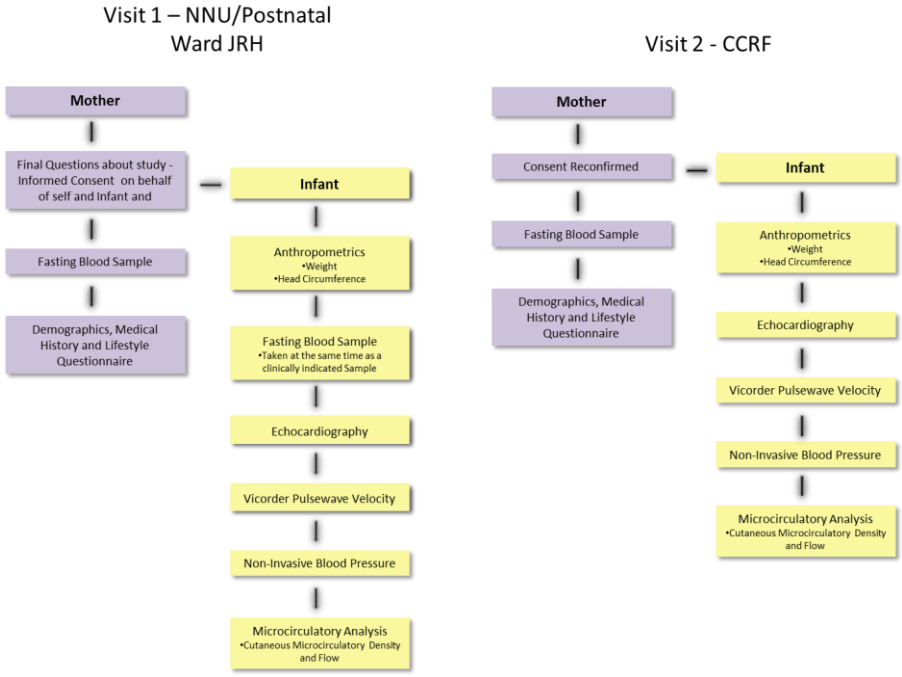


Figure 6.2: Summary of investigations undertaken in mothers and infants at birth and 3 months of age.

Investigations are described in detail in Chapter 7. Investigations at birth and 3 months included:

- Demographic data and lifestyle questionnaire (Section 7.2)
- Anthropometric measurements (Section 7.3)
- Blood sampling (Section 7.4)
- Echocardiography (Section 7.5)
- Non-invasive blood pressure measurement (Section 7.6)
- Microvascular structure analysis (Section 7.7)
- Pulsewave analysis and Pulsewave Velocity (Section 7.8)

6.2.9 RECRUITMENT TO THE EPOCH PILOT STUDY AND PARTICIPANT BASELINE DEMOGRAPHICS

46 infants have been recruited to the study to date. This has comprised of 16 infants born to normotensive pregnancies and 30 infants born to hypertensive pregnancies. Of these 30 infants 27 were born to preeclamptic pregnancies and 3 were born to hypertensive or superimposed preeclamptic pregnancies. In view of the small numbers subgroup analysis has not been undertaken and cases are pooled as 'hypertensive' offspring. The 36 mothers of these infants

have also consented to participate in the study. The perinatal characteristics of the mothers and infants participating in the study are presented in table 6.1 and 6.2

TABLE 6.2 CHARACTERISTICS OF MOTHERS RECRUITED TO THE EPOCH PILOT STUDY

	Normotensive (n = 12)	**Hypertensive** (n = 24)	**p-value**
Maternal Age (years)	33.58 (5.00)	31.92	0.44
Maternal BMI (kg/m^2)	23.12 (2.23) n =11	27.45 (5.12) n = 22	0.014
Maternal Birth Weight (g)	3312 (633) n = 5	2906 (585) n = 14	0.21
Primiparous (%)	50% (6)	88% (22)	0.012
Smoked During Pregnancy (%)	10% (1/10)	10% (2/20)	1.00
Ethnicity (% Caucasian)	100% (12)	100% (24)	1.00
Multiple Pregnancy (%)	33.3% (4/12)	20% (5/25)	0.38
Assisted Conception (%)	36.4% (4/11)	20% (4/20)	0.32
Booking Systolic Blood Pressure (mmHg)	108.3 (10.6) n = 11	120.1 (21.5) n = 20	0.099
Booking Diastolic Blood Pressure (mmHg)	63.6 (7.2)	70.8 (21.9)	0.30
Gestational Diabetes (%)	0% (0/11)	5% (1/20)	0.45

Table 6.1 Characteristics of mothers participating in the EPOCH pilot study.

TABLE 6.3 CHARACTERISTICS OF INFANTS RECRUITED TO THE EPOCH PILOT STUDY

	Normotensive (n = 16)	**Hypertensive (n = 30)**	**p-value**
Gestational Age (weeks)	33.02 (2.58)	34.03 (3.15)	0.28
Birth Weight (g)	1886 (513)	1942 (754)	0.79
Birth Weight Z score	-0.466 (0.753)	-1.026 (1.011)	0.06
Body Surface Area (m^2)	0.151 (0.029)	0.152 (0.434)	0.89
Sex (% Male)	43.8% (7)	46.7% (14)	0.85
AGPAR 1 Minute	8.62 (1.60)	8.26 (1.56)	0.59
APGAR 5 Minute	9.50 (0.755)	9.53 (1.17)	0.95
Length of Admission	26.37 (17.43)	26.37 (32.69)	0.99
ITU Admission	3.69 (5.11)	4.87 (11.11)	0.96
HDU Admission	6.06 (7.93)	8.57 (17.69)	0.59
LDU Admission	17.75 (11.90)	13.20 (13.31)	0.26
Total Days of Oxygen	13.68 (25.38)	15.03 (31.79)	0.88
Ventilation Days	0.75 (1.39)	1.90 (6.78)	0.46
Total Parentral Nutrition (Days)	2.37 (3.48)	2.60 (5.17)	0.88
Antenatal Steroids (% Received)	76.9%(10/13)	61.9% (13/21)	0.36
Sepsis (% Experienced)	56.2% (9)	40.0% (12)	0.38
Breastfed at Discharge (%)	57.5% (10/16)	50% (9/28)	0.71
SBP Birth (mmHg)	68.84 (10.17)	66.01 (10.15)	0.51
DBP Birth (mmHg)	34.95 (6.63)	36.90 (8.34)	0.56
SBP 3 months (mmHg)	89.10 (8.65)	88.60 (12.22)	0.90
DBP 3 months (mmHg)	53.68 (12.12)	50.13 (12.62)	0.47

As expected those mothers who went on to be hypertensive had significantly higher BMI's at booking than those who remained normotensive, with the mean BMI for these women falling into the overweight range. Consistent with previous data, mothers who went onto develop hypertension also tended to have higher blood pressure at booking than those who remained normotensive [240], although this did not reach statistical significance in this cohort. Otherwise there were no significant differences in maternal factors within the cohort.

The infants recruited to the study were well matched in terms of perinatal characteristics. There was a trend towards lower birth weight z score in those born to hypertensive pregnancies, although this was just short of statistical significance. With the exception of this, there were no significant differences in birth or admission characteristics. There was also as yet no difference in systolic or diastolic blood pressure in this population. Infants as premature as 27 weeks completed gestation; with birth weights as low as 636 grams were recruited into the study and successfully underwent study investigations in the neonatal period.

6.2.10 Challenges of the EPOCH Pilot Study

Despite the difficulties previously noted in undertaking research of this type I have had considerable success in recruiting 46 infants and their mothers into the EPOCH pilot study. For example over a six-month period 64 infants were identified to the study team by the direct care team. All were approached resulting in a recruitment of 16 infants. This recruitment rate of 25% is comparable both with other trials conducted in the John Radcliffe Hospital SCBU, and with other trials in the Division of Cardiovascular Medicine, including those trials being undertaken in adult populations.

However the challenges associated with recruitment and investigation in the perinatal period, particularly in the context of preterm born infants requiring high level care, are significant and should be considered by any group attempting similar studies. In particular it is important to consider the parental stress and feelings of powerlessness [278] associated with admission of a

newborn infant to a high dependency environment. Multiple approaches over a number of days were often required to allow parents to make a fully informed decision. Undertaking study measurements in the first days after delivery, while the parents were still coming to terms with their infant's condition, was often not possible.

There were also on occasions challenges associated with undertaking non-invasive measurements of vascular function in a neonatal population. Other researchers have also experienced this difficulty. Antonious and colleagues noted in their recent study of microvascular function in term born neonates that;

'We feel it is imperative to state here how difficult it proved to study these infants who often wake up when they are handled, and then it becomes difficult to proceed with the study as the infants become un-cooperative and their mothers restless and anxious and we often then had to abandon the study in as much as 25% of potential subjects [281]'

In undertaking the EPOCH study similar challenges were experienced. In particular for example efforts were made to keep the infants in a resting state during study investigations, however as Antonious found, infants often woke or became unsettled when handled. Infant's parents often become anxious during any perceived restlessness on the part of their infant and on occasions investigations had to be abandoned or abbreviated due to parental anxiety. This inherent challenge of research in the neonatal context needs to be considered in planning and sample size calculation for future work.

6.3 Conclusions

The EPOCH pilot study, a pilot case control study, examining the impact of hypertensive disorders of pregnancy on infant cardiovascular phenotype has been successfully recruiting for 2 years. 46 infants and their mothers have been recruited to date, with infants born up to 13 weeks prematurely weighing less than 700 grams participating successfully. This pilot has demonstrated

the feasibility of performing cardiovascular phenotyping in a preterm born population, requiring high-level medical care.

CHAPTER 7 – GENERAL METHODS

7.1 THESIS CONTEXT

This chapter describes the methods and analytical techniques relevant to the EPOCH Pilot Study and the data presented in Chapters 8 and 9.

7.2 QUESTIONNAIRES, DEMOGRAPHICS AND MEDICAL RECORD REVIEW

7.2.1 QUESTIONNAIRES AND DEMOGRAPHICS

Lifestyle data was obtained by way of a questionnaire. Data collected included a standard food frequency questionnaire, the smoking history of the mother and father, and the medical and family history relevant to preeclampsia and cardiovascular disease. Two questionnaires were completed. The first was provided and completed just after the delivery dealing with lifestyle factors during pregnancy. The second was completed at the three-month follow up visit to assess lifestyle factors over the post-natal period, as well as infant growth, development and feeding.

7.2.2 MATERNITY RECORD REVIEW

Data on pregnancy and antenatal care was obtained from maternity records. Basic demographics including age and occupation were recorded. Maternity booking information including booking gestation, weight, height, BMI and blood pressure were also recorded. Blood pressure and maternal weight were recorded in mid gestation (approximately 24 weeks), late gestation (approximately 32 weeks) and at the last antenatal visit. Fetal growth measures (head circumference, abdominal circumference, femur length and estimated fetal weight) were recorded from the 20-week fetal anomaly scan. Complications of pregnancy, including the gestation of diagnosis and severity of gestational hypertension and preeclampsia were noted. Details of delivery including method of delivery, gestation at delivery, delivery complications and details of the postnatal course were also recorded.

7.2.3 Infant Medical Record Review

Infants admitted to the SCBU had additional medical records relating to treatment in the post-natal period. These records were also reviewed with data collected including details of postnatal feeding (entral and paraentral), length of and nature of oxygen support, number of courses of antibiotics and length of post-natal stay.

7.3 Anthropometry

7.3.1 Weight:

In infants, weight was measured to the nearest gram using an electronic balance (Marsden Group, UK). Birth weights (to the nearest gram) were derived from clinical notes. During the inpatient stay infants were weighed daily and the weight recorded. In 3-month-old infants weight was measured to 10g accuracy in the undressed infant using an electronic balance (Marsden Group, UK).

7.3.2 Height

In the EPOCH study population birth length was not measured. Birth length is no longer routinely recorded in infants born at the John Radcliffe Hospital as crown-heel length measurements are known to be stressful for neonates and have a low accuracy [282, 283]. Variability in infant length measurement has previously been found to be sufficiently large to affect both clinical decisions and research findings [283]. More significantly, for the frequently preterm EPOCH study population, removal of infants from incubators for measurement was neither feasible or ethical.

7.3.3 Head Circumference

Head circumference was measured to the nearest 0.1cm using a non-stretchable tape measure.

7.3.4 Body Mass Index and Body Surface Area calculations

Body mass index in all adult populations was calculated using the standard formula.

BMI = weight in kg/ height in meters ^{2}.

7.3.5 Body Surface Area Calculations

In infants Body Surface area was calculated from birth weight using the Boyd formula.

$$BSA = 4.688 * Weight^{(0.8168 - 0.0154 * LogWeight)}$$

This formula has been demonstrated to deviate less than 10% from formulas using height and weight to calculate BSA [284].

7.4 Blood Sampling

In infant populations obtaining fasting blood samples for research purposes is neither practically nor ethically viable. In this study blood samples were taken as part of the routine clinical course and analyzed in clinical laboratories using routine validated assays with clinical-level quality controls.

7.5 Cardiac Structure and Function

Cardiac structure and function was assessed using echocardiography. A single study of children aged 5-8 years born to preeclamptic pregnancies demonstrated evidence of smaller hearts, increased heart rate and altered diastolic function [125]. More recently, young adults born prematurely to preeclamptic pregnancies have demonstrated alteration in cardiac strain compared to equally preterm individuals born to normotensive pregnancies [7].

