HYPERTENSIVE PREGNANCY AND FUTURE MATERNAL CARDIOVASCULAR PHENOTYPE

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DEDICATIONS

To Catriona, Theo and Fern with all my love

ABREVIATIONS

AIx	.....	Augmentation index
ARV	.....	Average real variability
AUC	.....	Area under curve
BMI	.....	Body mass index
BP	.....	Blood pressure
BPM	.....	Beats per minute
BSA	.....	Body surface area
Chol	.....	Cholesterol
CoV	.....	Coefficient of variance
DBP	.....	Diastolic blood pressure
EDV	.....	End diastolic volume
EF	.....	Ejection fraction
ESV	.....	End systolic volume
FA	.....	Fractional anisotropy
GH	.....	Gestational hypertension
HDL	.....	High density lipoprotein
HDP	.....	Hypertensive disorders of pregnancy
HF	.....	High frequency
HOMA IR	.....	Homeostatic model assessments for insulin resistance
HTN	.....	History of hypertensive pregnancy
LA	.....	Left atrium
LDA	.....	Linear discrimination analysis
LDL	.....	Low density lipoprotein
LF	.....	Low frequency
LV	.....	Left ventricle
LVM/EDV	.....	Left ventricular mass / end diastolic volume
MAP	.....	Mean arterial pressure
MD	.....	Mean diffusivity
NPD	.....	History of normotensive preterm delivery
NTN	.....	History of normotensive pregnancy
PCA	.....	Principal component analysis
PET	.....	Preeclampsia
PlGF	.....	Placental growth factor
pNN50	.....	Percentage of NN intervals with more than 50msec variation than the previous NN interval
PP	.....	Pulse pressure
PWV	.....	Pulse wave velocity
RMSSD	.....	The square root of the mean of the sum of the squares of differences between adjacent NN intervals
RV	.....	Right ventricle
RVM/EDV	.....	Right ventricular mass / end diastolic volume
SBP	.....	Systolic blood pressure
SD	.....	Standard deviation

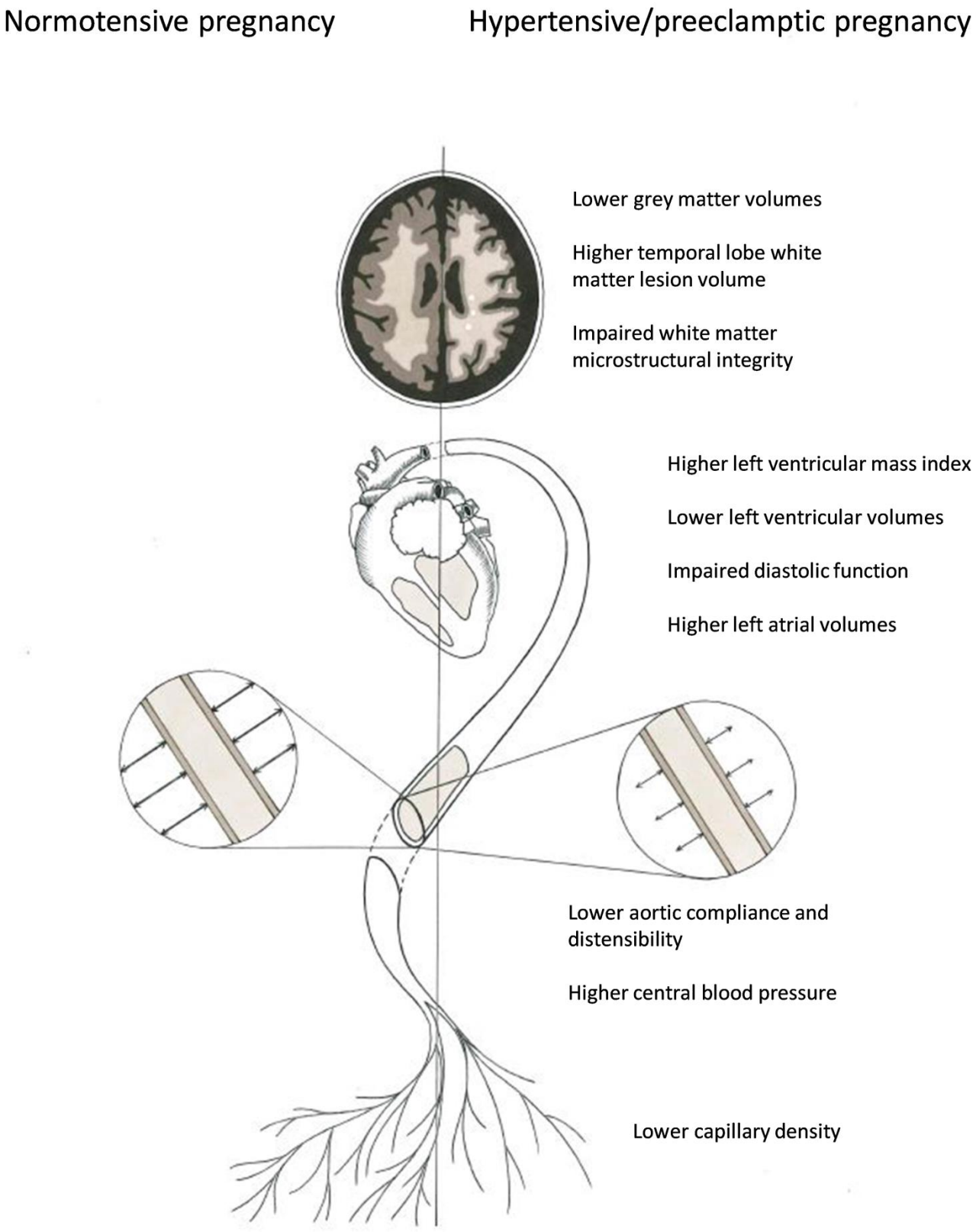
SDNNindex	……	Mean of standard deviations of all NN intervals
SDSD	……	Standard deviation of differences between adjacent NN intervals
sENG	……	Soluble endoglin
sFLT-1	……	Soluble fms-like tyrosine kinase-1
SSFP	……	Steady state free precession
SV	……	Stroke volume
TChol	……	Total cholesterol
TDI	……	Tissue Doppler imaging
TG	……	Triglycerides
UP	……	History of uncomplicated pregnancy
VEGF	……	Vascular endothelial growth factor
VHF	……	Very high frequency
VLF	……	Very low frequency
WMH	……	White matter hyperintensities
WML	……	White matter lesions
WSD	……	Weight standard deviation

Hypertensive pregnancy and future maternal cardiovascular phenotype

H Boardman, Green Templeton College, D.Phil submitted Trinity Term 2016

Hypertensive disorders of pregnancy are common and associated with persistent increased risk of maternal cardiovascular disease. However it is unclear which mechanisms are behind this increased risk and whether they are explained by traditional cardiovascular risk factors. I sought to characterise cardiovascular phenotypic patterns after hypertensive pregnancy and explore relevant relationships with risk factors and pregnancy characteristics. Women were identified through the John Radcliffe Hospital maternity records and recruited 5-10 years after hypertensive (cases) or normotensive pregnancy (controls). Detailed phenotyping to characterise cardiac, vascular and cerebral phenotypic differences was carried out using a range of techniques including magnetic resonance imaging, cardiac and vascular ultrasound, intravital video capillaroscopy and ambulatory monitoring. Women after hypertensive pregnancy were more likely to have adverse cardiac structure than women after normotensive pregnancy, with higher left ventricular mass and lower left ventricular volumes compared to women with a history of normotensive pregnancy. They were also more likely to have reduced aortic distensibility. Characterisation of cerebral structure demonstrated that women after preeclampsia had lower grey matter volumes and greater white matter damage compared to a control population. There were also distinct microvascular and blood pressure differences with capillary rarefaction and higher ambulatory blood pressure in women after hypertensive pregnancy compared to women after normotensive pregnancy. There were no significant differences in circulating angiogenic factors or autonomic function. Differences in left ventricular mass, cerebral white matter damage and capillary rarefaction were not explained by conventional risk factors, suggesting novel, disease-specific mechanisms may be responsible. This encompassing characterisation of postpartum maternal cardiovascular phenotype after hypertensive pregnancy highlights widespread adverse differences which are not fully explained by traditional risk factors. Understanding whether targeting these adverse phenotypic features during hypertensive pregnancy or in the early postpartum period affects clinical endpoints will be of future interest.

Figure 1. Illustration of phenotypic differences in women after hypertensive/preeclamptic pregnancy compared to normotensive pregnancy



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Introduction

Chapter 1

1.1 Cardiovascular disease in women

Cardiovascular disease is the most common cause of death in women and men both in high and low-middle income countries (1-3). It is also a major cause of morbidity across the world and is associated with a huge economic burden, both from direct health costs and indirect costs (4, 5). Although the mortality rate associated with ischaemic heart disease has fallen in recent years, the prevalence is rising with an increasing population living with cardiovascular disease, likely due to an ageing population and increasing prevalence of cardiovascular risk factors such as obesity and diabetes (6-9).

It has now been identified that there are subtle differences in cardiovascular disease between sexes in the presentation, management and outcomes (10, 11). Part of the explanation for these differences may be inherent differences in the pathophysiology between women and men (12).

In coronary artery disease, symptomatic women are less likely to have obstructive epicardial coronary artery disease and more likely to have plaque erosion and microvascular disease than symptomatic men (13). Thus diagnostic tests which focus on

identifying culprit obstructive lesions are less effective in women (14). Differences in the effectiveness of medication also exist, a meta-analysis of aspirin for primary prevention of cardiovascular disease, demonstrated a lack of evidence of efficacy in women for preventing myocardial infarction, whereas the evidence supported its use in men (15, 16). Women also tend to be less likely to be prescribed secondary prevention medication, such as lipid lowering agents and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and also less likely to be referred for revascularisation (11).

In addition to coronary artery disease, left ventricular hypertrophy, heart failure and stroke also exhibits sex differences. Women with left ventricular hypertrophy are at greater mortality risk than men (17). Women with symptoms of heart failure are more likely to have preserved ejection fraction (18) and there is some evidence that women have a lower risk of death, irrespective of ejection fraction (19, 20). The proportion of women dying as a result of a stroke is higher than in men (21) and female sex is a risk factor for stroke in individuals with atrial fibrillation (22) and is included in a commonly used stroke risk calculator (CHA_2DS_2-VASc) (23).

Part of the reason for sex differences seen in cardiovascular disease may be distinct pathophysiological factors which are associated with vascular function (24). Estrogen has been shown to have beneficial effects on lipid profile, vasodilation and prevention of atherosclerosis development (25-27). Ischaemic heart disease rates are low in women below the age of 50, the age of menopause in most women, but rapidly rise over the age of 60 (9). It has also been shown that early menopause is associated with increased cardiovascular risk (28, 29). Existing risk scores for evaluating risk of cardiovascular disease and death in patients with or without acute coronary syndrome do not currently include female specific risk factors such as age of menopause or menopausal status (30-

32). Current risk calculators have been developed from data collected from studies which have underrepresented women; a review of coronary heart disease studies conducted between 1994 and 2010 and including more than 75 000 patients showed only a third of participants were women (11). This need for updated risk scores, including female specific risk factors, to be better tailored to female patients has been increasingly highlighted in recent years (33-36).

1.2 Pregnancy is a time of unique haemodynamic and cardiovascular challenge

Pregnancy is also a sex-specific condition which is associated with significant cardiovascular challenge. During uncomplicated pregnancy cardiac output increases between 31-48%, peaking in early 3rd trimester (37, 38). This adaptation is thought to be driven by rises in oestrogen and relaxin levels which lead to a fall in systemic resistance, followed by a rise in heart rate (38-41). This increase in cardiac output is predominantly directed towards the increased demands from the uterus, kidneys and skin (42). In parallel with increases in stroke volume myocardial mass also increases, reaching a peak increase of 34-53% compared to non-pregnant levels in early 3rd trimester (37, 38). Cardiovascular changes appear to be greater in multiparous women and in women with twin pregnancies (43, 44).

The increase in stroke volume is typically matched by an increase in end diastolic volumes and usually ejection fraction does not change significantly through pregnancy (45). Reports of changes in more subtle markers of systolic function, such as long-axis displacement and tissue Doppler imaging, have been mixed with reports of both increased and decreased values during normal pregnancy (46, 47). Diastolic function however has been consistently reported to be decreased during pregnancy, measured by

both E: A ratio and tissue Doppler imaging (47-49) and both left and right atria are increased in size (50).

Global arterial compliance and distensibility are increased in normal pregnancy, 30% and 10% respectively (51-53). Augmentation index is reduced, reaching a nadir in the 2nd trimester and returning to early pregnancy levels in the latter half of the 3rd trimester (54, 55). Flow mediated vasodilatation, an indicator of endothelial function, is increased up to 47% compared to non-pregnant controls (56, 57). Systolic blood pressure does not change significantly through pregnancy, however diastolic blood pressure and mean arterial pressure start to fall during the 1st trimester, reaching a nadir around 20 weeks gestation, before rising in the 3rd trimester (37, 38, 51). Dermal capillary density has been shown to increase during pregnancy, reaching a peak in the 2nd trimester, 10-13% higher than non-pregnant controls, and mirroring the fall in diastolic blood pressure seen (58). During normal pregnancy parasympathetic function is attenuated with upregulation of sympathetic activity, manifesting in reduced heart rate variability (59-61). Literature reports of whether blood pressure variability is altered in normal pregnancy compared to non-pregnant controls are inconsistent (60).

Pregnancy related cardiovascular adaptations in normotensive pregnancies attenuate after delivery with the majority of cardiac, vascular and autonomic changes resolving by 6 months (37, 38, 44, 58, 62-66).

1.3 Hypertensive disorders of pregnancy

Pregnancy is common, affecting 80% of women during their lifetime in the UK and 74% in the United States (67) and pregnancy complicated by de novo hypertension (which include preeclampsia and gestational hypertension) is frequent and increasing in

incidence, affecting 6-10% of pregnancies (68-71). Hypertensive disorders of pregnancy are defined by the International Society for the Study of Hypertension in Pregnancy (ISSHP) as new onset hypertension (≥140 mmHg systolic or ≥90 mmHg diastolic) after 20 weeks gestation (72). This umbrella definition includes both gestational hypertension and preeclampsia. Preeclampsia is defined by ISSHP as: hypertension and the presence of one or more of the following new-onset conditions: proteinuria (≥300mg/day or "2+" on dipstick testing), maternal organ dysfunction (renal, liver, neurological or haematological) or uteroplacental dysfunction (characterised by fetal growth restriction) (72). Eclamptic seizures and haemolysis, elevated liver enzymes and thrombocytopaenia (often termed HELLP syndrome) can complicate more severe cases of preeclampsia. Gestational hypertension is new onset hypertension after 20 weeks gestation without any of these aforementioned conditions which define preeclampsia.

The main treatments of hypertensive disorders of pregnancy are antihypertensive medication and delivery. First line antihypertensive agents include labetalol, nifedipine and hydralazine (70). Delivery is recommended in severe preeclampsia or when control of hypertension is not achieved (27). After delivery blood pressure typically falls, this is more common in milder forms of hypertension during pregnancy, and many women no longer need antihypertensive medication after a few weeks (73). However a minority of women can develop preeclampsia during the postpartum periods and many women, particularly those with early onset preeclampsia, have blood pressure which stays elevated long term (74, 75).

1.4 History of hypertensive disorders of pregnancy identify a population at increased cardiovascular risk

In the past few years it has become established that a history of hypertensive disorder of pregnancy is a risk factor for cardiovascular disease in later life, with studies demonstrating this at a range of time points post-partum from 1 year (76), up to 37 years (77). Meta-analyses have shown that a history of preeclampsia has been associated with up to six fold risk of hypertension, twofold risk of ischaemic heart disease, almost fourfold risk of cerebrovascular disease, almost twofold risk of peripheral artery disease, 2-8 times risk of cardiovascular mortality, threefold risk of type 2 diabetes and a twofold risk of mortality of any cause (78-82). In fact one study using registry data from Norway reported a more than 8 fold increase in death from cardiovascular causes in women with preeclampsia and preterm delivery (81).

Women with a history of hypertensive disorders of pregnancy (not limited to preeclampsia) are also at increased risk of cardiovascular disease; almost 1.5 times increase in ischaemic heart disease, fivefold risk of hypertension, threefold risk of cardiovascular death, almost twofold risk of heart failure, twofold risk of stroke and threefold risk of type 2 diabetes (80, 82-84). Indeed there is evidence in populations of women with a history of gestational hypertension, excluding preeclampsia, of an almost twofold increased risk of cardiovascular disease and almost threefold increased risk of hypertension (85, 86).

There is also evidence of a dose response according to the severity of hypertensive disorder of pregnancy and the risk of cardiac disease compared to uncomplicated pregnancy. Across a spectrum of preeclampsia: uncomplicated preeclampsia (“mild”) is associated with a twofold risk, preeclampsia complicated by either eclampsia or poor fetal growth (“moderate”) with a threefold risk and preeclampsia complicated by

preterm delivery and/or fetal death ("severe") a fivefold risk (79). This dose effect is also seen between preeclampsia, with the greatest cardiovascular risk, and gestational hypertension; and within preeclampsia with early gestational onset of disease being associated with greater cardiovascular risk than later gestational onset (87, 88).

1.5 Some risk factors for hypertensive disorders of pregnancy are common to cardiovascular disease

One of the explanations for the association between hypertensive disorders of pregnancy and risk of cardiovascular disease in later life may be common risk factors; such as raised blood pressure, high maternal age, diabetes (both type 1 and gestational), obesity, renal disease and a history of hypertensive disorder of pregnancy, though difficult to explain is that smoking has been shown to be protective of preeclampsia (68, 89-94). However other risk factors for hypertensive disorders of pregnancy, such as low age (under 20 years of age), autoimmune disease, nulliparity or an increased interval between pregnancies (>10 years), twin birth, ethnicity (non-white), assisted conception and change in paternity are less well associated with cardiovascular disease and suggest a genetic and immunological component. In fact epidemiology studies have shown a genetic constituent to the pathophysiology of both preeclampsia and gestational hypertension, though this likely corresponds to small effects from multiple genes rather than a single gene (95, 96). The estimated heritability for preeclampsia and gestational hypertension, has been shown to range between 54% and 24% (97).

These common risk factors shared by both hypertensive disorders of pregnancy and cardiovascular disease raises the question of whether future cardiovascular risk associated with affected pregnancies relates to an underlying adverse cardiovascular

phenotype which predates the affected pregnancy or whether changes occur during the affected pregnancy itself influencing the future cardiovascular phenotype (98). Analysis of this relationship is complex, as retrospective analysis is not without limitations and adequately powered longitudinal studies from pre-conception to post-partum are limited. A rare study exploring this by Romundstad et al including over 3000 women, though less than 300 women had a history of hypertensive disorder of pregnancy, found that approximately 50% of differences in blood pressure several years after pregnancy were explained by blood pressure differences pre-pregnancy (99).

1.6 Pathophysiology of hypertensive disorders of pregnancy

The mechanisms responsible for hypertensive disorders of pregnancy are not fully elucidated though many potential key factors have been highlighted. One of the earliest manifestations which is widely supported to be important in the process is the impaired implantation of the placenta (100). The failure of the cytotrophoblast invasion into the spiral arteries leads to reduced uteroplacental perfusion and ischaemia (101-104). Histological specimens from the placentas of women with hypertensive disorders of pregnancy show the presence of acute atherosis, in some of the placental vessels, characterised by vascular necrosis and foam cell infiltration (104). These vascular findings often relate to areas of placental infarct (105) and resemble early atherosclerosis (106). The ischaemic placenta is thought to result in release of soluble placental factors into the maternal circulation which promote anti-angiogenesis and inflammation and lead to widespread endothelial dysfunction (107, 108). Reduction in nitric oxide synthesis and enhanced endothelin and thromboxane production have all been suggested as contributors to the resultant endothelial dysfunction (109-112). Inflammatory mediators, such as tumour necrosis factors-alpha (TNF-a) are also

increased in women with preeclampsia and are known to be associated with reduced endothelial function (113, 114).

Angiogenic factors have been shown to be altered 5-10 weeks prior to the onset of hypertension, with increased soluble fms-like tyrosine kinase 1 (sFLT-1) and reduced placental growth factor (PlGF) (115). The case for an important role of sFLT-1 and sEng (a related angiogenic factor) is supported by findings that exposure to pregnant rats with adenovirus expressing sFLT-1 and sEng causes a syndrome very similar to preeclampsia (116). sFLT-1 and sEng impair the binding of pro-angiogenic factors to their receptors, vascular endothelial growth factor (VEGF) and PlGF to the former and TGF-β to the latter (117). In concert with changes in angiogenic signalling markers structural changes of microvasculature also precede the onset of preeclampsia and are reduced compared to both normotensive pregnancy controls and non-pregnant controls (58, 118-120).

It has also been suggested that preeclampsia is not solely caused by the placenta and that underlying cardiovascular dysfunction may also be important (121). This might relate to the inability of the maternal cardiovascular system to adapt to a poorly implanted placenta and manage the associated cascade of antiangiogenic and inflammatory factors. This may be particularly apparent in early onset disease when cardiovascular reserve is exhausted at an earlier gestation. Cardiac dysfunction has been shown to be present in women in mid gestation, prior to the diagnosis of early onset preeclampsia, though the same findings were not identified in women who later developed late onset preeclampsia (122). The difference in cardiac findings between early and late onset preeclampsia may relate to the timing of investigation and diagnosis, with cardiac dysfunction developing just a few weeks prior to the onset of hypertension, or late onset preeclampsia being associated with a more subtle phenotype

of cardiac dysfunction, or it may relate to aetiological differences relating to cardiovascular dysfunction. Further support for the relevance of cardiovascular structure and function has been reported by Novelli et al (123) who demonstrated that adverse cardiac structure and function was far more prevalent, when measured at mid-gestation, in women who later developed complications during pregnancy such as hypertension, preterm delivery, growth restriction and placental abruption, than in women who did not develop these complications.

1.7 Cardiovascular adaptations during hypertensive disorder of pregnancy

The diagnostic criteria for preeclampsia and gestational hypertension include that hypertension should present after 20 weeks gestation; however vascular changes likely precede this, with early increases in arterial stiffness in both conditions (124-126). Reports of cardiac differences during hypertensive pregnancies have been mixed. A historic study using invasive measurements taken from women with untreated preeclampsia showed reduced cardiac index and increased vascular resistance compared to normotensive pregnant controls, though these findings were attenuated in women with treated preeclampsia (127). Similar findings have been shown in women with gestational hypertension, by Valensise et al with reduced cardiac output compared to normotensive pregnant women (128).

Left ventricular mass is increased disproportionately in women with a hypertensive disorder of pregnancy, this may well be an adaptive response to the increased afterload seen in subgroups of the hypertensive disorders with increased afterload, though if reduced afterload is present in certain groups the mechanism behind the exaggerated hypertrophy remains to be explained (128-130). Systolic function measured by ejection-phase parameters has been reported both increased, and reduced in women with hypertensive disorder of pregnancy (128, 129, 131). However more subtle markers of

systolic function using tissue Doppler indicate a reduction in function (130). Diastolic function is also reduced both in women with preeclampsia and gestational hypertension, with a greater reduction in earlier onset disease (128, 130, 131). In keeping with this left atrial dimensions are found to be increased (131).

1.7.1 Autonomic function during hypertensive disorder of pregnancy

Women with hypertensive disorders of pregnancy demonstrate reduced nocturnal blood pressure dipping and even reversal of diurnal blood pressure rhythms compared to normotensive pregnant controls (132, 133). Reports have also shown reduced heart rate variability, both in low and high frequency power bands, in women with preeclampsia compared to normotensive pregnant women (61). However systematic review data suggests overall there are no significant differences in blood pressure variability between women with preeclampsia and healthy pregnancies (60).

1.7.2 Cerebral changes during hypertensive disorder of pregnancy

Women with preeclampsia are at risk of eclampsia and death (134). The cause of eclampsia is not fully understood but it is thought that an important mechanism is disruption of the blood-brain barrier and development of cerebral oedema (135, 136). Indeed cerebral changes are noted in preeclampsia without eclampsia, characterised by increased cerebral blood flow and impaired autoregulation, which could be a risk factor for vasogenic oedema (137, 138). Magnesium sulphate has been shown to reduce middle cerebral artery pulsatility index in women with preeclampsia and might explain its mechanism of action in preventing and treating eclampsia in this population (139).

1.8 Cardiovascular differences after hypertensive disorders of pregnancy

Several studies have explored whether cardiovascular changes seen during hypertensive disorder of pregnancy persist after delivery. Identifying persistent adverse phenotypic changes is important for establishing relevant mechanisms leading to increased cardiovascular risk in later life.

At 3 months postpartum it has been shown that left ventricular wall thickness remains increased after preeclampsia compared to normotensive pregnancy, though no differences in left ventricular cavity dimensions or left ventricular mass index were detected (129). Melchiorre et al reported on a larger series of women during pregnancy and at 1 year postpartum and showed that cardiac dysfunction persisted in those with preeclamptic pregnancies compared to those after normotensive pregnancy, with the greatest difference found in those with preterm delivery (140). Preterm preeclampsia was associated with altered left ventricular geometry and stage B (asymptomatic) heart failure; whereas both preterm and term preeclampsia were associated with left ventricular systolic and diastolic dysfunction and right ventricular diastolic dysfunction at 1 year postpartum.

Only a few studies have explored cardiac differences several years after pregnancy; a small longitudinal study reported by Ghossein-Doha et al (141) found no differences in left ventricular structure or diastolic function 1 and 14 years after preeclamptic and uncomplicated pregnancies. However Strobl et al in a small study of women 13-18 years after pregnancy demonstrated impaired cardiac function in women with a history of preeclampsia using myocardial performance index, though individual measures of systolic and diastolic function, independent of loading conditions, were not different (142). More recently Scantlebury et al showed, in the largest of these studies, an

increased risk of left ventricular hypertrophy measured using echo in women decades after a hypertensive pregnancy (143).

Women with persistent cardiac dysfunction after preeclampsia have been shown to be at increased risk of further episodes of preeclampsia in future pregnancies (144). This might represent a causative role of poor cardiac reserve leading to further preeclamptic episodes, however it might also be explained by persistent cardiac dysfunction being a marker of severe preeclampsia and the causation might relate to an entirely different pathological mechanism.

It will be of interest to discover if there are both cardiac structural and functional differences in young healthy women after hypertensive pregnancy, which might explain the increased risk seen in this population. It is also relevant whether differences in cardiac structure are explained by differences in blood pressure or if other mechanisms are responsible.

1.8.1 Vascular differences after hypertensive disorders of pregnancy

A review by Lazdam et al (145) highlighted a range of differences in vascular function in the months and years after preeclampsia (Table 1) with flow mediated dilatation (FMD) reported reduced up to 3 years postpartum, reduced microvascular function 2 decades after delivery, though reports of arterial stiffness several years after delivery were inconsistent.

A meta-analysis of arterial stiffness during and after preeclampsia showed increased pulse wave velocity up to 2 years after affected pregnancy, though findings for

differences in augmentation pressure were mixed with the suggestion that it is increased after early, but not late, onset preeclampsia (146).

Reports of arterial stiffness after gestational hypertension or in populations including both preeclampsia and gestational hypertension are sparsely reported and study populations tend to be smaller than those reported for preeclampsia. A small study, including less than 50 women, studied 3 months postpartum showed no difference in arterial stiffness between women with a history of normotensive pregnancy and those with a history of hypertensive pregnancy; however this study may well have been underpowered to detect differences (147). In a slightly larger study Ehrenthal et al reported persistent increased arterial stiffness, measured using PWV and augmentation index, 1 year after hypertensive pregnancy, though differences in PWV were no longer significant after adjustment for blood pressure (148).

A meta-analysis of flow mediated dilatation (FMD) and preeclampsia reported reduced values before, during and up to 3 years after preeclamptic pregnancy compared to normotensive pregnancy, indicating impaired endothelial function (108). However this review also carried out a subgroup analysis of 4 studies comparing FMD in the "late" postpartum period (6-13 years after affected pregnancy) and showed no difference in FMD. FMD after gestational hypertension is much less reported, though in a small study Noori et al reported no differences compared to women with a history of normotensive pregnancy (149).

Whether long term differences in maternal arterial stiffness exist remote to hypertensive pregnancy is yet unclarified. If present arterial stiffness might represent a valuable surrogate marker for identifying high risk populations and potentially a therapeutic

target in those with a history of hypertensive pregnancy and at increased cardiovascular risk.

1.8.2 Cerebral structure differences after hypertensive disorders of pregnancy

White matter lesions are cerebral structural changes associated with ageing, raised blood pressure and are recognised risk factors for stroke (150). They have recently been demonstrated to be more common in women 5 years after preeclampsia compared to women after uncomplicated pregnancy (151). Women with a history of hypertensive disorder of pregnancy have also been shown to have reduced brain volumes and reduced cognitive performance compared to women with a history of normotensive pregnancy several decades after affected pregnancy (152). It is unclear if brain volumes are reduced throughout cerebral structure or if grey or white matter is preferentially affected. It is also not clear whether differences in brain structure are explained by differences in blood pressure and other cardiovascular risk factors or if a history of hypertension during pregnancy is additive.

1.8.3 Differences in microvascular structure and angiogenic factors after hypertensive disorders of pregnancy

There are limited reports of microvascular structure and angiogenic factors after hypertensive pregnancy. Nama et al reported lower dermal capillary density in women 10 weeks after delivery from preeclamptic pregnancy compared to normotensive pregnancy (118). Noori et al reported higher placental growth factor (PlGF) 12 weeks after hypertensive pregnancy (149). More remote from pregnancy higher levels of sFLT

have been reported 18 months and up to 8 years after preeclamptic pregnancy (153, 154).

Soluble FLT-1 is known to be associated with heart failure risk and prognosis (155, 156) and PlGF levels have been shown to have power to predict coronary heart disease in non-pregnant women (157). However it is not known if a range of angiogenic factors are altered long term after hypertensive disorders of pregnancy, how these relate to structural changes in microvasculature and organ damage, and whether they explain a proportion of the elevated cardiovascular risk seen in this population.

Table 1. Review of studies assessing vascular function after preeclampsia (145).

TABLE 1: Studies assessing the long-term impact of preeclampsia on vascular function. AIx (augmentation index) and PWV (pulse wave velocity) are robust surrogate markers of aortic stiffness. Endothelial-mediated vasodilatation was measured in conduit arteries by FMD (flow-mediated dilatation) and in the resistance arteries using VOP (venous occlusion plethysmography) to measure FBF (forearm blood flow) or peripheral arterial tonometry (PAT) to measure RHI (reactive hyperaemic index). Mircovascular endothelial responses were quantified in the microcirculation using LDF (laser Doppler flowmetry) and iontophoresis. SGA refers to small for gestational age.

Study	Subjects (Preeclampsia/control)	Interval after delivery	Vascular measures	Results in women with previous preeclampsia
Aortic stiffness				
Robb et al. 2009 [13]	15/22	7 weeks	AIx, PWV	Elevated PWV and AIx.
Yinon et al. 2010 [14]	24/16	6–24 months	AIx	Increased AIx in women with early onset preeclampsia.
Evans et al. 2011 [15]	18/50	6–36 months	PWV	No significant differences in central arterial stiffness.
Páez et al. 2009 [16]	20/20	2 years	PWV, AIx	Elevated PWV and AIx.
Elvan-Taşpinar et al. 2005 [17]	44/46	4–56 months	PWV	Elevated PWV.
Lampinen et al. 2006 [18]	30/21	5-6 years	AIx	No significant differences in AIx.
Subclinical atherosclerosis				
Blaauw et al. 2006 [19]	22/22	≥3 months	Femoral and carotid IMT	Increased IMT with early onset preeclampsia.
Conduit artery endothelial function				
Kuscu et al. 2003 [20]	15/11	2 and 6 weeks	FMD	Reduced FMD both during pregnancy and postpartum (no control data postpartum).
Noori et al. 2010 [21]	45/21	12 weeks	FMD	No significant differences in FMD compared to controls.
Yinon et al. 2010 [14]	24/16	6–24 months	FMD	Reduced FMD in women with early onset preeclampsia.
Hamad et al. 2007 [22]	18/17	15 ± 3 months	FMD	Reduced FMD and endothelial independent dilatation in women with severe preeclampsia.
Germain et al. 2007 [23]	25/22	16 ± 3.5 months	FMD	Reduced FMD.
Páez et al. 2009 [16]	20/20	2 years	FMD	Reduced FMD.
Chambers et al. 2001 [24]	113/48	3 years median	FMD	Reduced FMD particularly in women with recurrent preeclampsia and recovery of endothelial function with ascorbic acid.
Resistance artery endothelial function				
Lommerse et al. 2007 [25]	32/10	0.64–1.6 years	VOP	No significant difference in FBF.
Agatisa et al. 2004 [26]	16/14	9.9 ± 0.5 months	VOP	Reduced endothelium-mediated FBF.
Evans et al. 2011 [15]	18/50	6–36 months	VOP	Reduced FBF in response to mental stress.
Lampinen et al. 2006 [18]	30/21	5-6 yrs	VOP	Significantly reduced FBF to acetylcholine (ACh) and sodium nitroprusside (SNP).
Kvehaugen et al. 2011 [27]	26/17	5–8 years	PAT	RHI comparable between preeclamptic women and controls. Women with SGA baby had significantly lower RHI.
Cutaneous microvascular function				
Khan et al. 2005 [28]	15/54	6 weeks	LDF and iontophoresis	No significant differences between women in endothelial dependent or independent microvascular dilatation.
Blaauw et al. 2005 [29]	25/23	7.0 ± 2.8 months	LDF and iontophoresis	Greater microvascular vasodilator responses in preeclampsia.
Ramsay et al. 2003 [30]	10/10	16–25 years	LDF and iontophoresis	Reduced response to endothelial-dependent and -independent dilatation.