2D and 3D echocardiography was undertaken in neonates, and 3-month-old infants using a high-resolution ultrasound probe (Philips iE33 with 12MHz 2D sector array transducer or 7MHz Xmatrix 3D Array transducer). Infants were imaged either supine in their crib, or in their mother's arms according to tolerance as shown in Figure 7.1

Standard measurements of left ventricular structure (septal wall thickness, left ventricular internal diameter, posterior wall thickness, left ventricular apical-mitral valve length) were measured on 2D and/or M-Mode imaging. Left ventricular mass was calculated using the area-length formula on standard reporting software (Xcelera Phillips). Basic left ventricular systolic and diastolic function was assessed using standard methods including fractional shortening, modified Simpson's ejection fraction, ventricular volumes, and assessment of pulse wave doppler transmitral flow. Tissue doppler imaging of the lateral and sepal mitral annulus was used to assess myocardial relaxation in detail.

FIGURE 7.1 EXAMPLE OF INFANT UNDERGOING ECHOCARDIOGRAPHY



Figure 7.1 An example of an infant undergoing echocardiography while being held – infants were also imaged supine in cribs if settled.

Myocardial strain and deformation was assessed using speckle-tracking analysis software (Cardiac Performance Analysis, TomTec). Where image quality allowed, assessment of radial and circumferential strain and strain rate were assessed on mid short axis images, and longitudinal strain and strain rate on a 4 chamber image. Figure 7.2 demonstrates an example of a 4 chamber apical image with endocardial and epicardial borders defined for tracking and the resultant longitudinal strain curves.

FIGURE 7.2 EXAMPLE OF TOMTEC TRACKING AND STRAIN CURVES ON AN APICAL 4 CHAMBER IMAGE

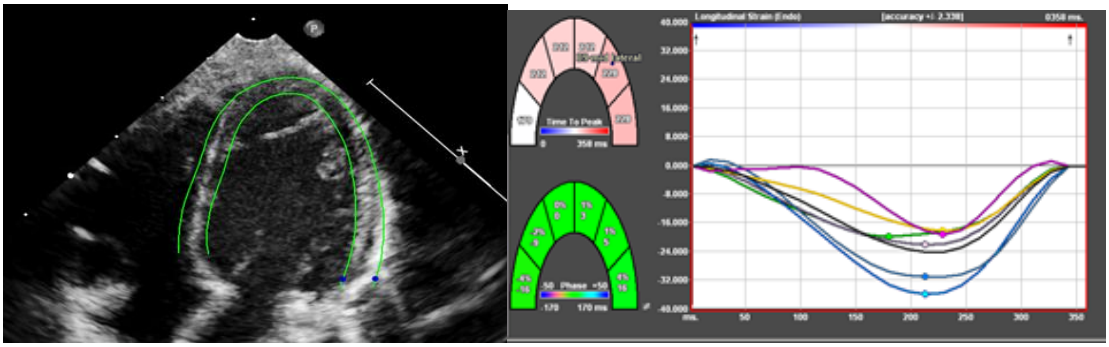


Figure 7.2 – Example of Tomtec tracking and derived longitudinal strain curves on an apical 4-chamber image from a 3-month-old infant. Segmental (coloured) and Global (Black line) longitudinal strain rates are derived for each subject.

Although speckle tracking technology has only been used occasionally in children, reference ranges have now been published [285, 286] and evidence of alteration of myocardial deformation and strain measured by 3D echocardiography has been demonstrated in children with cardiovascular risk factors such as obesity [287]. Inter and intra-observer coefficients of variance for this data are presented in Table 7.1.

TABLE 7.1 COEFFICIENT OF VARIANCE IN TOMTEC ANALYSIS

	Coefficient of Variance (Intra-Observer)	**Coefficient of Variance (Inter-Observer)**
Mid Ventricular Short Axis		
Radial Displacement	2.62%	5.32%
Velocity Systole	6.68%	5.20%
Velocity Diastole	4.25%	7.64%
Radial Strain	7.99%	17.14%
Strain Rate Systole	8.56%	13.04%
Strain Rate Diastole	10.08%	15.86%
Circumferential Strain	3.62%	6.69%
Strain Rate Systole	7.87%	11.59%
Strain Rate Diastole	8.98%	10.29%
4 Chamber		
EF	4.83%	2.70%
Longitudinal Displacement	10.15%	6.95%
Velocity Systole	8.62%	7.92%
Velocity Diastole	10.21%	9.31%
Longitudinal Strain	5.76%	8.13%
Strain Rate Systole	7.46%	9.21%
Strain Rate Diastole	5.40%	8.26%

Table 7.1 Intra observer and Inter-observer coefficients or variance for Tomtec measurements in the EPOCH study population.

7.6 BLOOD PRESSURE

Meta-analysis data suggests that preeclampsia is associated with an increase in blood pressure in childhood [62], however few reports exist as to its effect on blood pressure in the neonatal period. Blood pressure was measured in infants using a DINAMAP (GE, USA) non-invasive blood pressure monitor. This monitor has been validated against invasive blood pressures in a neonatal population with mean differences of 3.35mmHg for systolic blood pressure, -0.92mmHg for diastolic blood pressure, -0.80 mmHg for MAP and -0.17 bpm for heart rate [288]. NIBP measurements were taken with a cuff on the infant's upper arm using neonatal GE CRITIKONR SOFT-CUFR (GE, USA) cuff sizes 2, 3, 4, and 5 as appropriate. 3 NIBP measurements were undertaken with the infant resting at least 5 minutes after the application of the cuff. The average of three measurements was recorded for each infant.

7.7 MICROVASCULAR STRUCTURE

Abnormality of the microvascular system is common among conventional cardiovascular risk factors including hypertension, diabetes, obesity, and dyslipidemia and may be important in their pathenogensis [289]. In infants, imaging of the small vessel network was undertaken using Side Stream Dark Field (SDF) imaging device (MicroScan, Microvision Medical, Amsterdam). This device emits a LED light at a frequency (530nM) absorbed by hemoglobin in the microvasculature. This produces an image of dark red blood cells against a white/grey background as demonstrated in Figure 7.3. Quantification of these images can be undertaken using specialized software (AVA 3.0, MicroVision Medical) [290].

Infant subjects were imaged at rest, either in their mothers' arms, or in a crib (Figure 7.4). Imaging was undertaken on the upper inner arm in compliance with previous studies that have reported this as the optimal site to obtain images [291].

FIGURE 7.3 EXAMPLE OF MICROSCAN IMAGE TAKEN FROM THE UPPER INNER ARM OF A 3MONTH OLD INFANT

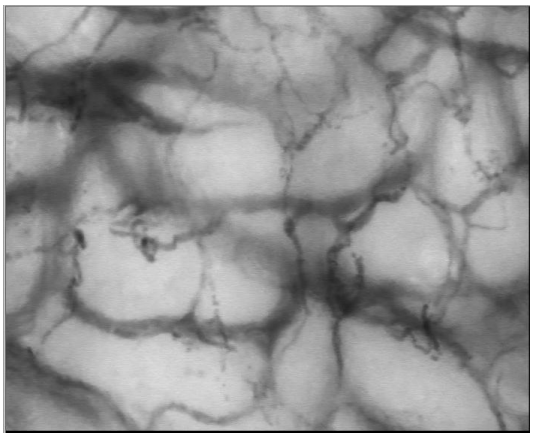


Figure 7.3 Example image taken by MicroScan on the upper inner arm of a 3 month old infant.

FIGURE 7.4 EXAMPLE OF MICROSCAN IMAGE ACQUISITION ON A NEONATE IN CRIB ON THE SPECIAL CARE BABY UNIT



Figure 7.4: An infant undergoing MicroScan imaging in the Special Care Baby Unit.

A minimum of 3 x 30 second images were taken, with additional images dependent on the compliance of the infant. Images were then divided into approximately 5-10s intervals for analysis. Total and perfused vessel density, proportion of perfused vessels (PPV = perfused vessel density/total vessel density), microvascular flow index (A semi-quantative assessment of flow state in the imaged vessels, each quadrant of the image is classed for flow characteristics as; No Flow = 0, Intermittent Flow = 1, Sluggish Flow = 2, Continuous Flow =3 and Hyperdynamic Flow =4, and the average of these is reported as the MFI) and De Barkers Score were measured on

each image. Three images were analysed for each infant and the mean of these was reported [292]. An example of an analysed image is shown below in Figure 7.5

Intra-Observer coefficient of variance was 7.7% for total vessel density, perfused vessel density 5.49% and De Backers 2.73%. Inter-observer coefficient of variance was 6.4% for total vessel density, 6.2% for perfused vessel density and 5.85% for De Backers Score.

FIGURE 7.5 EXAMPLE OF ANALYSIS OF MICROSCAN IMAGING FROM A NEONATAL SUBJECT.

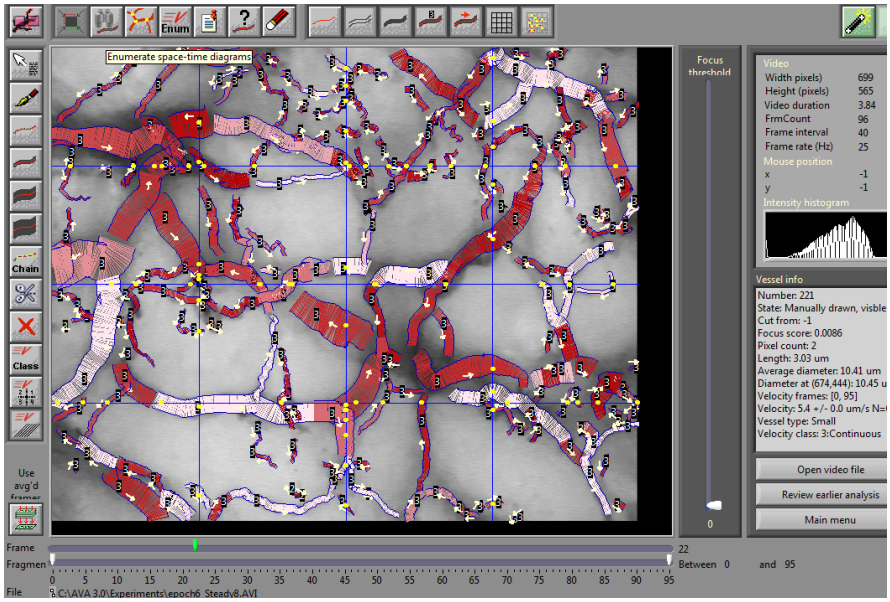


Figure 7.5 An example of an analysed MicroScan image. Capillaries are delineated in a semi-automated manner and flow characterized. Density of vessels, portion of perfused vessels and De Backers score is then automatically calculated by the software.

7.8 PULSE WAVE ANALYSIS AND PULSE WAVE VELOCITY

Pulse wave velocity (PWV), the velocity of the forward propagated arterial pressure wave [199], has become the gold standard in the measurement of arterial stiffness in adults [293]. PWV has been demonstrated to increase with increasing arterial stiffness and is strongly associated with atherosclerosis [294]. Several methods have been used to measure PWV including using pressure transducers, Doppler ultrasound, and applanation tonometry and oscillometric pressure cuffs. All methods generally require the measurement of the arterial pulse wave at two superficial arterial sites and the recording of the delay between the arrival of the waveform and the ECG R wave.

This combined with body surface measurements allows PWV to be calculated as distance over time [293]. In adults PWV has been demonstrated by meta-analysis to be a strong independent predictor of cardiovascular events and all cause mortality [295, 296]. It has also been shown to be increased in a variety of cardiovascular risk factors including in diabetes [297, 298], obesity [299-301], smoking [302, 303], the presence of the metabolic syndrome [304] and hyperlipidemia [305]. In children and adolescents increases in pulse wave velocity have been associated with being born small for gestational age [306], maternal alcohol consumption during pregnancy [307], diabetes [308] and obesity [309, 310].

PWV was measured using an oscillometric technique. Using a Vicorder (Smart Medical, Gloucestershire, United Kingdom) device the pulse wave was recorded at the femoral and brachial arteries using oscillometric cuffs. The cuff was automatically inflated to diastolic blood pressure and the corresponding oscillometric signal from each cuff was digitally analyzed for the pulse time delay. The distance between the two cuffs was measured on the body surface and the PWV computed by Vicorder software for each individual. Measurements were taken with the infant at rest after the application of the cuffs

The augmentation index (Aix) is a marker used to measure arterial stiffness. It is defined as the proportion of the central blood pressure attributable to the reflected pulse wave. As the arterial pulse is transmitted through the arterial tree it is reflected at sites of arterial bifurcation. This reflection augments the systolic pulse wave of the central aorta [311]. Increases in AIx has been shown to predict increases in cardiovascular morbidity and all cause mortality (RR 1.32 (95% CI 1.093–1.588) for cardiovascular events with a 10% absolute increase of AIx) [312]. Increases in AIx have been associated with impairment in glucose tolerance [313], hypercholesterolemia [314], smoking [315] and presence of the metabolic syndrome [316]. In children, increased AIx has been associated with extreme prematurity [317], type 2 diabetes [293], hypercholesterolaemia [318] and low birth weight [319].

In infants augmentation index was measured using the Vicorder (Smart Medical, Gloucestershire, United Kingdom) device. The pulse-wave form was recorded using a brachial oscillometric cuff and transformed to a central pulse waveform using a mathematical equation. Pulse waveform analysis was measured over 10-15 beats in each individual. Measurements were taken with the infant at rest after the application of the cuffs.

7.9 STATISTICS

Statistical analysis was carried out using SPSS version 20 (V20 Chicago USA). Statistical significance was determined as $p< 0.05$. Normality testing was undertaken by visual assessment of normality curves and the Shapiro-Wilk test. Non-normal data was log transformed before analysis, or analysed using non-parametric methods. Comparison between groups for means of continuous variables was performed with a 2-sided, independent-samples Student t test for normally distributed data, and the Mann-Whitney and Kruskal-Wallis tests for skewed data. For categorical variables, comparison was done with a $\chi 2$ test. Pearson correlations (r), or Spearmann's rho (r_s) were used for bivariate correlations as appropriate. Unstandardized regression coefficients (β) were used for bivariate and multivariate regression models. Additional statistical methods are described in detail in the appropriate chapter.

CHAPTER 8 – CARDIAC STRUCTURE AND FUNCTION IN INFANT OFFSPRING OF HYPERTENSIVE PREGNANCIES AT BIRTH AND 3 MONTHS.

8.1 THESIS CONTEXT

The offspring of preeclamptic pregnancies have recently been demonstrated to have altered cardiac function as young adults, with reduction in longitudinal cardiac strain, independent of degree of prematurity [7]. It is unclear whether these changes are present from birth or develop in early, or later, post-natal life. Altered cardiac structure or function could underlie some aspect of the increased cardiovascular risk seen in the offspring of preeclamptic pregnancies. This chapter explores cardiac structure and function in a cohort of infants born preterm to hypertensive pregnancies at birth and 3 months of age.

8.2 INTRODUCTION

The perinatal period is of particular importance to cardiac development and insults at this time are likely to have significant influence on cardiac structure and function throughout life. At birth cardiomyocytes switch from a predominately hyperplastic fetal state to a neonatal hypertrophic state [320]. In preterm born infants these changes may be more acute as relatively immature cardiomyocytes preferentially undergo hypertrophic change [7, 267, 320]. Additional insults around this time may have further effects on ventricular structure and function. It has been postulated, for example, that perinatal glucocorticoid exposure may reduce myocardial number and lead to abnormal hypertrophy in exposed individuals [5, 320].

Cardiac magnetic resonance imaging studies have demonstrated that prematurity itself has distinct effects on left ventricular structure and function in early adult life. Lewandowski and colleagues found that preterm born individuals had a 19g increase in left ventricular mass, shorter left ventricles with displaced apices, and reductions in longitudinal strain [7]. Those born to preeclamptic pregnancies had an additional reduction in global longitudinal systolic strain independent of any impact of prematurity [7]. Preterm born individuals also have increased right ventricular mass and reduced right ventricular function. Preeclampsia does not appear to independently impact on this phenotype [250].

Little additional work has considered cardiac function in the offspring of preeclamptic or hypertensive pregnancies. Fugelseth and colleagues assessed cardiac function assessed by echocardiography in a group of 5-8 year olds, 25 of whom were born to preeclamptic pregnancies and 15 whose mothers remained normotensive. In this cohort there were significant difference in both birth weight and gestational age between preeclamptic and control groups [125]. The study found that the offspring of preeclamptic pregnancies had significantly shorter left ventricles, which in view of Lewandowski et al's findings may be attributable to prematurity rather than preeclampsia [7, 125]. In addition the preeclampsia group had increased heart rate and increased late diastolic velocity (A'-wave) at mitral valve attachments when compared to a composite group of normotensive controls and offspring of diabetic pregnancies [125]. However in view of the disparities in gestation and birth weight between the groups it is difficult to conclude whether these changes can be attributed to the in utero effect of preeclampsia. In 1994 Himmelman and colleagues showed no difference in left ventricular mass between those born to hypertensive and normotensive pregnancies at ages 12 or 17 years [213]. Degree of prematurity and growth restriction was not considered in this analysis.

There is a relatively larger body of animal model based literature considering this topic. Studies using hypoxia to induce a preeclampsia like state have demonstrated evidence of both altered

cardiac structure and function in later life. At 4 months of age the offspring of Sprauge Dawley rats exposed to hypoxia (12% O_2) showed no differences in cardiac function compared to controls. However at 12 months of age male offspring demonstrated signs of left ventricular hypertrophy, and both male and female offspring showed impaired diastolic function and evidence of pulmonary hypertension [121]. In other studies hypoxia exposure in pregnancy led to abnormalities in cardiac appearances of a predominantly cardiomyopathic phenotype more commonly linked with development of heart failure. Rat offspring exposed to hypoxia during late gestation have increased cardiac size relative to body weight [109, 121, 122, 127] with reduced left ventricular wall thickness, reduced cardiomyocte proliferation and epicardial detachment [122, 123]. It has been hypothesised that this detachment of the epicardium in fetal life [123], may be mechanistically related to the changes in the myocardium, as the epicardium is a major source of growth factors during cardiac development [122, 123]. Higher levels of apoptotic proteins and induction of HIF1α are also evident and may drive some of this process [124, 128]. Additionally, offspring cardiac collagen content can be altered by maternal hypoxia, with rats exposed to hypoxia during late gestation having increased cardiac levels of collagen I and III [122, 212] and a complex array of alterations in extracellular matix regulation including alterations in levels of MMP 1, 2 and 13 [122, 212].

The animal model work, combined with the findings of Lewandowski et al suggest that in utero exposure to preeclampsia may have effects on cardiac structure or function distinct from that of other perinatal insults. Little work has however considered human hypertensive offspring with an equivalently preterm born and growth restricted control group, and this has limited the capacity of the current literature to distinguish changes associated with in utero exposure to hypertension from changes associated with prematurity and growth restriction. Furthermore, where distinct alterations have been established it remains unclear whether these changes are present from birth, develop in early post-natal life, or do not develop until later childhood or adolescence. I have therefore undertaken to analyse cardiac structure and function in the EPOCH study

population to determine the effects of maternal hypertension on cardiac phenotype in early postnatal life.

8.3 METHODS

Methods relevant to this chapter are described in detail in chapter 7. In particular:

- Collection of demographic, lifestyle and medical data is described in Section 7. 2.
- Anthropometric measurements and body surface area calculations are described in Section 7.3.
- Measurements of cardiac structure and function are described in Section 7.5.
- Measurement of blood pressure in the neonatal and infant population is described in Section 7.6.
- General statistical methods are described in Section 7.9.

8.4 POPULATION CHARACTERISTICS

The recruitment base for the EPOCH study is described in detail in Chapter 6.

46 infants have been recruited to date. Of these infants, 41 infants have undergone echocardiography and their data is presented in this chapter. 34 infants were scanned postnatally, and 30 at three months of age. 25 infants were scanned at both time points. 12 infants did not undergo echocardiography at birth. The reasons for this were; clinical instability prohibiting echocardiography (6 infants), discharge from hospital prior to acquisition of echocardiography (4 infants), and transfer from the John Radcliffe Hospital prior to acquisition of echocardiography (2 infants). Echocardiography was not undertaken at three months due to clinical instability in 1 participant, the other participants were not scanned as their mother declined 3-month follow up. At the time of writing 3 infants, all scanned at birth, had not yet reached 3 months of age.

The perinatal characteristics of infants who underwent echocardiography are presented in Table 8.1.

TABLE 8.1 PERINATAL CHARACTERISTICS OF INFANTS UNDERGOING ECHOCARDIOGRAPHY IN THE EPOCH PILOT STUDY

	Control (n=14)	**Hypertensive (n = 27)**	**p-value**
Gestational Age (weeks)	32.98 (2.24)	34.05 (2.85)	0.234
Birth Weight (g)	1854 (412)	1957 (765)	0.643
Birth weight Z score	-0.503 (.78)	-1.00 (1.08)	0.136
Sex (% Male)	42.9% (6)	48.1 (13)	0.747
Multiple Pregnancy (%)	57.1% (8)	37.0% (10)	0.219
Caesarean Delivery (%)	64.3% (9)	85.2% (23)	0.125
Antenatal Steroids (% received)	78.6% (11)	66.7% (16)	0.435
Days of Oxygen Support	8.50 (11.93)	13.29 (30.32)	0.574
Days of admission	25.50 (15.08)	24.52 (29.63)	0.910
Maternal Age (years)	33.92 (5.74)	32.55 (6.25)	0.498
Maternal BMI (kg/m^2)	23.68 (2.15)	27.00 (4.67)	0.005
Age at Birth Echocardiography (days)	9.00 (6.58)	7.23 (3.45)	0.302
Age at 3 month Echocardiography (days)	105.1 (14.69)	98.47 (16.67)	0.284
Weight at 3 months (g)	4907 (957)	4621 (1244)	0.514

Table 8.1 Perinatal and Maternal Characteristics of infants who underwent echocardiography at birth or 3 months.

With the exception of the expectedly higher BMI (p = 0.005) in those mothers who developed hypertension during pregnancy, there were no differences in the compared cohorts in any of the key perinatal characteristics or characteristics at 3 month follow-up.

8.5 LEFT VENTRICULAR STRUCTURE AND DIMENSIONS IN NEONATES AND INFANTS EXPOSED TO HYPERTENSIVE DISORDERS IN UTERO

There were no changes in cardiac structure or dimensions in neonates or 3-month-old infants exposed to hypertensive disorders in utero when compared to those whose mothers remained normotensive. Results are presented in Table 8.2.

To determine if other perinatal factors significantly influenced left ventricular mass bivariate correlation between left ventricular mass and a range of maternal, antenatal and postnatal variables were undertaken. As would be expected left ventricular mass correlated strongly with gestational age (Birth $r = 0.812$ $p < 0.001$ and 3 months $r = 0.607$ $p < 0.001$), birth weight (Birth $r = 0.932$ $p < 0.001$ and 3 months $r = 0.719$ $p < 0.001$) and birth weight z score (Birth $r = 0.672$ $p < 0.001$ and 3 months $r = 0.563$ $p = 0.001$) at both birth and 3 months of age (Figure 8.1). Similarly weight and body surface area at three months correlated strongly with left ventricular mass at this time point (weight $r = 0.821$ $p < 0.001$, BSA $r = 0.810$ $p < 0.002$).

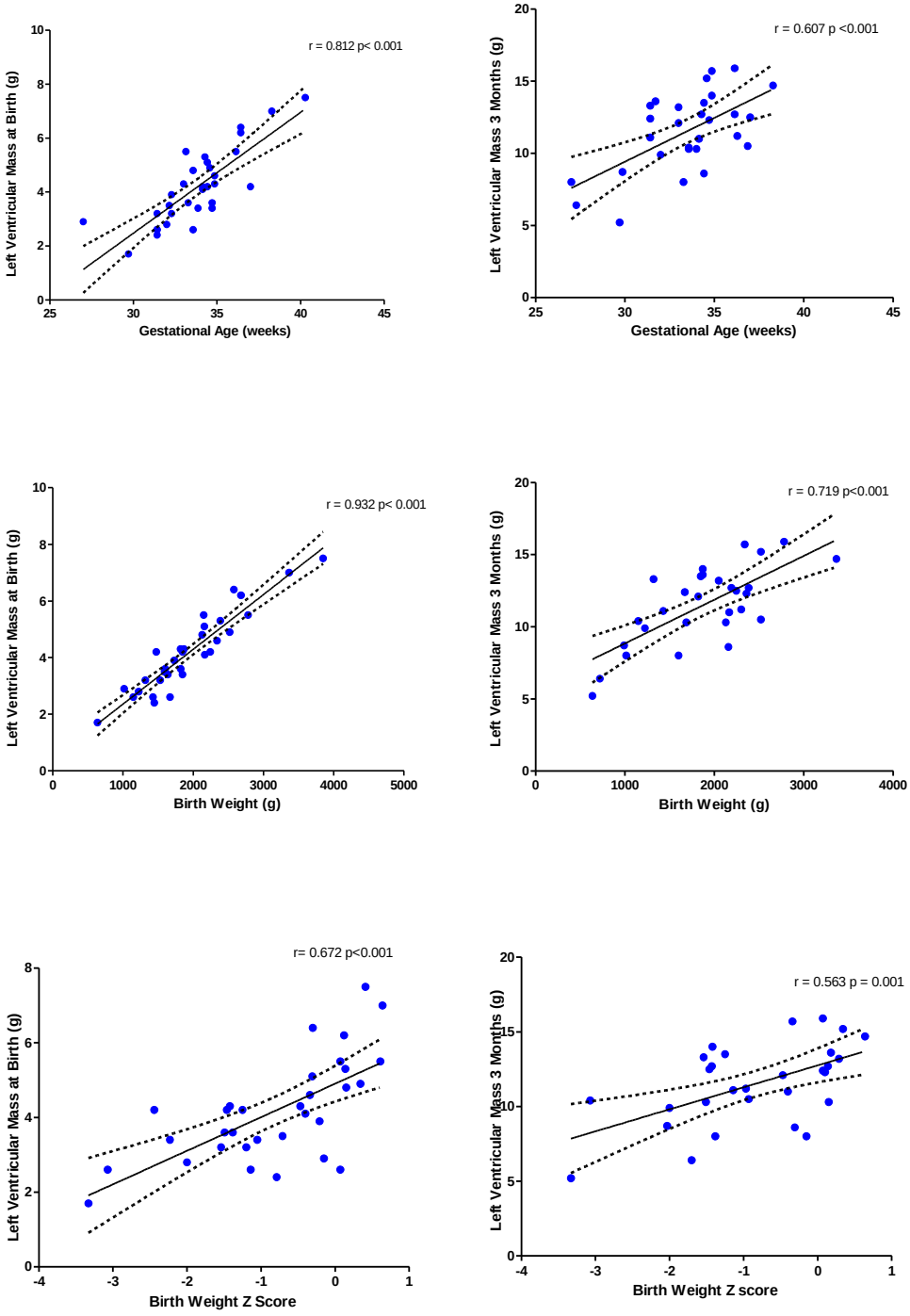
Perinatal factors including maternal age and BMI, maternal booking blood pressure, antenatal steroid administration, infant APGAR scores, days of admission, days of oxygen therapy and number of infectious episodes were also investigated. There was a negative correlation between administration of antenatal steroids and left ventricular mass at birth ($r = -0.524$ p 0.004), but not at three months of age ($r = -0.136$ $p = 0.49$). This correlation at birth was attenuated by correction for gestational age and birth weight ($r = -0.074$ $p = 0.714$). Additionally left ventricular mass at both time points correlated with days of admission (Birth $r = -0.697$ $p < 0.001$, 3 months -0.625 $p < 0.001$) and days of oxygen therapy (Birth $r = -0.456$ $p = 0.009$, and 3 months $r = -0.599$ $p < 0.001$). Both of these relationships were attenuated to non-significance by correction for birth weight and gestation at delivery and likely reflected the greater prematurity of those infants who went onto require a prolonged hospital stay.