1.8.4 Autonomic differences after hypertensive disorders of pregnancy

There are few reports of autonomic dysfunction after hypertensive pregnancy. Baroreceptor sensitivity in the first year after preeclamptic pregnancy has been shown to be lower than in a control population (158). Non-dipping blood pressure patterns and separately altered heart rate variability have been shown to be associated with organ damage in a non-pregnant hypertensive population (159, 160). Hypertensive disorders of pregnancy represent a distinct phenotype to chronic hypertension and are associated with autonomic dysfunction at the time of pregnancy (61). If this autonomic disruption persists it may explain a proportion of the future cardiovascular risk seen in this population.

1.9 The spectrum of hypertensive disorders of pregnancy

Hypertensive disorders of pregnancy describe a group of diseases with a spectrum of maternal risk (87). Some reports in the literature suggest they have distinct pathophysiological characteristics (161, 162). However preeclampsia and gestational hypertension share many common features, from diagnostic criteria (72), risk factors (68, 163, 164), anti-angiogenic associations (165), genetic factors (166) and cardiovascular manifestations, as detailed earlier in this chapter. In fact it is not uncommon for women with gestational hypertension to progress to preeclampsia and women with previous episodes of preeclampsia or gestational hypertension are at increased risk of both conditions in subsequent pregnancies (64, 91).

It is striking that, despite many trying to describe and understand it, a clear pathological mechanism has been elusive for both preeclampsia and gestational hypertension. This lends weight to the credence that hypertensive disorders of pregnancy might represent a common phenotype of similar and related diseases with subtle differences in pathophysiology (122, 167).

1.10 Current recommendations for reducing cardiovascular risk in women after hypertensive disorder of pregnancy

It is relevant that pregnancy complications occur relatively early in a woman's life allowing a potentially significant window for primary prevention (36) and several national and international guidelines highlight the increased cardiovascular risk associated with a maternal history of hypertensive pregnancy and some also offer recommendations that blood pressure and cardiovascular risk factors should be assessed postpartum (16, 168-171). However most guidelines do not offer detailed recommendations and leave the specifics on how to follow-up this population to the clinician (168).

1.11 Thesis aims and hypotheses

It is unclear whether all women with a history of hypertensive disorder of pregnancy are at increased cardiovascular risk or whether an identifiable high risk subgroup exist. It is also yet to be elucidated which surrogate markers of cardiovascular disease are altered long term in this population. Future randomised control trials powered to detect hard endpoints will be prohibitively costly in time and money and surrogate markers, at least initially, might allow a cost-effective and achievable route to test potential therapies to reduce cardiovascular risk in this population. Additionally improved knowledge of the long term cardiovascular adaptations will allow better understanding of the mechanisms driving the increase in cardiovascular risk and hopefully lead to clearer guidelines and trials assessing interventions on relevant cardiovascular targets.

Part A.

The aim of this work is to characterise cardiovascular and cerebral phenotypic differences 5-10 years after hypertensive disorder of pregnancy. Specifically I hypothesise that:

1. Left ventricular mass index will be higher in women with a history of hypertensive disorder of pregnancy compared to women with a history of normotensive pregnancy.

2. Arterial stiffness will be higher in women with a history of hypertensive disorder of pregnancy compared to women with a history of normotensive pregnancy.

3. A history of hypertensive disorder of pregnancy is associated with a distinct maternal cerebral phenotype, characterised by grey matter atrophy and altered white matter architecture.

4. A history of hypertensive pregnancy is associated with dermal capillary rarefaction and altered markers of angiogenesis compared to women with a history of normotensive pregnancy.

5. Autonomic function is impaired in women with a history of hypertensive disorder of pregnancy compared to women with a history of normotensive pregnancy.

Part B.

Where phenotypic differences are found I will explore whether these are explained by conventional risk factors.

Study population

Chapter 2

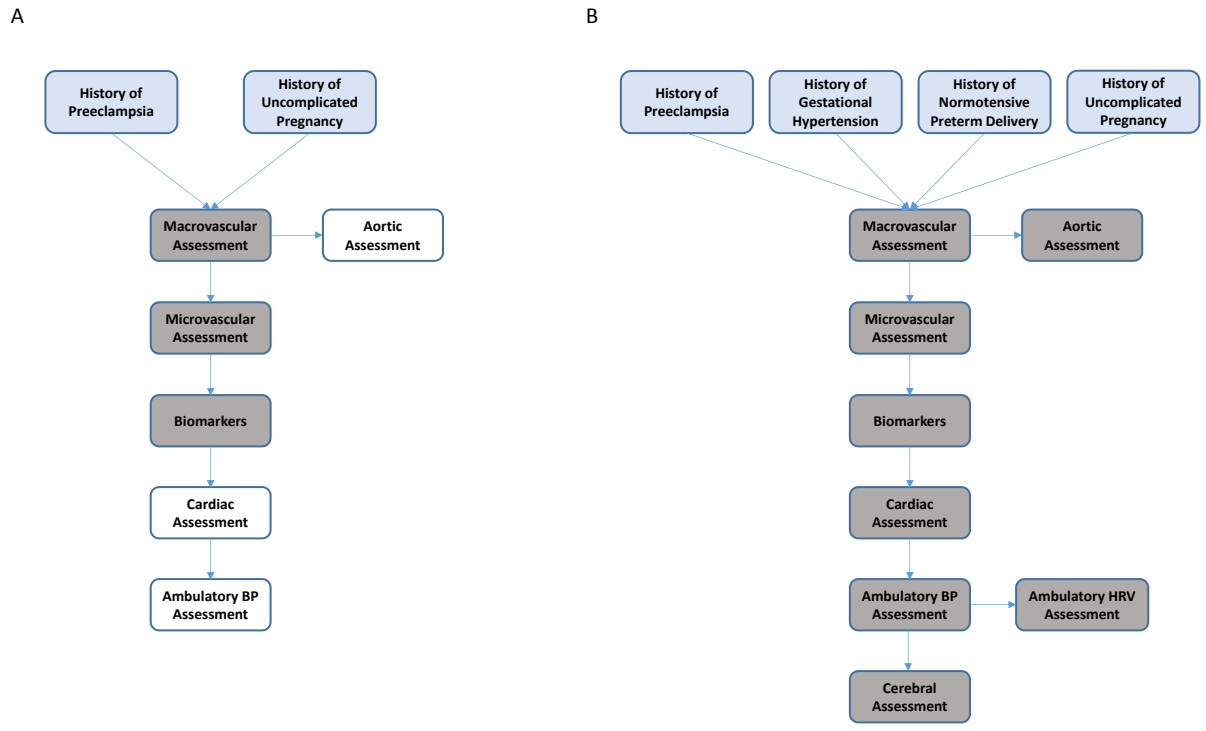
The study population included in this thesis incorporates 140 individuals (from a total of 268) who were recruited 2009 to 2012 by a previous DPhil candidate (Dr Merzaka Lazdam) prior to my starting my DPhil. The original cohort included individuals with a history of preeclampsia or uncomplicated pregnancy and all had assessment of blood pressure and vascular function. A subgroup of this original cohort (n=45) had assessment of cardiac and aortic structure and function using MRI and echo and had ambulatory monitoring of their blood pressure.

In addition to this original cohort I recruited 128 individuals between 2013 and 2015 and included women with a history of preeclampsia (26 with early onset preeclampsia and 26 with late onset preeclampsia), gestational hypertension (21), normotensive preterm delivery (29) and those with a history of uncomplicated pregnancy (26). In addition to the original measures of blood pressure and vascular function, these subjects also underwent assessment of cardiac and aortic structure and ambulatory assessment of blood pressure. In addition novel measures of cerebral structure using brain MRI and ambulatory heart rate variability were undertaken.

2.1 Subject selection

A search was undertaken of the Oxford University Hospitals Trust coding database to identify all individuals who had delivered at least 1 live child in the past 5-10 years and were coded with one of the following diagnoses of hypertensive disorders of pregnancy: preeclampsia (ICD10: O14), eclampsia (ICD10: O15) or gestational hypertension (ICD10: O13). In addition individuals who had delivered at least 1 live child in the past 5-10 years (ICD-10: O80, O81, O82, O83 and O84) without a hypertensive disorder of pregnancy and those who had delivered preterm (ICD: O60) without hypertension were also included. This was to allow exploration of the effect of preterm delivery independent of hypertension as the 2 often co-exist.

Figure 2. Overview of recruitment categories and assessments undertaken in original (A) and additional cohorts (B). Study measures carried out in whole cohort are represented in grey and those carried out in a subgroup in white.



The maternity notes of the individuals identified by the medical database search detailed above were reviewed and clinical details assessed to ascertain if the individual fulfilled the criteria for preeclampsia or gestation hypertension as set out by the International Society for the Study of Hypertension in Pregnancy (ISSHP) (72), full inclusion and exclusion criteria are detailed below (2.1.1 and 2.1.2).

In addition maternal and offspring electronic patient records were reviewed and potential recruits excluded if they or one of the offspring from the index pregnancy had subsequently died or had a life-limiting illness.

2.1.1 Inclusion criteria

A. Both controls (history of normotensive pregnancy) and cases (history of hypertensive pregnancy:

- Aged 21 to 50
- Able to give informed consent

B. Controls (normotensive pregnancy) only

Index pregnancy:

- Normal obstetric history (specifically no history of still birth or neonatal death, birthweight above the 5% centile (using standard gestational centile charts), no history of placental abruption, no history of recurrent (3 or more) miscarriages, no history of preeclampsia, gestational hypertension or gestational diabetes)
- Diastolic blood pressure consistently below 90mmHg
- Systolic blood pressure consistently less than 140mmHg

- No more than a trace of proteinuria

Preterm Delivery

- Delivery before 37 weeks of completed gestation in index pregnancy

C. Cases only (hypertensive pregnancy)

Index pregnancy:

Gestational Hypertension

- New onset hypertension after 20 weeks gestation in index pregnancy
- Diastolic blood pressure ≥90mmHg or systolic blood pressure ≥140mmHg on two separate occasions within a 24 hour period

Preeclampsia

- Raised blood pressure (as above)
- And new onset proteinuria (300mg/24h or more of protein in a 24 hour urine collection, or >30mg of protein/mmol of creatinine in a single urine sample, or at least 2+ protein at least twice on consecutive dipstick testing)

Hypertension with superimposed preeclampsia

- Diastolic blood pressure ≥90mmHg or systolic blood pressure ≥140mmHg on two separate occasions within a 24 hour period, prior to 20 weeks gestation, or an existing diagnosis of hypertension, made before pregnancy
- And new onset proteinuria (300mg/24h or more of protein in a 24 hour urine collection, or >30mg of protein/mmol of creatinine in a single urine sample, or at least 2+ protein at least twice on consecutive dipstick testing)

2.1.2 Exclusion criteria

A. Both controls and cases:

- Anyone who shows any doubt about taking part
- Serious mental illness
- Any other reason in the opinion of investigators why subject should not be entered into the study

At time of index pregnancy:

- Pre-existing renal disease
- Pre-existing cardiac or vascular disease
- Insulin dependent diabetics (Type I/II or gestational)

At time of study:

- Currently pregnant
- Significant medical history relevant to vascular measures including malignancy, vasculitides, systemic infection, recent major surgery/trauma, polycystic ovarian syndrome

2.2 Subject recruitment

Letters of invitation, including patient information sheets were sent to individuals who met the inclusion criteria, but not any of the exclusion criteria (both detailed above). The patient information leaflets (see Appendix) detailed all the proposed investigations in lay terms and gave contact details for the study investigators so individuals could ask questions. The letters included a brief form with a stamped addressed envelope to return if the individual was interested in taking part or wanted to find out more about the study. Those individuals who did not respond were sent one further invitation several weeks later. Non-responders to a second invitation were not contacted again.

Individuals who returned a form stating they were interested in the study were contacted and given the chance to ask any questions they might have and if still interested in taking part were invited to attend the study visit at the Cardiovascular Clinical Research Facility (CCRF) at the John Radcliffe Hospital, Oxford.

Figure 3. Flow chart of subject identification and recruitment, detailing the number of maternity records reviewed, invitation letters sent and participants recruited for the original cohort (blue), additional cohort (green) and study overall (grey).

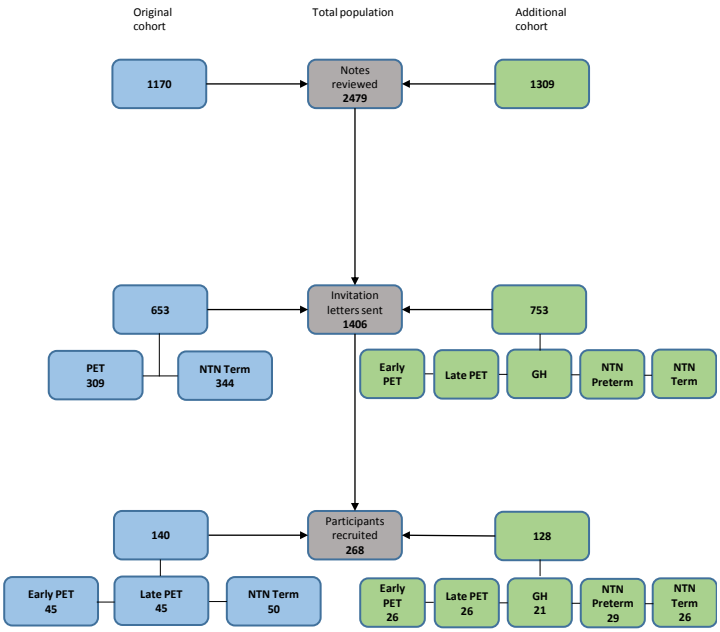
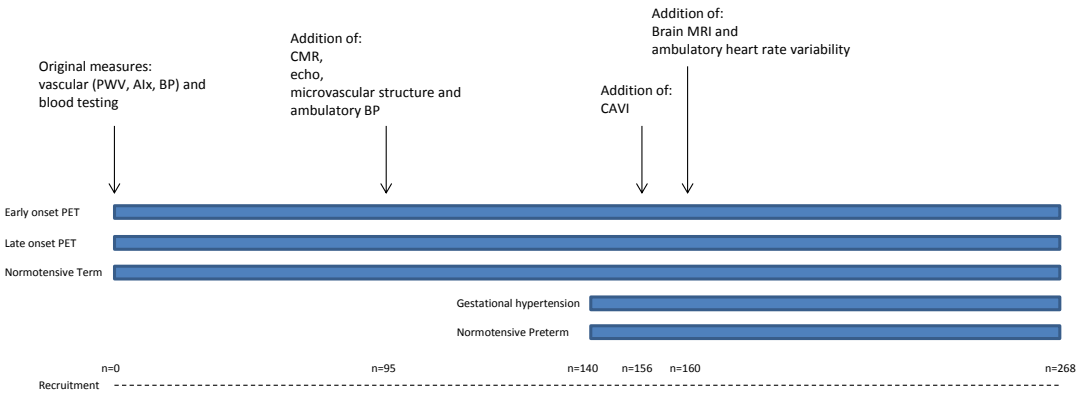


Figure 4 Flow chart detailing the study population, the measures employed and how this evolved as the study progressed.



Methods

Chapter 3

This chapter describes the methods utilised in this study. First I present an overview of the study visit, followed by the details of the investigations and methods of data collection undertaken, how they were analysed and their intra- and inter-observer coefficients of variation. Finally I describe the statistical methods employed.

3.1 Study visit outline

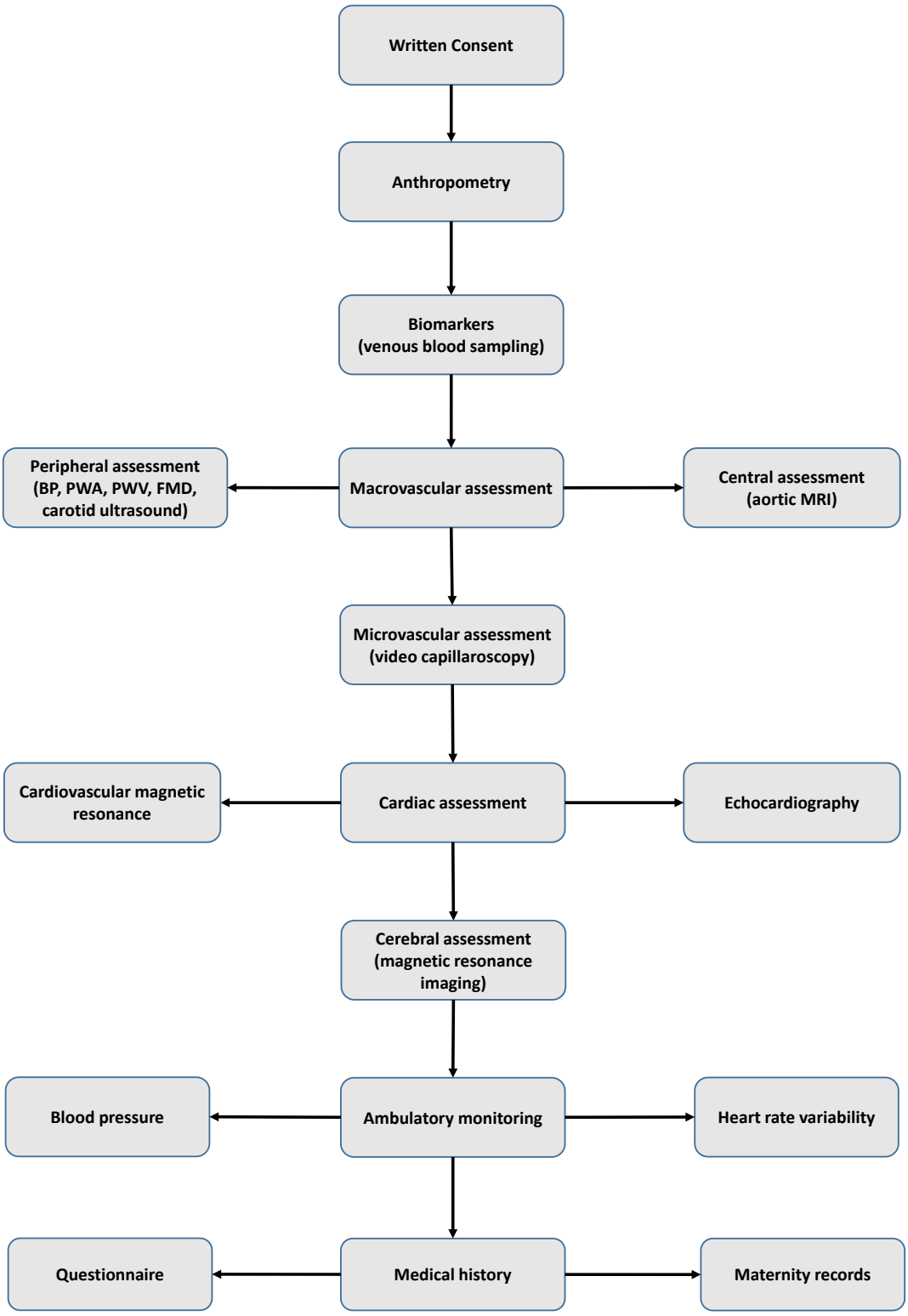
Potential recruits attended the Cardiovascular Clinical Research Facility (John Radcliffe Hospital, Oxford) after overnight fast (minimum 6 hours). The outline of the study visit (Figure 4) was explained in lay terms and a further opportunity to ask questions offered. Individuals who wished to take part in the study completed a written consent form and were offered a copy of this. Thereafter the data collected was anonymised and coded with study specific IDs (e.g. PVS1-001) to ensure confidentiality and blinded analysis.

All the statistical analysis in this thesis and the conclusions made within are my own work. As detailed in chapter 2 I am grateful to Merzaka Lazdam who recruited the

initial 140 participants and performed the assessments in these individuals. I recruited and performed the assessments on the additional 128 participants.

I performed the analysis on all the results, including the cardiac magnetic resonance, echocardiography, pulse wave velocity, augmentation index, flow mediated dilatation, central blood pressure, blood pressure variability, aortic compliance and distensibility, cerebral structure and volumes, capillary density and angiogenic markers, ambulatory blood pressure and heart rate variability. However I did receive some assistance with some of the analysis. Dr Lazdam's analysis of angiogenic markers from the individuals whom she recruited was used in the analysis in this thesis as it was not possible to repeat these ELISAs. For the analysis of subsequent samples I am grateful for the assistance of Charlotte Zwager whom joined the research group from Groningen medical school (Netherlands) and I supervised on a small research project. The CMR and echo images which Dr Lazdam collected I re-analysed myself as analysis software had changed and was more advanced when I undertook my analysis. The microvascular measures which Dr Lazdam performed she analysed and these results were pooled with the results from the additional cohort. For these additional participants I am grateful to Rhys Dore and Ajda Anžič who also undertook small research projects which I supervised and assisted in some of the analysis. Similarly I am grateful to Jane Francis who assisted with the analysis of aortic distensibility. Finally I am grateful for the guidance and assistance of Amy Bilderbeck and Timo Siepmann for guidance and assistance in the analysis of brain structure and Dr Pablo Lamata with assistance in cardiac shape analysis.

Figure 5. Flow chart of investigations and methods of data collection utilised for the study. (BP: blood pressure; PWA: pulse wave analysis; PWV: pulse wave velocity; FMD: flow mediated dilatation; MRI: magnetic resonance imaging)



3.2 Ethical standard

The study was approved by the Oxfordshire Research Ethics Committee A (Ethics reference number: 08/H0604/127). Written informed consent was obtained from all subjects prior to the start of the study.

3.3 Anthropometry

After removal of shoes and wearing light clothing only, subjects had their height and weight measured standing to the nearest centimetre and 0.1Kg respectively. Body surface area (BSA) was calculated using the Mosteller formula:

$$BSA\ (m^2) = \sqrt{([Height\ (cm) \times Weight\ (kg)]/ 3600)}$$

Body mass index (BMI) was calculated using the following formula:

$$BMI\ (kg/m^2) = Weight\ (kg)/[height\ (m)]^2$$

Waist and hip measurements were measured in centimetres and the waist: hip ratio calculated.

3.4 Biomarker collection

Participants had venepuncture performed whilst fasted using sterile technique, 20 mls of blood was collected. Samples were centrifuged at 3000 x G for 15 minutes at 4^0C. Serum, plasma and red cells were extracted and stored individually in cryovials at -80^0C.

3.4.1 Enzyme-linked immunosorbent assays

Quantikine enzyme-linked immunosorbent assays (ELISA) were performed to measure vascular endothelial growth factor A (VEGF-A), soluble fms-like tyrosine kinase-1 (sFLT-1) and soluble endoglin (sENG) concentrations. All ELISAs used colorimetric sandwich Quantikine technique (Quantikine, R & D Systems Europe, Abingdon, UK) and all samples, including standards, were plated in duplicate.

Analyte was reconstituted with deionized water to create a solution of known concentration and the solution was agitated to ensure homogeneity. Calibrator diluent was then mixed in incrementing proportions to create a series of reducing concentrations to be used as standards.

Frozen samples were thawed on ice and once thawed gently agitated to ensure homogeneity of sample. For VEG-F and sFLT-1 ELISAs 100µL of sample or standard were pipetted into each plate well (50µL for sENG). Plates were shaken gently on an automatic plate shaker at room temperature for 2 hours. This was followed by aspiration of the samples and standards and washing the plates using an automatic plate washer Hydroflex (Tecan, Männedorf, Switzerland). After washing, 200µL of conjugate solution was added to each well and the plate was incubated at room temperature for a further 2 hours. The wells were then washed using an automatic plate washer again and 200µL of substrate solution was added to each well and the plate was incubated at room temperature for 30 minutes in darkness. 50µL of stop solution was then added and the plates shaken gently on an automatic plate shaker for 5 minutes to ensure thorough mixing.

Optical density was measured at 450nm using a FLUOstar Omega microplate reader (BMG Labtech, KBioScience, USA) within 30 minutes of the stop solution being first

added and the results analysed using Omega Data Analysis software (BMG Labtech, KBioScience, USA). Duplicate readings for each sample and standard were compared and those with a coefficient of variation >15% were repeated. Standard curves were calculated using a four-parameter logistic curve-fit.

3.4.2 Biochemistry analysis

Fasting blood biochemistry was measured at the Oxford University Hospital Trust biochemistry laboratory using routine validated assays with clinical level quality controls. Total lipoprotein cholesterol, high density lipoprotein cholesterol and triglyceride levels were measured and low density lipoprotein cholesterol was calculated from these values using the Friedewald formula (172). Insulin and glucose levels were measured and homeostatic model assessments for insulin resistance (HOMA-IR) were calculated using the following formula:

$$\text{HOMA-IR} = \frac{\text{Glucose (mmol/L) X Insulin (mU/L)}}{22.5}$$

Creatinine and highly sensitive c reactive protein were also measured.

3.4.3 Urine collection and analysis

Participants were asked to provide a urine sample which was tested using a dipstick for the presence of microscopic blood, protein, leucocytes, nitrites and glucose. Women who were menstruating at the time of sample collection were excluded from urinalysis. Samples were analysed for albumin: creatinine ratios in the Oxford University Hospital Trust biochemistry laboratory using routine validated assays with clinical level quality controls.

3.5 Macrovascular assessment

Blood pressure and arterial stiffness are predictors of cardiovascular events (173, 174). These surrogate measures are of particular value in young and asymptomatic populations. Macrovascular structure and function were characterised non-invasively using a range of investigations including peripheral and central blood pressure, pulse wave analysis and velocity, carotid and brachial ultrasound and aortic magnetic resonance imaging. The specific methods employed for each technique are detailed below.

3.5.1 Peripheral blood pressure

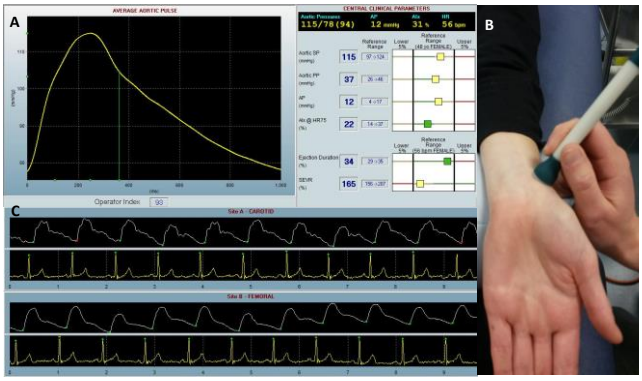
Participants rested in a supine position for 10 minutes prior to blood pressure being recorded. An automatic blood pressure monitor (A & D Instruments Ltd., Japan) was used to measure left brachial blood pressure and heart rate 3 times and the mean value recorded. Brachial blood pressure was also recorded whilst the participants lay supine inside the MRI scanner, part way through the scan, using an oscillometry device (Vicorder, Skidmore Medical, Taunton, UK).

3.5.2 Pulse wave analysis and central blood pressure

Aortic blood pressure was measured using two distinct methods. Firstly using applanation tonometry (SphygmoCor, AtCor Medical, West Ryde, Australia) on the left radial pulse. The hand was rest supinated with the wrist slightly extended and supported by a cushion. The hand was steadied to reduce movement and the tonometer held gently partially compressing the centre of the radial artery against the head of the radius. Pressure waveforms from the artery were seen in real time and recording was continued

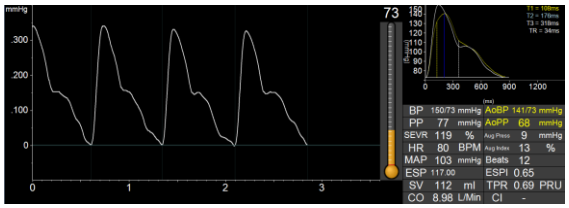
to include 10 waveforms with similar characteristics from which to calculate a mean waveform. This waveform was used to generate the ascending aortic waveform using a transfer function. Aortic blood pressure was then calculated based on this waveform and the previously measured brachial blood pressure. Augmentation index at an average rate of 75 beats per minute was also measured using the same technique.

Figure 6. Pulse wave analysis and pulse wave velocity using SphygmoCor. Radial artery pressure waveform used to calculate central blood pressure (A), demonstration of the positioning of the transducer to measure the radial artery pressure waveform (B) and the pressure waveforms measured at the carotid and femoral arteries in order to calculate carotid-femoral pulse wave velocity (C).



In addition aortic blood pressure was measured whilst the participants lay supine in the MRI scanner. The measurements were taken mid-way through the scan, between sequences to measure aortic distensibility, using a brachial cuff connected to an oscillometry device (Vicorder, Skidmore Medical, Taunton, UK).

Figure 7. Pulse wave analysis using Vicorder. Brachial artery pressure waveform used to derive central blood pressure.



3.5.3 Pulse wave velocity

Pulse wave velocity was measured both between carotid and femoral arteries and separately between brachial and femoral arteries. For carotid-femoral pulse wave velocity the distance was measured from sternal notch to left carotid pulse and sternal notch to left femoral pulse. Then applanation tonometry (SphygmoCor; AtCor Medical, West Ryde, Australia) was used to obtain pressure waveforms of the carotid and femoral pulse, by holding the tonometer gently to partially compress the corresponding artery and record 10 consecutive pressure waveforms. A mean pressure waveform was calculated and the time difference from the R wave on the ECG recording to the pulse arrival time at each arterial measurement site used to derive the pulse wave velocity (PWV) between the 2 sites.

For brachial-femoral pulse wave velocity brachial and femoral cuffs were fitted (a known distance apart) and connected to an oscillometry device (Vicorder, Skidmore Medical, Taunton, UK). The cuffs were partially inflated and used to detect pulse arrival and thus derive pulse wave velocity from the difference between the cuffs. This technique was used whilst subjects lay supine in the MRI scanner.

3.5.4 Cardio ankle vascular index

Cardio-ankle Vascular Index (CAVI) was measured with VaSera VS-1500 (Fukuda Denshi Co. Ltd, Tokyo, Japan). CAVI is a "global" index of arterial stiffness, from the origin of the aorta to the ankle of the lower leg. The measurement involves attaching a phonocardiograph onto the subject's sternum and blood pressure cuffs to all 4 limbs. The time delay from aortic valve closure to arterial waveform measured in the limbs is

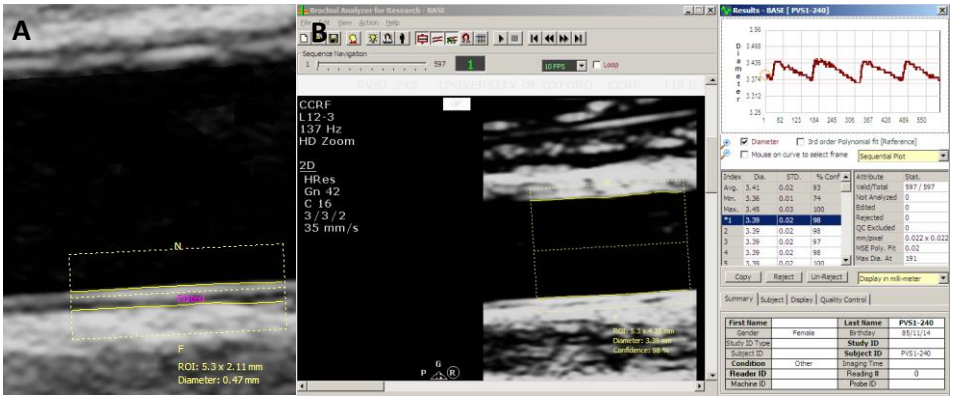
used to calculate stiffness parameter β, which is independent of blood pressure at time of measurement

3.5.5 Flow mediated dilatation

The participant lay supine with their right arm supported at right angles to their body on a table with small fixed rests designed specifically for this test. An uninflated cuff was attached to the proximal right forearm and 2D ultrasound was performed proximal to this to acquire ECG gated images of the brachial artery with clear and parallel vessel borders to the ultrasound probe. Settings were adjusted for maximal contrast of the vessel borders. Once a high quality image was obtained the probe was fixed in position using an adjustable clamp and the participant asked not to move until the test was finished. A baseline image and Doppler flow from the brachial artery was acquired before inflation of the forearm cuff to 250mmHg. After 4.5 minutes an image of the brachial artery was acquired. At 5 minutes the cuff was released and Doppler flow characteristics measured, repeated again at 1 minute. At 15 second intervals after the cuff was released 2D images were acquired, for a total of 2 minutes, to measure flow mediated dilatation.

Images were analysed offline using Brachial analyser version 5.6.15 (Medical Imaging Applications LLC, Coralville, Iowa, USA) to measure the vessel diameter at different time points. A region of interest was selected to include both proximal and distal vessel walls, the semi-automated software then tracked these borders through the cardiac cycle. The automated tracking was reviewed and manually adjusted if errors in tracking were found. Flow mediated dilatation was calculated from the percentage change in diameter of the brachial artery from baseline (before cuff inflation) to 60 seconds after cuff deflation.

Figure 8. Carotid and brachial ultrasound. Measurement of intima media thickness in the carotid artery (A) and measurement of flow mediated dilatation of the brachial artery diameter using Brachial analyser (B).



3.5.6 Carotid ultrasound

Ultrasound was performed of both left and right carotid arteries using a CX-50 ultrasound machine and L12-3 linear probe (Philips, Amsterdam, Netherlands). ECG gated cine loops were acquired of the common carotid artery 1cm proximal to the bifurcation whilst the patient lay supine. Settings were adjusted for maximal contrast of the intima media borders. Image analysis was performed offline using Brachial analyser version 5.6.15 (Medical Imaging Applications LLC, Coralville, Iowa, USA) to measure the intima media thickness at end diastole. A region of interest was selected to include the intima media interface and intima luminal border, the software then tracked these borders through the cardiac cycle. The automated tracking was reviewed and manually adjusted if errors in tracking were found.