TABLE 8.2 LEFT VENTRICULAR STRUCTURE AND DIMENSIONS IN NEONATES AND INFANTS EXPOSED TO HYPERTENSIVE DISORDERS IN UTERO

	Birth			3 months		
	Normotensive (n = 13)	**Hypertensive (n = 22)**	**p-value**	**Normotensive (n=11)**	**Hypertensive (n=20)**	**p-value**
Cardiac Wall Thickness						
IVS Thickness (cm)	0.31 (0.07)	0.30 (0.08)	0.91	0.42 (0.07)	0.43 (0.12)	0.87
IVS adjusted for BSA (cm/m^2)	2.17 (0.74)	2.04 (0.50)	0.55	1.44 (0.29)	1.53 (0.38)	0.50
PW Thickness (cm)	0.29 (0.04)	0.32 (0.06)	0.18	0.48 (0.08)	0.42 (0.10)	0.11
PW Thickness adjusted for BSA (cm/m^2)	2.03 (0.49)	2.16 (0.55)	0.48	1.64 (0.30)	1.60 (0.44)	0.80
Left Ventricular Dimension						
IDD (cm)	1.20 (0.17)	1.28 (0.22)	0.25	1.81 (0.26)	1.85 (0.28)	0.66
IDD adjusted for BSA (cm/m^2)	8.22 (1.35)	8.78 (2.48)	0.46	6.20 (1.29)	6.66 (0.98)	0.26
IDS (cm)	0.85 (0.15)	0.90 (0.15)	0.33	1.23 (0.17)	1.27 (0.22)	0.64
IDS adjusted for BSA (cm/m^2)	5.78 (0.91)	6.23 (1.84)	0.42	4.22 (0.84)	4.54 (0.65)	0.25
Left Ventricular Length (cm)	2.37 (0.31)	2.25 (0.36)	0.38	3.42 (0.34)	3.19 (0.38)	0.10
LVL adjusted for BSA (cm/m^2)	16.55 (2.40)	15.42 (3.78)	0.36	11.72 (1.83)	11.68 (2.16)	0.96
EDV (ml)	2.06 (0.60)	2.24 (0.88)	0.55	7.38 (1.15)	6.59 (2.23)	0.31
EDV adjusted for BSA (ml/m^2)	14.26 (3.89)	14.60 (4.84)	0.84	25.02 (3.57)	22.91 (5.48)	0.28
Left Ventricular Mass (LVM)						
LVM (g)	3.97 (1.03)	4.29 (1.57)	0.542	11.50 (1.93)	11.57 (2.99)	0.95
LVM adjusted for BSA (g/m^2)	27.27 (4.74)	26.74 (3.10)	0.704	38.82 (6.03)	39.90 (5.30)	0.63
LVM to Body Weight Ratio	0.0022 (0.00041)	0.0021 (0.0023)	0.400	0.0024 (0.00047)	0.0025 (0.00033)	0.32

Table 8.2 This table presents left ventricular structure and dimensions by maternal hypertension status at birth and 3 months of age. BSA = Body Surface Area, IDD = Internal Diameter Diastole, IDS = Internal Diameter Systole IVS = Intra-Ventricular Septum, LVL = Left Ventricular Length, LVM = Left Ventricular Mass, PW = Posterior Wall

FIGURE 8.1 RELATIONSHIP BETWEEN LEFT VENTRICULAR MASS AND GESTATIONAL AGE, BIRTH WEIGHT AND BIRTH WEIGHT Z SCORE AT BIRTH AND 3 MONTHS



Relationship between gestational age, birth weight or birth weight z score and left ventricular mass at neonatal (left panel) and 3 month (right panel) echocardiography.

Finally the relationship between left ventricular mass and haemodynamic factors at the time of measurements were considered. The results of this analysis are presented in Table 8.3.

8.3 CORRELATIONS BETWEEN HAEMODYNAMIC FACTORS AND LEFT VENTRICULAR MASS AT BIRTH AND 3 MONTHS

	Birth		3 months	
	r	**p-value**	**R**	**p-value**
SBP (mmHg)	0.229	0.282	0.483	0.008
DBP (mmHg)	0.229	0.222	0.323	0.088
MAP (mmHg)	0.226	0.220	0.437	0.018
Heart Rate (bpm)	-0.381	0.073	-0.205	0.421
Pulsewave Velocity (m/s)	-0.104	0.671	-0.284	0.458

Table 8.3 This table presents the correlation (r) between left ventricular mass and haemodynamic factors (SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, and heart rate) a birth and 3 months of age with associated p-values.

Left ventricular mass already correlated with mean systolic (p = 0.008) and mean blood pressure (p = 0.018) at three months of age. This relationship with MAP was attenuated by correction for gestational age (p = 0.47) and birth weight (p=0.261). There was no correlation between heart rate and left ventricular mass at either time point.

8.6 LEFT VENTRICULAR FUNCTION IN NEONATES EXPOSED TO HYPERTENSIVE DISORDERS IN UTERO

At birth, cardiac function was assessed by calculation of ejection fraction, trans-mitral doppler flows, tissue doppler imaging and assessment of global cardiac strain using speckle tracking (see Chapter 7.5). Ejection fraction (Modified Simpsons Ejection Fraction 61.95± 6.27 vs. 60.09 ± 4.49 % p = 0.378), and fractional shortening (30.7 ± 4.28 vs. 29.03 ± 5.26 % p = 0.313) did not significantly differ between neonates born to hypertensive or normotensive pregnancies at birth. Transmitral doppler flow imaging was accomplished in 28 infants at birth and there were no significant differences in any transmitral doppler parameter, or in the mitral valve E/A ratio between groups (MV E Max Velocity p = 0.595, MV A Max Velocity p = 0.690 and MV E/A ratio p = 0.580).

Tissue Doppler imaging at the mitral valve annulus was successfully undertaken at birth in 27 infants at the septal annulus and 22 infants at the lateral annulus. Results are presented in Table 8.4.

TABLE 8.4 TISSUE DOPPLER IMAGING PARAMETERS AT THE MITRAL VALVE ANNULUS AT BIRTH

	Septal			Lateral		
	Normotensive	**Hypertensive**	**p- value**	**Normotensive**	**Hypertensive**	**p- value**
S (cm/s)	4.83 (.44)	4.26 (.49)	0.005	5.04 (.86)	4.32 (.81)	0.058
E' (cm/s)	5.67 (1.74)	5.24 (1.43)	0.499	6.09 (1.61)	6.98 (1.42)	0.183
A' (cm/s)	6.68 (1.83)	5.84 (1.12)	0.179	6.53 (0.96)	5.94 (1.36)	0.274
E/E'	9.28 (2.32)	9.67 (2.66)	0.703	8.39 (2.28)	7.59 (2.02)	0.411

Table 8.4 This table presents the Tissue Doppler imaging parameters in infants who underwent echocardiography at birth. (S = peak early systolic myocardial velocity, E' = early diastolic myocardial velocity, A' = late diastolic myocardial velocity, E/E' = ratio of early transmitral flow to early diastolic myocardial velocity).

At birth the offspring of hypertensive pregnancies already showed a reduction in early systolic peak velocity at the septal mitral valve annulus (p = 0.005) with a strong trend toward reduction at the lateral annulus (p = 0.058). This reduction persisted after correction for sex (p = 0.006) gestational age (p = 0.007), birth weight (p = 0.006), birth weight z score (p = 0.008), mean blood pressure (p = 0.028) and heart rate (p = 0.022) at the time of measurement. There was no correlation between septal early systolic velocity and a variety of perinatal factors including maternal age (p = 0.274), mode of delivery (p = 0.721), multiple pregnancy (p = 0. 837), 5 minute APGAR score (p = 0.176), days of oxygen dependence (p = 0.156) or days of total neonatal admission (p = 0.079). Early septal velocity did correlate with maternal BMI (p = 0.021). However, preeclampsia exposure remained an independent predictor in a model where maternal BMI was taken into account (p = 0.037).

There was no difference at birth in global endocardial circumferential or longitudinal strain assessed by speckle tracking between those born to normotensive, and those born to hypertensive pregnancies. Data is presented in Table 8.5.

TABLE 8.5 – SPECKLE TRACKING ASSESSMENT OF CIRCUMFERENTIAL AND LONGITUDINAL STRAIN AT BIRTH

	Normotensive	**Hypertensive**	**p- value**
Mid Short Axis	(n= 12)	(n=17)	
Circumferential Strain (%)	-31.87 (4.96)	-32.17 (6.56)	0.90
SRS (%/s)	-4.49 (0.89)	04.29 (0.95)	0.56
SRD (%/s)	5.89 (1.35)	5.85 (1.42)	0.95
	Normotensive	**Hypertensive**	**p- value**
4-Chamber	(n=9)	(n=16)	
Longitudinal Strain (%)	-22.81 (5.94)	-22.90 (3.32)	0.96
SRS (%/s)	-3.07 (1.09)	-3.02 (0.39)	0.85
SRD (%/s)	3.91 (1.24)	3.85 (0.23)	0.88

Table 8.5 This table reports speckle tracking derived mid short axis circumferential strain and 4-chamber longitudinal strain in EPOCH infants scanned at birth. Associated systolic and diastolic strain rates are also reported. (SRS = systolic strain rate, DSR = diastolic strain rate).

8.7 LEFT VENTRICULAR FUNCTION IN INFANTS EXPOSED TO HYPERTENSIVE DISORDERS IN UTERO

At three months of age there was a small, though borderline significant, reduction in modified Simpsons ejection fraction in the offspring of hypertensive pregnancies (60.3 ± 3.80 vs 63.77 ± 5.15 % p = 0.048). However there was no corresponding reduction in Fractional Shortening (31.73 ± 5.89 vs 30.76 ± 7.26 % p = 0.69) or ejection Fraction derived by Teichholz formula (62.54 ± 8.77 vs 61.68 ± 9.64 % p = 0.80). There were no differences in transmitral doppler parameters (MV E Max Velocity p = 0. 22, MV A Max Velocity p = 0.83 and MV E/A ratio p = 0.53). Regional early systolic peak velocity at the mitral valve annulus did not differ between groups at three months (Septal p = 0.28 Lateral p = 0.86).

Global endocardial longitudinal strain on a 4-chamber image was calculated in 30 infants (19 born to hypertensive pregnancies) at three months of age (Mean age 100.9 ± 16.04 days). Peak systolic longitudinal strain was reduced by 3.74% in those born to hypertensive pregnancies when compared to controls (-25.08 ± 2.70 vs -21.34 ± 3.48 % p = 0.005, Figure 8.2). This reduction persisted after correction for sex (p = 0.003), gestational age (p = 0.002), birth weight z score (p =

0.014), length of hospitalisation at birth (p = 0.002) age at follow up (p = 0.010), weight at follow up (p = 0.007) and mean arterial pressure at 3 months of age (p = 0.008).

FIGURE 8.2 PEAK GLOBAL LONGITUDINAL STRAIN IN OFFSPRING OF HYPERTENSIVE AND NORMOTENSIVE PREGNANCIES AT 3 MONTHS OF AGE

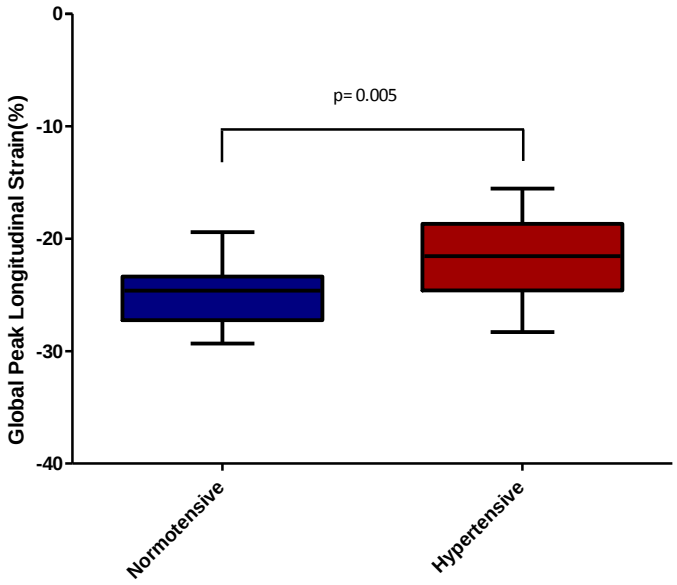


Figure 8.2 This figure demonstrates longitudinal peak systolic strain in the offspring of normotensive and hypertensive pregnancies at 3 months of age. Those born to hypertensive pregnancies had significantly less negative peak systolic strain values at this time (p = 0.005)

Systolic and diastolic longitudinal systolic strain rate (SSR = Systolic strain rate, DSR = diastolic strain rate) was also significantly reduced in the offspring of hypertensive pregnancies (SSR -2.53 ± .57 vs -3.20 ± .59 %/s p=0.003, DSR 4.19 ± 1.14 vs 5.11 ± 1.01 %/s p=0.041 Figure 8.3). Reductions in strain rate also persisted after adjustment for infant sex (SSR p= .003, DSR p=0.041), gestation at delivery (SSR p= 0.002, DSR p= 0.039), birth weight (SSR p=0.002, DSR p = 0.045), birth weight z score (SSR p = 0.007, DSR p = 0.040), age at follow up (SSR p= 0.003, DSR p = 0.032), and weight at 3 month follow up (SSR p= 0.004, DSR p = 0.051). The relationship between systolic strain rate and hypertension in pregnancy persisted after correction for days of admission (p= 0.004), and mean arterial pressure at follow up (p = 0.004), but the relationship to diastolic strain rate was moderated slightly by adjustment for these factors (days of admission p = 0.062 and MAP p = 0.064).

FIGURE 8.3 PEAK SYSTOLIC AND DIASTOLIC LONGITUDINAL STRAIN RATES AT 3 MONTHS POST-NATAL AGE.

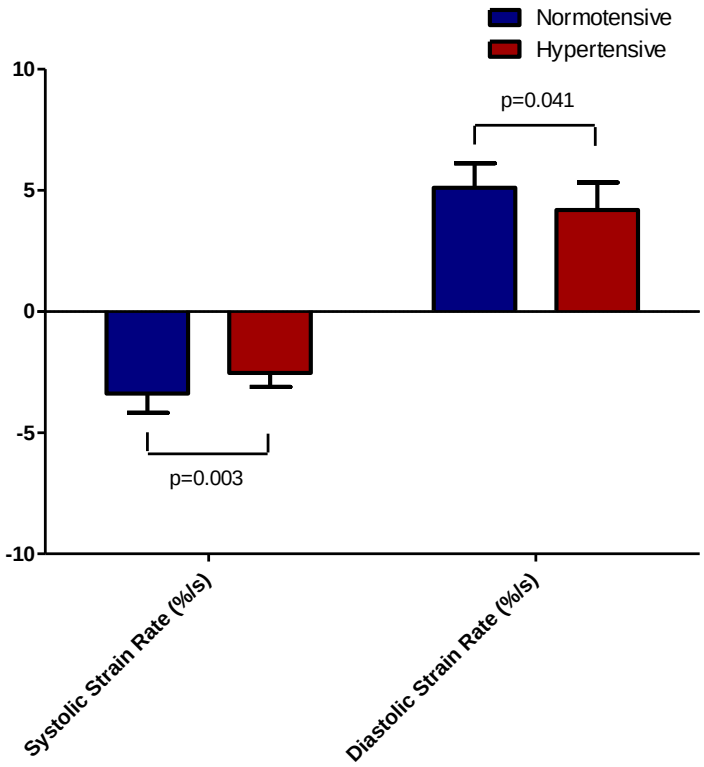


Figure 8.3 This figure demonstrates longitudinal systolic and diastolic strain rates in the offspring of hypertensive and normotensive pregnancies at 3 months. Those born to hypertensive pregnancies had significant reductions in both systolic and diastolic strain rates at this time point.

To determine if other perinatal factors significantly influenced strain, bivariate correlation between longitudinal peak systolic strain and a range of maternal, antenatal and postnatal variables were undertaken. These are presented in Table 8.6. Where a significant, or borderline significant relationship was found its effect on the relationship between hypertensive disorders of pregnancy was tested using bivariate regression. The adjusted p-vales from these models are also presented in Table 8.6.

TABLE 8.6 RELATIONSHIP OF PERINATAL VARIABLES WITH GLOBAL PEAK SYSTOLIC LONGITUDINAL STRAIN AT 3 MONTHS.

	r	p-value for correlation with longitudinal strain.	Adjusted p-value (Preeclampsia and longitudinal strain)	Adjusted p-value (Preeclampsia and systolic strain rate)	Adjusted p-value (Preeclampsia and Diastolic strain rate)
Maternal Age	.136	.474	-	-	-
Maternal BMI	.233	.243	-	-	-
Antenatal Steroids	-.190	.435	-	-	-
Multiple Pregnancy	-.107	.574	-	-	-
C-Section Delivery	.134	.481	-	-	-
APGAR 1 minute	-.250	.302	-	-	-
APGAR 5 minutes	-.190	.435	-	-	-
Days of ITU admission	.361	.083	-	-	-
Days of Oxygen Therapy	.420	.042	0.001	0.002	0.033
Number of Infectious Episodes	.284	.178	-	-	-
Days of TPN	.101	.640	-	-	-
Discharge Weight	.210	.362	-	-	-
Breast Fed	-.204	.340	-	-	-
Heart Rate at Follow-up	-.307	.119	-	-	
Left Ventricular Mass	-.085	.661	-	-	-
Left Ventricular length	-0.340	.066	0.020	0.014	0.078
Pulsewave Velocity	-0.076	0.846	-	-	-

This table shows the correlation coefficients for the relationship between perinatal factors and infant global longitudinal strain at 3 months of age. Significant or borderline significant relationships were identified between strain and days of oxygen therapy, and left ventricular length. The relationship between in utero exposure to hypertensive disorders of pregnancy and strain and systolic strain rate persisted after correction for these factors. The relationship between diastolic strain rate and hypertensive disorders of pregnancy was attenuated by correction for left ventricular length.

Circumferential strain was calculated on mid short axis images in 29 infants at three months of age. There were no significant differences in peak systolic circumferential strain (-33.59 ± 6.40 vs -

32.51 ± 7.64 p = 0.705), systolic (p = 0.302) or diastolic (p= 0.473) strain rate. No significant correlation was found between circumferential strain and any of the perinatal factors considered above.

8.7 DISCUSSION

This work has demonstrated for the first time that infants born preterm to hypertensive pregnancies already show subtle alterations in cardiac function when compared to an equally preterm and equally low birth weight group whose mothers remained normotensive. In particular these infants demonstrate reduced early systolic peak velocity at the mitral valve annulus at birth, and reduced global peak longitudinal systolic strain and strain rates at three months of age. These changes persist after adjustment for gestation at delivery, birth weight, birth weight z score and a variety of perinatal factors, and are present in the absence of any alteration in myocardial structure or clinically overt changes in cardiac function. This reduction in peak global longitudinal systolic strain has previously been demonstrated in a cohort of young adults born preterm to preeclamptic pregnancies [7] and the alterations demonstrated in the EPOCH cohort are comparable in magnitude to those seen in young adult life. These observations highlight the perinatal period as key in the development of persistent subclinical changes in cardiac functions associated with in utero exposure to maternal hypertensive disorders.

Prematurity alone has previously been associated with changes in longitudinal, rotational and circumferential systolic function, although longitudinal changes associated with preeclampsia appear to be independent of gestation both in young adulthood [7] and within the EPOCH cohort. Human studies indicate that longitudinal movement of the myocardium is derived from the subendocardial and subepicardial fibres and slightly precede radial movements [267, 320]. These longitudinal fibres are thought to be particularly susceptible to ischemia, and animal work undertaken in hypoxeamic piglets demonstrates hypoxia has specific effects on neonatal longitudinal cardiac function which exceed those on radial and circumferential strain [252]. As

preeclampsia is a condition characterised by relative placental and fetal hypoxia from early gestation, it is plausible that these in utero conditions lead to specific changes in longitudinal function in the offspring of such pregnancies [265, 267, 320].

Speckle tracking echocardiographic studies of neonatal cardiac strain remain relatively rare. Marcus et al have reported reference ranges for cardiac strain in a group of 24 infants at a mean age of 3 months, reporting a global longitudinal strain of -18.3% and a global circumferential strain of -18.6% [257]. The mean strain values achieved in the EPOCH population are somewhat lower than those reported by Marcus with an average across the entire cohort of -22.71% for longitudinal strain and -32.88% for circumferential strain at 3 months of age. Although it is possible that perinatal differences between this cohort and the EPOCH participants may underlie some of this difference, it is also equally plausible that this variation may be attributable to differences in the vendor software used as there is significant inter-vendor variation in 2-dimensional strain values.

Persistent reductions in cardiac strain have been reported in neonates and children with a variety of cardiac conditions including congenital aortic stenosis [268], left ventricular non-compaction [214], and Kawasaki's disease [321]. In addition global longitudinal systolic strain has been demonstrated to correlate negatively with BMI in obese children and adolescence in the absence of additional cardiovascular risk factors [256, 322]. In adult populations subclinical reductions in longitudinal strain have been found in women with preeclampsia during the effected pregnancy [269]. Decreases have also been seen in adult populations with traditional cardiovascular risk factors such as hypertension [67, 323] and non-insulin dependent diabetes mellitus [324]. Finally longitudinal systolic strain independently predicts mortality in heart failure patients and those with chronic ischaemic cardiomyopathy [239, 251].

Despite the association of decreased longitudinal strain with this variety of conditions and risk factors, the long-term consequences of a reduction in cardiac strain present from early postnatal

life are unclear. The findings of Lewandowski and colleagues suggest we can be fairly confident that this reduction in strain decreases into young adult life [7], but the implications of this for the long term health of offspring of hypertensive pregnancies requires further investigation. Animal models of preeclampsia suggest that a potential link between *in utero* hypoxia and later cardiovascular disease is through alterations in cardiac-ischaemia reperfusion mechanisms. Specifically, rats exposed to hypoxia in late gestation have alterations in cardiac proton production, and increased myocardial production of acetyl-CoA during reperfusion [127, 128]. In fetal life such animals also have significant reduction in cardiac PKCε, possibly secondary to increased methylation at the PKCε promoter site [129]. PKCε plays a critical role in cardioprotection during ischaemia [325] and it is plausible that reduction in this may lead to increased susceptibility to subendocardial ischemia and associated reduction in longitudinal strain in the offspring of preeclamptic pregnancies.

There are several potential limitations to this analysis. Firstly, it was not possible to undertake echocardiographic assessment of all infants recruited into the study. At birth a portion of recruits were not clinically stable enough to allow investigation, and another group were discharged or transferred prior to echocardiography being undertaken. At 3 months a portion of mothers declined to attend the follow up appointment. The numbers who were not scanned at any time point are however relatively small. Additionally there was no significant difference in the gestational age, birth weight or birth weight z score between those who were scanned at birth only, at three months only and those who were scanned at both time points, which suggests that no systemic bias has been introduced by this loss to follow up. However due to the small sample size it is impossible to exclude subtle changes in cardiac structure or left ventricular mass between groups which may only become apparent in a large cohort.

Secondly due to the relatively small sample size I have been unable to subdivide cases born to hypertensive pregnancies by severity of maternal preeclampsia and have had to group all

maternal hypertensive disorders for analysis, these factors may have significant effects on the offspring cardiac phenotype. The sample size has also required a conservative approach to the number of factors included in multiple regression analyses. In the future a larger sample size will allow the effects of the multitude of potentially relevant perinatal factors to be considered concurrently, rather than separately as they have been in this analysis. Having said that, the lack of correlation between the majority of perinatal factors and longitudinal strain, or strain rates suggests that this analysis has been able to account for the most important sources of variance. Finally I have not addressed whether infants born to hypertensive pregnancies have alterations in left ventricular radial strain or right ventricular structure or function. The geometrical complexities of the right ventricle, particularly in the context of the perinatal transitional circulation, make analysis challenging. However it is anticipated that as the sample included in the EPOCH study structure and function of the right ventricle will be addressed. Left ventricular radial strain is at present poorly reproducible and this cohort average inter and intra observer coefficients of variance were greater than 10% (See chapter 7). In view of this it has not been considered in this research, however, as speckle-tracking technology improves a consideration of radial strain in the offspring of hypertensive pregnancies may be of considerable interest.

8.8 Conclusions

Infants born preterm to hypertensive pregnancies show evidence of alteration of cardiac function during the first three months of life compared to preterm infants whose mothers were normotensive. The changes are comparable to those observed in young adult life and highlight the perinatal period as potentially important in the development of the long-term cardiac changes associated with in utero exposure to hypertensive pregnancies. As preeclampsia affects between 2% and 5% of all pregnancies, and accounts for 20% of premature deliveries and hypertensive disorders of pregnancy may effect up to 10% of all pregnancies [16, 17, 20, 22] these findings are of significant long-term public health interest. In particular understanding

whether perinatal interventions can prevent these subclinical impairments of cardiac function may play an important role in reducing the excess cardiovascular risk in the offspring of hypertensive pregnancies.

CHAPTER 9 – MICROVASCULAR STRUCTURE IN INFANT OFFSPRING OF HYPERTENSIVE PREGNANCIES AT BIRTH AND 3 MONTHS

9.1 THESIS CONTEXT

As outlined in Chapters 4 and 5, term and moderately preterm born offspring of preeclamptic pregnancies have increased blood pressure in young life that is independent of birth weight and gestation. Dysfunction of the microvasculature has been consistently associated with hypertension, and it is plausible that microcirculatory changes underlie some aspect of the increase in blood pressure seen in the offspring of hypertensive pregnancies. As the perinatal period is a time of rapid growth and development of microcirculation, insults during this period could lead to lifelong changes in vascular function and cardiovascular risk. This chapter therefore explores the microvasculature of infants born to hypertensive pregnancies at birth and 3 months of age.