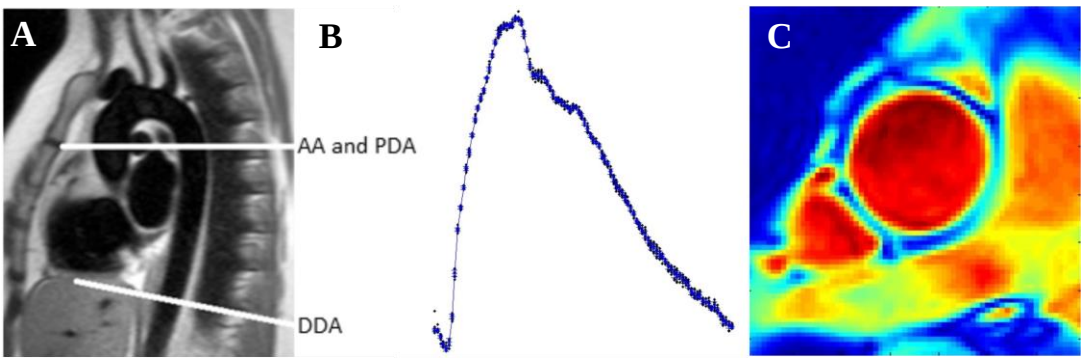
3.5.7 Aortic magnetic resonance imaging

Aortic structure and function was assessed at multiple levels using cardiovascular magnetic resonance (1.5T scanner, Siemens, Munich, Germany). Cine images of the

thoracic aorta at the level of the pulmonary artery bifurcation, both in the ascending and proximal descending aorta (AA and PDA respectively); additionally images of the distal descending aorta (DDA) 12 cm distal to the proximal descending aorta perpendicular to the lumen and in the abdominal aorta at the level of the 2nd lumbar vertebra (AbA). A retrospective ECG gated sequence at end breath hold with the following parameters was used: repetition time 31.92 ms, echo time 1.12 ms, flip angle 45^0, temporal resolution 10 ms, in plane resolution 1.8 x 1.8 mm, slice thickness 8.0 mm.

Maximal and minimum aortic area was measured using semi-automated edge detection algorithms (Matlab, Mathworks Inc., Massachusetts, USA). Compliance was calculated by dividing the change in aortic area by minimum aortic area. Distensibility was calculated by dividing compliance by central pulse pressure measured by Vicorder during the CMR scan (Skidmore Medical, Taunton, UK). Global distensibility was calculated from the mean value of local distensibility measured throughout the aorta.

Figure 9. Aortic distensibility analysis. Oblique sagittal image of thoracic aorta showing sites of measurement (ascending aorta – AA, proximal descending aorta – PDA, distal descending aorta – DDA, abdominal aorta – AbA is not included in this image (A), a trace of aortic luminal area over 1 cardiac cycle (B) and a cross-sectional image of the ascending aorta viewed in Matlab (C).



3.6 Microvascular assessment – intravital capillary video microscopy

Microvascular structure was assessed using intravital video capillaroscopy. A Leica Stereo Microscope (X200 magnification) and Schott light emitting diode light were used to image the dorsum of the middle phalanx of left hand. The wave length of the light was consistent with the absorption spectrum of haemoglobin (450-610 nm) which allowed clear discrimination of capillaries filled with blood. A Bosch Dinion CCD Analogue Camera was used to relay the images, which were reviewed in real time Norban Vista black and white high-resolution monitor and digitally recorded using a Sony DVO-1000MD digital video disc recorder. A 1.0 mm glass stage graticule (Knight Optical UK) was used for calibration.

A cradle of plasticene was placed on a platform controlled by micrometers under the microscope lens. The participants' middle phalanx was positioned in the cradle to reduce movement artefact. Six distinct image fields were recorded for 1 minute each; movement between fields was controlled with micrometers. During each 1 minute recording the image focus was gradually adjusted to allow clear visualisation of capillary vessels across the whole field of view, which due to the nature of the phalanx is not flat.

A small cuff was then inflated at the base of the phalanx to 50 mmHg and after 4 minutes the same image fields recorded again. The inflated cuff was to reduce venous return and ensure capillary engorgement with blood, thereby allowing measurement of the structural, also known as maximal, capillary density.

Image analysis was performed offline using Image ProPlus (MediaCybernetics, Rockville, MD, USA). A circle of known area (0.4 mm^2) was overlaid on the centre of the field of view at the start of a recording. All capillaries that appeared within this

circle were tracked, tagged and counted for each field of view. The mean capillary density per mm^2 was calculated from the 6 fields for both baseline (functional) capillary density and venous occlusion (structural) capillary density. Capillary recruitment was calculated as the percentage increase in capillary density from baseline assessment to structural assessment with venous occlusion.

Table 2. Inter-observer coefficients of variation for capillary density

Measurement	Intra-observer CoV (%)	Inter-observer CoV (%)
Functional capillary density (capillaries/mm^2)	2.3	5.6
Structural capillary density (capillaries/mm^2)	2.1	7.3

CoV: coefficient of variance

3.7 Cardiac assessment

3.7.1 Cardiovascular magnetic resonance

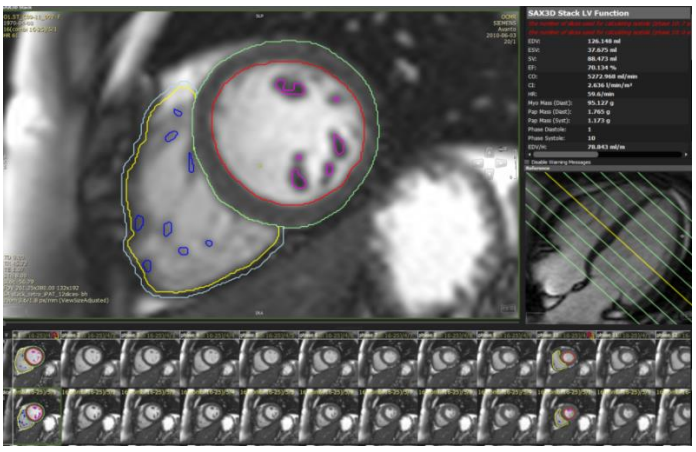
Cardiac structure and function was assessed using cardiovascular magnetic resonance (1.5T scanner and 12 channel body and spine array, Siemens, Munich, Germany). Images were retrospectively ECG gated with a precordial three lead ECG.

3.7.1.1 Ventricular structure (volumes, mass, wall thickness)

Steady state free precession (SSFP) images during breath hold at end expiration were acquired with the following sequence parameters: repetition time 45.72 ms, echo time 1.07 ms, flip angle 60^0, calculated phases 25 (temporal resolution 40 ms at a heart rate of 60 beats per minute), in plane resolution 2.8 x 2.0 mm, slice thickness 8.0 mm. Using 2, 3 and 4 chamber views to plan images in line with the atrio-ventricular valves a stack of short-axis images were acquired at 1cm intervals to include the entire left and right ventricles.

Image analysis was performed using CVI42 versions 5.1.1 (Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada). Left and right ventricular endocardial and epicardial borders were manually contoured at end-diastole and endocardial borders only in end-systole. Papillary muscles and trabeculations were included in the myocardial mass for the relevant ventricle. Myocardial mass was calculated from the sum of myocardial area in the stack of images multiplied by 1.05g/cm^3 (specific gravity of myocardium per cm^3). End-diastolic and end-systolic volumes were also calculated from the sum of ventricular areas in the stack of images. Stroke volume was the difference between end-diastolic and end-systolic volume. Ejection fraction was calculated stroke volume divided by end-diastolic volume. Wall thickness was measured at mid ventricular level in 6 segments as are represented in standard international guidelines (175) and mean values were calculated from these.

Figure 10. Cardiac analysis using CMR. Stack of short axis CMR images viewed in CMR42 with contouring of myocardium to calculate ventricular volumes and myocardial mass



3.7.1.2 Myocardial T1 mapping

Myocardial T1 values were measured from short modified look locker images during breath hold at end expiration with the following sequence parameters: repetition time

198.77 ms, echo time 1.07 ms, flip angle 35^0, in plane resolution 1.8 x 1.8 mm, slice thickness 8.0 mm. A modified stack of 3 short-axis images corresponding to the slices acquired for ventricular volumetric analysis were acquired of the base, mid and apical levels of the left ventricle.

Image analysis was performed using MC_ROI software (Oxford Centre for Clinical Magnetic Resonance, developed by Dr Stefan Piechnik). Endocardial and epicardial borders were manually delineated to include normal myocardium. Mean T1 values were calculated for each slice and then combined to describe the mean T1 value for all 3 slices.

Table 3. Intra- and Inter-observer coefficients of variation for cardiac structure

Measurement	Intra-observer CoV (%)	Inter-observer CoV (%)
Volumes and mass		
LV end diastolic volume	6.0	1.1
LV end systolic volume	4.5	3.0
LV stroke volume	7.9	1.1
LV ejection fraction	4.3	1.1
LV myocardial mass	11.5	4.5
RV end diastolic volume	7.9	1.9
RV end systolic volume	7.9	1.7
RV stroke volume	10.7	2.4
RV ejection fraction	3.1	1.2
RV myocardial mass	18.5	12.9
Wall thickness		
Anterior	23.9	24.7
Lateral	15.5	22.9
Inferolateral	17.2	24.0
Inferior	26.0	18.3
Inferoseptal	11.8	7.4
Anteroseptal	21.9	20.4
Mean wall thickness	13.2	17.2

CoV: coefficient of variance; LV: left ventricle; RV: right ventricle

3.7.1.3 Left atrial volumes

SSFP images at breath hold during end expiration were acquired in 2 and 4 chamber views, with the following sequence parameters: repetition time 39 ms, echo time 1.1 ms, flip angle 60^0, calculated phases 25 (temporal resolution 40 ms at a heart rate of 60 beats per minute), in plane resolution 1.9 x 1.9 mm, slice thickness 7.0 mm. Mean left atrial volumes was calculated using standard and recognised methods (176). In brief, maximal left atrial volumes were contoured in 2 and 4 chamber views. Left atrial appendage was included where seen and pulmonary veins excluded. Left atrial volume index was calculated by dividing mean left atrial volume by body surface area (Mosteller equation). Maximal transverse and longitudinal atrial dimensions were also made in 2 and 4 chamber views.

Figure 11. Left atrial structural analysis. Volume and dimension measurement using CVI42 software in 2 chamber and 4 chamber views.

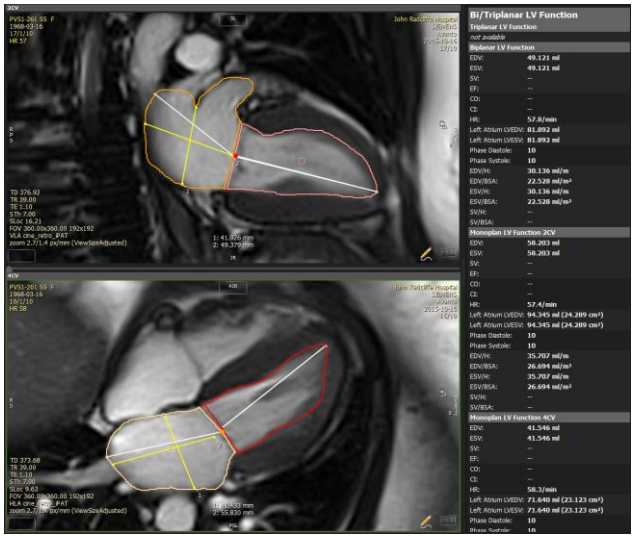


Table 4. Intra- and Inter-observer coefficients of variation for left atrial volumes and dimensions

Measurement	Intra-observer CoV (%)	Inter-observer CoV (%)
LA volume	19.5	5.5
LA transverse dimension (4ch view)	8.3	5.4
LA longitudinal dimension (4ch view)	9.2	18.6
LA transverse dimension (2ch view)	8.3	6.1
LA longitudinal dimension (2ch view)	6.9	4.3

CoV: coefficient of variance; LA: left atrial; 4ch view: 4 chamber view; 2ch view: 2 chamber view

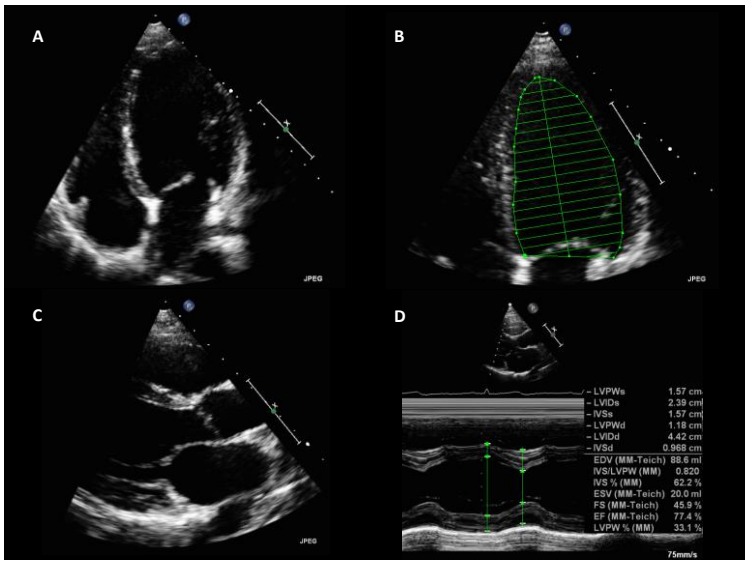
3.7.2 Echocardiography

Echocardiography was performed using a Toshiba Artida (original cohort) or Philips iE33 (additional cohort) equipped with a 2D phased array transducer in the left lateral decubitus position.

3.7.2.1 Cardiac structure (echocardiography)

Left ventricular (LV) dimensions were measured using M-mode in a parasternal long axis view in both end diastole and end systole, including: interventricular septum, left ventricle internal diameter, posterior wall thickness. LV volumes were measured in both end diastole and end systole in apical 2 and 4 chamber views and the mean calculated. Maximal left atrial dimensions were measured in 3 and 4 chamber views and volumes measured in a 4 chamber view. All atrial and ventricular volumes were indexed to body surface area (Mosteller equation).

Figure 12. Echo ventricular analysis. Apical 4 chamber view (A), measurement of LV end diastolic volume (B), parasternal long axis view of heart (C) and M-mode measurement of LV end diastolic and end systolic dimensions (D).



3.7.2.2 Cardiac function (echocardiography)

Stroke volume was calculated from the difference between end diastolic and end systolic volumes. Ejection fraction was calculated from the following equation:

$$\text{LV Ejection fraction (\%)} = \frac{\text{LV end diastolic volume} - \text{LV end-systolic volume}}{\text{LV end-diastolic volume}} \times 100$$

Doppler flow was assessed across all valves to assess function. A pulsed wave Doppler sample volume was placed at the mitral valve leaflet tips in a 4 chamber view to record peak early (E) and late (A) velocities, early (E) wave deceleration time was also measured. A continuous wave Doppler sample volume through the tricuspid valve leaflets quantified peak velocity of any tricuspid regurgitation. The pressure difference across the tricuspid valve was calculated using the Bernoulli equation:

$$\text{Pressure difference} = 4 \times \text{velocity}^2$$

Subcostal views of the inferior vena cava (IVC) where acquired during expiration and inspiration to measure dimension changes with respiration. Right atrial pressure was estimated using standard techniques based on the dimension changes of the IVC with respiration (177). Pulmonary systolic pressure was calculated by adding right atrial pressure to the pressure difference measured across the tricuspid valve. Tissue Doppler was used to measure systolic (s'), early (e') and late (a') at the right ventricle free, septum and LV lateral wall, all at the level of atrioventricular valves.

Figure 13. Echo Doppler analysis. Measurement of mitral valve inflow velocities (A) and measurement of septal wall velocity using tissue Doppler imaging (B).

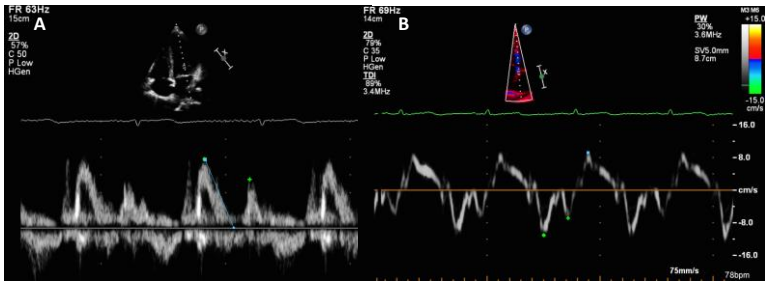


Table 5. Intra- and Inter-observer coefficients of variation for echo assessment of cardiac structure and function

Measurement	Intra-observer CoV (%)	Inter-observer CoV (%)
Septal thickness (diastole)	9.9	4.1
LV posterior wall thickness (diastole)	14.9	12.0
LV end diastolic volume	10.3	13.4
LV end systolic volume	16.8	24.9
LV stroke volume	10.4	15.3
Ejection fraction (Simpson biplane)	5.9	11.0
Septal wall S'	5.4	13.9
Lateral wall S'	4.2	12.0
Mitral valve peak E wave velocity	2.5	5.6
Mitral valve peak A wave velocity	4.2	7.4
E/A	6.7	4.5
Mitral valve E wave deceleration time	14.0	12.0
Septal wall e'	5.5	12.6
Septal wall E/e'	7.5	10.1
Lateral wall e'	4.4	6.6
Lateral wall E/e'	5.7	7.4
LA volume	13.1	31.9
LA transverse dimension (4 ch view)	7.0	10.1
LA transverse dimension (3 ch view)	3.5	5.5
TAPSE	6.8	10.2
RV free wall S'	2.6	5.7
RV systolic pressure	8.8	3.6

CoV: coefficient of variance; LV: left ventricle; E: early; A: atrial; LA: left atrial; 4 ch view: 4 chamber view; 3 ch view: 3 chamber view; TAPSE: Tricuspid annular plane systolic excursion; RV: right ventricle.

3.7.2.3 Echocardiography speckle tracking analysis

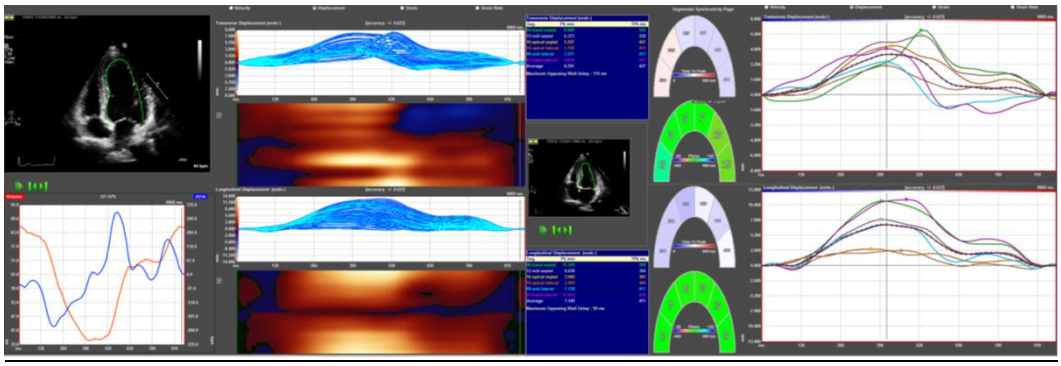
Assessment of strain by speckle tracking was undertaken offline using semi-automated 2D Cardiac Performance Analysis Software (TomTec, Munich, Germany). Apical 4 chamber 2D images were acquired and the endocardial border delineated in end diastole. The endocardial border automatically tracked through a single cardiac cycle, tracking was visually inspected and manually corrected if poorly correlated with myocardial margin. Peak global values of longitudinal and transverse strain and displacement in systole and velocity and strain rate in systole and diastole were measured.

Table 6. Intra- and Inter-observer coefficients of variation for echo assessment of cardiac strain

Measurement	Intra-observer CoV (%)	Inter-observer CoV (%)
Longitudinal		
Systolic velocity	14.0	15.4
Displacement	4.0	12.4
Strain	6.7	10.4
Systolic strain rate	8.1	10.6
Diastolic velocity	8.2	12.6
Diastolic strain rate	12.2	13.8
Transverse		
Systolic velocity	9.5	7.0
Displacement	6.1	9.2
Strain	27.7	36.1
Systolic strain rate	29.6	35.6
Diastolic velocity	6.3	15.1
Diastolic strain rate	21.2	25.3

CoV: coefficient of variance

Figure 14. Echo strain analysis (Tomtec).



3.7.3 Cardiac shape analysis

Cardiac atlases were developed in collaboration with Dr Pablo Lamata at the Department of Biomedical Engineering, King's College London. The aim was to explore if there were subtle differences in cardiac shape, according to pregnancy history, which were not identified using simple volumes and dimensions. Differences in shape could be valuable in identifying distinct cardiac phenotypes which might aid our

understanding of disease specific patterns of cardiac remodelling and relevant mechanisms which relate to future cardiovascular disease.

Cardiovascular magnetic resonance short axis stack images of the left ventricle (previously described in 3.7.1.1) including the manually drawn contours of the endocardium and epicardium were combined into a single DICOM file. A mesh was fitted to the contours and this mesh was described by 3456 nodal variables. Principal component analysis (PCA) was undertaken to define the modes of anatomical variation that capture the largest part of the shape variation within the total study population. Furthermore linear discrimination analysis (LDA) was then undertaken in an attempt to identify a remodelling pattern that discriminated between women with a history of normotensive pregnancy and those with a history of hypertensive pregnancy. The generality of the classification of the LDA was assessed using a leave-1 out cross-validation experiment. Subgroup analysis was also undertaken to explore differences in subgroups of women with a history of hypertensive pregnancy.

3.7.4 Definition of heart failure stage B

Heart failure stage B is described by American Heart Association guidelines as structural heart disease without signs or symptoms of heart failure (178). It was defined as the absence of signs or symptoms of heart failure and the presence of one or more of the following characteristics: previous myocardial infarction, left ventricular hypertrophy (LV mass index >95g/m^2), concentric remodelling (regional wall thickness >0.42 [see below]), impaired systolic function (ejection fraction <55%) or asymptomatic valvular disease.

$$\text{Regional wall thickness} = \frac{2 \times \text{posterior wall thickness}}{\text{LV internal dimension}}$$

3.8 Cerebral assessment – MRI

Magnetic resonance imaging with a Siemens 1.5T scanner and 12 element head matrix coil was used to assess brain structure. Analysis was performed in collaboration with Dr Amy Bilderbeck at the Oxford Centre for Clinical Magnetic Resonance Research and the Department of Pyschiatry, both at the University of Oxford.

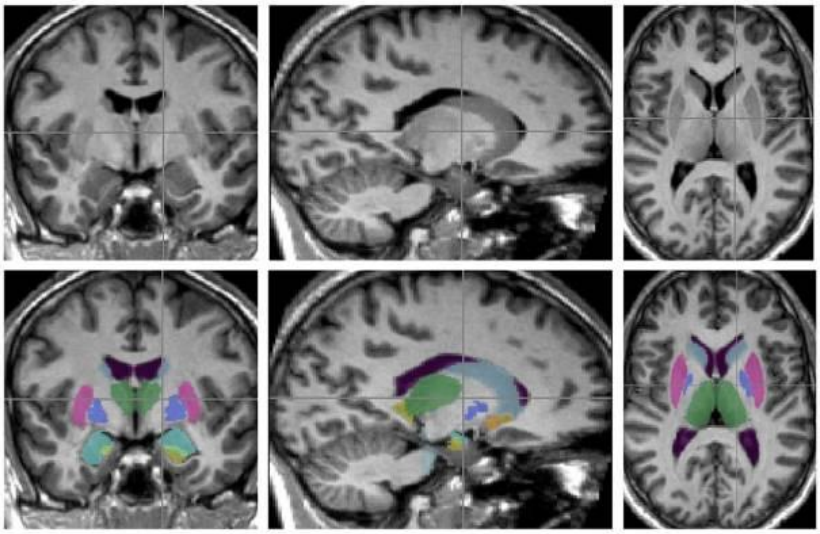
3.8.1 Brain volumes

Overall brain volumes and specifically grey matter volumes are closely associated with cardiovascular risk. Brain weight at death has been shown to be inversely associated with blood pressure in midlife (179). Grey matter volumes are reduced in those with a higher prevalence of cardiovascular risk factors (180), furthermore accelerated grey matter atrophy has been shown in hypertensive individuals despite treatment (181).

T1 weighted sequences were applied to include the entire cortex and cerebellum. Magnetic resonance scan parameters were as follows: repetition time 12 ms, echo time 5.65 ms, in plane resolution 1.0 x 1.0 mm, slice thickness 1.0 mm. Brain tissue volume, normalised for skull size, was estimated using SIENAX software (182, 183) (Oxford Centre for Functional MRI of the Brain, University of Oxford, UK). The voxels representing the brain were first extracted (184) and affine-registered to Montreal Neurological Institute 152 space (MNI152 space), a validated standard brain atlas template derived from 152 structural images (185) with the skull size used to determine registration scaling. Tissue segmentation was then used to differentiate grey and white matter and cerebrospinal fluid (CSF) (186). Total brain volume was the sum of grey and white matter and ventricular CSF.

Absolute volumes of subcortical brain structures were estimated using FMRIB's Integrated Registration and Segmentation Tool (FIRST) software (187) (Oxford Centre for Functional MRI of the Brain, University of Oxford, UK). This model-based segmentation tool uses models manually segmented by the Centre for Morphometric Analysis (Massachusetts General Hospital, Boston, USA). Based on learned models, combinations of shape modes of variation were searched and structures identified according to shape characteristics.

Figure 15. Example of subcortical segmentation using FIRST (187)

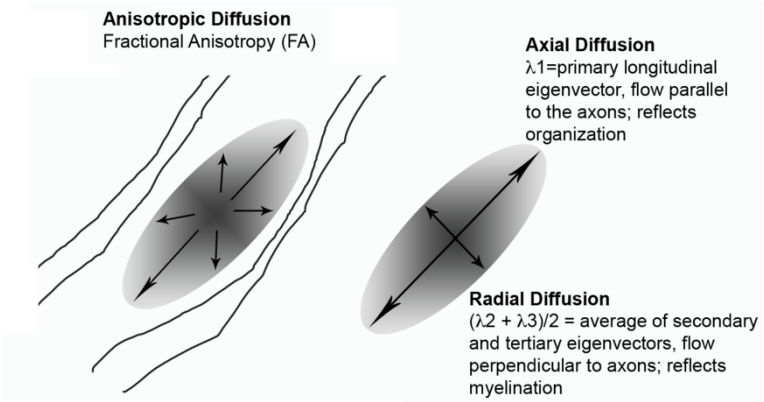


3.8.2 White matter structure

White matter lesions or "hyperintensities", shown as increased signal intensity on T2 weighted fluid attenuated inversion recovery (FLAIR) images, are a sign of subclinical cerebrovascular disease and are a risk factor for stroke (150). They have also been shown to be more prevalent in women several years after preeclampsia (151) and eclampsia (188). Impairment of white matter microstructural integrity, assessed through the measurement of water molecule diffusion in fibre tracts, typically precedes

development of white matter hyperintensities (189) and has also been shown to be associated with vascular risk (190). Microstructure integrity can be measured by fractional anisotropy which describes the directionality of water molecule diffusion and diffusivity which describes the magnitude of diffusion. Higher values of fractional anisotropy (closer to 1) are associated with greater integrity of tract microstructure. Diffusivity can in turn be measured in two different planes, perpendicular to the tracts (radial diffusivity) or parallel to the tracts (axial diffusivity), the combination is termed mean diffusivity (Figure 15).

Figure 16. Illustration of fractional anisotropy and diffusion (191)



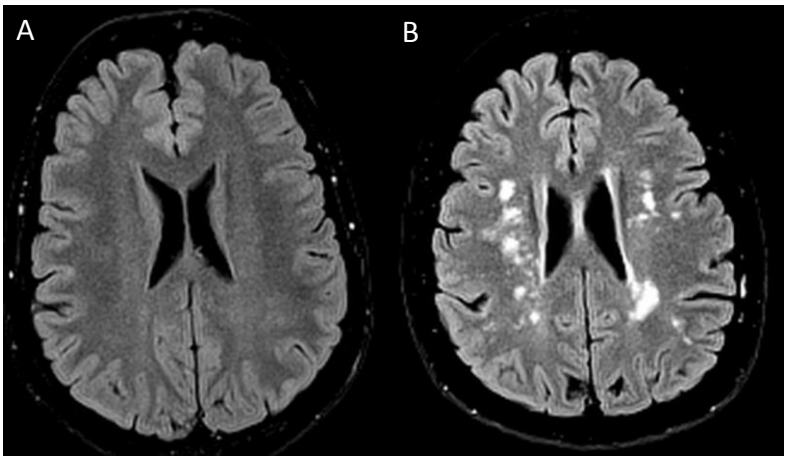
3.8.2.1 White matter hyperintensities

T2 weighted fluid attenuated inversion recovery (FLAIR) images were acquired with the following image parameters: repetition time 9000 ms, echo time 80 ms, flip angle 180^0, in plane resolution 1.0 x 1.0 mm, slice thickness 3.0 mm. Assessment of FLAIR images for the presence of white matter hyperintensities was done both visually and using automated voxel based segmentation.

Visual analysis was performed using CVI42 versions 5.1.1 (Circle Cardiovascular Imaging Inc. (Calgary, Alberta, Canada). Two reviewers, each with several years' experience of reviewing brain MRI imaging (neurologist: TS and radiologist: DM), reviewed the images independently. White matter hyperintensities were identified, if

there was an identifiable area of increased signal intensity in the white matter. The independent reviewers quantified the number of lesions overall in the brain and according to predefined regions (frontal lobe, parietal lobe, occipital lobe, temporal lobe, infratentorial or basal ganglia). Fazekas score was measured for each set of brain images. This validated score is used for quantifying the global extent and severity of white matter lesions (192, 193). The score rates periventricular hyperintensities as 0=absent, 1=”caps” or pencil-thin lining, 2= smooth “halo”, 3=irregular periventricular hyperintensities extending into the deep white matter; deep white matter lesions are score separately: 0= absent, 1= punctate foci, 2= beginning confluence of foci, 3=large confluent areas (194).

Figure 17. White matter hyperintensities. Examples of brain MRI pictures using FLAIR sequences in 2 subjects: one without white matter hyperintensities (A) and one with extensive white matter hyperintensities.

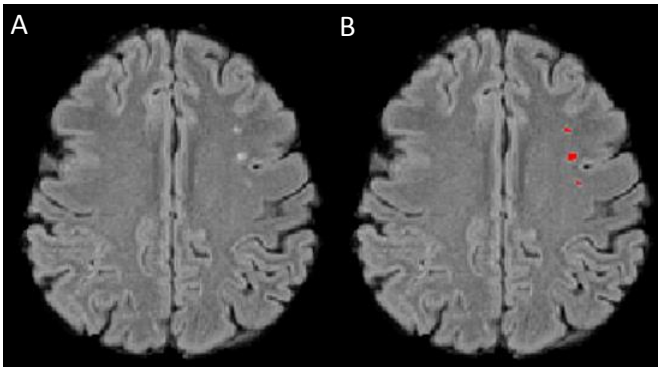


Voxel based segmentation analysis was also undertaken in collaboration with Dr Ludovica Griffanti at the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, University of Oxford, to measure white matter hyperintensity volumes. This was undertaken using recently developed automated software known as Brain Intensity AbNormality Classification Algorithm (BIANCA) [unpublished – Griffanti et al]. Firstly a training set of FLAIR brain images (n=9) from the study population was

assessed by one of the reviewers (TS) and all white matter hyperintensities were identified and semi-automatically segmented using Jim software (Xinapse Systems, Leicester, UK; http://www.xinapse.com/Manual/). The pre-classified voxels from the defined lesions in the training set were compared to all voxels across the entire cohort of FLAIR images. Probability maps were created according to the likelihood of each voxel representing white matter hyperintensity. Voxels which had ≥0.9 probability were accepted, this threshold was selected to offer the best balance of sensitivity and specificity and showed good correlation with visual assessment by an experienced reviewer (TS). A minimum number of clustering voxels to classify a white matter hyperintensity lesion as present was also set. This was to minimise image noise leading to overestimation of lesions. A comparison of 3 and 5 voxel minimums were compared and 5 selected as the closest agreement to visual assessment.

The total volume of white matter hyperintensities was calculated based on these thresholds. The regional volumes of white matter hyperintensities in the different lobes (frontal, parietal, temporal and occipital) were calculated within masks generated in MNI space and registered to the subjects' FLAIR images.

Figure 18. Voxel based segmentation analysis (BIANCA) of white matter hyperintensities. An example of a brain MRI image using FLAIR sequence in a subject; before BIANCA analysis the lesions appear white (A) BIANCA identifies all 3 lesions (highlighted in red) and allows quantitative volumetric analysis (B).



3.8.2.2 White matter diffusion

Diffusion tensor imaging sequences were acquired twice, with 180^0 rotation in phase between sequences, with the following parameters: repetition time 7300 ms, echo time 81 ms, in plane resolution 2.0 x 2.0 mm, slice thickness 2.0 mm.

Voxelwise statistical analysis of the diffusion data was carried out using Tract-Based Spatial Statistics (TBSS) (195). Firstly raw diffusion data was fitted to a tensor model and then the brain extracted using Brain extraction tool (BET)(184). All subjects' diffusion data (fractional anisotropy, axial diffusivity, radial diffusivity and mean diffusivity) was then aligned using a nonlinear registration tool and a mean image created with a diffusion value for each voxel. The image was then thinned, to create a skeleton illustrating the central white matter tracts for the cohort. Each subjects' individual diffusion data was then projected onto this skeleton and compared voxelwise across the whole brain and in predefined regions (frontal, parietal, temporal and occipital lobes). Using this technique fractional anisotropy, axial diffusivity, radial diffusivity and mean diffusivity were compared between groups.

3.9 Ambulatory monitoring

Participants were fitted with two ambulatory monitors at the end of the study visit, to measure their blood pressure and heart rate. They were given a stamped addressed envelope to return them along with a diary card to record their sleeping and exercise periods during the recording period.

3.9.1 Blood pressure

A size appropriate blood pressure cuff was attached to the participant's left upper arm and connected to a digital ambulatory blood pressure recording device (TM-2430, A&D Instruments, Abingdon, UK) and worn for 24 hours. Measurements were taken every 30 minutes during the day and every 60 minutes during the night. Day time was defined as between 07:00 and 23:00 and night time as between 23:00 and 07:00. Normal daily activities were encouraged, with participants asked to keep their left arm relaxed and still when measurements were taking place. The reading, editing and analysis of blood pressures were done using ABPM Data Analysis Software for Windows (version 2.40, A&D Instruments, Abingdon, UK).

Blood pressures were extracted and mean and standard deviations for the entire recording duration, as well as, awake and asleep periods were calculated. Weighted standard deviation (SD) of systolic (SBP) and diastolic blood pressure (DBP) over 24 hours was calculated with the following formula:

$$\text{Weighted SD of BP over 24-hours} = \frac{(\text{SD of awake BP} \times \text{awake hours}) + (\text{SD of asleep BP} \times \text{asleep hours})}{\text{Total recording length (hours)}}$$

Average real variability (ARV) was calculated using the following formula:

$$ARV = \frac{1}{N-1}\sum_{k=1}^{N-1}|BP_{k+1} - BP_k|$$

3.9.2 Heart rate variability

Heart rate variability is a marker of autonomic function and decreased heart rate variability is a risk factor for cardiovascular morbidity and mortality (196, 197). It can be measured over short monitored periods in a controlled environment, or over longer ambulatory periods which are not typically monitored (198). Heart rate variability can be analysed using a number of different methods, the most commonly used are time domain and frequency domain analyses. Time domain analysis uses simple mathematical techniques to describe the variability between heart beats in a specified recording period (199). Frequency domain analysis using more advanced mathematical techniques such as fast Fourier transformation and compares the variation at different frequencies (199). Commonly reported heart rate variability parameters are described below (Table 7).

Towards the end of the study visit, after skin preparation to ensure good electrode contact, 3 ECG stickers were attached to the participant's thorax and connected to a holter monitor (Lifecard CF, Spacelabs, Snoqualmie, Washington, USA). A high resolution ECG (1024 Hz) was recorded continuously for 24 hours. Subjects were asked to record their sleep periods and any exertional activity on a diary card. A stamped addressed envelope was provided to return the monitor and diary card at the end of the recording period.

The ECG recordings were analysed using Pathfinder SL version 1.7.1.4557 (Spacelabs, Snoqualmie, Washington, USA). Recordings were reviewed both manually and using automated techniques to identify and then remove artefact, ectopy and arrhythmia. To further ensure analysis of only normal beats, beat to beat intervals >120% or <80% of the previous interval were excluded, as these were more likely to represent artefact,

ectopy or arrhythmia. Mean heart rate for the entire recording duration, as well as, awake and asleep periods were calculated.

Heart rate variability was measured for the duration of edited recording and separately for a period of 10 minutes at 2am when participants were asleep. Time and frequency domain analyses were measured over 5 minute epochs and averaged over the recording period. Frequency domain analyses were further fast Fourier transformed for each epoch to assess differences in frequency power bands.

Table 7. Definition and description of common used parameters of heart rate variability

Variable	Units	Definition/description
Time Domain		
SDNNindex	msec	Mean of standard deviations of all NN intervals for all epochs for the recording
RMSSD	msec	The square root of the mean of the sum of the squares of differences between adjacent NN intervals
SDSD	msec	Standard deviation of differences between adjacent NN intervals
pNN50	%	Percentage of NN intervals with more than 50msec variation than the previous NN interval
Triangular index		Total number of NN intervals divided by the peak of the histogram of NN intervals
Frequency Domain		
Very low frequency	$msec^2$	0.017-0.05Hz
Low frequency	$msec^2$	0.05-0.15Hz
High frequency	$msec^2$	0.15-0.35Hz
Very high frequency	$msec^2$	0.35-0.50Hz
Low frequency: high frequency		Ratio of high frequency to low frequency

3.10 Medical history

Medical and pregnancy history was collected both from a subject completed questionnaire and from detailed extraction from the maternity records.

3.10.1 Maternity records

Maternity records were reviewed for all subjects. Clinical data was extracted for the duration of the pregnancy including haemodynamic changes, treatment and delivery details including birthweight and gestation. Diagnostic details were confirmed as corresponding with international guidelines for the classification of hypertensive disorders of pregnancy (72).

3.10.2 Questionnaire

Data was collected on demographic details, past medical history, medication, family history, pregnancy history, diet, smoking and alcohol intake using a validated questionnaire (see Appendix).

3.11 Calculation of lifetime risk of cardiovascular disease

Lifetime risk of cardiovascular disease, including stroke, transient ischaemic attack, angina and heart attack was calculated using a validated risk calculator *QRISK®-lifetime cardiovascular risk calculator* (200). The risk score estimates the lifetime risk for individuals aged 30 years or older. In the few cases where subjects were aged below 30 their age was adjusted to 30.

3.12 Statistical analysis

Statistical analysis was undertaken using Statistical Product and Service Solutions (SPSS) Version 20 (Armonk, New York, U.S). Normality was assessed using visual assessment of histograms and Shapiro-Wilk test. Comparison between two groups for continuous variables was performed using a two-sided, independent-samples student T-

test (parametric data) and Mann Whitney test (non-parametric data). Comparison between two groups for categorical variables was performed using a Chi-squared test. Results are reported as mean ±standard deviation (continuous variables), and number and percentage (categorical variables). Univariate linear regression was carried out to identify relevant associations between cardiovascular phenotypes and both pregnancy characteristics and cardiovascular risk factors. Multivariable modelling was then performed using a stepwise method using factors which demonstrated a significance level $p<0.1$ in univariate regression, including a history of hypertensive pregnancy if found to be significantly different between cohorts. Thereafter factors which remained significant but that were felt clinically to be confounders of other included factors were removed one by one and the model retested using a forced entry method to find the model with the best fit. Standardised and unstandardized regression coefficients (β), along with the associated 95% confidence intervals, were reported. Analysis of left ventricular shape variation was performed using non-paired t-tests of axes determined by principle component analysis (PCA); in addition linear discrimination analysis was undertaken to assess which combination of modes maximised the area under the curve (AUC) of the receiver operating characteristic. P-values <0.05 were considered statistically significant. Power calculations for this study are described in further statistical analysis sections in each results chapter 4-8.

Hypertensive Disorders of Pregnancy and Maternal Cardiac Phenotype

Chapter 4

4.1 Abstract

Introduction - A history of hypertensive pregnancy is known to be associated with myocardial infarction and heart failure. Relative left ventricular (LV) hypertrophy and dysfunction is disproportionately present during pregnancies complicated by hypertension but it is not known whether these cardiac changes persist long term.

Methods – Women with a history of hypertensive (n=103) and normotensive (n=70) pregnancy were studied 5-10 years after affected pregnancy. They underwent detailed cardiovascular phenotyping with cardiovascular magnetic resonance (CMR) imaging (1.5T scanner) and 2D echocardiography to allow detailed assessment of cardiac structure and function.

Results – Women with a history of hypertensive pregnancy had increased LV mass index (49.9±7.1 g/m^2 vs 46.0±6.5 g/m^2; p=0.001), which was still significant after

adjustment for systolic blood pressure (p=0.01). They also demonstrated greater wall thickness (5.5±0.9 mm vs 5.2±0.7 mm; p=0.02) and reduced LV volumes (LV end-diastolic volume index: 70.3±9.0 ml/m^2 vs 74.0±9.5 ml/m^2; p=0.02). They also had altered LV systolic function, with reduced systolic strain (global longitudinal strain: -18.31±4.46 % vs -19.94±3.59 %; p=0.02) and reduced diastolic function (E:A: 1.34±0.35 vs 1.52±0.45; p=0.003).

Conclusions – This is the first study using CMR to show evidence of long term increased LV mass index associated with hypertensive pregnancy, independent of blood pressure. LV mass is strongly associated with all-cause mortality, particularly in women and may, in part, explain the increased future cardiovascular risk in this population.

4.2 Introduction

In uncomplicated pregnancy maternal cardiac structure adapts to the physiological challenge of pregnancy with increased left ventricular (LV) mass and volumes, which resolve after delivery (201, 202). Functional changes in normal pregnancy have also been reported, with reduced diastolic function (202), though changes in systolic function have been more variable with increases, decreases and no change reported through pregnancy (51, 202).

In pregnancies complicated by hypertension LV hypertrophy, systolic and diastolic dysfunction have been reported to be more prevalent than in uncomplicated pregnancy, with a dose response found according to disease onset and severity (203-205). Some of these changes are attenuated, but are still present, at 1 and 2 years postpartum, particularly in early onset preeclampsia (140).

It has been hypothesised that the myocardial hypertrophic response in preeclamptic pregnancy may be an adaptive response to increased systemic afterload (203). Failure for this hypertrophy to completely resolve postpartum may in part explain the increase in future cardiovascular risk with which it is associated (206). It is also established that women with a history of hypertensive disorder of pregnancy (HDP) have higher blood pressure 6-13 years after pregnancy (75, 207). Target organ damage, such as left ventricular hypertrophy, is a sequelae of longstanding hypertension (208). In addition few studies have looked at changes in the right ventricle after hypertensive pregnancy, but those that have report increased rates of hypertrophy and reduced systolic and diastolic function (140) .

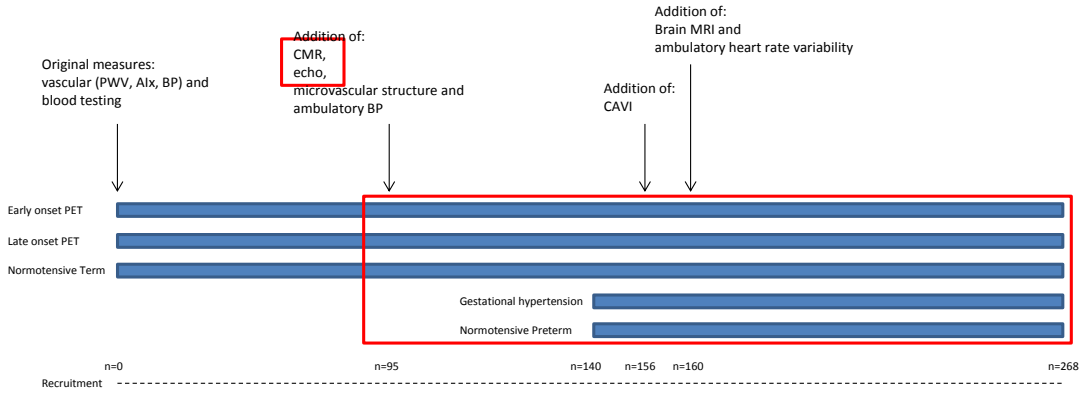
I have used complimentary imaging modalities (CMR and 2D echo) to assess whether structural differences, such as LV hypertrophy are present in women with a history of hypertensive disorder of pregnancy (HDP) compared to women with a history of normotensive pregnancy and if this relates to differences in blood pressure. I have also measured systolic and diastolic cardiac function to assess if differences exist.

4.3 Methods

4.3.1 Study population

This chapter includes 173 participants, from a total cohort size of 268, who underwent cardiac imaging. Details of this cohort and how they were recruited are set out in Study population (chapter 2).

Figure 19. Flow chart detailing the study population and the measures employed in chapter 4.



4.3.2 Study visit

All participants attended the research department after overnight fast and had blood collected. All had the following assessments and analysis, which are described in detail in Methods (chapter 3):

- CMR (see page 61)
    - Cardiac cine acquisition
    - LV mass and volumes
    - LV wall thickness
    - T1 values
    - LV Shape analysis
    - LA volumes
    - LA dimensions
    - RV mass and volumes
- 2D echo (see page 65)
    - LV volumes and EF
    - LV wall thickness
    - LV Systolic function assessment
    - LV Diastolic function assessment
    - LV speckle tracking strain assessment
    - RV Systolic function assessment

- Medical notes data extraction (see page 79)
- Medical history and lifestyle questionnaire (see page 79)
- Anthropometry (see page 51)
- Blood sampling and cardiovascular risk biomarker analysis (see page 51)
- Brachial blood pressure measurements (see page 54)

4.3.3 Statistics

For full description of statistical methods please see statistics section in Methods (Chapter 3, page 79). In brief statistical analysis was carried out using SPSS version 22 (IBM, NY, United States). A p-value <0.05 was used to identify significant differences.

Previously reported differences in systolic blood pressure between women with a history of hypertensive compared to normotensive pregnancies were 10 mmHg at a similar time after affected pregnancy [unpublished data]. The Multiethnic Study of Atherosclerosis (MESA) study (209) which reported associations between LV mass and blood pressure suggested differences in LV mass of 4.8 g would be expected with this difference in systolic blood pressure between groups. Seventy individuals in each group provided 80% power at P=0.01 to identify differences of this size between groups.

Retrospective analyses of sample sizes of the subgroups were undertaken to ensure that groups and subgroups were appropriately powered to detect differences in LV mass index. Seventy individuals in the normotensive group and 103 individuals in the hypertensive group provided 80% power at p=0.05 to identify a 0.44 standard deviation difference between groups, equivalent to 3.05 g/m^2 in LV mass index. Seventy individuals in the normotensive group and 38 individuals in the early onset preeclampsia group provided 80% power at p=0.05 to identify a 0.69 standard

deviation difference between groups, equivalent to 4.69 g/m^2 in LV mass index. Seventy individuals in the normotensive group and 44 individuals in the late onset preeclampsia group provided 80% power at p=0.05 to identify a 0.50 standard deviation difference between groups, equivalent to 3.41 g/m^2 in LV mass index. Seventy individuals in the normotensive group and 21 individuals in the gestational hypertension (GH) group provided 80% power at p=0.05 to identify a 0.85 standard deviation difference between groups, equivalent to 5.27 g/m^2 in LV mass index.

4.4 Results

4.4.1 Study cohort characteristics

The cohorts of normotensive and hypertensive pregnancies were well matched in age and proportion of current smokers, though duration since index pregnancy was significantly lower in women with a history of normotensive pregnancy (see Table 8). Gestation and birthweight were significantly lower in the hypertensive pregnancy cohort. Women with a history of hypertensive pregnancy also had fewer pregnancies and a greater number of complicated pregnancies. Women with a history of HDP had higher blood pressures and were more likely to be taking antihypertensive medications. Despite no significant differences in body mass index women with a history of HDP also had a distinct metabolic profile with lower HDL cholesterol and raised HOMA-IR levels.

The characteristics of the cardiac subgroup (shown in Table 8) were, in the main, representative of the entire cohort (n=268). The only significant differences were that the normotensive group who had cardiac imaging were studied earlier after the index pregnancy (8.3±1.9 years vs 9.0±1.9 years, p=0.02) and had a higher waist: hip ratio than the entire normotensive group (0.882±0.073 vs 0.858±0.076, p=0.04).

Table 8. Characteristics of cardiac subgroup

	NTN (n=70)	HTN (n=103)	p-value
Demographics			
Age (years)	41.0±5.1	42.1±4.7	0.17
Duration since index pregnancy (yrs.)	8.3±1.9	9.3±2.2	**0.02**
Smokers, n (%)	9 (13)	7 (7)	0.19
Taking antihypertensive Rx, n (%)	0 (0)	8 (8)	**0.02**
Pregnancy Characteristics			
Gestation of index pregnancy (wks.)	35.7±5.2	35.8±3.4	0.21
Index offspring birthweight (g)	2729±1137	2512±960	0.14
Index twin pregnancies, n (%)	7 (11)	6 (6)	0.25
Total number of live births	2.3±1.0	1.9±0.9	**0.005**
Total number of complicated births	0.4±0.6	1.4±0.6	**<0.001**
Anthropometrics			
Height (m)	1.65±0.06	1.64±0.06	0.23
Weight (kg)	70.5±14.5	71.8±14.4	0.35
BMI (Kg/m^2)	25.8±5.3	26.7±5.5	0.22
BSA (m^2)	1.79±0.19	1.80±0.18	0.50
Waist: hip	0.882±0.073	0.870±0.096	0.18
Haemodynamics			
SBP (mmHg)	115.9±14.6	123.3±14.3	**<0.001**
DBP (mmHg)	74.2±8.7	80.6±10.3	**<0.001**
MAP (mmHg)	88.1±10.2	94.8±11.0	**<0.001**
Heart rate (bpm)	59.0±8.6	61.8±9.4	**0.03**
Biochemistry			
Total Cholesterol (mmol/L)	4.97±0.84	4.84±0.80	0.34
LDL (mmol/L)	2.94±0.79	2.89±0.65	0.74
HDL (mmol/L)	1.64±0.39	1.51±0.32	**<0.05**
TChol:HDL	3.18±0.86	3.32±0.76	0.23
Triglyceride (mmol/L)	0.83±0.34	0.93±0.45	0.20
HsCRP (mg/L)	2.28±3.94	2.15±3.52	0.58
HOMA-IR	1.57±1.42	2.08±1.67	**0.01**

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as $p<0.05$. BMI, body mass index; BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; T Chol: total cholesterol; HsCRP, highly sensitive C reactive protein; HOMA IR, homeostatic model assessment of insulin resistance.

4.4.2 Increased left ventricular mass after hypertensive pregnancy

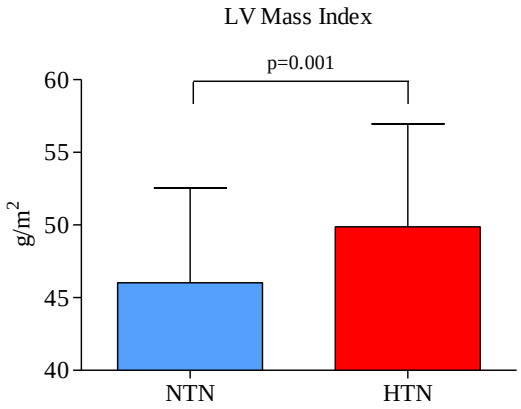
Women with a history of HDP had higher LV mass index compared to women with a history of normotensive pregnancy (49.9±7.1 g/m^2 vs 46.0±6.5 g/m^2, p=0.001) (figure 20). This was also demonstrated through overall higher mean LV wall thickness (5.5±0.9 mm vs 5.2±0.7 mm, p=0.02) (table 9). However, in the main, individual wall dimensions were not different, measured either by CMR or echo. There was also no difference between cohorts in native T1 values to suggest an underlying difference in myocardial tissue characteristic to accompany the difference in mass.

Table 9. Left ventricular mass, wall thickness and volumes

	NTN	HTN	p-value
Mass and Wall Thickness			
CMR	(n=63)	(n=90)	
LV mass index (g/m^2)	46.0±6.5	49.9±7.1	**0.001**
Wall thickness			
Anterior (mm)	4.6±1.0	4.7±1.2	0.87
Lateral (mm)	4.6±0.9	5.0±1.2	0.07
Inferolateral (mm)	4.3±1.0	4.8±1.2	**0.01**
Inferior (mm)	5.0±1.1	5.2±1.2	0.22
Inferoseptum (mm)	6.5±1.1	6.9±1.2	0.08
Anteroseptum (mm)	5.9±1.2	6.3±1.5	0.12
Mean wall thickness (mm)	5.2±0.7	5.5±0.9	**0.02**
T1 values (msec)	965±17	970±26	0.39
Echo	(n=67)	(n=97)	
Septum (mm)	9.6±2.0	9.3±2.1	0.29
LV posterior wall (mm)	8.6±1.4	8.7±1.5	0.83
Volumes			
CMR	(n=63)	(n=90)	
LV EDV Index (ml/m^2)	74.0±9.5	70.3±9.0	**0.01**
LV ESV Index (ml/m^2)	26.9±5.0	24.3±5.1	**0.002**
LVM/EDV	0.63±0.08	0.71±0.09	**<0.001**
2D Echo	(n=67)	(n=90)	
LV EDV Index (ml/m^2)	46.1±10.2	42.7±8.5	**0.02**
LV ESV Index (ml/m^2)	16.6±5.0	15.4±4.1	0.14

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as p<0.05. LV: left ventricle; EDV: end diastolic volume; ESV: end systolic volume; LVM/EDV: left ventricular mass/end diastolic volume.

Figure 20. Bar graph showing higher left ventricular mass indexed to body surface area (LV Mass Index) in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (49.9±7.1 g/m2 vs 46.0±6.5 g/m2, p=0.001).

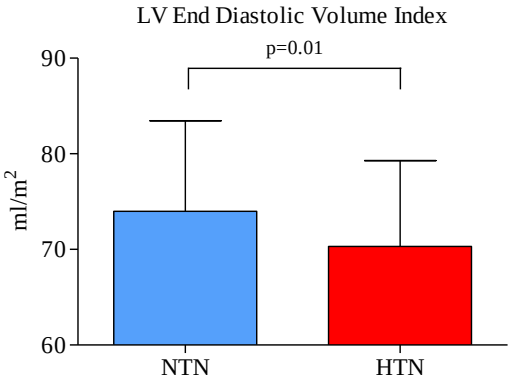


4.4.3 Reduced left ventricular volumes after hypertensive pregnancy

Women with a history of HDP had reduced LV volumes, measured by CMR, compared to women with a history of normotensive pregnancy, with lower end diastolic volume

index (70.3±9.0 ml/m^2 vs 74.0±9.5 ml/m^2, p=0.01) (figure 21) and end systolic volume index (24.3±5.1 ml/m^2 vs 26.9±5.0 ml/m^2, p=0.002) (table 9). Echocardiographic measurement of LV end diastolic volume index was significantly reduced (42.7±8.5 ml/m^2 vs 46.1±10.2ml/m^2, p=0.02) but LV end systolic volume index was not significantly reduced (15.4±4.1 ml/m^2 vs 16.6±5.0 ml/m^2, p=0.14).

Figure 21. Bar graph showing lower LV end diastolic volume index in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (70.3±9.0 ml/m^2 vs 74.0±9.5 ml/m^2, p=0.01)



Furthermore I explored how LV mass and volume together related between cohorts. Women with a history of HDP had an increased ratio of LV mass: end diastolic volume (0.71±0.09 vs 0.63±0.08, p<0.001) suggesting the relative difference in LV hypertrophy was concentric.

In addition I explored which factors were associated with the differences in LV mass index using univariate linear regression. I found that blood pressure was significantly associated with LV mass index (see table 10). I went on to test a multivariable model of left ventricular mass index and showed that a history of hypertensive disorder of pregnancy, current smoking status and systolic blood pressure were all significantly and independently associated with LV mass index (table 11).

Table 10. Relationship of variables with left ventricular mass index using univariate regression

LV Mass Index	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
SBP (mmHg)	0.17	0.10	0.25	**<0.001**
DBP (mmHg)	0.15	0.03	0.26	**0.01**
MAP (mmHg)	0.18	0.08	0.29	**0.001**
Central SBP (mmHg)*	0.13	0.06	0.21	**0.001**
Central DBP (mmHg)*	0.13	0.02	0.24	**0.02**
Central MAP (mmHg)*	0.15	0.05	0.25	**0.003**
Gestation (weeks)	-0.06	-0.33	0.21	0.65
Offspring birthweight (g)	<0.01	<-0.01	<0.01	0.54
Subject birthweight (g)	<0.01	<-0.01	<0.01	0.23
Number of pregnancies	0.45	-0.81	1.70	0.48
Years since index pregnancy	0.39	-0.21	1.00	0.20
Twin pregnancies	0.63	-4.09	5.35	0.79
Smoking (current status)	3.71	-0.34	7.76	0.07
Total Cholesterol (mmol/L)	0.12	-1.28	1.51	0.87
LDL (mmol/L)	-0.29	-1.88	1.31	0.72
HDL (mmol/L)	1.22	-2.32	4.75	0.50
Triglyceride (mmol/L)	0.54	-1.84	2.92	0.65
Glucose (mmol/L)	1.02	-1.70	3.73	0.46
HsCRP (mg/L)	0.05	-0.26	0.36	0.75
Creatinine (micromol/L)	-0.08	-0.25	0.10	0.40
Weight (kg)	0.03	-0.04	0.11	0.37

Statistical significance was defined as p<0.05. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; *central blood pressure measured using SphygmoCor; ǂ booking blood pressure was the blood pressure recorded at the first ante-natal appointment in the index pregnancy; LDL: low density lipoprotein; HDL: high density lipoprotein; T Chol: total cholesterol; HsCRP: highly sensitive C reactive protein; BMI: body mass index.

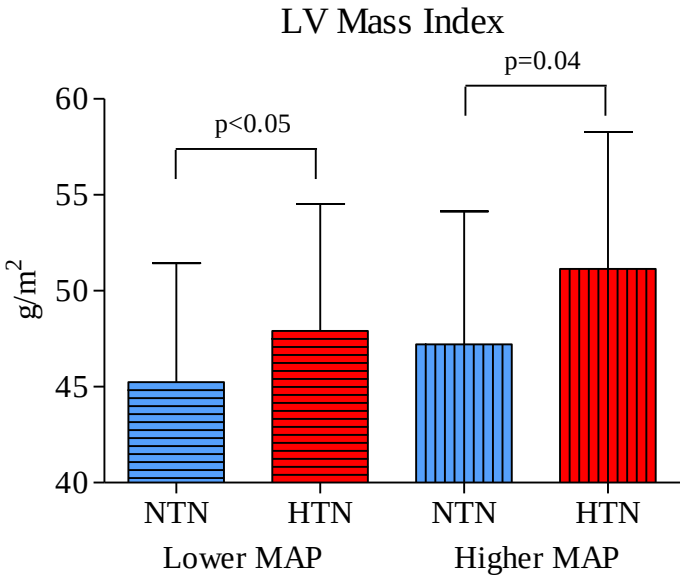
Table 11. Model of left ventricular mass index

LV Mass Index Model	Standardized β Coefficient	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
History of HDP	0.213	3.11	0.86	5.35	0.007
Current smoking status	0.176	4.44	0.69	8.20	0.02
SBP (mmHg)	0.291	0.15	0.07	0.23	<0.001

Statistical significance was defined as p<0.05. HDP: hypertensive disorder of pregnancy; SBP: systolic blood pressure.

To test whether a history of HDP was associated with LV mass index across a spectrum of blood pressure, I split the cohort in half according to mean arterial pressure and compared groups in those with mean arterial pressure (MAP) below the cohort mean and separately in those with MAP above the cohort mean. This showed the difference in LV mass index between individuals with a history of HDP compared to individuals with a history of normotensive pregnancy remained in both subgroups of lower and higher mean arterial pressure (see Figure 22).

Figure 22. Bar graph showing higher LV mass index in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) both in individuals with mean arterial pressure below the cohort mean (horizontal lines) (47.7±6.8 g/m^2 vs 44.6±5.7 g/m^2, p<0.05) and in individuals with mean arterial pressure above the cohort mean (vertical lines) (51.1±7.0 g/m^2 vs 47.8±7.1 g/m^2, p=0.04).



I further explored the relationship between the number of affected pregnancies and LV mass index, by comparing LV mass index separately between women who had experienced 1 normotensive pregnancy with those who had experienced 1 hypertensive pregnancy and also between women who had experienced 2 or 3 normotensive pregnancies with 2 or 3 hypertensive pregnancies. Women with 4 or more normotensive or hypertensive pregnancies were rare and therefore excluded from this analysis.

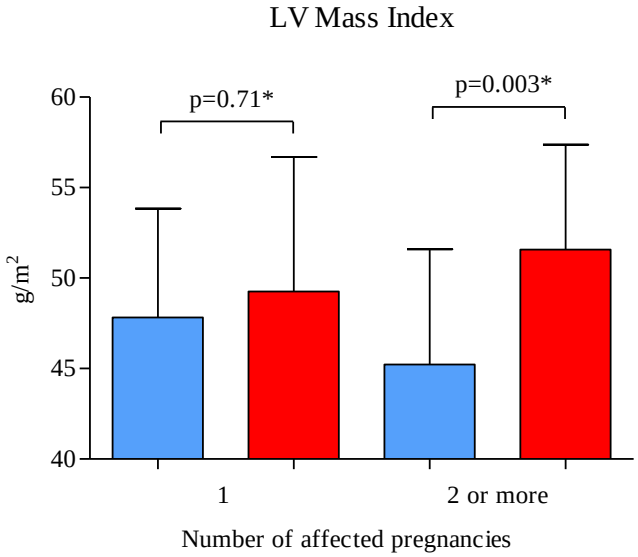
There was no statistically significant difference in LV mass index between women with a history of 1 normotensive pregnancy and those with a history of 1 hypertensive pregnancy, adjusted for age (47.82±6.03 g/m2 vs 49.26±7.44 g/m2, p=0.58) (Figure 23). However LV mass index was significantly greater in women with a history of 2 or 3 hypertensive pregnancies than in women with 2 or 3 normotensive pregnancies,

adjusted for age and systolic blood pressure (51.57±5.81 g/m2 vs 45.23±6.36 g/m2, p<0.001).

Sensitivity analysis showed that differences in left ventricular mass index were still present between groups after excluding women with a history of normotensive preterm delivery (p=0.003) and separately excluding women currently taking antihypertensive medication (p=0.001).

However a sensitivity analysis showed that left ventricular mean wall thickness was no longer significantly different between groups after excluding participants with a history of normotensive preterm delivery, but showed a strong trend to differences (p=0.05). Similarly exclusion of participants currently taking antihypertensive medication demonstrated a non-significant trend to differences in mean wall thickness between groups (p=0.06).

Figure 23. Bar graph demonstrating the relationship between the number of affected pregnancies and LV mass index. There was no difference in LV mass index between women with a history of 1 hypertensive pregnancy compared to women with a history of 1 normotensive pregnancy (49.26±7.44 g/m^2 vs 47.82±6.03 g/m^2, p=0.71*), however women with a history of 2 or more hypertensive pregnancies had significantly greater LV mass index compared to women with a history of 2 or more normotensive pregnancies (51.57±5.81 g/m^2 vs 45.23±6.36 g/m^2, p=0.003*). *adjusted for age and blood pressure.



I then used linear regression to explore which relevant factors were associated with LV end diastolic volume index (Table 12). I found that blood pressure (measured during the study visit), current smoking and several metabolic markers (HDL cholesterol, triglyceride and HOMA-IR) were all significantly associated with LV end diastolic volume index.

Table 12. Relationship of variables with left ventricular end diastolic volume index using univariate regression

LVEDV Index	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
SBP (mmHg)	-0.09	-0.19	0.02	0.10
DBP (mmHg)	-0.22	-0.37	-0.08	**0.003**
MAP (mmHg)	-0.18	-0.31	-0.04	**0.01**
Central SBP (mmHg)*	-0.10	-0.20	<0.01	0.06
Central DBP (mmHg)*	-0.16	-0.31	-0.02	**0.03**
Central MAP (mmHg)*	-0.15	-0.28	-0.01	**0.03**
Booking SBP (mmHg) †	0.02	-0.11	0.14	0.81
Booking DBP (mmHg) †	0.05	-0.13	0.24	0.57
Booking MAP (mmHg) †	0.04	-0.06	0.13	0.44
Gestation (wks.)	0.15	-0.21	0.50	0.41
Offspring birthweight (g)	<0.01	<0.01	<0.01	0.10
Subject birthweight (g)	<0.01	<0.01	<0.01	**0.02**
Number of pregnancies	1.47	-0.17	3.11	0.08
Duration since index pregnancy (yrs.)	-0.45	-1.25	0.34	0.26
Twin pregnancies	-1.34	-7.54	4.87	0.67
Smoking	6.11	0.83	11.40	**0.02**
Total Cholesterol (mmol/L)	0.10	-1.74	1.94	0.91
LDL (mmol/L)	-0.73	-2.83	1.36	0.49
HDL (mmol/L)	8.08	3.66	12.51	**<0.001**
Triglyceride (mmol/L)	-4.99	-7.99	-1.99	**0.001**
HOMA-IR	-1.20	-2.34	-0.06	**0.04**
HsCRP (mg/L)	-0.27	-0.68	0.14	0.19
Creatinine (micromol/L)	-0.03	-0.27	0.20	0.78
Weight (kg)	-0.02	-0.12	0.08	0.68

Statistical significance was defined as p<0.05. LVEDV: left ventricle end diastolic volume; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; *central blood pressure measured using SphygmoCor; † booking blood pressure was the blood pressure recorded at the first ante-natal appointment in the index pregnancy; LDL: low density lipoprotein; HDL: high density lipoprotein; T Chol: total cholesterol; HsCRP: highly sensitive C reactive protein; HOMA IR: homeostatic model assessment of insulin resistance; BMI: body mass index.

I went on to test a multivariable model of LV end diastolic volume index. Diastolic blood pressure, current smoking status, HDL cholesterol and subject birthweight were

all significantly and independently associated with LV end diastolic volume index (Table 13).

Group differences in left ventricular end diastolic volume index remained after excluding women with a history of normotensive preterm delivery (p=0.005) and separately excluding those currently taking antihypertensive medication (p=0.02).

Table 13. Model of left ventricular end diastolic volume index using univariate regression

LVEDV Index Model	Standardized β Coefficient	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
Subject birthweight (g)	0.188	0.003	<0.01	0.01	0.03
DBP (mmHg)	-0.199	-0.188	-0.35	-0.03	0.02
Current smoking status	0.186	6.143	0.62	11.66	0.03
HDL (mmol/L)	0.283	7.38	3.02	11.75	0.001

Statistical significance was defined as p<0.05. LVEDV: left ventricle end diastolic volume; DBP: diastolic blood pressure; HDL: high density lipoprotein.

In order to assess whether differences in LV mass index and volumes found in women with a history of HDP differed according to the severity of HDP, I looked at three subgroups of HDP: early onset preeclampsia (diagnosed before 34 weeks gestation), late onset preeclampsia (diagnosed at or after 34 weeks gestation) and gestational hypertension. Firstly I explored demographic characteristics of the HDP subgroups were comparable to whole HDP group (see Table 14). Characteristics of the subgroups of women with a history of HDP were similar to the combined group of women with a history of HDP, with a few notable exceptions. Compared to women with a history of normotensive pregnancy, women with a history of late onset preeclampsia tended to be older and both women with a history of late onset preeclampsia and gestational hypertension were studied later after their index pregnancy. Women with a history of early and late onset preeclampsia were more likely to be on antihypertensive treatment and women with a history of early onset preeclampsia were more likely to have lower

gestation of their index pregnancy and lower offspring birthweight. Women with a history of preeclampsia were more likely to have a lower number of total live births and all subgroups of women with a history of HDP were more likely to have a higher number of complicated pregnancies and higher blood pressures. Women with a history of late onset preeclampsia were more likely to have higher heart rates, an adverse metabolic profile and raised highly sensitive CRP, though women with a history of early onset preeclampsia also tended to have higher HOMA-IR.