9.2 INTRODUCTION

It has been posited that alteration to, and inadequacy of, the microvasculature underlie the tissue and organ dysfunction in chronic conditions such as hypertension, diabetes and obesity, all of which increase cardiovascular risk [289]. Capillary rarefaction, or reduced capillary number or length per volume of tissue, has consistently been demonstrated in hypertensive adults [289] and has also been shown in young adults predisposed to high blood pressure, suggesting that an abnormality in capillary density may be associated with the etiology of essential hypertension [289, 326]. Microcirculatory dysfunction also relates to general cardiovascular risk, with Framingham Risk Score correlating negatively with capillary density and capillary function [327],

and abnormal microcirculatory responses to ischaemia associated with adverse cardiac remodeling and hypertrophy [328].

In addition to traditional cardiovascular risk factors, perinatal factors have been demonstrated to impact on capillary density. Preterm birth has been associated with reductions in functional capillary density in childhood [329] and low birth weight infants have been found to have impaired microcirculatory reactions at three months of age [330]. Interestingly however, low birth weight infants born to normotensive mothers demonstrate increased, rather than reduced structural capillary density at birth [331]. Furthermore, despite the well reported relationship between low birth weight and future cardiovascular risk, a study of children aged 6 to 16 years and young adults aged 24 years demonstrated no relationship between birth weight and dermal capillary density in either group [332].

Limited additional data exists on capillary density in neonatal life or in the offspring of hypertensive pregnancies possibly due to the technical challenges of capillary imaging in this population. Despite the limited data available to date, however, the strong relationship between hypertensive disorders of pregnancy and increased blood pressure in the offspring [67, 240], combined with the general association between hypertension and capillary rarefaction, suggests that the offspring of hypertensive pregnancies may demonstrate alterations in their capillary structure or function. I have therefore undertaken to investigate small vessel structure in the EPOCH study cohort to determine the effects of maternal hypertension on microvascular phenotype in early postnatal life.

9.3 METHODS

Methods relevant to this chapter are described in detail in Chapter 7. In particular:

- Collection of demographic, lifestyle and medical data is described in Section 7. 2.

- Anthropometric measurements and body surface area calculations are described in Section 7.3.
- Measurements of the microvasculature by MicroScan are described in Section 7.7.
- Measurement of blood pressure and arterial stiffness are described in Sections 7.6 and 7.8.
- General statistical methods are described in Section 7.9.

9.4 POPULATION CHARACTERISTICS

The recruitment base for the EPOCH Pilot study is described in detail in Chapter 6.

As noted 46 infants have been recruited to date. Of these 39 have undergone microvasculature assessment by MicroScan at birth or 3 months. 28 infants were scanned at birth and 25 at 3 months of age. 15 infants were scanned at both time points. As with assessment of cardiac function, (Chapter 8) the most common reasons that infants were not scanned at birth were; clinical instability prohibiting MicroScan assessment (4 infants), discharge from hospital prior to acquisition of MicroScan Imaging (5 infants), and transfer from the John Radcliffe Hospital prior to acquisition of Microscan Imaging (4 infants). At 3 months one infant was not scanned due to clinical instability, and 13 infants did not return for 3-month follow up. At the time of writing 3 infants, all scanned at birth, had not yet reached 3 months of age. In the remainder of cases where no data is available MicroScan imaging was unsuccessful due to infant movement.

The perinatal characteristics of infants who underwent MicroScan Imaging are presented in Table 9.1.

TABLE 9.1 CHARACTERISTICS OF INFANTS UNDERGOING MICROSCAN IN THE EPOCH PILOT STUDY

	Control (n=14)	**Hypertensive (n = 25)**	**p-value**
Gestational Age (weeks)	32.98 (2.24)	33.66 (3.15)	0.48
Birth Weight (g)	1854 (412)	1878 (788)	0.93
Birth weight Z score	-.503 (.78)	-1.08 (1.05)	0.08
Sex (% Male)	42.9% (6)	52.0% (13)	0.58
Multiple Pregnancy (%)	57.1% (8)	36.0% (9)	0.20
Caesarean Delivery (%)	64.3% (9)	84.0% (23)	0.16
Antenatal Steroids (% received)	78.6% (11)	66.7% (14/21)	0.44
Days of Oxygen Support	8.50 (11.93)	17.72 (34.92)	0.34
Days of admission	25.50 (15.08)	35.02 (35.01)	0.70
Maternal Age (years)	33.92 (5.74)	31.84 (6.24)	0.31
Maternal BMI (kg/m^2)	23.68 (2.15)	27.50 (4.52)	0.006
Age at Birth MicroScan (days)	11.1 (8.19)	10.22 (5.80)	0.74
SBP Birth (mmHg)	66.84 (10.17)	66.01 (10.15)	0.51
DBP Birth (mmHg)	34.95 (6.63)	36.90 (8.34)	0.56
Age at 3 month MicroScan (days)	105.1 (14.7)	94.47 (7.50)	0.044
Weight at 3 months (g)	4907 (957)	4537 (1279)	0.42
SBP 3 months (mmHg)	89.10 (8.64)	88.59 (12.22)	0.90
DBP 3 months (mmHg)	53.68 (12.12)	50.13 (12.62)	0.47

Table 9.1 Characteristics of infants who underwent MicroScan Imaging at birth or 3 months.

As previously noted mothers who went on to develop hypertension in pregnancy had higher BMI's than those who remained normotensive. There were no other significant differences in factors describing the perinatal course. Scanned offspring of mothers who remained normotensive were slightly older at 3 month follow up (105.1 ± 14.7 vs 94.47 ± 7.50 p = 0.044), however as there was no correlation between age and vessel density at this time point (r = -0.193 p = 0.366) this was not considered to unduly impact the analysis.

9.5 MICROVASCULATURE IN NEONATES EXPOSED TO HYPERTENSIVE DISORDERS IN UTERO

There were no differences in small, large or total vessel density in those infants exposed to hypertensive disorders in utero compared to those whose mothers remained normotensive. Results are presented in Table 9.2.

TABLE 9.2 – MEASUREMENTS OF MICROVASCULAR STRUCTURE AND FUNCTION IN NEONATES EXPOSED TO HYPERTENSIVE DISORDERS OF PREGNANCY

	Normotensive (n=11)	**Hypertensive** (n=17)	**p-value**
Small Vessel Density (mm/mm^2)	27.20 (2.45)	27.66 (2.69)	0.65
Large Vessel Density (mm/mm^2)	0.46 (0.60)	0.78 (0.79)	0.25
Total Vessel Density (mm/mm^2)	27.69 (2.49)	28.50 (2.62)	0.42
Proportion of Perfused Vessels (%)	98.50 (1.85)	98.37 (1.87)	0.93
Microvascular Flow Index	2.98 (0.055)	2.99 (0.032)	0.84
De Backers Score (1/mm)	16.73 (1.50)	16.87 (1.87)	0.83

Table 9.2: Microvascular structural and functional measurements in offspring of hypertensive and normotensive pregnancies at birth. Small vessels = diameter <25µm, Large Vessels = Diameter > 25µm.

To determine if other perinatal factors significantly influenced microvascular structure at birth, bivariate correlations between total vessel density and a range of maternal, antenatal and postnatal variables were undertaken.

Interestingly there was no correlation between total microvascular density at birth and any perinatal factor considered including; gestation ($p = 0.78$), birth weight ($p = 0.45$), birth weight z score ($p = 0.24$), multiple pregnancy ($p = 0.69$), antenatal steroid administration ($p = 0.72$), APGAR score ($p = 0.103$) length of neonatal admission ($p = 0.91$) or days of oxygen dependency ($p = 0.94$). In the offspring of hypertensive pregnancies there was a trend towards a relationship with maximal maternal diastolic blood pressure ($r = 0.576$, $p = 0.066$), but no relationship with maximal systolic ($p = 0.30$) or booking blood pressures. There was no relationship with maternal age ($p = 0.33$) or maternal BMI ($p = 0.32$).

The relationships between microvasculature and haemodynamic factors at birth were then investigated. There was no relationship between systolic (p = 0.43), diastolic (p = 0.41) or mean (p = 0.25) blood pressure and total vessel density during the neonatal period. Nor was there any correlation between Vicorder derived central blood pressure (systolic p = 0.36, diastolic p = 0.91), arterial stiffness measured by pulse wave velocity (p = 0.68), or augmentation index (p = 0.74) and microvascular density in the neonatal period.

9.6 Microvascular Structure and Function in Infants exposed to hypertensive disorders in Utero

MicroScan imaging was successfully undertaken in 25 infants (15 born to hypertensive pregnancies) at 3 months of age. Infant movement had a greater impact at 3 months and therefore a larger number of infants were not scanned successfully at this age. Mean microvascular parameters at 3 month follow up are presented in table 9.3.

TABLE 9.3 – Measurements of Microvascular Structure and Function in Infants exposed to Hypertensive Disorders of Pregnancy

	Normotensive (n=10)	**Hypertensive** (n=15)	**p-value**
Small Vessel Density (mm/mm^2)	21.75 (2.05)	22.43 (1.91)	0.40
Large Vessel Density (mm/mm^2)	0.077 (0.16)	0.43 (0.82)	0.19
Total Vessel Density (mm/mm^2)	21.83 (2.10)	22.86 (1.85)	0.21
Proportion of Perfused Vessels (%)	97.97 (2.28)	96.49 (3.38)	0.24
Microvascular Flow Index	2.98 (0.042)	2.96 (0.77)	0.60
De Backers Score (1/mm)	12.30 (1.25)	13.34 (1.33)	0.062

Table 9.3: Microvascular structural and functional measurements in offspring of hypertensive and normotensive pregnancies at 3 months of age. Small vessels = diameter <25µm, Large Vessels = Diameter > 25µm.

There was a strong trend towards an increased De Backers Score in the offspring of hypertensive pregnancies at 3 months of age (p = 0.062) but no significant differences in vessel density or perfusion. To determine if other perinatal factors significantly influenced microvascular structure at birth, correlation between small vessel density, total vessel density and De Backers Score with

a range of maternal, antenatal and postnatal variables were undertaken. The results of this analysis are presented in Table 9.4.

TABLE 9.4: CORRELATIONS BETWEEN MICROVASCULAR STRUCTURE AT 3 MONTHS AND PERINATAL FACTORS

	Small Vessel Density		**Total Vessel Density**		**De Backers Score**	
	R	p-value	r	p-value	R	p-value
Infant Sex	-0.085	0.68	-0.034	0.87	0.17	0.42
Birth Weight (g)	-0.422	0.036	-0.395	0.051	-0.115	0.59
Gestational Age	-0.338	0.098	-0.319	0.120	0.013	0.95
Birth Weight z score	-0.316	0.124	-.304	0.140	-0.223	0.28
5 minute APGAR score	-0.387	0.113	-.351	0.15	-0.290	0.24
Mode of Delivery	-0.141	0.50	-0.218	0.29	-0.077	0.71
Antenatal Steroid Administration (%)	0.139	0.53	0.091	0.68	0.068	0.76
Days of Neonatal Admission	0.240	0.25	0.257	0.21	0.085	0.69
Days of Oxygen Dependence	0.144	0.49	0.192	0.36	0.141	0.50
Neonatal Infections	0.139	0.51	0.073	0.73	-0.050	0.81
Maternal Age	0.192	0.36	0.080	0.70	-0.257	0.21
Maternal BMI	0.073	0.74	0.151	0.48	0.237	0.26
Weight at 3 months	-0.42	0.04	-0.44	0.03	-0.288	0.16

Table 9.4: Correlations between microvascular structure at 3 months of age and perinatal factors.

With the exception of birth weight which correlated negatively with small ($p = 0.036$) and total vessel density ($p = 0.051$), perinatal factors did not appear to have a significant relationship with microvascular structure at 3 months of age. Weight at three months of age also correlated negatively with small and total vessel density although these were attenuated to non-significance if corrected for birth weight.

The relationship between microvascular structure and haemodynamic factors at 3 months was also investigated. Small and total vessel density already demonstrated a strong negative correlation with mean (SVD $r = -0.584$ $p = 0.002$, TVD $r = -0.501$ $p = 0.011$) and diastolic (SVD $r = -0.709$ $p<0.001$, TVD $r = -0.607$ $p = 0.001$) blood pressure at 3 months of age (Figure 9.1). The correlations between blood pressure and small vessel density remained significant after correction for weight at three month follow up (SVD and DBP $r = -0.632$ $p = 0.001$, SVD and MAP r

= -0.458 p = 0.22) and birth weight (SVD and DBP r = -0.635 p = 0.001 and SVD and MAP r = -0.450 p = 0.027). Interestingly there was also a positive correlation between augmentation index and small vessel density at this time point (r = 0.751 p = 0.012) although the numbers of infants with both measurements was small (n = 10). In the small number of infants with Vicorder data at 3 months of age (n = 10) there was no association between pulsewave velocity (p = 0.431) or derived central blood pressure parameters and vessel density. Correction for haemodynamic factors did not affect the relationship between hypertensive disorders of pregnancy and small vessel parameters.

FIGURE 9.1 CORRELATIONS BETWEEN MICROVASCULAR DENSITY AND HAEMODYNAMIC PARAMETERS AT 3 MONTHS OF AGE.

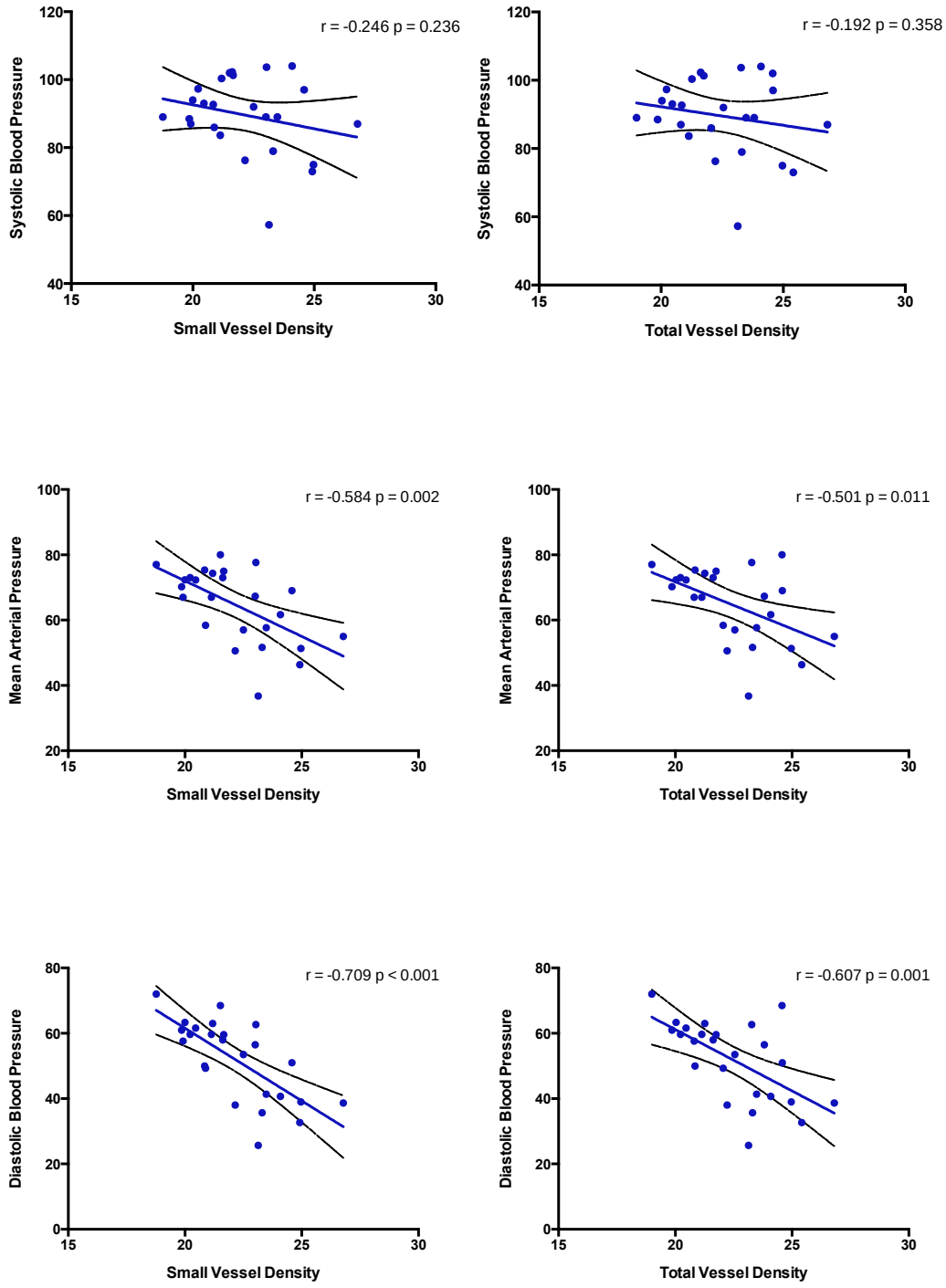


Figure 9.1 This figure shows the correlations between small vessel density (left) or total vessel density (right) and blood pressure at 3 months of age. Small and total vessel density correlated significantly with mean and diastolic blood pressure.

9.7 DISCUSSION

In this cohort there were no differences in small or total microvascular vessel density between those born to hypertensive pregnancies and those whose mothers remained normotensive at either birth or three months of age. There was also no correlation between micovascular density and birth weight or gestation at birth. At three months of age however, birth weight correlated negatively with cutaneous capillary density. In addition a strong negative correlation between diastolic, mean blood pressure and small vessel density was present at this time point. Capillary rarefaction has been consistently demonstrated in hypertensive and pre-hypertensive adults [289, 326] and this data suggests that this relationship extends back into early postnatal life. In view of the fact that the offspring of hypertensive pregnancies will go onto have higher blood pressure as children and young adults, this relationship suggests that they may eventually demonstrate lower microvascular density, even if such changes are not yet evident in the very young EPOCH cohort.

In term born infants, the first weeks of life are a period of rapid development of the capillary network. At birth a disordered microvascular network exists which develops into an ordered network by the 14^{th} to 17^{th} week of life [333]. A study of buccal mucosa capillary density in children under the age of three years suggested that there is a rapid reduction in functional capillary density after the first week of life [334]. It is possible, however, that these changes are less acute in a preterm population as a study measuring cutaneous vessel density daily over the first 30 days of life in infants born at less than 30 weeks gestation found that microvascular parameters were not dependent on postnatal age or gestation [335]. In the EPOCH cohort there was no correlation between age in days at measurement and microvascular structure at birth consistent with previous work in preterm infants. There was however a reduction in mean vessel density between birth and 3 months suggesting maturation of the microvascular network had occurred during that period.

As previously noted limited work has considered the relationship between maternal hypertension and offspring microcirculatory structure in early life. A recent pilot study using OPS imaging found lower capillary density in those born at term to hypertensive mothers, while preterm born low birth weight offspring of hypertensive mothers demonstrated a higher capillary density in early life [281]. Previous work has suggested that low birth weight infants born to normotensive mothers also demonstrate increased structural capillary density at birth [331] and therefore this increase in capillary density is unlike to be solely attributable to the unique in utero environment of a preeclamptic pregnancy. A further study of neonates born to preeclampic mothers suggests that such infants have altered baseline microvascular flow and that this may display a sexually dimorphic pattern [336]. The EPOCH Study has not demonstrated difference in structural capillary density, or functional flow related to maternal hypertensive disorders, although it is possible that early trends towards increased perinatal capillary density in the offspring of hypertensive pregnancies are indicated by the raised De Backer score, which is evident at 3 months of age. Larger sample sizes will allow these estimates to be refined.

There are several limitations to this analysis. Firstly as with echocardiography (Chapter 8), it was not possible to undertake microvascular assessment of all infants recruited into the study. At birth a number of recruits were not clinically stable enough to allow investigation, and another group were discharged or transferred prior to imaging being undertaken. At 3 months of age a number of mothers declined to attend the follow up appointment. In addition to this microvascular measurements are far more susceptible to movement artifact than cardiac or macrovascular measurements. In some infants attempts at microvascular imaging had to be abandoned, or resultant images were unable to be analysed due to movement artifact. Due to these factors it was difficult to exclude subtle differences in small vessel density that were not apparent in the relatively small sample of the EPOCH cohort. Despite this the sample size of the EPOCH Study is similar to those of several other published studies of infant microcirculatory

function [291, 330, 337], suggesting these results are informative in the context of the current literature.

In addition I was unable to relate microvascular state to circulating levels of angiogenic and anti-angiogenic factors. Previous work undertaken in our laboratory has suggested that sEng levels correlate with reduced capillary density in young adult life, and offspring of preeclamptic pregnancies have persisting elevation of sFLT-1 at this point (Lewandowski et al *In review)*. As preeclamptic pregnancies are characterized by significant elevations in circulating anti-angiogenic factors the investigation of the relationship between these factors and microcirculatory status would be of considerable interest. However although blood was collected in some neonates at the same time as routine clinical testing, insufficient infants had both blood samples and MicroScan imaging obtained to make testing feasible. Current European Union guidelines allow no more than 1% of total blood volume to be drawn for research purposes, equating to approximately 2-3mL in a term neonate and less in those born preterm [338]. As neonates are relatively polycythaemic the volume of serum or plasma obtainable from such samples was insufficient to allow individuals testing of multiple angiogenic and anti-angiogenic factors. It is possible that with larger samples sizes this analysis will become possible in the future.

9.8 CONCLUSIONS

In this study population there were no differences in microvascular structure or perfusion associated with maternal hypertensive disorders of pregnancy. Capillary density correlated negatively with both birth weight and current weight at three months of age. This analysis has also demonstrated a strong negative correlation between mean and diastolic blood pressure and microvascular vessel density, which persists after correction for birth weight, and which is already present as early as three months of age. In view of the fact that the offspring of hypertensive pregnancies are likely to have higher blood pressure throughout childhood and into adult life, it is plausible that differences in capillary density may develop as these children age. Larger future

studies will allow this hypothesis to be explored and consider the transition high capillary densities in early life to capillary rarefaction in adult life.

CHAPTER 10 – CONCLUSIONS AND FUTURE DIRECTIONS

10.1 SUMMARY

This thesis has provided a holistic view of the impact of preeclampsia and other hypertensive disorders of pregnancy on offspring cardiovascular phenotype from infancy to early adult life. In addition it has begun to separate and distinguish the effects of hypertensive disorders of pregnancy from those of the frequently associated perinatal insults of fetal growth restriction and premature delivery. This was accomplished firstly by summarising what is known about the impact of preeclampsia on offspring cardiovascular health. The next step was to build on this by the investigation of cardiovascular phenotype in two large birth cohorts with follow up into young adult life. Finally it involved the detailed examination of the cardiovascular phenotype of a group of neonates born to pregnancies complicated by preeclampsia. The major findings of this program of work are summarised in Figure 10.1

FIGURE 10.1 SUMMARY OF MAJOR FINDINGS

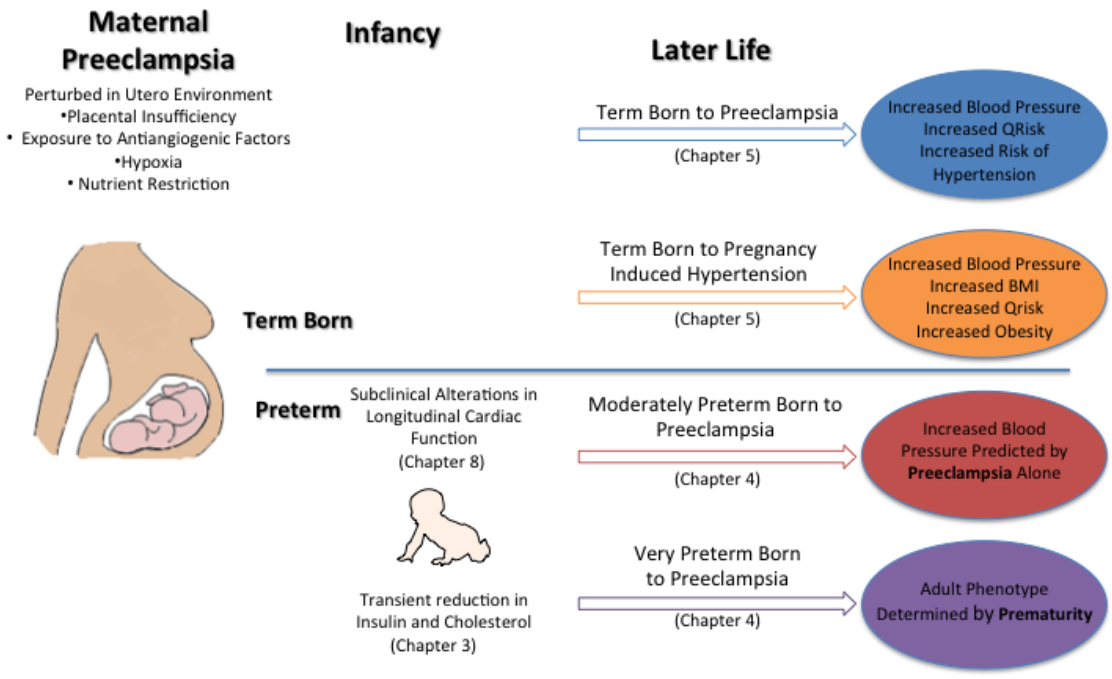


Figure 10.1 Summary of the major findings presented in this thesis.

The first objective of this thesis was to summarise the known impact of preeclampsia on offspring cardiovascular health through systematic review and meta-analysis. This detailed understanding of cardiovascular phenotype then underpins the experimental work presented. The decision to consider the body of literature as a systematic review and meta-analysis was made because of the established advantages of systematic review as an efficient scientific technique. These advantages include the ability to consolidate a large body of literature and the encouragement of scientific efficiency by providing a clear summary of deficits in current knowledge [339]. In particular the rigorous requirements of a systematic review and meta-analysis allows for more reliable and precise estimates of effect size than individual studies. The systematic review and meta-analysis presented in this thesis provides insight into the impact of preeclampsia on offspring cardiovascular risk, as distinct from previous reviews that have incorporated studies considering both preeclampsia and gestational hypertension. This data has confirmed the widely held belief that the offspring of preeclamptic pregnancies have subtle increases in blood pressure and BMI as children and young adults. Perhaps more interestingly the review also highlighted that the increases in blood pressure shown to date are insufficient to explain the demonstrated increase in cardiovascular events in this population. This finding illustrates the need for greater understanding of cardiovascular phenotype associated with being born to preeclamptic pregnancies in order to characterise whether there are other changes in phenotype that may underlie this risk, or whether particular subgroups of offspring demonstrate risk that is not seen in the population as a whole.