I found that differences in LV mass index found in the group of women with a history of HDP were present in women from all subgroups of HDP (early onset preeclampsia: $p=0.02$, late onset preeclampsia: $p=0.02$, GH: $p=0.002$) (see table 15). Differences in LV end systolic volumes were present between women with a history of normotensive pregnancy and those with a history of early and late onset preeclampsia but not GH ($p=0.02$, $p=0.002$ and $p=0.24$ respectively). Also, differences in LV mass: end diastolic volume remained between women with a history of normotensive pregnancy and all three subgroups of women with a history of HDP ($p<0.001$ for all 3 subgroups). Mean wall thickness was higher in women with a history of late onset preeclampsia and GH compared to a history of normotensive pregnancy ($p<0.05$ and $p=0.03$ respectively), however there was only a non-significant trend to higher wall thickness in women with a history of early onset preeclampsia ($p=0.08$).

Table 14. Characteristics of cardiac subgroup separated according to severity of hypertensive disorder of pregnancy.

	NTN (n=70)	Early PET (n=39)	Late PET (n=43)	GH (n=21)	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)	NTN vs GH (p-value)
Demographics							
Age (years)	41.0±5.1	41.0±4.8	43.0±4.7	42.1±4.5	0.98	**<0.05**	0.35
Years since index pregnancy	8.3±1.9	8.6±2.2	9.6±2.4	9.7±1.6	0.37	**0.005**	**0.005**
Smokers, n (%)	9 (13%)	3 (9)	1 (2)	3 (15)	0.46	0.05	0.84
Antihypertensive Rx, n (%)	0 (0)	5 (13)	3 (7)	0 (0)	**0.002**	**0.03**	>0.99
Pregnancy Characteristics							
Gestation of index pregnancy (wks.)	35.7±5.2	32.5±2.8	37.6±1.6	38.3±2.2	**0.001**	0.75	0.16
Index offspring birthweight (g)	2729±1137	1650±750	2934±615	3088±850	**<0.001**	0.57	0.28
Index twin pregnancies, n (%)	7 (11)	4 (11)	1 (2)	1 (5)	0.94	0.11	0.41
Total number of live births	2.3±1.0	1.9±0.9	1.9±0.9	2.1±0.7	**0.04**	**0.007**	0.33
Total number of complicated births	0.4±0.6	1.4±0.7	1.2±0.5	1.6±0.8	**<0.001**	**<0.001**	**<0.001**
Anthropometrics							
Height (m)	1.65±0.06	1.64±0.06	1.64±0.06	1.65±0.07	0.18	0.33	0.88
Weight (kg)	70.5±14.5	71.8±16.9	71.2±14.0	73.1±10.2	0.75	0.57	0.14
BMI (Kg/m^2)	25.8±5.3	26.9±6.4	26.3±5.0	27.0±4.8	0.49	0.29	0.29
BSA (m^2)	1.79±0.19	1.80±0.21	1.79±0.18	1.83±0.12	0.86	0.79	0.17
Haemodynamics							
SBP (mmHg)	115.9±14.6	121.9±14.4	125.3±14.2	121.9±14.4	**0.02**	**<0.001**	0.06
DBP (mmHg)	74.2±8.7	79.5±9.2	81.6±10.2	80.5±12.7	**0.007**	**<0.001**	**0.03**
MAP (mmHg)	88.1±10.2	93.6±10.5	96.2±10.6	94.3±12.7	**0.009**	**<0.001**	**0.03**
Heart rate (bpm)	59.0±8.6	60.9±10.8	62.8±8.0	61.7±9.5	0.23	**0.02**	0.22
Biochemistry							
Total Cholesterol (mmol/L)	4.97±0.84	4.88±0.81	4.88±0.82	4.67±0.74	0.64	0.61	0.20
LDL (mmol/L)	2.94±0.79	2.92±0.68	2.91±0.63	2.80±0.68	0.92	0.86	0.51
HDL (mmol/L)	1.64±0.39	1.52±0.41	1.50±0.28	1.51±0.25	0.11	0.10	0.30
TChol:HDL	3.18±0.86	3.38±0.90	3.31±0.56	3.23±0.88	0.31	0.21	0.86
Triglyceride (mmol/L)	0.83±0.34	0.97±0.53	0.96±0.37	0.81±0.47	0.23	0.05	0.31
HOMA-IR	1.57±1.42	1.96±1.27	2.36±2.12	1.69±1.21	**0.03**	**0.01**	0.76
HsCRP (mg/L)	2.28±3.94	1.95±2.19	2.74±4.84	1.24±1.45	0.74	0.41	0.92

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as $p<0.05$. PET: history of preeclampsia; GH: history of gestational hypertension; BMI, body mass index; BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; T Chol: total cholesterol; HOMA IR, homeostatic model assessment of insulin resistance; HsCRP, highly sensitive C reactive protein.

Table 15. Left ventricular structure and severity of hypertensive disorder of pregnancy.

	NTN	Early PET	Late PET	GH	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)	NTN vs GH (p-value)
CMR	(n=63)	(n=34)	(n=38)	N=18)			
LV Mass Index (g/m^2)	46.0±6.5	49.4±6.8	49.6±7.8	51.4±6.1	**0.02**	**0.02**	**0.002**
LVEDV Index (ml/m^2)	74.0±9.5	69.1±8.0	70.4±9.5	72.5±9.5	**0.01**	0.07	0.56
LVESV Index (ml/m^2)	26.9±5.0	24.3±5.3	23.8±5.1	25.3±4.9	**0.02**	**0.002**	0.24
LVM:LVEDV	0.625±0.079	0.721±0.104	0.709±0.084	0.713±0.082	**<0.001**	**<0.001**	**<0.001**
Wall thickness (mm):							
Anterior	4.6±1.0	4.8±1.1	4.7±1.4	4.4±0.7	0.49	0.96	0.54
Lateral	4.6±0.9	4.8±1.4	5.1±1.2	4.9±0.9	0.74	**0.03**	0.12
Inferolateral	4.3±1.0	4.6±1.3	4.8±1.2	5.1±0.9	0.32	**0.02**	**0.002**
Inferior	5.0±1.1	5.1±1.2	5.2±1.1	5.4±1.3	0.57	0.26	0.22
Inferoseptum	6.5±1.1	6.8±1.3	6.8±1.1	7.0±1.3	0.13	0.25	0.08
Anteroseptum	5.9±1.2	6.5±1.7	6.1±1.3	6.5±1.3	**0.04**	0.55	0.10
Mean wall thickness	5.2±0.7	5.5±1.0	5.5±0.9	5.6±0.8	0.08	**<0.05**	**0.03**
T1 values (msec)	965±17	970±27	975±31	965±19	0.38	0.19	0.95
Echo	(n=67)	(n=35)	(n=41)	(n=21)			
Septum (mm)	9.6±2.0	9.3±2.3	9.0±2.1	9.9±1.6	0.43	0.10	0.58
LV posterior wall (mm)	8.6±1.4	8.5±1.4	8.6±1.7	9.1±1.4	0.83	0.73	0.15
LVEDV Index (ml/m^2)	46.1±10.2	43.2±9.5	41.3±6.7	44.0±9.4	0.18	**0.02**	0.42
LVESV Index (ml/m^2)	16.6±5.0	15.3±4.5	14.8±3.9	16.4±3.6	0.18	0.08	0.90

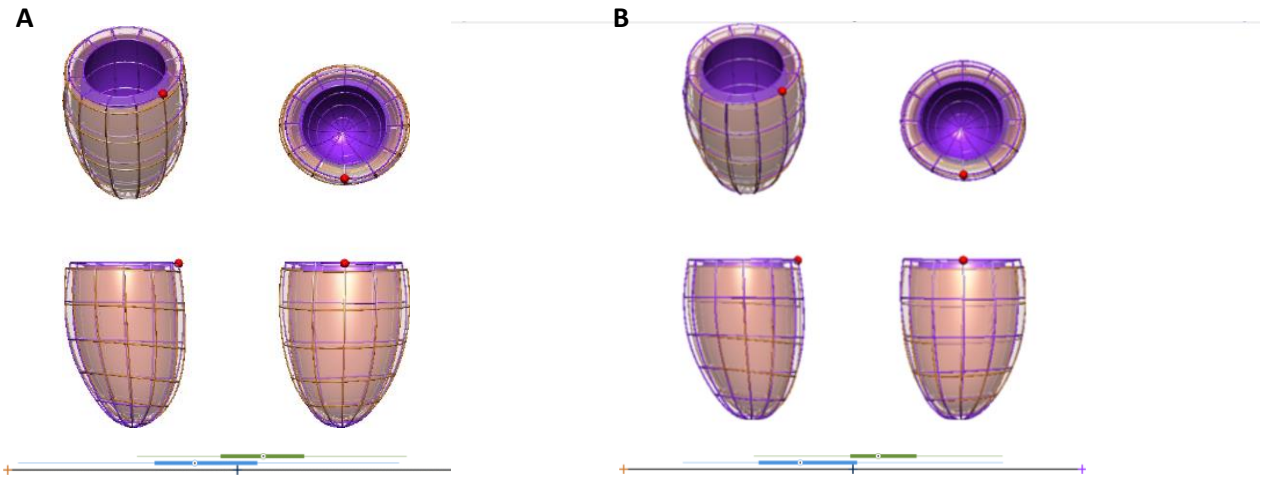
Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as $p<0.05$. PET: history of preeclampsia; GH: history of gestational hypertension; LV: left ventricle; EDV: end diastolic volume; ESV: end systolic volume; LVM/EDV: left ventricular mass/end diastolic volume.

4.4.4 Left ventricular shape analysis

An average fitting accuracy of 1.39 mm was achieved in the process of automatic fitting of the meshes to the manually drawn cardiac contours. The first 7 principal component analysis (PCA) modes of anatomical variation combined explained 89% of the LV anatomical variance in the entire population. The first mode related to the positioning of the basal slice and orientation of the RV, which related to the acquisition of CMR images. Modes 2 through 7 predominantly described differences in wall thickness and diameter which closely related to differences in wall thickness and volumes shown through simple manual analysis. Mode 6 (wall thickness) was the single best PCA mode for distinguishing between groups (area under curve [AUC]=0.73, $p<10^{-6}$) (Figure 22A). No other individual mode significantly differentiated the two groups of normotensive versus hypertensive mothers.

Further exploration of additional modes was undertaken to assess if a combination of modes by linear discrimination analysis (LDA) would reveal further anatomical differences. The combination of modes 3 to 7 demonstrated the greatest classification ability in the cross-validation scenario (AUC=0.78, p= $p<10^{-6}$) (Figure 22B). Any LDA combination that contained the mode 6 was predominantly weighted by this mode, and therefore qualitatively very similar to it (compare Figure 24 A and B). Nevertheless, there were further combinations without mode 6 that revealed differences, specifically modes 1 to 4 (AUC=0.62, $p<0.001$), which also captured significant phenotypic differences between early and late onset preeclampsia using the combination of modes 1 through 4 ($p=0.009$). This highlighted that women with a history of late onset preeclampsia have more eccentric remodelling whereas women with a history of early onset preeclampsia have more concentric remodelling.

Figure 24. Linear discrimination analysis using cardiac atlases. Linear discrimination between extreme hypertensive (orange) and normotensive (purple) phenotypes by mode 6 (Figure 21A) and linear discrimination by modes 3-7 (Figure 21B). The box plots demonstrate the median, quartile range and overall range against the x axis.



4.4.5 Left ventricular systolic function and history of hypertensive pregnancy

Having compared structural differences according to a history of HDP I proceeded to compare LV function between groups. Women with a history of HDP had higher LV ejection fractions (EF) measured by CMR compared to women with a history of normotensive pregnancy (CMR LVEF: 65.6±5.4 % vs 63.7±4.3 %, p=0.03) (figure 25), however this was not found when measured with 2D echo (2D echo LVEF: 64.0±6.5 % vs 64.1±6.5 %, p=0.91) (see table 16). There was no difference in stroke volume index between groups measured by CMR (p=0.41), however when measured by echo women with a history of HDP had significantly lower values compared to women with a history of normotensive pregnancy (echo stroke volume index: 27.3±6.2 ml/m^2 vs 29.4±6.9 ml/m^2, p<0.05). There were no significant differences in S' measured by tissue Doppler imaging of the septum or lateral wall (p=0.24 and p=0.09 respectively).

Global longitudinal strain and displacement were both reduced in women with a history of HDP compared to women with a history of normotensive pregnancy (global longitudinal strain: -18.31±4.46 % vs -19.94±3.59 %, p=0.02; global longitudinal

displacement: 5.21±1.89 mm vs 5.96±1.62 mm, p=0.02) (figure 26). Also, global transverse displacement was reduced (3.79±1.27 mm vs 4.30±1.02 mm, p=0.01).

Sensitivity analysis showed that differences in left ventricular ejection fraction were still present between groups after excluding individuals with a history of normotensive preterm delivery (p=0.04) but only showed a strong non-significant trend to differences after excluding women currently taking antihypertensive medication (p=0.05).

Group differences in left ventricular global longitudinal strain were no longer significant between groups after excluding women with a history of normotensive preterm delivery (p=0.15) however significant differences remained after excluding women currently taking antihypertensive medication (p=0.02).

Table 16. Left ventricular systolic function

	NTN	**HTN**	**p-value**
CMR	(n=63)	(n=90)	
Ejection Fraction (%)	63.7±4.3	65.6±5.4	**0.03**
Stroke Volume Index (ml/m^2)	47.1±6.5	46.5±7.8	0.41
Heart rate during CMR (bpm)	60.7±8.1	64.7±9.3	**0.007**
Cardiac index (ml/m^2)	2846±433	2999±504	0.07
2D echo	(n=65)	(n=86)	
Ejection Fraction (%)	64.1±6.5	64.0±6.5	0.91
Stroke Volume Index (ml/m^2)	29.4±6.9	27.3±6.2	**<0.05**
Cardiac index (ml/m^2)	182±412	1702±406	0.09
Septal S' (cm/sec)	9.4±1.6	9.2±1.7	0.24
Lateral S' (cm/sec)	11.9±3.0	11.2±2.6	0.09
Speckle Tracking (2D echo)			
2D Longitudinal			
Systolic velocity (cm/sec)	3.36±1.00	3.09±1.03	0.09
Strain (%)	-19.94±3.59	-18.31±4.46	**0.02**
Systolic strain rate (%/sec)	-1.14±0.24	-1.07±0.28	0.05
Displacement (mm)	5.96±1.62	5.21±1.89	**0.02**
2D Transverse			
Systolic velocity (cm/sec)	2.42±0.42	2.27±0.59	0.09
Strain (%)	20.55±10.84	19.99±11.83	0.64
Systolic strain rate (%/sec)	1.55±0.49	1.60±0.61	0.65
Displacement (mm)	4.30±1.02	3.79±1.27	**0.01**

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as p<0.05.

Figure 25. Bar graph showing higher left ventricular ejection fraction (LV Ejection Fraction) in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (65.6±5.4 g/m2 vs 63.7±4.3 g/m2, p=0.03).

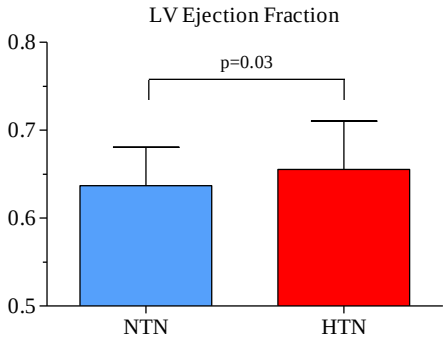
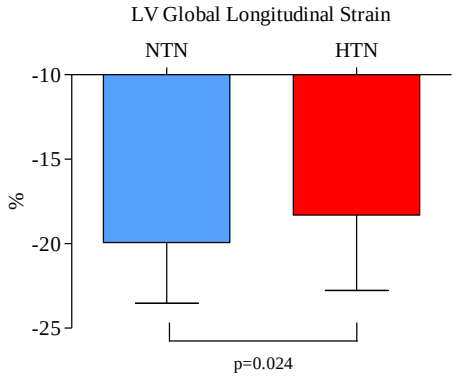


Figure 26. Bar graph showing lower left ventricular global longitudinal strain (LV Global Longitudinal Strain) in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (-18.31±4.46 % vs -19.94±3.59 %, p=0.02).



I then explored whether LV systolic function was different between women with a history of normotensive pregnancy and those with a history of early onset preeclampsia, late onset preeclampsia or GH separately. There were no significant differences in women with a history of early onset preeclampsia (all p>0.05). Women with a history of late onset preeclampsia had higher LV ejection fraction, heart rate and cardiac index, all measured by CMR (p=0.02, p<0.001 and p=0.006 respectively). However global longitudinal strain and displacement were reduced (p=0.04 and p=0.003 respectively). Women with a history of GH did not have significantly different LV ejection fraction measured by CMR (p=0.25), however they did have reduced global longitudinal strain (p=0.006) (see Table 17).

Table 17. Left ventricular systolic function according to severity of hypertensive disorder of pregnancy.

	NTN	Early PET	Late PET	GH	NTN vs Early PET p-value	NTN vs Late PET p-value	NTN vs GH p-value
CMR	(n=63)	(n=34)	(n=38)	(n=18)			
Ejection Fraction (%)	63.7±4.4	65.1±5.5	66.2±5.7	65.2±5.3	0.18	**0.02**	0.25
Stroke Volume Index (ml/m^2)	47.1±6.5	44.9±5.6	47.4±9.4	47.5±7.4	0.09	0.91	0.84
Heart rate during CMR (bpm)	60.7±8.1	62.9±8.1	67.1±8.7	63.1±11.6	0.21	**<0.001**	0.32
Cardiac index (ml/m^2)	2846±433	2835±366	3172±583	2943±455	0.79	**0.006**	0.45
2D echo	(n=65)	(n=33)	(n=32)	(n=21)			
Ejection Fraction (%)	64.1±6.5	64.8±6.0	64.2±7.1	62.4±6.3	0.61	0.94	0.28
Stroke Volume Index (ml/m^2)	29.4±6.9	27.9±6.5	26.5±5.2	27.5±7.5	0.29	**0.03**	0.29
Cardiac index (ml/m^2)	1821±412	1710±408	1710±383	1679±458	0.22	0.22	0.19
Septal S' (cm/sec)	9.4±1.6	9.3±1.8	9.0±1.8	9.3±1.1	0.49	0.25	0.87
Lateral S' (cm/sec)	11.9±3.0	11.5±3.3	10.9±2.1	11.2±2.2	0.19	0.08	0.62
Speckle Tracking Analysis (2D echo)							
2D Longitudinal							
Systolic velocity (cm/sec)	3.36±1.00	3.32±0.95	2.75±0.86	3.35±1.34	0.79	**0.007**	0.99
Strain (%)	-19.94±3.59	-19.44±4.01	-18.08±4.35	-16.66±5.18	0.57	**0.04**	**0.006**
Systolic strain rate (%/sec)	-1.14±0.24	-1.14±0.27	-1.06±0.31	-0.95±0.21	0.70	0.21	**0.008**
Displacement (mm)	5.96±1.62	5.42±1.57	4.79±1.83	5.64±2.47	0.16	**0.003**	0.55
2D Transverse							
Systolic velocity (cm/sec)	2.42±0.42	2.37±0.46	2.22±0.58	2.16±0.82	0.58	0.07	0.09
Strain (%)	20.55±10.84	25.18±13.23	18.20±10.25	14.30±8.73	0.10	0.31	**<0.05**
Systolic strain rate (%/sec)	1.55±0.49	1.60±0.47	1.60±0.65	1.58±0.79	0.14	0.82	0.74
Displacement (mm)	4.30±1.02	4.18±1.41	3.57±1.07	3.49±1.29	0.67	**0.003**	**0.01**

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as $p<0.05$.

4.4.6 Left ventricular diastolic function and history of hypertensive disorder of pregnancy

Women with a history of HDP had reductions in diastolic function compared to women with a history of normotensive pregnancy, with lower E:A (1.34±0.35 vs 1.52±0.45, p=0.003) (figure 27), lower septal e' velocity (10.47±2.68 cm/sec vs 11.36±2.20 cm/sec, p=0.03) and lower lateral e' velocity (13.74±3.25 cm/sec vs 15.07±3.23 cm/sec, p=0.01) (see Table 18). However deceleration time, septal and lateral E/e' were not significantly different (p=0.88, p=0.74 and p=0.67 respectively). I then proceeded to explore if there were structural changes in the left atrium in keeping with the finding of impaired diastolic function in women with a history of HDP. I found that left atrial volume index measured by CMR was increased in women with a history of HDP compared to women with a history of normotensive pregnancy (40.4±9.2 ml/m^2 vs 37.3±7.3 ml/m^2, p=0.03) (figure 28). However left atrial index measured by echo was not significantly different (27.3±9.4 ml/m^2 vs 29.7±9.9 ml/m^2, p=0.12).

Sensitivity analysis showed that differences in E:A persisted between groups after excluding individuals with a history of normotensive preterm delivery (p=0.008) and separately excluding those currently taking antihypertensive medication (p=0.004). However differences in left atrial volume index were no longer significant between groups after excluding participants with a history of normotensive preterm delivery (p=0.11) though significant differences remained after excluding those currently taking antihypertensive medication (p=0.02).

Figure 27. Bar graph showing lower mitral valve inflow velocity E:A ratio (E:A) in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (1.34±0.35 vs 1.52±0.45, p=0.003).

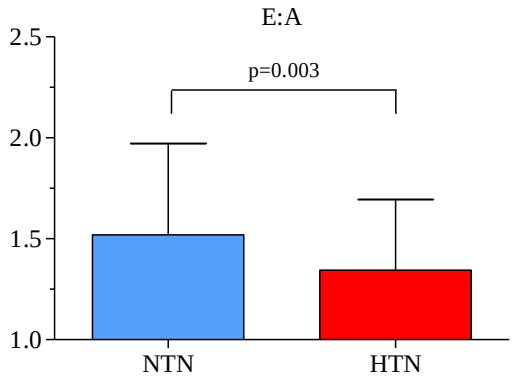
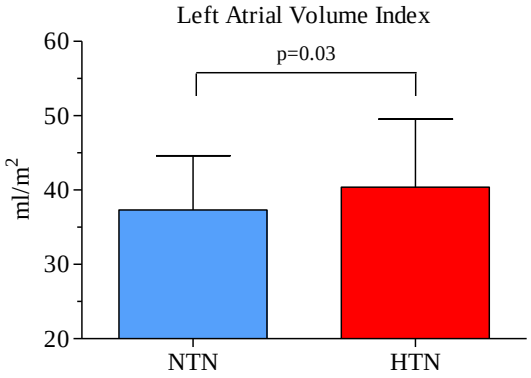


Table 18. Left ventricular diastolic function

	NTN	HTN	p-value
Diastolic Function			
2D echo	(n=67)	(n=97)	
E (cm/sec)	86.6±17.1	80.6±15.4	**0.01**
A (cm/sec)	60.6±17.5	62.3±14.3	0.35
E:A	1.52±0.45	1.34±0.35	**0.003**
E:A ratio <1, n (%)	5 (7)	12 (12)	0.33
Deceleration Time (msec)	210±48	209±41	0.88
Septal e' (cm/sec)	11.36±2.20	10.47±2.68	**0.03**
Septal E/e'	7.78±1.91	8.25±2.91	0.74
Lateral e' (cm/sec)	15.07±3.23	13.74±3.25	**0.01**
Lateral E/e'	5.99±1.69	6.27±2.21	0.67
Speckle Tracking (2D echo)			
Longitudinal			
Velocity (cm/sec)	-4.53±1.56	-3.70±1.32	**0.001**
Strain rate (%/sec)	1.49±0.49	1.31±0.42	**0.01**
Transverse			
Velocity (cm/s)	-2.88±0.79	-2.52±0.80	**0.01**
Strain rate (%/sec)	-1.80±0.68	-1.83±0.72	0.83
Atrial Volumes and Dimensions			
CMR	(n=63)	(n=90)	
Volume index (ml/m^2)	37.3±7.3	40.4±9.2	**0.03**
4Ch transverse (mm)	42.3±4.5	43.5±5.9	0.16
4Ch longitudinal (mm)	54.6±5.3	54.5±6.5	0.70
2Ch transverse (mm)	45.8±4.9	46.1±5.3	0.73
2Ch longitudinal (mm)	47.3±5.1	48.4±7.4	0.10
2D echo	(n=67)	(n=97)	
Volume index (ml/m^2)	29.7±9.9	27.3±9.4	0.12
4Ch transverse (mm)	44.6±5.9	43.2±7.3	0.16
3Ch transverse (mm)	30.9±4.7	30.9±5.4	>0.99

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as p<0.05. E: early; A: atrial; 4Ch: 4 chamber view; 2Ch: 2 chamber view; 3Ch: 3 chamber view.

Figure 28. Bar graph showing higher left atrial volume index in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (40.4±9.2 ml/m^2 vs 37.3±7.3, p=0.03).



I then explored whether LV diastolic function was different between women with a history of normotensive pregnancy and those with a history of early onset preeclampsia, late onset preeclampsia or GH separately (Table 19). Compared to women with a history of normotensive pregnancy, women with a history of early onset preeclampsia had lower left atrial volume index measured using echo (p=0.04). Women with a history of late onset preeclampsia had evidence of diastolic impairment with lower E velocities (p=0.01), E:A ratios (p<0.001), lateral e' velocity (p=0.02), global longitudinal diastolic velocity and strain (p=0.001 and p=0.04 respectively), global transverse diastolic velocity (p=0.03) and higher left atrial volume index measured using CMR (p=0.04). Women with a history of GH also had evidence of diastolic impairment with lower septal e' velocity (p=0.03), lateral e' velocity (p=0.02) and lateral E/e' (p=0.01). In addition women with a history of GH had lower global longitudinal diastolic strain rate (p=0.04), global transverse diastolic velocity (p=0.04), higher left atrial volume and 2 chamber longitudinal dimensions, measured by CMR (p=0.009 and p=0.01 respectively).

I also compared the rates of LV structural and functional disease and calculated the prevalence of stage B heart failure which was not significantly different between

women with a history of HDP compared to women with a history of normotensive pregnancy (32% vs 24%, p=0.27). Furthermore there were no significant differences when comparing individual subgroups of HDP with women with a history of normotensive pregnancy (early preeclampsia: p=0.31, late preeclampsia: p=0.67, GH: p=0.21).

Table 19. Left ventricle diastolic function according to severity of hypertensive disorder of pregnancy.

	NTN	Early PET	Late PET	GH	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)	NTN vs GH (p-value)
2D echo	(n=67)	(n=35)	(n=41)	(n=21)			
E (cm/sec)	86.6±17.1	79.9±13.3	78.4±16.2	86.3±17.5	0.05	**0.01**	0.94
A (cm/sec)	60.6±17.5	60.8±15.8	62.8±13.2	63.7±14.4	0.91	0.16	0.45
E:A	1.52±0.45	1.38±0.33	1.28±0.30	1.43±0.46	0.21	**<0.001**	0.21
E:A ratio <1, n (%)	5 (7)	6 (20)	3 (7)	3 (14)	0.15	0.95	0.34
Deceleration Time (msec)	210±48	215±45	211±36	194±42	0.91	0.60	0.18
Septal e' (cm/sec)	11.36±2.20	10.85±3.08	10.29±2.38	10.22±2.65	0.36	0.08	**0.03**
Septal E/e'	7.78±1.91	8.21±3.57	8.07±2.91	8.66±1.43	0.86	0.67	0.06
Lateral e' (cm/sec)	15.07±3.23	14.59±3.27	13.48±2.94	12.99±3.70	0.31	**0.02**	**0.02**
Lateral E/e'	5.99±1.69	5.93±2.28	6.13±2.30	7.02±1.82	0.51	0.77	**0.01**
Speckle Tracking (2D echo)							
Longitudinal							
Velocity	-4.53±1.56	-3.84±1.25	-3.53±1.29	-3.80±1.55	0.05	**0.001**	0.10
Strain rate	1.49±0.49	1.37±0.37	1.31±0.43	1.19±0.49	0.22	**0.04**	**0.04**
Transverse							
Velocity	-2.88±0.79	-2.59±0.92	-2.53±0.78	-2.36±0.59	0.19	**0.03**	**0.04**
Strain rate	-1.80±0.68	-1.97±0.87	-1.76±0.66	-1.72±0.53	0.39	0.72	0.93
Atrial Volumes and Dimensions							
CMR	(n=63)	(n=34)	(n=38)	(n=18)			
Volume index (ml/m^2)	37.3±7.3	38.3±9.5	41.0±9.0	42.8±8.4	0.42	**0.04**	**0.009**
4Ch transverse (mm)	42.3±4.5	43.8±7.0	43.0±5.5	44.0±4.5	0.35	0.27	0.15
4Ch longitudinal (mm)	54.6±5.3	53.1±5.8	55.7±6.0	54.6±8.4	0.24	0.33	0.96
2Ch transverse (mm)	45.8±4.9	46.0±5.9	46.8±5.5	44.8±3.7	0.69	0.48	0.44
2Ch longitudinal (mm)	47.3±5.1	46.1±8.7	49.5±6.4	50.4±6.1	0.80	0.14	**0.01**
Echo	(n=67)	(n=35)	(n=41)	(n=21)			
Volume index (ml/m^2)	29.7±9.9	25.7±7.5	26.4±9.6	31.6±10.7	**0.04**	0.11	0.48
4Ch transverse (mm)	44.6±5.9	44.4±8.5	42.6±7.2	42.5±5.0	0.08	0.22	0.15
3Ch transverse (mm)	30.9±4.7	30.7±6.3	30.9±4.3	31.0±6.1	0.43	0.45	0.89

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as $p<0.05$. E: early; A: atrial; 4Ch: 4 chamber view; 2Ch: 2 chamber view; 3Ch: 3 chamber view.

4.4.7 History of hypertensive pregnancy and right ventricular structure

Women with a history of HDP demonstrated a non-significant trend to increased right ventricular (RV) mass index compared to women with a history of normotensive pregnancy (15.6±3.2 g/m^2 vs 14.8±4.5 g/m^2, p=0.06). However RV volumes, both end diastolic volume index and end systolic volume index were not significantly different (p=0.37 and p=0.09 respectively). However women with a history of HDP showed increased RV mass: end diastolic volume (0.203±0.033 vs 0.186±0.048, p<0.001) (figure 29), suggesting that there is a degree of concentric remodelling not elucidated by comparison of individual measures of mass or volume alone (see Table 20).

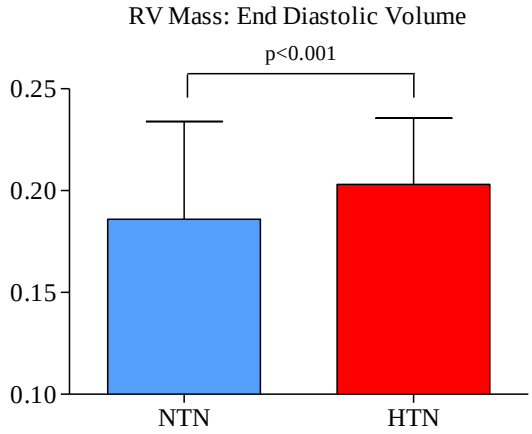
I then explored whether RV structure was different between women with a history of normotensive pregnancy and those with a history of early onset preeclampsia, late onset preeclampsia or GH separately. Compared to women with a history of normotensive pregnancy women with a history of early onset preeclampsia had increased RV mass index (15.8±2.5 g/m^2 vs 14.8±4.5 g/m^2, p<0.05). Women with a history of late onset preeclampsia had lower RV end systolic volume index (28.2±6.5 ml/m^2 vs 31.3±6.9 ml/m^2, p=0.03). Differences in RV mass: volumes were present in both groups of women with a history of either early or late onset preeclampsia (p=0.001 and p=0.02) but there was only a trend to difference in women with a history of GH which did not reach significance (p=0.06) (see Table 24).

Table 20. Right ventricular mass and volumes

	NTN	HTN	p-value
CMR	(n=63)	(n=90)	
RV Mass Index (g/m^2)	14.8±4.5	15.6±3.2	0.06
RV EDV Index (ml/m^2)	78.8±10.5	77.1±11.4	0.37
RV ESV Index (ml/m^2)	31.3±6.9	29.4±6.6	0.09
RVM/EDV	0.186±0.048	0.203±0.033	**<0.001**

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as p<0.05. RV: right ventricle; EDV: end diastolic volume; ESV: end systolic volume; RVM/EDV: right ventricular mass/end diastolic volume.

Figure 29. Bar graph showing higher RV mass: end diastolic volume in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (0.203±0.033 ml/m2 vs 0.186±0.048, p<0.001).



Furthermore I explored which relevant factors were significantly associated with RV mass index using univariate linear regression. I found that delivery gestation of index pregnancy and index offspring birthweight both had significant associations with RV mass index (see table 21).

Table 21. Relationship of variables with right ventricular mass index using univariate regression

RV Mass Index	Unstandardized β coefficient	95% confidence Lower limit	95% confidence Upper limit	p-value
Number of live births	-0.36	-1.03	0.31	0.29
Number of hypertensive pregnancies	0.22	-0.51	0.95	0.55
Gestation (weeks)	**-0.34**	**-0.48**	**-0.21**	**<0.001**
Offspring birthweight (g)	**<-0.01**	**<-0.01**	**<-0.01**	**0.001**
Subject birthweight (g)	<0.01	<-0.01	<0.01	0.83
Smoking	1.01	-1.17	3.20	0.36
RVSP (mmHg)	-0.03	-0.15	0.10	0.67
SBP (mmHg)	-0.01	-0.05	0.03	0.64
DBP (mmHg)	-0.03	-0.09	-0.04	0.40
MAP (mmHg)	-0.02	-0.08	0.04	0.48

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as p<0.05. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; RVSP: right ventricular systolic pressure.

I also explored which variables were significantly associated with RV end diastolic volume index. I found that subject birthweight and blood pressure were significantly associated (see table 22).