The next objective was to investigate the impact of in utero exposure to preeclampsia on cardiometabolic and cardiovascular phenotype. This was undertaken by investigation of follow up data in two large birth cohorts with prospective follow up in in infancy, adolescence and young adulthood. The use of these cohorts allowed consideration of phenotype separately in those born preterm and those born at term and also allowed differentiation of the effects of maternal hypertension from those of prematurity and growth restriction. Individuals born very preterm

(<32 weeks gestation) to hypertensive pregnancies have transient alterations in glucose and lipid metabolism during the first weeks of life (Chapter 3). However as these very preterm infants age, maternal hypertensive disorders do not have an additional impact to that of prematurity in determining offspring blood pressure or metabolism (Chapter 4). Conversely in the moderately preterm born (32-37 weeks gestation) prematurity does not appear to have long-term impacts on cardiovascular phenotype, but in utero exposure to maternal hypertensive disorders independently predicts an 8mmHg increase in systolic blood pressure (Chapter 4). Finally for those born at term, exposure to any hypertensive disorder of pregnancy predicts an increased lifetime cardiovascular risk score at age 20 years. The detailed cardiovascular phenotype underling this increased risk varies, however, depending on whether the mother had true preeclampsia or non-protienuric gestational hypertension. At age 20 years offspring of non-protienuric hypertensive pregnancies demonstrated an increase in systolic blood pressure associated with increased BMI and risk of obesity. Conversely those whose mothers were preeclamptic remained lean, but demonstrated a similar magnitude of increase in blood pressure and were more likely to have clinically apparent hypertension (Chapter 5). In particular the association demonstrated in this thesis between in utero exposure to preeclampsia, increased lifetime QRISK and risk of hypertension has not been previously reported. This is presumably because the data presented in this thesis represents the results of two of the largest birth cohorts with sufficient lengths of follow-up to allow for clinical risk to be assessed. This highlights the unique advantages of the use of two large birth cohorts with detailed perinatal information to investigate these questions.

The final experimental objective of this thesis was to investigate the cardiovascular phenotype of infants born preterm to hypertensive pregnancies in order to determine whether alterations in cardiovascular phenotype are present from birth, develop in early post-natal life or do not develop until childhood or adolescence. Chapters 6 and 7 describe the study design and methods that were used to phenotype the newborn infants in the EPOCH study and demonstrate the

feasibility of detailed and comprehensive cardiovascular phenotyping in a premature born infant population. The selection of methods was made to allow for detailed structural and functional cardiovascular imaging and detailed microvascular assessment together with measurement of blood pressure, anthropometry and highly detailed perinatal data collection. These methods have been demonstrated in Chapter 7 to create reproducible data on infant cardiovascular phenotype. As with the long term follow up cohorts, a unique aspect of this data is the detailed perinatal information that was prospectively collected on all participants. This, combined with cardiovascular phenotyping at two time points, has allowed for a detailed consideration of the alterations of cardiovascular structure and function associated with the in utero insult of hypertensive disorders of pregnancy. In infancy the offspring of hypertensive pregnancies already demonstrate reductions in longitudinal cardiac strain independent of birth weight and gestation at delivery. The demonstrated reductions are comparable to those demonstrated in young adult life [7] and may relate to the more hypoxic in utero environment associated with a preeclamptic pregnancy (Chapter 8). There were no alterations in microvascular structure or function associated with being born to hypertensive pregnancies at either birth or three months of age. However by 3 months of age microvascular density already demonstrated a strong negative correlation with mean blood pressure (Chapter 9). As the offspring of hypertensive pregnancies will go on to have higher blood pressures as children and adolescents, follow-up in later life may reveal microvascular changes prior to the onset of overt hypertension.

10.2 Future Directions

There are several other avenues of investigation that could not be pursued over the course of this thesis but which would nonetheless be of future interest. For example it may be of considerable interest to undertake detailed cardiovascular phenotyping of those individuals who fall into high-risk categories, for example those who are already hypertensive or above the 75th centile for lifetime cardiovascular risk at age 20 years. As these individuals may well represent the subgroup

of those born to preeclamptic pregnancies at most extreme risk, a detailed understanding of their phenotype, and review of their perinatal course, may well be informative in understanding the relationship between preeclampsia and future cardiovascular risk.

Additionally the data on alteration in cardiac function presented in Chapter 8 of this thesis was undertaken using two-dimensional speckle tracking software and only circumferential and longitudinal strain were considered. The reproducibility of radial strain and rotational cardiac displacement are currently relatively low, however as speckle-tracking technology improves these may prove interesting avenues for investigation. Additionally three-dimensional speckle tracking and cardiac strain analysis is an emerging technique which may prove more reproducible and representative of true cardiac mechanics due to its ability to account for three dimensional cardiac movement [340, 341]. In the EPOCH Pilot study three-dimensional echocardiographic images were obtained on a number of the study participants, however analysis proved problematic primarily due to the rapid heart rate of the study population. With further improvement in this technology it may be possible to gain a truly global understanding of cardiac strain in the offspring of preeclamptic pregnancies. Finally it would be of substantial interest to investigate whether the changes in cardiac strain I have identified at three months of age are present in utero. Crispi and colleagues have previously demonstrated subclinical longitudinal systolic dysfunction in growth restricted foetuses in utero which persists to 5 years of age [342]. It is notable that a significant number of these 'growth restricted' foetuses were exposed to preeclampsia in utero. It would be interesting to consider whether these findings could be replicated in a purely preeclamptic cohort and differentiated from those of non-preeclamptic growth restriction.

10.3 CONCLUSIONS

In conclusion this work has begun to highlight the distinctive cardiovascular phenotype associated with being born to a preeclamptic pregnancy as one of chronically elevated blood pressure and

increased risk of clinically apparent hypertension from early adult life. These changes are present without significant alterations in glucose and lipid metabolism or adiposity, which are more closely associated with prematurity and late onset non-proteinuric hypertensive disorder of pregnancy respectively. Moreover the presence of early changes in cardiac function in those born to early onset hypertensive disorders of pregnancy, highlights the perinatal period as potentially important in the development of the long-term changes in cardiac phenotype. As preeclampsia affects between 1 and 5% of pregnancies globally, it is an easily identifiable and relevant factor in the cardiovascular health of up to three hundred million women and their three hundred million children worldwide. The long-term goal of this, and other work investigating the impact of perinatal factors on later cardiovascular health, is to reduce the progression of this large subgroup of individuals to overt cardiovascular disease by identifying groups at increased risk early in life to allow maximal impact of primary preventative strategies. The work of this thesis highlighting the importance of early life in determining the cardiovascular phenotype associated with hypertensive pregnancies suggests that interventions in this period could have long term benefits for effected individuals. At present further research is required to understand the mechanism underlying the observed changes in cardiovascular structure and function. This mechanistic understanding will then underpin future preventative interventions. While significant work remains to be done in order to fully elucidate the mechanisms linking maternal hypertensive disorders of pregnancy and later cardiovascular events, this thesis has highlighted the key importance of in utero exposure to preeclampsia in determining cardiovascular phenotype and risk. Importantly it identifies the offspring of preeclamptic pregnancies as those who would benefit from early and targeted cardiovascular primary prevention strategies that would likely result in both benefit to the individual and contribute to reductions in the population burden of cardiovascular disease in this group.

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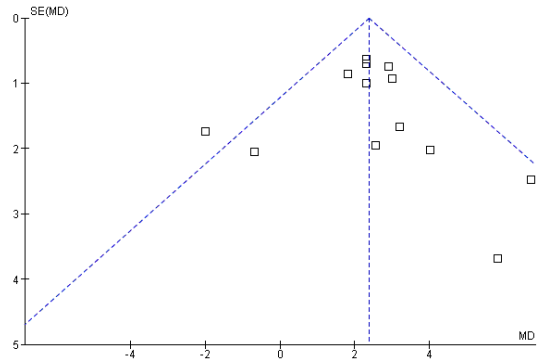
APPENDICES

APPENDIX 1 – SEARCH STRATEGY FOR SYSTEMATIC REVIEW AND META-ANALYSIS

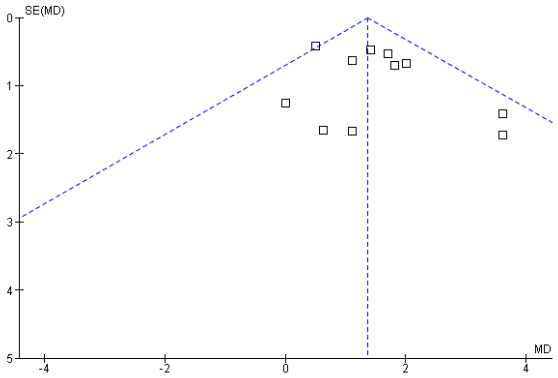
1. preeclampsia or pre-eclampsia or toxaemia or toxemia or gestation* hypertension or pregnancy induced hypertension or eclampsia
2. limit 1 to human
3. children or offspring or neonates or infants
4. limit 3 to human
5. 2 and 4
6. myocardial ischaemia or coronary atheroclerosis or coronary disease or cardiovascular disease or cardiovascular risk or myocardial infarction or angina.
7. limit 6 to human
8. 5 and 7
9. peripheral artery disease or vascular disease or arterial occlusive disease or cerebrovascular disease or cerebrovascular accident or stroke
10. limit 9 to human
11. 5 and 10
12. hypertension or high blood pressure or elevated blood pressure or increased blood pressure or blood pressure
13. limit 12 to human
14. 5 and 13
15. metabolic syndrome or metabolic syndrome X
16. limit 15 to human
17. 5 and 16
18. anthropometry or BMI or body mass index or weight or waist hip ratio
19. limit 18 to human
20. 5 and 19
21. insulin or glucose or serum insulin or serum glucose or insulin resistance or imapired glucose tolerance or diabetes
22. limit 21 to human
23. 5 and 22
24. lipids or lipoproteins or serum cholesterol or total Cholesterol or LDL or low density lipoprotein or HDL or high density lipoprotein or TG or Triglyceride
25. limit 24 to human
26. 5 and 25
27. arterial stiffness or aortic stiffness or aorta or pulse wave velocity or central pulse wave or augmentation index or endothel* or endothelial dysfunction or flow mediated dilalatation or endothelial mediated vasodilatation or intima media thickness or microvascular function or laser doppler flowmetry or laser doppler fluximetry
28. limit 27 to human
29. 5 and 28 3
30. 8 or 11 or 14 or 17 or 20 or 23 or 26 or 29

APPENDIX 2 – FUNNEL PLOTS FOR META-ANALYSIS

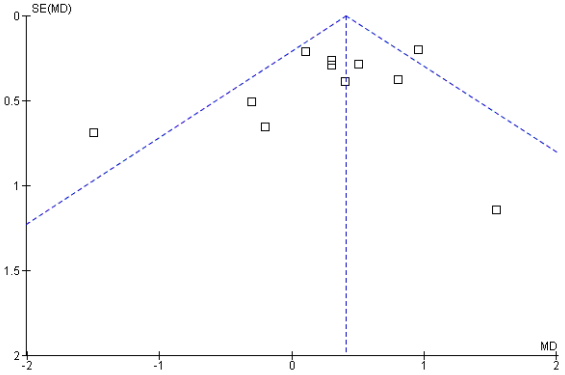
FUNNEL PLOT - SYSTOLIC BLOOD PRESSURE



FUNNEL PLOT - DIASTOLIC BLOOD PRESSURE



FUNNEL PLOT – BODY MASS INDEX



APPENDIX 3: RESULTS OF RANDOM EFFECTS VS FIXED EFFECTS META-ANALYSIS

Outcome	Fixed Effect Model	Random Effect Model
SBP	2.39 (1.73 -3.05) p < 0.00001	2.37 (1.55 – 3.20) p < 0.00001
SBP Age <10	2.91 (1.55-4.27) p < 0.0001	2.92 (1.06 – 4.77) p = 0.002
SBP Age > 10	2.24 (1.49 – 2.98) p < 0.00001	2.18 (1.24 – 3.12) p < 0.00001
SBP Male	2.48 (1.18 – 3.79) p = 0.0002	2.48 (1.18 – 3.79) p = 0.0002
SBP Female	2.41 (1.29 – 3.53) p < 0.0001)	2.24 (0.78 – 3.70) p = 0.003)
SBP Term Normal Birth weight	2.26 (1.35 – 3.16) p < 0.00001	1.94 (0.4 – 3.48) p = 0.01
DBP	1.35 (0.9 – 1.80) p < 0.00001	1.58 (0.90 – 2.26) p < 0.0001
DBP Age <10	2.06 (0.99-3.12) p = 0.0002	2.06 (0.99-3.12) p = 0.0002
DBP Age > 10	1.19 (0.69 – 1.70) p < 0.00001	1.43 (0.60 – 2.25) p = 0.0007
DBP Male	0.67 (-0.12 – 1.45) p = 0.1	1.60 (-1.31 – 4.51) p = 0.28
DBP Female	1.70 (0.89 – 2.51) p < 0.0001	1.70 (0.89 – 2.51) p < 0.0001
DBP Term Normal Birth weight	1.48 (0.82 – 2.14) p < 0.0001	1.48 (0.82 – 2.14) p < 0.0001
BMI	0.62 (0.41 – .84) p < 0.00001	0.56 (0.25- 0.87) p = 0.0005
BMI Age <10	0.15 (-0.34 – 0.64) p= 0.55	0.14 (-.039 – 0.66) p = 0.61
BMI Age >10	0.73 (0.50 – 0.97) p < 0.00001	0.70 (0.39 – 1.01) p < 0.0001
BMI term Normal Birth Weight	0.75 (0.38 – 1.12) p = 0.001	0.69 (0.22 – 1.16) p = 0.004