Table 22. Relationship of variables with right ventricular end diastolic volume index using univariate regression

RV EDV Index	Unstandardized B coefficient	95% confidence Lower limit	95% confidence Upper limit	p-value
Number of live births	-0.53	-1.43	2.48	0.60
Number of hypertensive pregnancies	-1.07	-3.19	1.05	0.32
Gestation (weeks)	-0.08	-0.50	0.34	0.70
Offspring birthweight (g)	<0.01	<-0.01	<0.01	0.47
Subject birthweight (g)	0.01	<0.01	0.01	**0.01**
Smoking	3.61	-2.71	9.94	0.26
RVSP (mmHg)	0.23	-0.12	0.58	0.19
SBP (mmHg)	-0.13	-0.25	-0.01	**0.04**
DBP (mmHg)	-0.23	-0.41	-0.06	**0.01**
MAP (mmHg)	-0.21	-0.37	-0.04	**0.02**

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as $p<0.05$. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; RVSP: right ventricular systolic pressure.

4.4.8 History of hypertensive pregnancy and right ventricular function

Having compared RV structure according to a history of HDP I proceeded to compare RV function between groups. There was no statistically significant differences in RV ejection fraction or stroke volume index measured by CMR between women with a history of HDP and those with a history of normotensive pregnancy (RVEF: 62.1±5.5 % vs 60.5±5.8 %, p=0.09; RV stroke volume index: 47.8±7.4 ml/m^2 vs 47.5±6.8 ml/m^2, p=0.86) (Table 23). However compared to women with a history of normotensive pregnancy women with a history of HDP demonstrated increased heart rate (60.7±8.1 bpm vs 64.7±9.3 bpm respectively, p=0.007) and RV cardiac index (2877±486 ml/m^2 vs 3116±557 ml/m^2, p=0.007).

Table 23. Right ventricular systolic function

	NTN	HTN	p-value
CMR	(n=63)	(n=90)	
RV Ejection Fraction (%)	60.5±5.8	62.1±5.5	0.09
RV SV Index (ml/m^2)	47.5±6.8	47.8±7.4	0.86
Heart rate during CMR (bpm)	60.7±8.1	64.7±9.3	**0.007**
RV Cardiac Index (ml/m^2)	2877±486	3116±557	**0.007**
2D Echo	(n=51)	(n=55)	
TAPSE (cm)	2.5±0.4	2.5±0.37	0.98
RV S' (cm/sec)	15.2±2.7	15.1±2.3	0.85
RV systolic pressure (mmHg)	15.4±7.4	14.7±6.2	0.84

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as $p<0.05$. RV: right ventricle; SV: stroke volume; TAPSE: tricuspid annular plane systolic excursion.

I then explored whether RV function differed between individual subgroups of HDP (early onset preeclampsia, late onset preeclampsia or GH separately) and women with a history of normotensive pregnancy (see table 24). Compared to women with a history of normotensive pregnancy, both women with a history of early onset preeclampsia and separately those with a history of GH, had no significant differences in RV systolic function (all $p>0.05$). However women with a history of late onset preeclampsia had higher RV ejection fraction, heart rate and cardiac index, all measured using CMR ($p=0.02$, $p<0.001$ and $p<0.001$ respectively).

Table 24. Right ventricular structure and function according to severity of hypertensive disorder of pregnancy

	NTN	Early PET	Late PET	GH	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)	NTN vs GH (p-value)
Structure							
CMR	(n=63)	(n=34)	(n=38)	(n=18)			
RV Mass Index (g/m^2)	14.8±4.5	15.8±2.5	15.3±3.6	15.9±3.8	**<0.05**	0.31	0.21
RVEDV Index (ml/m^2)	78.8±10.5	76.1±10.6	76.9±11.7	79.7±12.4	0.23	0.41	0.74
RVESV Index (ml/m^2)	31.3±6.9	29.8±6.7	28.2±6.5	31.1±6.4	0.31	**0.03**	0.94
RV Mass: Volume	0.186±0.048	0.210±0.025	0.198±0.035	0.201±0.038	**0.001**	**0.02**	0.06
Function							
CMR	(n=63)	(n=34)	(n=38)	(n=18)			
RV Ejection Fraction (%)	60.5±5.8	61.2±5.6	63.4±5.8	61.1±4.0	0.58	**0.02**	0.69
RV SV Index (ml/m^2)	47.5±6.8	46.4±7.1	48.7±8.2	48.4±7.4	0.41	0.46	0.63
Heart rate during CMR (bpm)	60.7±8.1	62.9±8.1	67.1±8.7	63.1±11.6	0.21	**<0.001**	0.32
RV Cardiac Index (ml/m^2)	2877±486	2922±392	3334±636	3025±507	0.65	**<0.001**	0.26
2D Echo	(n=51)	(n=19)	(n=16)	(n=20)			
TAPSE (cm)	2.5±0.4	2.5±0.4	2.5±0.3	2.6±0.4	0.57	0.85	0.52
RV S' (cm/sec)	15.2±2.7	15.3±2.0	15.3±2.7	14.6±2.5	0.82	0.90	0.45
RV systolic pressure (mmHg)	15.4±7.4	14.6±6.7	11.4±4.6	17.2±5.9	0.71	0.10	0.17

Values as mean ± standard deviation unless otherwise stated. Statistical significance was defined as $p<0.05$. RV: right ventricle; EDV: end diastolic volume; ESV: end systolic volume; LVM/EDV: left ventricular mass/end diastolic volume; SV: stroke volume; TAPSE: tricuspid annular plane systolic excursion.

4.5 Discussion

The results presented in this chapter provide the first evidence of long term cardiac structural changes associated with HDP using CMR, which is the gold standard technique for measuring cardiac structure. The findings that LV mass is increased in women with a history of HDP is further supported by increased wall thickness dimensions. I also show that these increases in mass are associated with lower end-diastolic and end-systolic volumes which suggest that the relative hypertrophy is concentric in nature. I show that a statistically significant proportion of the differences found in LV mass between women with a history of HDP or normotensive pregnancy are independent of blood pressure. Furthermore there is a mixed picture of differences in LV systolic function but that diastolic function is overall reduced in women with a history of HDP. Although the RV structure is less affected than the LV, the findings of increased mass: volume ratio are similar; though without detectable differences in function.

Clinical Relevance

Left ventricular hypertrophy has been shown to be strongly associated with mortality of any cause, independent of blood pressure (17) and the finding of 8% higher LV mass in women with a history of HDP may explain a proportion of the increase in cardiovascular risk seen in this population (78, 210). In the Multi-Ethnic Study of Atherosclerosis, a large CMR based study of men and women free of cardiovascular disease, a 10% increase in LV mass correlated with a 40% increased risk of heart failure events (211). Recently Tsao et al (212) reported on the associations in a large Framingham cohort between both LV mass index and LVM: LVEDV and the future risk of cardiovascular disease over a median follow up of 8.4 years. They found that for every $10g/m^2$ increase in LV mass index there was a 36% increase in cardiovascular

disease and for every 0.2g/ml increase in LVM: LVEDV there was an increased risk of 40%. The differences seen in the hypertensive pregnancy group in the cohort I report had changes approximately half those reported in this Framingham cohort and if the relationship is linear might suggest that the LV geometrical differences correspond to a relative increase in cardiovascular disease of 14-20%.

Interestingly the association between LV hypertrophy and mortality is much stronger in women than men (relative risk of death from all causes 2.01 vs 1.49) (17) suggesting that the differences in LV mass found may be of particular importance in a female population. Specifically in a population of women with a history of HDP the presence of increased LV mass might also have value in identifying women who are at greater risk of future hypertension. Melchiorre et al (140) reported that in a cohort of women the majority of those with a history of preeclampsia and whom delivered preterm had moderate-severe LV abnormalities and of this subgroup 40% went on to develop hypertension within 2 years of delivery.

The finding of higher LV ejection fraction in women with a history of HDP may be due to women with a history of HDP having increased sympathetic activation, known to be present during hypertensive pregnancy (213), which persists many years later and drives the increase in LV ejection fraction. However a more likely explanation may be that the ejection fraction reflects a smaller concentrically remodelled LV with preserved stroke volume. The finding across a range of Doppler diastolic measures and longitudinal measures of systolic and diastolic strain in women with a history of HDP suggesting reduced function implies that this higher ejection fraction might be masking a ventricle under strain. Measures of function by tissue Doppler and by speckle tracking typically predate less sensitive markers of systolic function such as ejection fraction (214). It is known that diastolic dysfunction predicts clinical heart failure (215) and an increased

risk of death (216). This is supported by Savitz et al (76) showing gestational hypertension is associated with an increased relative risk of heart failure (RR 2.6) however longitudinal follow-up data would be needed to confirm this association.

Explanation for these findings

We are not able to draw conclusions from this cross-sectional study about when differences in LV geometry first develop. Differences in cardiac geometry may relate to genetic differences or birth history of the mother. Those born preterm or to preeclampsia have increased LV mass and smaller LV volumes (217). It is also established that individuals born preterm are more likely to develop HDP whilst pregnant themselves (218) and those born from a pregnancy complicated by preeclampsia are more likely to develop preeclampsia themselves (217). It is also known that risk factors for preeclampsia (219), such as hypertension, obesity and diabetes are also risk factors for LV hypertrophy (220).

If present prior to pregnancy a relative thicker and stiffer heart might be relevant in the development of HDP as it is less able to adapt to the physiological challenges of pregnancy. In normotensive pregnancy diastolic function is reduced (202, 221) with greater dysfunction found in pregnancies complicated by preeclampsia (130). If less diastolic reserve is available prior to conception this might be a key factor in the development of an adverse cardiovascular phenotype during pregnancy.

Alternatively differences in cardiac structure might occur during pregnancy when accelerated hypertension might lead to an adaptive myocardial hypertrophy in response to increased afterload. Several studies report higher LV mass in women during pregnancy who have preeclampsia. Hamad et al (131) showed greater LV mass index compared to normotensive pregnant cohort (60 g/m^2 vs 45 g/m^2) together with reduced

diastolic function. In a small echo based study Simmons et al (129) (n=71) explored longitudinal changes in LV mass between women with normotensive and preeclamptic pregnancies and found that in normotensive pregnancies LV mass index increased from 66±13 g/m^2 in the first trimester to a peak of 76±16 g/m^2 in 3rd trimester before returning to 67±11 g/m^2 at 13 weeks postpartum. Comparison was made to women with preeclampsia (recruited in the 3rd trimester) who had the greatest LV mass index of 90±18 g/m^2 which fell to 74±15 g/m^2 at 13 weeks postpartum and was not significantly different from normotensive controls postpartum.

Melchiorre et al (140) studied whether cardiac structural changes persist at 1 year postpartum in a larger echo based study and reported a higher prevalence of altered LV geometry in women with a history of preeclampsia and delivering preterm but not term compared to women with a history of normotensive pregnancy. The difference in postpartum findings between Melchiorre and Simmons may relate to Melchiorre's larger study population which were divided according to severity of pregnancy complication and measuring more sensitive markers of LV geometry.

Interestingly the attenuation of LV mass index in the early postpartum period in women with a history of preeclampsia reported by Simmons et al (129) suggests 2 possible explanations. Either that LV structural differences associated with hypertensive pregnancy are attenuated after delivery, with the long term cumulative effects of blood pressure and other factors such as vascular resistance driving the differences seen at 9 years postpartum. Alternatively there are distinct phenotypes within the group of hypertensive pregnancies, those at lower cardiovascular risk whom have resolution of LV mass after delivery and those at higher risk who maintain elevated LV mass. This is supported by a study by Ghossein et al (222) who reported that elevated LV mass index measured at 9 months postpartum in women with initial normalisation of blood pressure

after a history of preeclampsia was predictive of developing hypertension by 6 years postpartum.

Most of the studies to date describing cardiovascular phenotypic changes postpartum are cross-sectional or have limited longitudinal data. This lack of sizeable longitudinal datasets prevents us from drawing strong conclusions of the course of LV structural changes. However it is likely that the explanation of LV geometrical differences at 9 years postpartum is multifactorial with a combination of factors from pre-conception, adaptation during pregnancy and postpartum all contributing.

Comparison with previous studies

My findings confirm those of previous studies. Scantlebury et al (143) reported a large echo based study which included 2637 women when the first hypertensive pregnancy was more than 25 years prior to the study echo. As in the cohort I report they found that those women with a history of HDP had higher LV mass index and greater wall thickness. They also found that a history of HDP was associated with lower E:A ratios and greater left atrial dimensions, but no difference in systolic function measured by ejection fraction. The population studied by Scantlebury were 15 years older, however the difference in LV mass index (2.6 g/m^2) was smaller than I found in my cohort (3.9 g/m^2). This may be due to the greater sensitivity of CMR compared to echo, with studies comparing the reproducibility of both modalities suggesting that comparable differences in LV mass can be detected with CMR using 93% lower sample size compared to echo (223). The smaller difference found by Scantlebury et al may also relate to the older age of individuals in this cohort and also the fact that the study population were all recruited due to their strong family history of hypertension, many had subclinical hypertension and approximately half were on antihypertensive medication. Furthermore characterisation of hypertensive pregnancy complication was

done by questionnaire remote to the pregnancy, whereas in my study I was able to perform detailed extraction from maternity records to characterise the presence and severity of HDP without limitation of recall bias. In addition to confirming the differences in LV mass shown by Scantlebury I was able to demonstrate for the first time that a history of HDP, independent of blood pressure, is significantly associated with increased LV mass index.

Melchiorre et al undertook an echo assessment of women closer to delivery (1 year after preeclamptic and uncomplicated pregnancy) (140). They found that in women with a history of preeclampsia, 70% of those who delivered preterm and 24% of those who delivered at term had stage B (asymptomatic) heart failure. They also reported that biventricular structural disease was more common in women with a history of preeclampsia delivering preterm and biventricular functional reduction more common in women with a history of preeclampsia (whether they delivered preterm or at term) compared to controls. These findings are similar to those in my cohort, however I did not find a dose response according to the severity of HDP with differences in the left and right ventricular function, though it is possible not all subgroups were powered to detect these differences. This may relate to a difference in the description of severity, with Melchiorre et al characterising preeclampsia according to preterm or term delivery. I characterised HDP according to GH and preeclampsia diagnosed either before (early onset) or after (late onset) 34 weeks gestation. This was due to recognition of differences in maternal metabolic and vascular phenotypes associated with the timing of preeclamptic diagnosis, with women diagnosed with preeclampsia before 34 weeks more likely to have had adverse uterine Doppler profiles at 24 weeks gestation and women diagnosed at or after 34 weeks gestation with preeclampsia more likely to have a higher BMI then those diagnosed earlier (162). However the lack of a clear dose

response may be due to attenuation of these subgroup differences between 1 and 9 years postpartum.

Other smaller studies have not found quite such consistent results. Evans et al (224) studied 18 women with a history of preeclampsia and 50 women with uncomplicated pregnancies 16 months after delivery and found no differences in LV mass, wall thickness, volumes or fractional shortening measured by echo. However diastolic function and more sensitive markers of systolic function such as strain were not reported. Ghossein-Doha et al (141) in a small longitudinal study, performing echo on women at 1 and 14 years after preeclamptic and uncomplicated pregnancies, found no differences in LV dimensions, mass or diastolic function at either time point. However these two studies with relatively small total populations of 68 and 28 women respectively were likely insufficiently powered to detect differences in these parameters.

Strobl et al (142) showed impaired cardiac function by measuring myocardial performance index in a small group of women (n=48) 15 years after HELLP, preeclamptic pregnancy and uncomplicated pregnancy, matched for blood pressure. However they did not find differences in other markers of systolic or diastolic function and did not report on cardiac structure. There is very limited data on postpartum right ventricular structural or functional changes associated with HDP. Melchiorre et al (140) reported right ventricular hypertrophy together with systolic and diastolic dysfunction present 1 year after preeclampsia with preterm delivery but not with term delivery compared to a control population.

Strengths of the study

This is the first study to use both CMR and echo, utilising the strengths of each modality to collect a comprehensive set of measurements encompassing structural and

functional measurements of both the RV and LV in a population with a history of HDP. Although other studies have explored LV geometrical changes remote to PET, this is the first to explore changes across the spectrum of HDP severity, from GH through to early onset preeclampsia. This has allowed me to explore differences in subgroups according to disease severity. Another strength of this study is that I was able to access maternity records for each participant and was therefore able to ensure the accuracy and timing of diagnosis as well as key characteristics of maternal haemodynamics and fetal well-being during the index pregnancy.

Limitations of the study

There are some limitations in this study which I acknowledge. The study design is retrospective and cross-sectional in design, so I cannot draw conclusions about longitudinal relationships. However very detailed data collection from medical records and patient questionnaires allows exploration of relationships between pregnancy characteristics and cardiovascular phenotypic differences 9 years later. In addition to reporting cardiac variation according to a history of hypertensive pregnancy or normotensive pregnancy I have reported results according to the subgroup of hypertensive pregnancy. The study may not be adequately powered to detect differences in these smaller subgroups and therefore may not identify differences which might be detected in a larger study powered for these subgroups. Also I did not include women with a history of diabetes or cardiovascular disease. However the prevalence in a population of this age is generally low and relatively few cases might have a large effect on the results and prevent understanding of the true relevance of HDP in an asymptomatic and well population.

4.6 Conclusions

In summary this work demonstrates that women with a history of HDP have altered LV structure and function many years remote to pregnancy. Of importance is that a statistically significant proportion of the higher LV mass seen in women with a history of HDP is independent of differences in blood pressure. Differences in cardiac structure and function may in part explain the increased cardiovascular risk in this population. Future longitudinal studies using state of the art imaging will be of value in revealing when cardiac differences first present. Hopefully this will lead to a timely intervention which might, at least in part, mitigate future cardiovascular disease associated with HDP.

Hypertensive Pregnancy and Maternal Vascular Phenotype

Chapter 5

5.1 Abstract

Introduction – During uncomplicated pregnancy maternal arterial stiffness is reduced. However in pregnancies complicated by hypertension reductions in arterial stiffness are attenuated. Women with a history of hypertensive pregnancy are at increased risk of cardiovascular disease later in life however it is not known if differences in arterial stiffness persist and may, in part, explain this increased risk. I therefore explored whether arterial stiffness is increased in women with a history of hypertensive pregnancy; and if so whether this was explained by variations in blood pressure.

Methods – Vascular function was comprehensively assessed in healthy women, free from cardiovascular disease and diabetes, 5-10 years after hypertensive (n=164) and normotensive pregnancy (n=104) (mean: 9 years). Distensibility was measured at multiple levels of the aorta using magnetic resonance imaging (MRI) and in the carotid

arteries by ultrasound. Pulse wave velocity (PWV) was measured between carotid-femoral and brachial-femoral arteries.

Results – Women with a history of hypertensive pregnancy had no significant differences in carotid distensibility, carotid-femoral PWV or brachial-femoral PWV compared to women with a history of normotensive pregnancy (p=0.12, p=0.09 and p=0.41 respectively). However women with a history of hypertensive pregnancy did demonstrate reduced global aortic compliance and distensibility (aortic compliance: 0.240±0.053 vs 0.258±0.063, p<0.05; aortic distensibility: 4.95±1.66 $mmHg^{-1}10^3$ vs 5.71±2.09 $mmHg^{-1}10^3$, p=0.02). Differences in global aortic compliance and distensibility were not significant after adjustment for both age and blood pressure (p=0.26 and p=0.15 respectively).

Conclusions –Aortic compliance and distensibility was reduced in women with a history of hypertensive pregnancy however this was not independent from differences in blood pressure.

5.2 Introduction

Women with a history of hypertensive pregnancy are at increased risk of cardiovascular disease, including hypertension, in later life (85, 225-227). It is established that during normotensive pregnancy there are changes in maternal vascular function, with vasodilation and reduced arterial stiffness (228, 229). These changes are attenuated during pregnancies complicated by preeclampsia and gestational hypertension (GH) (146, 230). However it is not clear if these findings relate to differences in arterial function or are confounded by alterations in blood pressure, which is known to change in normotensive and hypertensive pregnancy (231).

Reports of differences in aortic stiffness, typically assessed by pulse wave velocity (PWV), after hypertensive pregnancy have been mixed (232, 233). However this technique is known to be closely affected by blood pressure at the time of measurement (234) and it is not known if there are differences in arterial stiffness distinct from alterations in blood pressure. Persistent differences in arterial stiffness long term after hypertensive pregnancy, independent of differences in blood pressure, might in part explain the increased cardiovascular risk seen in women with a history of hypertensive pregnancy.

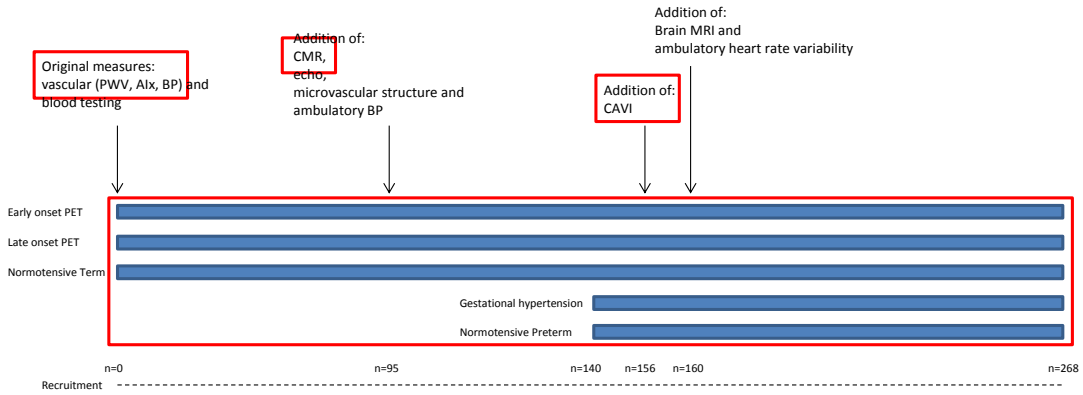
I therefore set out to determine, using methods adjusting for blood pressure at the time of measurement, if several years after pregnancy maternal aortic stiffness is increased in women with a history of hypertensive pregnancy compared to normotensive pregnancy.

5.3 Methods

5.3.1 Study population

This chapter includes the entire study population of 268 participants, all had assessment of blood pressure and arterial stiffness, using carotid-femoral pulse wave velocity (PWV) (SphygmoCor) and carotid ultrasound. A subgroup of 173 individuals also underwent further assessment using MRI to image of their aorta at multiple levels and brachial-femoral PWV (Vicorder). Details of this cohort and how they were recruited are set out in Study population (chapter 2).

Figure 30. Flow chart detailing the study population and the measures employed in chapter 5.



5.3.2 Study visit

All participants attended the research department after overnight fast and had blood collected. The following assessments and analysis, these are described in detail in Methods (chapter 3):

- CMR (page 58)
    - Aortic cine acquisition at multiple levels
- Brachial blood pressure measurements (office) (page 54)
- SphygmoCor (page 54 and 56)
    - Central blood pressure measurements
    - carotid-femoral pulse wave velocity
    - Augmentation pressure and index
- Vicorder (page 54 and 56)
    - Brachial blood pressure (MRI scanner)
    - Central blood pressure measurements (MRI scanner)
    - Brachial-femoral pulse wave velocity
    - Augmentation pressure and index
- Cardio-ankle vascular index (page 56)

- Carotid ultrasound (page 58)
- Flow mediated dilatation (page 57)
- Ambulatory blood pressure (page 76)
    - Blood pressure variability
- Medical notes data extraction (page 79)
- Medical history and lifestyle questionnaire (page 79)
- Anthropometry (page 51)
- Blood sampling and cardiovascular risk biomarker analysis (page 51)

5.3.3 Statistics

For full description of statistical methods please see statistics section in Methods (Chapter 3, page 79). In brief statistical analysis was carried out using SPSS version 22 (IBM, NY, United States). A p-value <0.05 was used to identify significant differences.

Previously reported differences in systolic blood pressure between women with a history of hypertensive or normotensive pregnancies suggested differences of 10 mmHg [unpublished data]. A large CMR study (235) reporting normal ranges in aortic distensibility found a 1.9 $mmHg^{-1}10^3$ for 12mmHg difference in systolic blood pressure. Therefore a difference of 1.58 $mmHg^{-1}10^3$ would be expected with a 10mmHg difference in blood pressure between groups. 38 individuals in each group provided 80% power at p=0.01 to identify differences of this size between groups.

Retrospective analysis of sample sizes of the subgroups was undertaken to ensure that groups were appropriately powered to detect differences in aortic distensibility. 65 individuals in the normotensive pregnancy group and 91 individuals in the hypertensive

pregnancy group provided 80% power at p=0.05 to identify a 0.21 standard deviation differences between groups, equivalent to 0.4 $mmHg^{-1}10^3$ in aortic distensibility.

5.4 Results

5.4.1 Study cohort characteristics

The cohorts of women were well matched in age and proportion of smokers (Table 25). However there was a significantly greater proportion of women with a history of hypertensive pregnancy taking antihypertensive medications than those with a history of normotensive pregnancy ($p<0.001$). Pregnancy characteristics such as delivery gestation, offspring birthweight and number of complicated pregnancies were significantly different between cohorts. Women with a history of hypertensive pregnancy were on average shorter with higher body mass index. They also had higher blood pressure, heart rate and an adverse metabolic phenotype with lower HDL and higher triglycerides and HOMA IR.

Table 25. Whole study population characteristics

	NTN (n=104)	HTN (n=164)	p-value
Demographics			
Age (years)	40.8±4.6	41.1±5.2	0.48
Yrs. since index pregnancy	9.0±1.9	9.3±2.0	0.66
Smokers, n (%)	11 (11)	11 (7)	0.31
Taking antihypertensive medications, n (%)	0 (0)	12 (7)	**0.005**
Pregnancy Characteristics			
Gestation (weeks)	37.2±4.8	35.9±3.2	**<0.001**
Offspring birthweight (g)	3042±1038	2512±895	**<0.001**
Index twin pregnancies, n (%)	7 (7)	10 (6)	0.75
Total number of live births	2.4±1.0	2.0±0.8	**<0.001**
Total number of complicated pregnancies	0.3±0.6	1.4±0.7	**<0.001**
Anthropometrics			
Height (m)	1.65±0.06	1.63±0.06	**0.005**
Weight (kg)	70.1±14.8	72.7±15.4	0.11
BSA (m^2)	1.79±0.19	1.81±0.19	0.30
BMI (Kg/m^2)	25.6±5.5	27.2±6.0	**0.01**
Waist: Hip	0.858±0.076	0.849±0.091	0.23
Haemodynamics			
SBP (mmHg)	114.0±13.0	121.5±13.9	**<0.001**
DBP (mmHg)	73.7±8.3	79.3±10.1	**<0.001**
MAP (mmHg)	87.2±9.4	93.4±10.7	**<0.001**
Heart rate (bpm)	59.5±8.5	61.9±9.6	**0.02**
Biochemistry			
Total Cholesterol (mmol/L)	4.84±0.87	4.87±0.93	0.94
LDL (mmol/L)	2.82±0.77	2.89±0.80	0.49
HDL (mmol/L)	1.64±0.37	1.50±0.34	**0.004**
TChol:HDL	3.09±0.85	3.38±0.84	**0.003**
Triglyceride (mmol/L)	0.87±0.41	1.02±0.59	**0.01**
HsCRP (mg/L)	2.13±4.01	2.53±4.08	0.14
HOMA IR	1.71±1.31	2.16±1.54	**0.007**

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at $p<0.05$. BSA: body surface area; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; TChol: total cholesterol; HsCRP: highly sensitive C reactive protein HOMA IR: homeostatic model assessment of insulin resistance.

Table 26. Whole study population characteristics according to subgroups of HDP

	NTN (n=104)	Early PET (n=63)	Late PET (n=80)	GH (n=21)	Early PET vs NTN (p-value)	Late PET vs NTN (p-value)	GH vs NTN (p-value)
Demographics							
Age (years)	40.8±4.6	40.0±5.3	41.6±5.2	42.1±4.5	0.35	0.20	0.21
Yrs. since index pregnancy	9.0±1.9	8.9±2.0	9.5±2.0	9.7±1.6	0.90	0.24	0.29
Smokers, n (%)	11 (11)	6 (11)	2 (3)	3 (15)	0.94	**0.04**	0.60
Taking antihypertensive medications, n (%)	0 (0)	7 (11)	5 (6)	0 (0)	**0.001**	**0.01**	>0.99
Pregnancy Characteristics							
Gestation (weeks)	37.2±4.8	33.0±2.7	37.6±1.6	38.3±2.2	**<0.001**	**0.006**	0.75
Offspring birthweight (g)	3042±1038	1765±739	2897±615	3088±850	**<0.001**	**0.01**	0.67
Index twin pregnancies, n (%)	7 (7)	7 (11)	2 (3)	1 (5)	0.38	0.16	0.71
Total number of live births	2.4±1.0	2.0±0.8	2.0±0.9	2.1±0.7	**0.001**	**0.001**	0.07
Total number of complicated births	0.3±0.6	1.4±0.7	1.3±0.6	1.6±0.8	**<0.001**	**<0.001**	**<0.001**
Anthropometrics							
Height (m)	1.65±0.06	1.63±0.06	1.63±0.05	1.65±0.07	**0.03**	**0.004**	0.73
Weight (kg)	70.1±14.8	73.9±18.0	71.6±14.4	73.1±10.2	0.23	0.33	0.09
BSA	1.79±0.19	1.82±0.22	1.79±0.18	1.83±0.12	0.37	0.72	0.12
BMI (Kg/m^2)	25.6±5.5	27.7±6.7	26.9±5.7	27.0±4.8	**<0.05**	**0.04**	0.17
Waist: Hip	0.858±0.076	0.842±0.085	0.836±0.094	0.921±0.060	0.23	**0.017**	**0.001**
Haemodynamics							
SBP (mmHg)	114.0±13.0	121.7±14.6	121.2±13.3	121.9±14.4	**<0.001**	**<0.001**	**0.01**
DBP (mmHg)	73.7±8.3	79.7±9.7	78.7±9.8	80.5±12.7	**<0.001**	**0.001**	**0.01**
MAP (mmHg)	87.2±9.4	93.7±10.8	92.9±10.3	94.3±12.7	**<0.001**	**<0.001**	**0.009**
Heart rate (bpm)	59.5±8.5	61.7±9.9	62.2±9.6	61.7±9.5	0.07	**0.03**	0.29
Biochemistry							
Total Cholesterol (mmol/L)	4.84±0.87	4.92±0.93	4.89±0.97	4.67±0.74	0.84	0.98	0.45
LDL (mmol/L)	2.82±0.77	2.92±0.81	2.89±0.84	2.80±0.68	0.45	0.57	0.98
HDL (mmol/L)	1.64±0.37	1.48±0.37	1.51±0.34	1.51±0.25	**0.01**	**0.02**	0.22
TChol:HDL	3.09±0.85	3.48±0.91	3.34±0.77	3.23±0.88	**0.007**	**0.01**	0.47
Triglyceride (mmol/L)	0.87±0.41	1.12±0.79	1.00±0.38	0.81±0.47	**0.02**	**0.006**	0.21
HsCRP (mg/L)	2.13±4.01	2.61±3.56	2.78±4.79	1.24±1.45	0.26	0.18	0.52
HOMA IR	1.71±1.31	2.16±1.32	2.27±1.76	1.69±1.21	**0.008**	**0.01**	0.72

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at $p<0.05$. BSA: body surface area; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; TChol: total cholesterol; HsCRP, highly sensitive C reactive protein HOMA IR, homeostatic model assessment of insulin resistance;.

5.4.2 No difference in pulse wave velocity in women with a history of hypertensive pregnancy

Women with a history of hypertensive pregnancy had no difference in either carotid-femoral pulse wave velocity (PWV) or brachial-femoral PWV compared to women with a history of normotensive pregnancy (6.46±1.18 m/sec vs 6.15±0.98 m/sec, p=0.09 and 9.57±1.62 m/sec vs 9.68±2.51 m/sec, p=0.41 respectively). However women with a history of HDP did have higher augmentation index and pressure (radial augmentation index: 22.03±9.35 % vs 18.95±9.13 %, p=0.03 and brachial augmentation pressure: 8.79±4.30 mmHg vs 6.63±5.30 mmHg, p=0.002) (figure 31 and table 27). Flow mediated dilatation was not different between women with a history of hypertensive pregnancy and women with a history of normotensive pregnancy (6.40±4.33 % vs 5.83±4.40 %, p=0.59). However carotid intima media thickness was greater in women with a history of HDP compared to those with a history of normotensive pregnancy (0.55±0.07 mm vs 0.52±0.06 mm, p<0.001).

Figure 31. Bar graph showing higher augmentation index measured using SphygmoCor in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (22.03±9.35 % vs 18.95±9.13 %, p=0.03).

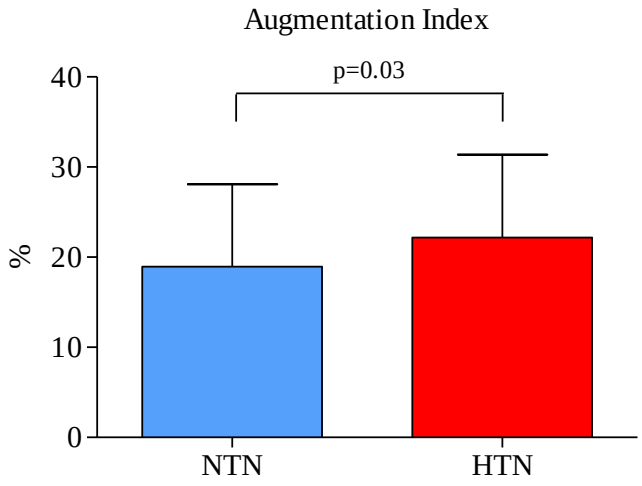


Table 27. Peripheral measures of arterial stiffness

	NTN	HTN	p-value
	(n=99)	(n=145)	
Carotid compliance	0.296±0.092	0.289±0.091	0.63
Carotid distensibility $mmHg^{-1}10^3$	7.48±2.16	6.99±2.00	0.12
Carotid-femoral PWV (m/sec)	6.15±0.98	6.46±1.18	0.09
FMD (%)	5.83±4.40	6.40±4.33	0.59
Radial AIx (%)	18.95±9.13	22.03±9.35	**0.03**
	(n=68)	(n=89)	
Brachial-femoral PWV (m/sec)	9.68±2.51	9.57±1.62	0.41
Brachial AIx (%)	12.15±7.87	15.75±5.90	**0.001**
Brachial augmentation Pressure (mmHg)	6.63±5.30	8.79±4.30	**0.002**
	(n=54)	(n=54)	
CAVI	6.62±0.89	6.81±0.69	0.22

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at p<0.05. PWV: pulse wave velocity; AIx: augmentation index; CAVI: cardio-ankle vascular index; FMD: flow mediated dilatation.

I then explored whether peripheral measures of arterial structure and function were different between women with a history of normotensive pregnancy and those with a history of early onset preeclampsia, late onset preeclampsia or GH separately. Compared to women with a history of normotensive pregnancy women with a history of early onset preeclampsia demonstrated greater carotid intima media thickness ($p<0.05$). Women with a history of late onset preeclampsia demonstrated increased arterial stiffness with reduced carotid distensibility ($p=0.04$), higher carotid-femoral PWV ($p=0.04$), higher brachial augmentation index and pressure (both $p=0.006$) and also greater intima media thickness ($p<0.001$) than women with a history of normotensive pregnancy (Table 28). Women with a history of GH had higher brachial augmentation pressure ($p=0.04$) and carotid intima media thickness ($p<0.05$) than comparable women after normotensive pregnancy.

Table 28. Peripheral measures of arterial stiffness according to subgroup of hypertensive disorder of pregnancy

	NTN	Early PET	Late PET	GH	Early PET vs NTN (p-value)	Late PET vs NTN (p-value)	GH vs NTN (p-value)
	(n=99)	(n=53)	(n=72)	(n=20)			
Carotid compliance	0.296±0.092	0.283±0.084	0.281±0.091	0.347±0.106	0.47	0.37	0.06
Carotid distensibility	7.48±2.16	6.96±1.95	6.78±2.01	8.16±1.90	0.20	**0.04**	0.18
PWV (cf)	6.15±0.98	6.60±1.44	6.47±0.99	6.05±1.00	0.12	**0.04**	0.51
FMD (%)	5.83±4.40	7.41±5.30	6.00±3.28	4.47±3.97	0.21	0.78	0.17
Radial AIx (%) SphygmoCor	19.0±9.1	21.51±10.2	22.1±8.7	23.5±9.3	0.15	0.05	0.09
Brachial AIx (%) Vicorder	12.2±7.9	15.3±7.1	16.2±5.6	15.8±4.0	0.06	**0.006**	0.05
Augmentation Pressure (mmHg)	6.6±5.3	8.8±5.5	9.3±3.9	8.1±3.1	0.06	**0.006**	**0.04**
	(n=68)	(n=33)	(n=36)	(n=20)			
PWV (bf)	9.68±2.51	9.61±1.66	9.74±1.66	9.20±1.51	0.49	0.27	0.82
	(n=54)	(n=20)	(n=15)	(n=19)			
CAVI	6.62±0.89	6.81±0.54	6.95±0.86	6.70±0.69	0.37	0.21	0.74

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at $p<0.05$. PET: history of preeclampsia; GH: history of gestational hypertension; PWV: pulse wave velocity; AIx: augmentation index; CAVI: cardio-ankle vascular index; FMD: flow mediated dilatation.

I then explored which relevant factors were associated with augmentation index measured radially. Several factors were significantly associated including: age, blood pressure, antihypertensive medication usage and metabolic characteristics such as cholesterol and BMI (Table 29).

Table 29. Univariate regression of augmentation index and related factors

Radial AIx	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
Age	0.71	0.50	0.93	**<0.001**
Gestation (wks.)	-0.15	-0.44	0.15	0.33
Offspring birthweight (g)	<-0.01	<-0.01	<0.01	0.21
SBP (mmHg)	0.31	0.24	0.38	**<0.001**
DBP (mmHg)	0.48	0.38	0.58	**<0.001**
MAP (mmHg)	0.45	0.36	0.54	**<0.001**
PP (mmHg)	0.20	0.07	0.33	**0.003**
Current antihypertensive medication usage	6.98	0.96	13.00	**0.02**
Number of pregnancies	-0.57	-1.84	0.70	0.38
Twin pregnancies	-0.51	-5.25	4.22	0.83
Smoking	3.47	-0.70	7.64	0.10
Total Cholesterol (mmol/L)	1.99	0.65	3.34	**0.004**
LDL (mmol/L)	1.94	0.39	3.48	**0.01**
HDL (mmol/L)	-0.86	-4.33	2.61	0.62
Triglyceride (mmol/L)	1.86	-0.47	4.18	0.12
HOMA IR	0.87	0.02	1.73	**<0.05**
HsCRP (mg/L)	0.26	-0.05	0.56	0.10
Creatinine (micromol/L)	-0.22	-0.43	<0.01	0.05
Weight (kg)	0.03	-0.05	0.10	0.48

Statistically significance was defined as p<0.05. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; T Chol: total cholesterol; HsCRP: highly sensitive C reactive protein; HOMA IR: homeostatic model assessment of insulin resistance.

I then tested a multivariable model and showed that age and mean arterial pressure were independent predictors of augmentation index but history of hypertensive pregnancy was not.

Table 30. Multivariable regression analyses of augmentation index and related factors

Augmentation Index Model	Standardized β Coefficient	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
MAP (mmHg)	0.46	0.40	0.25	0.56	<0.001
Age (yrs.)	0.29	0.55	0.22	0.88	<0.001

Statistical significance was defined as p<0.05. MAP: mean arterial pressure.

5.4.3 Aortic imaging

In a subgroup of women with a history of HDP (n=91) and normotensive pregnancy (n=65) CMR was used to assess aortic compliance and distensibility at multiple levels. The characteristics of the aortic subgroup (shown in Table 31) were, in the main, representative of the entire cohort (shown in Table 25). However there were a few notable exceptions; the normotensive group who had aortic imaging were studied earlier after the index pregnancy when compared to the entire normotensive group (8.2±1.8 years vs 9.0±1.9 years, p=0.007), they also had lower delivery gestations and offspring birthweight of the index pregnancy (35.5±5.3 weeks vs 37.2±4.8 weeks, p=0.03 and 2687±1149g vs 3042±1038g, p=0.04 respectively). In addition the normotensive group who had aortic imaging had greater number of complicated pregnancies compared to the entire normotensive group (0.5±0.6 vs 0.3±0.6, p=0.04) and a higher waist: hip ratio (0.886±0.074 vs 0.858±0.076, p=0.02). The hypertensive pregnancy group who had aortic imaging (shown in Table 31) compared to the entire hypertensive pregnancy group (shown in Table 25) had no significant differences in population characteristics, except higher waist: hip ratios (0.872±0.085 vs 0.849±0.091, p<0.05).

5.4.3.1 Reduced aortic compliance in women with a history of hypertensive pregnancy

Women with a history of hypertensive pregnancy had no differences in aortic luminal area at several levels of the aorta (all p>0.05), however they demonstrated significantly reduced global compliance and distensibility (compliance: 0.240±0.053 vs 0.258±0.063, p<0.05; and distensibility: 4.95±1.66 $mmHg^{-1}$ vs 5.71±2.09 $mmHg^{-1}$, p=0.02) (figure 32, 33 and table 32). In regional analysis of the aorta, compliance and distensibility were reduced in the thoracic aorta (compliance: AA, p<0.05; PDA: p=0.02 and

distensibility: AA, p=0.01; PDA: 0.004) but not in the abdominal aorta (compliance: DDA, p=0.55; AbA: p=0.37 and distensibility: DDA, p=0.06; AbA: 0.11).

A sensitivity analysis was performed which showed that global aortic distensibility remained significantly different between groups after excluding participants with a history of normotensive preterm delivery (p=0.001) and separately excluding those currently on antihypertensive medication (p=0.02).

Table 31. Characteristics of subgroup with aortic imaging

	NTN (n=65)	HTN (n=91)	p-value
Demographics			
Age (years)	40.8±5.3	41.9±4.8	0.20
Yrs. since index pregnancy	8.2±1.8	9.1±2.0	**0.02**
Smokers, n (%)	9 (14)	6 (7)	0.14
Taking antihypertensive medications, n (%)	0 (0)	7 (8)	**0.02**
Pregnancy Characteristics			
Gestation (weeks)	35.5±5.3	35.7±3.5	0.31
Offspring birthweight (g)	2687±1149	2461±982	0.22
Index twin pregnancies, n (%)	7 (12)	6 (7)	0.30
Total number of live births	2.2±1.0	1.9±0.9	**0.03**
Total number of complicated pregnancies	0.5±0.6	1.3±0.6	**<0.001**
Anthropometrics			
Height (m)	1.65±0.06	1.64±0.06	0.14
Weight (kg)	70.3±14.6	71.3±14.2	0.45
BSA (m^2)	1.79±0.19	1.79±0.18	0.68
BMI (Kg/m^2)	25.7±5.4	26.5±5.3	0.21
Waist: Hip	0.886±0.074	0.872±0.085	0.24
Haemodynamics			
SBP (mmHg)	116.1±14.7	122.9±14.2	**0.001**
DBP (mmHg)	74.5±8.9	80.0±10.4	**0.001**
MAP (mmHg)	88.3±10.4	94.3±11.1	**0.001**
Heart rate (bpm)	58.9±8.8	61.9±9.5	0.03
Biochemistry			
Total Cholesterol (mmol/L)	4.94±0.84	4.88±0.81	0.63
LDL (mmol/L)	2.90±0.79	2.92±0.65	0.80
HDL (mmol/L)	1.65±0.39	1.51±0.32	**0.04**
TChol:HDL	3.15±0.87	3.33±0.74	0.12
Triglyceride (mmol/L)	0.84±0.35	0.94±0.46	0.27
HsCRP (mg/L)	2.24±3.99	1.86±2.49	0.61
HOMA IR	1.56±1.45	1.92±1.56	**0.03**

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at $p<0.05$. BSA: body surface area; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; TChol: total cholesterol; HsCRP: highly sensitive C reactive protein HOMA IR: homeostatic model assessment of insulin resistance.

Figure 32. Bar graph showing lower global aortic compliance in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (0.240±0.053 vs 0.258±0.063, p<0.05).

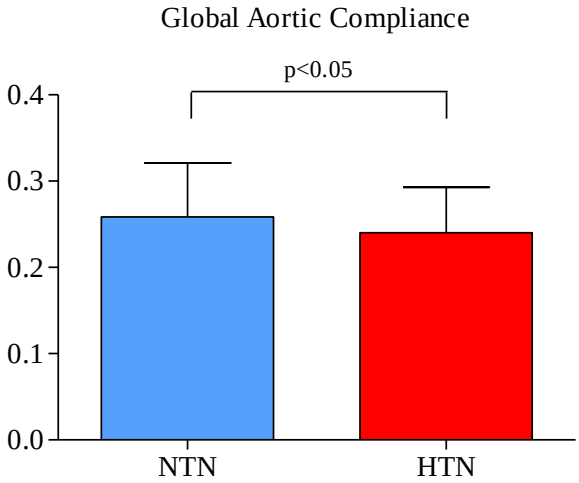


Figure 33. Bar graph showing lower global aortic distensibility in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (4.95±1.66 $mmHg^{-1}10^3$ vs 5.71±2.09 $mmHg^{-1}10^3$, p=0.02).

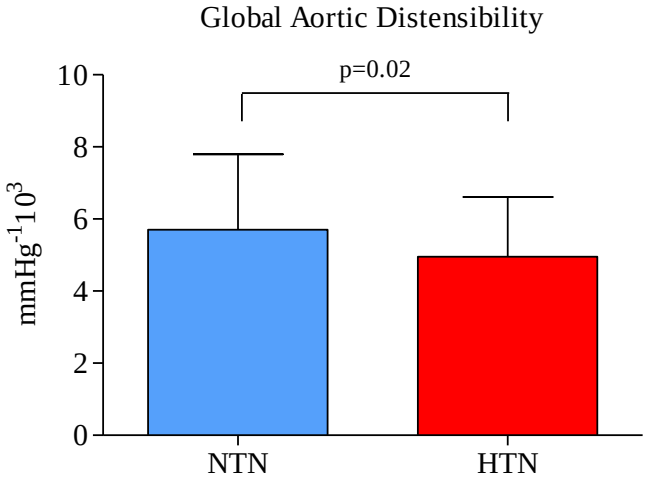


Table 32. Aortic structure and function

	NTN (n=65)	HTN (n=91)	p-value
Aortic area			
Global (mm^2)	304±54	312±67	0.55
AA (mm^2)	531±108	581±180	0.08
PDA (mm^2)	290±57	299±44	0.20
DDA (mm^2)	222±50	227±39	0.55
AbA (mm^2)	151±38	152±29	0.87
Aortic Compliance			
Global	0.258±0.063	0.240±0.053	**<0.05**
AA	0.225±0.073	0.203±0.063	**<0.05**
PDA	0.232±0.052	0.214±0.042	**0.02**
DDA	0.327±0.080	0.320±0.070	0.55
AbA	0.247±0.104	0.223±0.080	0.37
Aortic Distensibility			
Global ($mmHg^{-1}10^3$)	5.71±2.09	4.95±1.66	**0.02**
AA ($mmHg^{-1}10^3$)	5.00±2.13	4.23±1.87	**0.01**
PDA ($mmHg^{-1}10^3$)	5.10±1.73	4.31±1.20	**0.004**
DDA ($mmHg^{-1}10^3$)	7.22±2.56	6.51±2.06	0.06
AbA ($mmHg^{-1}10^3$)	5.52±3.00	4.62±2.12	0.11

Mean ± standard deviation, level of significance p<0.05. Global: mean of values throughout the aorta, AA: ascending aorta, PDA: proximal descending aorta, DDA: distal descending aorta and AbA: abdominal aorta.

I then explored whether CMR measures of aortic compliance and distensibility were different between women with a history of normotensive pregnancy and those with a history of early onset preeclampsia, late onset preeclampsia or GH separately. Compared to women with a history of normotensive pregnancy women with a history of early onset preeclampsia had lower distensibility in the PDA, but not in other regions (p=0.03) (Table 33). Women with a history of late onset preeclampsia had lower global compliance, which was also shown in the AA and PDA (p=0.04, p=0.02 and p=0.009 respectively). Women with a history of late onset preeclampsia also had lower global distensibility, which was also shown in the PDA (p=0.04 and p=0.008 respectively). Women with a history of GH had lower compliance in the AA and PDA (p<0.05 and p=0.02), as well as reduced global distensibility, also shown in the AA and PDA (p=0.01, p=0.02 and p=0.001).

Table 33. Aortic function according to subgroup of hypertensive pregnancy

	NTN (n=65)	Early PET (n=34)	Late PET (n=37)	GH (n=19)	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)	NTN vs GH (p-value)
Aortic Compliance							
Global	0.258±0.063	0.247±0.052	0.233±0.050	0.241±0.059	0.37	**0.04**	0.050
AA	0.225±0.073	0.217±0.069	0.191±0.059	0.201±0.057	0.58	**0.02**	**<0.05**
PDA	0.232±0.052	0.217±0.046	0.206±0.036	0.224±0.044	0.17	**0.009**	**0.02**
DDA	0.327±0.080	0.329±0.063	0.307±0.074	0.329±0.076	0.91	0.22	0.55
AbA	0.247±0.104	0.227±0.092	0.218±0.074	0.227±0.075	0.64	0.26	0.81
Aortic Distensibility							
Global ($mmHg^{-1}10^3$)	5.71±2.09	5.15±1.78	4.86±1.56	4.75±1.69	0.14	**0.04**	**0.01**
AA ($mmHg^{-1}10^3$)	5.00±2.13	4.35±1.62	4.28±2.26	3.87±1.44	0.12	0.05	**0.02**
PDA ($mmHg^{-1}10^3$)	5.10±1.73	4.35±1.25	4.23±1.17	4.40±1.24	**0.03**	**0.008**	**0.001**
DDA ($mmHg^{-1}10^3$)	7.22±2.56	6.66±1.92	6.38±2.13	6.51±2.25	0.27	0.09	0.059
AbA ($mmHg^{-1}10^3$)	5.52±3.00	4.77±2.38	4.56±2.05	4.49±1.82	0.32	0.14	0.26

Mean ± standard deviation, level of significance $p<0.05$. PET: history of preeclampsia; GH: history of gestational hypertension; Global: mean of values throughout the aorta, AA: ascending aorta, PDA: proximal descending aorta, DDA: distal descending aorta and AbA: abdominal aorta.

Furthermore I explored whether relevant factors were associated with differences in global aortic compliance (Table 34); age, both peripheral and central measures of blood pressure and several biochemical measures of metabolic function were all significantly associated.

Table 34. Univariate regression of aortic compliance and related factors

Global Aortic compliance	**Unstandardized β Coefficient**	**95% Confidence**		**p-value**
		Lower limit	**Upper limit**	
Age	-0.007	-0.008	-0.005	**<0.001**
Smoking	0.017	-0.016	0.050	0.32
Number of pregnancies	0.003	-0.007	0.013	0.60
Twin pregnancies	-0.023	-0.061	0.015	0.23
Gestation (wks.)	<0.001	-0.003	0.002	0.84
Offspring birthweight (g)	<0.001	<-0.001	<0.001	0.99
SBP (mmHg)	-0.002	-0.002	-0.001	**<0.001**
DBP (mmHg)	-0.002	-0.003	-0.001	**<0.001**
MAP (mmHg)	-0.002	-0.003	-0.001	**<0.001**
PP (mmHg)	-0.002	-0.003	-0.001	**<0.001**
Central SBP* (mmHg)	-0.002	-0.002	-0.001	**<0.001**
Central DBP* (mmHg)	-0.002	-0.003	-0.001	**<0.001**
Central MAP* (mmHg)	-0.003	-0.004	-0.002	**<0.001**
Central PP* (mmHg)	-0.002	-0.003	-0.001	**<0.001**
Current antihypertensive medication usage	0.01	-0.041	0.056	0.75
Weight (kg)	-0.001	-0.001	<-0.001	0.04
Total Cholesterol (mmol/L)	-0.019	-0.03	-0.008	**0.001**
LDL (mmol/L)	-0.019	-0.032	-0.007	**0.003**
HDL (mmol/L)	0.001	-0.028	0.029	0.96
Triglyceride (mmol/L)	-0.028	-0.046	-0.009	**0.004**
HOMA IR	<0.001	-0.007	0.007	0.99
HsCRP (mg/L)	<0.001	-0.002	0.003	0.87
Creatinine (micromol/L)	0.001	-0.001	0.002	0.43

Statistical significance was defined as p<0.05. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; *central blood pressure measured using SphygmoCor; † booking blood pressure was the blood pressure recorded at the first ante-natal appointment in the index pregnancy; LDL: low density lipoprotein; HDL: high density lipoprotein; T Chol: total cholesterol; HsCRP: highly sensitive C reactive protein; HOMA IR: homeostatic model assessment of insulin resistance; BMI: body mass index.

I tested a model of relevant factors; age, mean arterial pressure and triglyceride level were all significantly and independently associated with global aortic compliance.

Table 35. Multivariable regression analyses of aortic compliance and related factors

Aortic Compliance Model	Standardized β Coefficient	Unstandardized β Coefficient	95% Confidence		p-value
			Lower limit	Upper limit	
Age (yrs.)	-0.51	-0.01	-0.01	<-0.01	<0.001
MAP (mmHg)	-0.25	<-0.01	<-0.01	<-0.01	0.001
Triglyceride (mmol/L)	-0.15	-0.02	-0.03	<-0.01	<0.05

Statistical significance was defined as p<0.05. MAP: mean arterial pressure.

5.4.4 Higher central blood pressure in women after hypertensive pregnancy

Women with a history of hypertensive pregnancy had higher central blood pressure, measured using applanation tonometry (SphygmoCor), compared to those with a history of normotensive pregnancy (systolic blood pressure: 114.3±14.9 mmHg vs 107.1±14.1 mmHg, p<0.001 and diastolic blood pressure: 80.6±10.7 mmHg vs 75.4±8.9 mmHg, p<0.001) (Table 36 and figure 34). Similar differences were found using an oscillometry device (Vicorder) whilst participants lay supine in a CMR scanner.

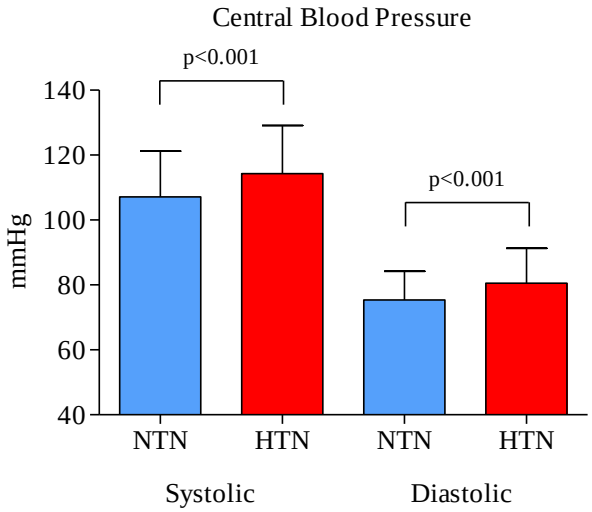
Differences in central systolic and diastolic blood pressure measured using SphygmoCor were still present between groups after excluding women with a history of normotensive preterm delivery (both p<0.001) and also in a separate analysis excluding women currently taking antihypertensive medication (both p<0.001).

Table 36. Central blood pressure

	NTN	HTN	p-value
SphygmoCor	(n=101)	(n=160)	
SBP (mmHg)	107.1±14.1	114.3±14.9	**<0.001**
DBP (mmHg)	75.4±8.9	80.6±10.7	**<0.001**
MAP (mmHg)	85.9±10.1	91.8±11.4	**<0.001**
PP (mmHg)	31.7±8.1	33.7±9.2	0.10
Vicorder	(n=67)	(n=95)	
SBP (mmHg)	114.7±14.0	121.6±13.2	**<0.001**
DBP (mmHg)	66.3±7.1	70.2±8.4	**0.003**
MAP (mmHg)	82.5±8.6	87.4±9.2	**<0.001**
PP (mmHg)	48.4±10.5	51.1±9.4	**0.03**

Mean ± standard deviation, level of significance p<0.05. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure.

Figure 34. Bar graph showing central blood pressure measured using SphygmoCor in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) both for systolic (114.3±14.9 mmHg vs 107.1±14.1 mmHg, p<0.001) and diastolic blood pressure (80.6±10.7 mmHg vs 75.4±8.9 mmHg, p<0.001).



5.4.5 Blood pressure variability

Women with a history of hypertensive pregnancy had lower day time diastolic blood pressure standard deviations (14.60±5.69 mmHg vs 17.49±8.37 mmHg, p=0.04) (table 37). However all other measures of blood pressure variability did not differ between groups (all p>0.05).

Table 37. Blood pressure variability

	NTN (n=65)	HTN (n=97)	p-value
SBP			
24hr SD mmHg	19.07±7.17	18.05±5.86	0.49
Day SD mmHg	18.42±7.19	17.07±6.19	0.28
Night SD mmHg	11.49±8.03	12.62±8.96	0.24
Weighted SD mmHg	16.09±6.41	15.62±5.51	0.86
ARV mmHg	16.66±6.88	16.60±7.62	0.91
DBP			
24hr SD mmHg	17.37±7.91	14.86±5.03	0.06
Day SD mmHg	17.49±8.37	14.60±5.69	**0.04**
Night SD mmHg	8.77±6.59	8.22±4.35	0.61
Weighted SD mmHg	15.00±7.86	12.54±4.45	0.12
ARV mmHg	15.59±8.22	13.30±5.41	0.15

Mean ± standard deviation, level of significance p<0.05. SBP: systolic blood pressure; DBP: diastolic blood pressure; SD: standard deviation; ARV: average real variability.

5.5 Discussion

Aortic compliance and distensibility were lower in women with a history of hypertensive pregnancy compared to women with a history of normotensive pregnancy. Blood pressure was strongly associated with these measures and was independently associated with aortic compliance in models. However a history of hypertensive pregnancy was not associated with aortic compliance independently from age and blood pressure. This suggests that differences in aortic compliance seen between cohorts are not independent of age and blood pressure. In fact the magnitude of differences seen between groups in blood pressure and compliance appear proportional, 7% in each case. Findings of higher augmentation index and central blood pressure support the findings of increased arterial stiffness in this population. However peripheral measures of central stiffness such as PWV were not different and blood pressure variability was not increased in women with a history of hypertensive pregnancy.

Clinical Relevance

Stiffer arteries expose organs to greater variation in blood pressure and increased arterial stiffness is a risk factor for left ventricular hypertrophy (236), renal (237) and cerebrovascular disease (174, 238). Aortic stiffness is also independently associated with cardiac events and all-cause mortality (174, 239, 240) and guidelines recommend its use for stratifying a population at increased cardiovascular risk (241). Augmentation index and pressure are also valid and reproducible vascular measures of cardiovascular risk (242-244).

Explanation for these findings

The findings in this cohort confirm previous findings that women with a history of hypertensive pregnancy have increased blood pressure and rates of hypertension (75,

78, 85). Blood pressure has a close and complex relationship with arterial stiffness (245) and there is controversy over the temporal relationship between arterial stiffness and blood pressure (246, 247).

Raised blood pressure is a risk factor for hypertensive pregnancy (248). However to my knowledge there is no study exploring whether arterial stiffness is itself a risk factor and it is possible differences in arterial stiffness may precede the affected pregnancies. There have been several studies reporting increased aortic stiffness during pregnancies complicated by hypertension (124, 146) and the findings in this cohort suggest that these differences persist years after delivery.

The findings that differences in proximal aortic stiffness were significantly different, whereas distal regions were not, is in keeping with previous reports that more proximal aortic regions are more sensitive to ageing (249) and might suggest accelerated vascular ageing in this population. The greater susceptibility of the proximal vessel is thought to be due to distinct differences in the distribution of elastin and collagen along the aorta thereby varying its elastic properties according to region (250) and thus the sensitivity of the vessel to ageing.

The explanation for differences found in compliance and distensibility but not in PWV may relate to the greater sensitivity of MRI, particularly in individuals under 50 years of age (251). It is also less affected by operator bias (252). In addition to its close relationship with blood pressure, PWV relies on the body surface measurement of the vascular distance between the arterial sites of measurement which can introduce errors, particularly in larger individuals. It may also relate to differences occurring preferentially in the proximal aorta where the most significant differences were found. The ascending aorta is not included in either carotid-femoral or brachial-femoral PWV

assessment. PWV does however include smaller more muscular vessels such as femoral and carotid, or brachial arteries. Smaller arteries have been shown to demonstrate a compensatory increase in distensibility in response to increased aortic stiffness in hypertensive individuals (253) and a paradoxical reduction in stiffness in these arteries may cancel out any differences present in aortic stiffness and explain the lack of difference seen in PWV in this cohort.

Comparison with previous studies

In keeping with my results other studies have also reported increased arterial stiffness in women with a history of hypertensive pregnancy. Hausvater et al (146) reported a systematic review showing arterial stiffness is increased after pregnancy complicated by preeclampsia compared to normotensive pregnancy. It included two studies reporting at 4 months (254) and 2 years (255) after pregnancy showing significantly increased PWV after preeclampsia. However one of these two studies also analysed whether these differences were still present after adjusting PWV for blood pressure and found no significant difference remained between groups (254). This is an important consideration as it has been long established that PWV has a close and dependent relationship with blood pressure (234).

Other studies comparing PWV in similar populations have found no differences in PWV. Polonia et al (232) studied 100 women, approximately 8 years after affected pregnancy, and reported higher augmentation index but no difference in PWV after preeclampsia compared to normotensive pregnancy. Al-Nashi et al assessed 31 women 11 years after affected pregnancy, finding no difference in carotid-radial or carotid-femoral PWV (233). In a larger study by Ehrenthal et al 74 women were assessed 1 year after hypertensive disorder of pregnancy or uncomplicated pregnancy, with no

difference was found in PWV (148). In addition Yuan et al (256) measured PWV in 50 women both during pregnancy and 18 months postpartum. They reported significantly higher PWV during late onset preeclampsia compared to normotensive pregnancy, however this was no longer significant after adjustment for blood pressure. Furthermore 18 months after delivery PWV was not significantly different between groups.

Strengths of the study

This study is the one of the largest to study arterial stiffness after hypertensive pregnancy and to my knowledge the first to assess aortic distensibility using CMR in this population. The use of additional modalities, such as PWV and carotid ultrasound allowed a comprehensive description of vascular function in this population and comparison with other studies. MRI is a well validated and accurate modality for measuring aortic distensibility (249) and is thought to be the most sensitive measure for arterial ageing in individuals under 50 years of age (251).

PWV remains the most popular method for characterising arterial stiffness and has the most extensive literature reporting its association with cardiovascular disease and mortality (239). However it has a close and dependent relationship with blood pressure (234). This limits its utility in distinguishing true differences in arterial stiffness between populations with distinct blood pressures. The relationship between blood pressure and arterial stiffness is complex and no technique currently in use is without limitation, however direct visualisation of the arterial wall through the cardiac cycle, coupled with simultaneously measured central blood pressure allows accurate assessment of the individual relevant factors.

Limitations of the study

There are some limitations with this study. The characteristics of the study population might suggest that those included have lower rates of smoking, lower proportion using antihypertensive medication and lower BMI than we might expect for a typical female population aged 40. Furthermore the mean blood pressures both in the group of women with a history of hypertensive pregnancy and those with a history of normotensive pregnancy were well below the threshold for diagnosing hypertension and also below what might be expected in a typical population of this age. If selection bias has influenced our study population and they are healthier than would be representative of the general population then absolute values of arterial stiffness might be underestimated.

Due to the design of this retrospective cross-sectional study we cannot draw conclusions on when differences in arterial stiffness first present. Also the lack of differences in aortic stiffness between subgroups of hypertensive pregnancy and normotensive pregnancies may be due to lack of statistical power.

5.6 Conclusions

In conclusion, women with a history of hypertensive pregnancy have reduced aortic compliance and distensibility. These differences are in proportion to higher peripheral and central blood pressures seen in this population.

Preeclamptic Pregnancy and Maternal Cerebral Phenotype

Chapter 6

6.1 Abstract

Introduction – Women with a history of preeclampsia have an increased risk of stroke later in life which is not fully explained by traditional cardiovascular risk factors. Preeclampsia is known to be associated with acute cerebral changes at the time of pregnancy which may be associated with long term structural damage and increased stroke risk. The study described in this chapter sought to explore if differences exist in total brain and grey matter volumes and white matter structure in women with a history of preeclampsia. Also to identify whether potential differences are explained by traditional cerebrovascular risk factors or if there might be novel mechanisms relating to preeclampsia itself which are responsible.

Methods – A cohort of women with a history of preeclampsia (n=32, aged 42.8±5.1 years, mean ± standard deviation) or normotensive pregnancy (n=46, 40.6±5.5 years) underwent detailed cerebral phenotyping 5-10 years after index pregnancy. Brain

magnetic resonance imaging was performed. Volumes of total brain and grey matter were measured using voxel based analysis of T1 weighted images with adjustment for skull size. Global and regional analysis of white matter structure was undertaken using diffusion tensor imaging to assess microstructural integrity and fluid attenuation inversion recovery sequences to assess white matter lesions

Results – Women with a history of preeclampsia had lower grey matter volumes (663±38 ml vs 686±54 ml, p=0.04) than women with a history of normotensive pregnancy, but there was no difference in total brain volumes (p=0.23). Women with a history of preeclampsia also demonstrated greater damage to white matter structure predominantly in the temporal lobe, with high white matter lesion volume (p=0.04), lower fractional anisotropy (p=0.03), and higher mean diffusivity (p=0.02) and radial diffusivity (p=0.008). History of preeclampsia was associated with white matter damage, independent of age and blood pressure (temporal lobe white matter lesion volume: p=0.02, temporal lobe radial diffusivity: p<0.05), reduced grey matter volume was not independent of age (p=0.13) or systolic blood pressure (p=0.08).

Conclusion. This data shows grey matter atrophy and temporal lobe white matter damage in women with a history of preeclampsia. It also suggests that preeclampsia may be additive to conventional cardiovascular risk factors and novel disease-specific mechanisms may explain why this population is at increased risk of stroke in later life.

6.2 Introduction

Women with a history of preeclampsia have a two-fold increased risk of stroke several years after affected pregnancy, even after adjusting for traditional risk factors such as hypertension (78, 79, 84). Preeclampsia is associated with increased permeability of the

blood brain barrier and risk of stroke at the time of pregnancy (257, 258). These acute changes during pregnancy may represent a distinct cerebral phenotype associated with preeclampsia which explains the increased risk of stroke seen in this population. Asymptomatic cerebral structural changes often predate and predict stroke presentation in patients at increased cardiovascular risk (150). Moreover, it remains to be answered whether there is a disease specific pattern of structural damage to the brain, the encompassing characterisation of which might help improve our understanding of the mechanistic link between preeclampsia and stroke.

Therefore I set out to assess total brain and grey matter volumes and white matter structure in women with a history of preeclampsia compared to those with a history of normotensive pregnancy and investigate whether there are distinct patterns of damage related to the disease.

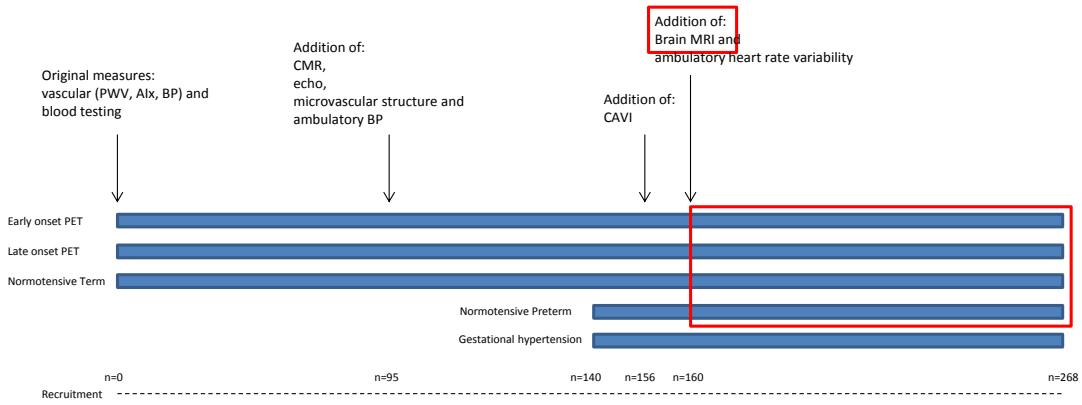
6.3 Methods

6.3.1 Study population

The analysis reported in this chapter compares the brain structure of women with a history of preeclampsia with those with a history of normotensive pregnancy. I specifically selected these study groups based on existing literature indicating differences in brain structure at the time of pregnancy in some preeclamptic women and the increased risk of stroke in later life. The analysis in this chapter includes 78 out of 82 participants who underwent brain imaging (32 women with a history of preeclampsia and 46 control subjects with normotensive pregnancy). Four subjects were excluded from this analysis as their brain scans exhibited pathological appearances suggestive of inflammatory brain disease. Demographic details of this population are described below

and further description of the entire study population and how they were recruited are set out in Study population (chapter 2).

Figure 35. Flow chart detailing the study population and the measures employed in chapter 6.



6.3.2 Study visit

All participants attended the research department in the morning after overnight fast and had blood collected.

The participants had the following assessments and analysis, which are described in detail in Methods (chapter 3):

- Brain MRI (page 70)
    - T1 weighted sequence
    - Fluid attenuated inversion recovery (T2 weighted) sequence
    - Diffusion tensor imaging
- Brachial blood pressure measurements (office) (page 54)
- Medical notes data extraction (page 79)
- Medical history and lifestyle questionnaire (page 79)
- Anthropometry (page 51)
- Blood sampling and cardiovascular risk biomarker analysis (page 51)

6.3.3 Statistics

For full description of statistical methods please see statistics section in Methods (Chapter 3, page 79). In brief, statistical analysis was carried out using SPSS version 22 (IBM, NY, United States). A p-value <0.05 was used to identify significant differences.

Previously reported differences in systolic blood pressure between women with a history of preeclampsia and those with a history of normotensive pregnancy show differences of 10 mmHg [unpublished data]. In a study of white matter damage in hypertension, Hannesdottir et al (259) reported a difference in mean fractional anisotropy of 0.01 for 25mmHg difference in systolic blood pressure. Therefore a difference of 0.004 in fractional anisotropy would be expected with a 10mmHg difference in systolic blood pressure between groups. Sixteen individuals in each group provided 80% power at p=0.01 to identify differences of this size between groups.

Retrospective analysis of sample sizes of the subgroups was undertaken to ensure that groups were appropriately powered to detect differences in mean fractional anisotropy. Thirty two individuals in the normotensive pregnancy group and 46 individuals in the preeclamptic pregnancy group provided 80% power at p=0.05 to identify a 0.65 standard deviation differences between groups, equivalent to 0.008 in mean fractional anisotropy.

6.4 Results

6.4.1 Study cohort characteristics

The cohorts of women were not significantly different in terms of age and proportion of current smokers (Table 38). However those with a history of preeclampsia were studied later after affected pregnancy (8.7±2.3 vs 7.8±1.7, p=0.03) and were more likely to be taking antihypertensive medication (9% vs 0%, p=0.03). Pregnancy characteristics

were, in the main, similar across groups, though women with a history of preeclampsia had more complicated pregnancies (1.3±0.7 vs 0.6±0.7, p<0.001). Anthropometrics were not different between groups. However blood pressure was higher in women with a history of preeclampsia (systolic blood pressure: 123.2±13.0 mmHg vs 116.8±15.1 mmHg, p=0.01). Biochemistry was not different between groups, except HDL cholesterol which was lower in women with a history of preeclampsia (1.46±0.38 mmol/L vs 1.62±0.38 mmol/L, p<0.05).

Table 38. Baseline characteristics of brain imaging subgroup.

	NTN (n=46)	**PET (n=32)**	**p-value**
Demographics			
Age (years)	40.6±5.5	42.8±5.1	0.06
Yrs. since index pregnancy	7.8±1.7	8.7±2.3	**0.03**
Smokers, n (%)	6 (13)	1 (3)	0.16
Taking antihypertensive medications, n (%)	0 (0)	3 (9)	**0.03**
Pregnancy Characteristics			
Gestation (weeks)	34.1±5.3	34.2±3.7	0.85
Offspring birthweight (g)	2332±1117	2042±945	0.40
Index twin pregnancies, n (%)	7 (16)	2 (6)	0.19
Total number of live births	2.3±1.0	2.0±0.9	0.26
Total number of complicated pregnancies	0.6±0.7	1.3±0.7	**<0.001**
Anthropometrics			
Height (m)	1.66±0.06	1.64±0.06	0.11
Weight (kg)	70.7±15.3	71.6±11.6	0.39
BSA	1.79±0.20	1.80±0.15	0.63
BMI (Kg/m^2)	25.8±5.7	26.7±4.5	0.17
Waist: Hip	0.903±0.048	0.915±0.072	0.38
Haemodynamics			
SBP (mmHg)	116.8±15.1	123.2±13.0	**0.01**
DBP (mmHg)	74.9±8.8	79.9±8.8	**0.01**
MAP (mmHg)	88.9±10.5	94.4±9.7	**0.007**
Heart rate (bpm)	58.4±8.1	61.6±8.5	0.05
Biochemistry			
Total Cholesterol (mmol/L)	5.00±0.88	4.87±0.84	0.55
LDL (mmol/L)	2.99±0.85	2.90±0.68	0.66
HDL (mmol/L)	1.62±0.38	1.46±0.38	**<0.05**
TChol: HDL	3.25±0.94	3.46±0.82	0.38
Triglyceride (mmol/L)	0.84±0.35	1.03±0.55	0.10
HsCRP (mg/L)	2.63±4.25	2.54±2.96	0.13
HOMA IR	1.65±1.68	1.75±1.09	0.39

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at p<0.05. BSA: body surface area; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; HsCRP: highly sensitive C reactive protein; HOMA IR: homeostatic model assessment of insulin resistance.

The characteristics of the subgroup who underwent brain imaging (shown in table 38) were, in the main, representative of the total study population (described in Chapter 5, page 127). However there were a few notable exceptions: the normotensive women in brain imaging subgroup were studied earlier after the index pregnancy than the normotensive women overall in the study (7.8±1.7 years vs 9.0±1.9 years, p<0.001) and had lower gestation of index pregnancy (normotensive pregnancy: 34.1±5.3 weeks vs 37.2±4.8 weeks, p<0.001; preeclamptic pregnancy: 34.2±3.7 weeks vs 35.9±3.2 weeks, p=0.008) and birthweight of index pregnancy (normotensive pregnancy: 2332±1117g vs 3042±1038g, p<0.001 and preeclamptic pregnancy: 2042±945g vs 2512±895g, p=0.008). The brain imaging subgroup also had higher waist: hip ratios (normotensive pregnancy: 0.903±0.048 vs 0.858±0.076, p<0.001 and preeclamptic pregnancy: 0.915±0.072 vs 0.849±0.091, p<0.001). In addition, women with a history of normotensive pregnancy who had brain imaging each had a greater number of complicated births than the women with normotensive pregnancies overall (0.6±0.7 vs 0.3±0.6, p=0.007).

6.4.2 Reduced grey matter volumes in women with a history of preeclampsia

Women with a history of preeclampsia had lower total grey matter volumes compared with women with a history of normotensive pregnancy (663±38 ml vs 686±54 ml, p=0.04) (Table 39 and figure 36). Women with previous preeclampsia also had lower cortical grey matter volumes (523±30 ml vs 544±45 ml, p=0.04) (figure 37). There were no differences in total brain or white matter volumes (all p>0.05).

A sensitivity analysis was performed which showed that differences in total grey matter volume were no longer significant between groups after excluding women with a history of normotensive preterm delivery (p=0.13) however significant differences were

still present after excluding women currently taking antihypertensive medication (p=0.03).

Figure 36. Bar graph showing lower total grey matter volumes in women with a history of preeclampsia (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (663±38 ml vs 686±54 ml, p=0.04).

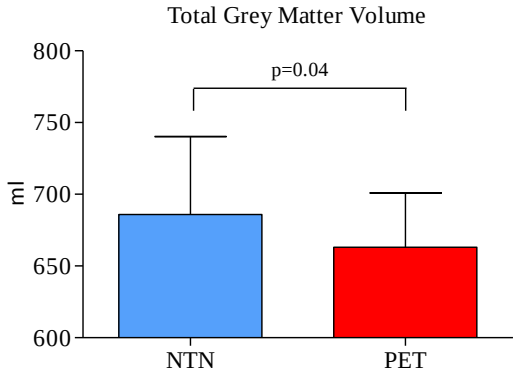


Figure 37. Bar graph showing lower cortical grey matter volumes in women with a history of preeclampsia (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (523±30 ml vs 544±45 ml, p=0.04).

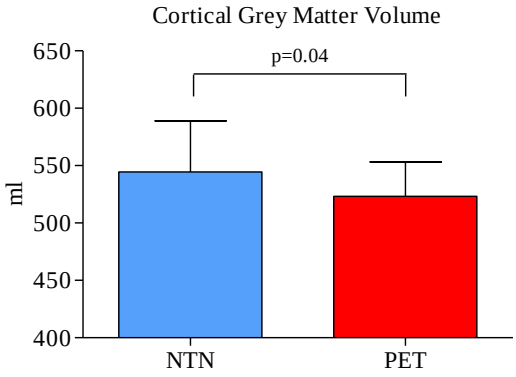


Table 39. Total and regional brain volumes in women with a history of normotensive and preeclamptic pregnancy

	NTN (n=46)	**PET (n=32)**	**p-value**
Total brain volume (ml)	1621±71	1600±83	0.23
Total grey matter (ml)	686±54	663±38	**0.04**
Cortical grey matter (ml)	544±45	523±30	**0.04**
Total white matter (ml)	901±58	905±55	0.79

Mean ± standard deviation, bold indicates significant group difference at p<0.05

When separating the cohort according to the severity of preeclampsia (early and late onset) there was a non-significant trend to reduced total grey matter volumes in women with a history of early onset preeclampsia (p=0.07) and there were also significantly lower cortical grey matter (521±27 mm^3 vs 544±45 mm^3, p=0.04) (Table 40). There were no significant differences in total brain volume in early onset preeclampsia; or in total brain, or grey matter volumes in late onset preeclampsia (all p>0.05).

Table 40. Total and regional brain volumes in women with a history of normotensive, early onset and late onset preeclamptic pregnancy

	NTN (n=46)	Early PET (n=19)	Late PET (n=13)	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)
Total brain volume (ml)	1621±71	1602±85	1598±83	0.35	0.31
Total grey matter (ml)	686±54	661±37	666±40	0.07	0.22
Cortical grey matter (ml)	544±45	521±27	526±35	**0.04**	0.27
Total white matter (ml)	901±58	910±58	897±49	0.58	0.81

Mean ± standard deviation, bold indicates significant group difference at p<0.05. PET: history of preeclampsia; GH: history of gestational hypertension.

There were no differences between groups in volumes of subcortical brain structure (Table 41).

Table 41. Subcortical regional brain volumes in women with a history of normotensive and preeclamptic pregnancy

	NTN (n=46)	PET (n=32)	p-value
Thalamus (ml)	15.7±1.0	15.5±1.0	0.24
Caudate (ml)	6.6±0.6	6.6±0.7	0.71
Putamen (ml)	9.0±0.8	8.9±0.6	0.70
Pallidus (ml)	3.4±0.2	3.3±0.2	0.20
Hippocampus (ml)	6.5±0.8	6.5±0.6	0.94
Amygdala (ml)	2.4±0.3	2.5±0.4	0.79
Accumbens (ml)	0.9±0.2	0.8±0.1	0.29
Brainstem (ml)	20.2±1.9	20.1±1.8	0.78

Mean ± standard deviation, bold indicates significant group difference at p<0.05.

In univariate regression the only relevant factor which emerged as a significant predictor of grey matter volume was age (see Table 42).

Table 42. Univariate regression associations with total grey matter volumes

Total grey matter volume (ml)	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
Age (yrs)	-3.34	-5.130	-1.56	**<0.001**
Body surface area (m^2)	-6.44	-56.52	43.64	0.80
Smoking	30.10	-3.94	64.15	0.08
Number of pregnancies	3.44	-7.29	14.17	0.53
Years since index pregnancy	-0.13	-5.19	4.94	0.96
Twin pregnancies	-23.15	-62.76	16.46	0.25
Gestation (wks.)	0.04	-2.37	2.44	0.98
Offspring birthweight (g)	<0.01	-0.01	0.01	0.72
SBP (mmHg)	-0.50	-1.17	0.17	0.14
DBP (mmHg)	-0.26	-0.71	1.22	0.60
MAP (mmHg)	-0.13	-1.03	0.76	0.77
Current antihypertensive medication usage	6.30	-43.74	56.34	0.80
Weight (kg)	0.07	-0.55	0.70	0.82
Total Cholesterol (mmol/L)	-4.56	-16.89	7.77	0.46
LDL (mmol/L)	-3.47	-17.58	10.64	0.63
HDL (mmol/L)	-8.52	-39.81	22.77	0.59
Triglyceride (mmol/L)	-4.91	-26.00	16.17	0.64
HOMA IR	2.47	-5.40	10.35	0.53
HsCRP (mg/L)	-0.73	-3.48	2.03	0.60
Creatinine (micromol/L)	0.57	-0.68	1.82	0.37

Statistically significance was defined as $p<0.05$. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; T Chol: total cholesterol; HsCRP: highly sensitive C reactive protein; HOMA IR: homeostatic model assessment of insulin resistance; BMI: body mass index.

In multivariable analysis a history of preeclampsia was predictive of reduced grey matter volumes independent of age (table 43).

Table 43. Multivariable regression analyses of grey matter volumes

Grey Matter Model	Standardized β Coefficient	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
Age (yrs.)	-0.36	-3.31	-5.25	-1.37	0.001
History of PET	-0.23	-20.96	-40.40	-1.52	0.04

Statistical significance was defined as $p<0.05$. PET: preeclampsia.

6.4.3 White matter lesions (manual count) in women with a history of preeclampsia

There was no difference in the prevalence of white matter hyperintensities between women with a history of preeclamptic or normotensive pregnancy (97% vs 88%, p=0.16) (Table 44). In addition neither total number of lesions (7.0±8.0 vs 4.5±3.0 respectively, p=0.44) or Fazekas score differed between groups (1.6±0.9 vs 1.4±0.8 respectively, p=0.29). There were also no differences in number of white matter lesions in regional analysis (all p>0.05).

Table 44. White matter hyperintensities (manual count) in women with a history of normotensive and preeclamptic pregnancy

	NTN (n=46)	PET (n=32)	p-value
Global			
Presence of WMH, n (%)	42 (88)	30 (97)	0.16
Number of lesions, mean ± SD	4.5±3.0	7.0±8.0	0.44
Fazekas score, mean ± SD	1.4±0.8	1.6±0.9	0.29
Number of lesions			
Frontal, mean ± SD	2.8±1.7	5.2±6.1	0.12
Parietal, mean ± SD	1.2±1.1	0.9±1.3	0.16
Occipital, mean ± SD	0.6±0.9	0.9 ±1.1	0.25
Temporal, mean ± SD	<0.1±0.3	0.2±0.8	0.15

Mean ± standard deviation, bold indicates significant group difference at p<0.05. WMH: white matter hyperintensities; SD: standard deviation.

There were also no significant differences in the presence or number of white matter lesions in women with either early or late onset preeclampsia, in both global and regional analysis (Table 45).

Table 45. White matter hyperintensities (manual count) in women with a history of normotensive, early onset and late onset preeclamptic pregnancy

	NTN (n=46)	Early PET (n=19)	Late PET (n=13)	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)
Global					
Presence of WMH, n (%)	42 (88)	19 (95)	11 (100)	0.35	0.22
Number of lesions, mean ± SD	4.5±3.0	6.5±7.2	7.8±9.5	0.43	0.71
Fazekas score, mean ± SD	1.4±0.8	1.7±1.0	1.6±0.7	0.41	0.38
Number of lesions					
Frontal, mean ± SD	2.8±1.7	4.7±5.2	6.3±7.7	0.21	0.23
Parietal, mean ± SD	1.2±1.1	0.7±1.2	1.2±1.4	0.08	0.83
Occipital, mean ± SD	0.6±0.9	0.8±1.0	0.9±1.4	0.28	0.50
Temporal, mean ± SD	<0.1±0.3	0.3±0.9	0.1±0.3	0.15	0.29

Mean ± standard deviation, bold indicates significant group difference at p<0.05. WMH: white matter hyperintensities; SD: standard deviation.

6.4.4 White matter lesions (voxel-based analysis) in women with a history of preeclampsia

There were significantly higher volumes of white matter lesions in the temporal lobes of women with a history of preeclampsia compared to women with a history of normotensive pregnancy (23.2±24.9 μl vs 10.9±15.0 μl, p=0.04) (Table 46 and figure 38). There was no such difference in volumes in all other regions analysed (all p>0.05). There were also no differences in the total number and volume of lesions in the entire brain between groups (both p>0.05).

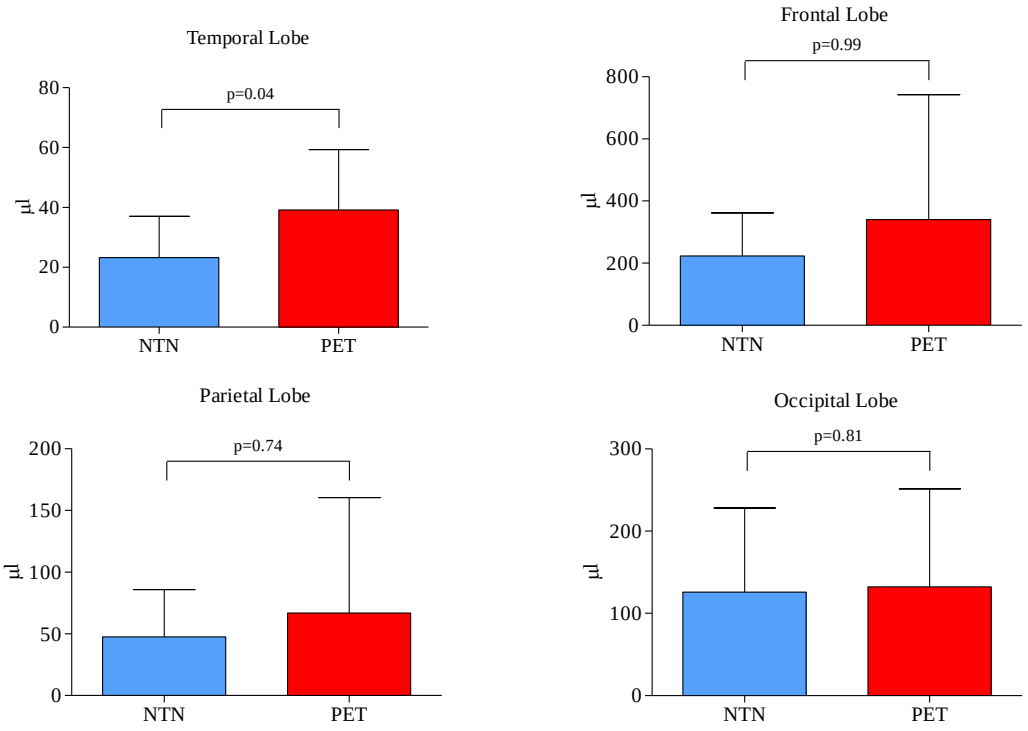
Sensitivity analysis showed that differences in temporal lobe white matter lesion volume were no longer significant between groups after excluding women with a history of normotensive preterm delivery (p=0.09) however significant differences were still present after excluding women currently taking antihypertensive medication (p<0.05).

Table 46. Automated voxel-based analysis of white matter hyperintensities using BIANCA in women with a history of normotensive and preeclamptic pregnancy

	NTN (n=46)	PET (n=32)	p-value
Global			
Number of lesions	7.4±3.2	8.8±5.4	0.44
Volume of lesions (µl)	412.6±257.5	546.6±570.8	0.75
Regional volume of lesions (lobes)			
Temporal (µl)	10.9±15.0	23.2±24.9	**0.04**
Frontal (µl)	223.2±138.3	318.8±398.1	0.99
Parieto (µl)	21.4±34.8	27.2±67.1	0.74
Occipital (µl)	95.0±104.1	103.2±118.6	0.81

Mean ± standard deviation, bold indicates significant group difference at p<0.05

Figure 38. Bar graph showing higher volume of white matter lesions in the temporal lobes in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (23.2±24.9 µl vs 10.9±15.0 µl, p=0.04), however there were no differences in white matter lesion volumes in other lobes (frontal: 318.8±398.1 µl vs 223.2±138.3 µl, p=0.99; parietal lobe: 27.2±67.1 µl vs 21.4±34.8 µl, p=0.74; occipital: 103.2±118.6 µl vs 95.0±104.1 µl, p=0.81).



When separating the cohort according to the severity of preeclampsia (early and late onset) there was a significantly higher volume of white matter lesions in the temporal lobe of women with a history of early onset preeclampsia compared to women with a

history of normotensive pregnancy (25.7±25.2 μl vs 10.9±15.0 μl, p=0.02) (Table 47). There were no differences in volume of white matter lesion in the temporal lobe between women with a history of late onset preeclampsia compared to women with a history of normotensive pregnancy (p>0.05). There were also no differences in white matter lesion count or volume in the entire brain or volume of white matter lesions in other regions of the brain in either women with a history of early onset or late onset preeclampsia and those with a history of normotensive pregnancy (all p>0.05).

Table 47. Automated voxel-based analysis of white matter hyperintensities using BIANCA in women with a history of normotensive and early onset and late onset preeclampsia

	NTN (n=46)	**Early PET (n=19)**	**Late PET (n=13)**	**NTN vs Early PET (p-value)**	**NTN vs Late PET (p-value)**
Global					
Number of lesions	7.4±3.2	9.1±4.7	8.3±6.5	0.25	0.93
Volume (μl)	412.6±257.5	556.4±609.0	532.4±533.9	0.69	0.94
Regional (lobes) volume of lesions					
Temporal (μl)	10.9±15.0	25.7±25.2	19.6±24.8	**0.02**	0.48
Frontal (μl)	223.2±138.3	354.0±472.4	267.4±264.3	0.82	0.76
Parieto (μl)	21.4±34.8	29.5±81.6	23.8±40.1	0.62	0.97
Occipital (μl)	95.0±104.1	79.5±95.7	137.8±143.0	0.59	0.27

Mean ± standard deviation, bold indicates significant group difference at p<0.05

In univariate regression blood pressure emerged as a significant predictor of volume of white matter lesions in the temporal lobes (Table 48).

Table 48. Univariate regression associations with volume of white matter lesions in temporal lobes

Volume of white matter lesions in temporal lobes (voxel-based analysis)	Unstandardized β Coefficient	95% Confidence Lower limit	Upper limit	p-value
Age (yrs)	-0.05	-0.89	0.80	0.91
Smoking	-0.11	-16.53	16.30	0.99
Gestation (wks.)	-0.25	-1.22	0.72	0.61
Offspring birthweight (g)	<-0.01	-0.01	<0.01	0.75
SBP (mmHg)	0.33	0.03	0.63	**0.03**
DBP (mmHg)	0.60	0.12	1.08	**0.02**
MAP (mmHg)	0.52	0.10	0.94	**0.02**
Current antihypertensive medication usage	9.77	-14.45	33.99	0.42
Weight (kg)	-0.03	-0.32	0.25	0.81
Total Cholesterol (mmol/L)	-1.86	-7.97	4.25	0.54
LDL (mmol/L)	-2.56	-9.23	4.10	0.45
HDL (mmol/L)	-1.76	-15.48	11.95	0.80
Triglyceride (mmol/L)	-3.45	-15.42	8.53	0.57
HOMA IR	-0.34	-4.06	3.39	0.86
HsCRP (mg/L)	0.17	-1.56	1.22	0.81

Statistical significance was defined as p<0.05. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; T Chol: total cholesterol; HsCRP: highly sensitive C reactive protein; HOMA IR: homeostatic model assessment of insulin resistance.

Multivariable regression of relevant factors revealed that a history of preeclampsia was a significant predictor of temporal lobe white matter lesion volume after adjustment for blood pressure (Table 49).

Table 49. Multivariable regression model of volume of white matter lesions in temporal lobes

Temporal Lobe White Matter Lesion Volume Model	Standardized β Coefficient	Unstandardized β Coefficient	95% Confidence Lower limit	Upper limit	p-value
History of PET	0.30	12.73	3.64	21.82	0.007

Statistical significance was defined as p<0.05. PET: preeclampsia.

6.4.5 White matter diffusion in women with a history of preeclampsia

Fractional anisotropy was reduced in women with a history of preeclampsia compared to those with a history of normotensive pregnancy in the temporal and occipital lobe (p=0.03 and p=0.04 respectively) and there was a non-significant trend to reduced values in the entire brain overall (p=0.10) (Table 50). Mean diffusivity was increased in women with a history of preeclampsia compared to those with a history of normotensive

pregnancy in the temporal lobe (p=0.02) and there was a non-significant trend to increased values in the occipital and parietal lobe as well as the entire brain (all p=0.10). There were no significant differences between groups in axial diffusivity. Radial diffusivity was increased in subjects with a history of preeclampsia compared to those with a history of normotensive pregnancy in the temporal, occipital and parietal lobes (p=0.008, p=0.04 and p<0.05 respectively) and demonstrated a non-significant trend to increased values in the entire brain overall (p=0.06).

Table 50. White matter diffusion in women with a history of normotensive and preeclamptic pregnancy

	NTN (n=46)	**PET (n=32)**	**p-value**
Global			
Fractional anisotropy	0.491±0.011	0.486±0.012	0.10
Mean diffusivity ($x10^{-3}$ mm^2/sec)	0.700±0.014	0.705±0.016	0.10
Axial diffusivity ($x10^{-3}$ mm^2/sec)	1.11±0.017	1.11±0.021	0.40
Radial diffusivity ($x10^{-3}$ mm^2/sec)	0.496±0.014	0.503±0.016	0.06
Regional Analysis			
Fractional anisotropy			
Frontal	0.484±0.012	0.480±0.013	0.21
Parietal	0.510±0.013	0.504±0.013	0.14
Occipital	0.459±0.017	0.450±0.018	**0.04**
Temporal	0.464±0.013	0.457±0.014	**0.03**
Mean diffusivity			
Frontal ($x10^{-3}$ mm^2/sec)	0.689±0.017	0.694±0.017	0.20
Parietal ($x10^{-3}$ mm^2/sec)	0.701±0.015	0.707±0.019	0.10
Occipital ($x10^{-3}$ mm^2/sec)	0.749±0.018	0.757±0.022	0.10
Temporal ($x10^{-3}$ mm^2/sec)	0.719±0.018	0.730±0.019	**0.02**
Axial diffusivity			
Frontal ($x10^{-3}$ mm^2/sec)	1.08±0.021	1.09±0.024	0.83
Parietal ($x10^{-3}$ mm^2/sec)	1.13±0.018	1.14±0.024	0.45
Occipital ($x10^{-3}$ mm^2/sec)	1.16±0.026	1.17±0.029	0.69
Temporal ($x10^{-3}$ mm^2/sec)	1.10±0.022	1.11±0.024	0.14
Radial diffusivity			
Frontal ($x10^{-3}$ mm^2/sec)	0.493±0.016	0.498±0.017	0.17
Parietal ($x10^{-3}$ mm^2/sec)	0.485±0.016	0.493±0.019	**<0.05**
Occipital ($x10^{-3}$ mm^2/sec)	0.542±0.020	0.552±0.023	**0.04**
Temporal ($x10^{-3}$ mm^2/sec)	0.526±0.018	0.538±0.019	**0.008**

Mean ± standard deviation, bold indicates significant group difference at p<0.05

Differences in temporal lobe radial diffusivity between groups remained after excluding women with a history of normotensive preterm delivery (p=0.02) and also separately excluding women currently taking antihypertensive medication (p=0.01).

Subgroup analysis revealed that fractional anisotropy was reduced in women with a history of late onset preeclampsia in the entire brain overall and in the occipital lobe compared with women with a history of normotensive pregnancies ($p<0.05$ and p=0.02 respectively) but there were no such differences in any other brain regions (Table 51). Compared to women with a history of normotensive pregnancy, women with a history of late onset preeclampsia had higher mean diffusivity in the temporal lobes (p=0.01) and a non-significant trend to increased mean diffusivity in the entire brain overall (p=0.06). Women with late onset preeclampsia also had higher radial diffusivity in the entire brain, and separately in the occipital and temporal lobes (p=0.03, p=0.03 and p=0.01 respectively). There were no differences between women with a history of early onset preeclampsia and those with a history of normotensive pregnancy in fractional anisotropy or diffusivity in the entire brain or in regional analysis.

Table 51. White matter diffusion in women with a history of normotensive pregnancy and early onset and late onset preeclamptic pregnancy

	NTN (n=46)	Early PET (n=19)	Late PET (n=13)	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)
Global					
Fractional anisotropy	0.491±0.011	0.488±0.012	0.483±0.011	0.45	**<0.05**
Mean diffusivity ($x10^{-3}$ mm^2/sec)	0.700±0.14	0.703±0.16	7.08±0.16	0.39	0.06
Axial diffusivity ($x10^{-3}$ mm^2/sec)	1.106±0.017	1.108±0.021	1.112±0.020	0.64	0.34
Radial diffusivity ($x10^{-3}$ mm^2/sec)	0.496±0.014	0.500±0.016	0.507±0.016	0.42	**0.03**
Regional Analysis					
Fractional anisotropy					
Frontal	0.484±0.012	0.483±0.013	0.477±0.012	0.67	0.09
Parietal	0.510±0.013	0.507±0.014	0.501±0.014	0.45	0.09
Occipital	0.459±0.017	0.453±0.017	0.446±0.018	0.27	**0.02**
Temporal	0.464±0.013	0.458±0.015	0.456±0.012	0.12	0.06
Mean diffusivity					
Frontal ($x10^{-3}$ mm^2/sec)	0.689±0.017	0.693±0.018	0.696±0.018	0.47	0.17
Parietal ($x10^{-3}$ mm^2/sec)	0.701±0.015	0.706±0.017	0.710±0.022	0.27	0.11
Occipital ($x10^{-3}$ mm^2/sec)	0.749±0.018	0.754±0.019	0.760±0.025	0.32	0.08
Temporal ($x10^{-3}$ mm^2/sec)	0.719±0.018	0.727±0.021	0.732±0.016	0.13	**0.01**
Axial diffusivity					
Frontal ($x10^{-3}$ mm^2/sec)	1.082±0.021	1.086±0.024	1.086±0.024	0.53	0.99
Parietal ($x10^{-3}$ mm^2/sec)	1.133±0.018	1.137±0.021	1.137±0.029	0.46	0.62
Occipital ($x10^{-3}$ mm^2/sec)	1.163±0.026	1.166±0.025	1.166±0.034	0.76	0.74
Temporal ($x10^{-3}$ mm^2/sec)	1.105±0.022	1.110±0.27	1.117±0.019	0.46	0.07
Radial diffusivity					
Frontal ($x10^{-3}$ mm^2/sec)	0.493±0.016	0.496±0.017	0.502±0.016	0.50	0.07
Parietal ($x10^{-3}$ mm^2/sec)	0.485±0.016	0.490±0.018	0.496±0.022	0.21	0.05
Occipital ($x10^{-3}$ mm^2/sec)	0.542±0.020	0.549±0.021	0.557±0.026	0.25	**0.03**
Temporal ($x10^{-3}$ mm^2/sec)	0.526±0.018	0.536±0.021	0.541±0.017	0.07	**0.01**

Mean ± standard deviation, bold indicates significant group difference at $p<0.05$.

In univariate regression blood pressure and HDL emerged as significant predictors of radial diffusivity in the temporal lobes (Table 52).

Table 52. Univariate regression associations with radial diffusivity in the temporal lobes

Radial diffusivity in temporal lobes	Unstandardized β Coefficient	95% Confidence		p-value
		Lower limit	Upper limit	
Age	<0.01	<0.001	0.001	0.14
Smoking	0.01	-0.002	0.029	0.08
Gestation (wks.)	<0.01	-0.001	0.001	0.86
Offspring birthweight (g)	<-0.01	<0.001	<0.001	0.91
SBP (mmHg)	<0.01	<0.001	0.001	**0.04**
DBP (mmHg)	<0.01	<0.001	0.001	**0.02**
MAP (mmHg)	<0.01	<0.001	0.001	**0.02**
Current antihypertensive medication usage	<0.01	-0.016	0.031	0.55
Weight (kg)	<0.01	<0.01	<0.01	0.34
Total Cholesterol (mmol/L)	<0.01	-0.007	0.005	0.80
LDL (mmol/L)	<0.01	-0.005	0.008	0.73
HDL (mmol/L)	-0.01	-0.027	-0.001	**0.03**
Triglyceride (mmol/L)	0.01	-0.006	0.017	0.32
HOMA IR	<0.01	-0.001	0.006	0.11
HsCRP (mg/L)	<0.01	<0.001	0.002	0.20

Mean ± standard deviation, bold indicates significant group difference at p<0.05

Multivariable regression revealed that a history of preeclampsia was a significant predictor of radial diffusivity in the temporal lobes independent of both HDL and diastolic blood pressure (Table 53).

Table 53. Multivariable regression models of radial diffusivity in the temporal lobes

Temporal Lobe Radial Diffusivity Model	Standardized β Coefficient	Unstandardized β Coefficient	95% Confidence		p-value
			Lower limit	Upper limit	
History of PET	0.30	<0.01	<0.01	<0.01	0.01

Statistical significance was defined as p<0.05. PET: preeclampsia.

6.5 Discussion

The results of this study indicate that women several years after preeclampsia have lower grey matter volumes and greater white matter damage compared to women with a history of normotensive pregnancy. Furthermore greater white matter damage and reduced grey matter volumes are not fully explained by differences in conventional cardiovascular risk factors which suggest there may be novel disease-specific mechanisms responsible which are yet to be determined. This is the first study to comprehensively describe changes in both white and grey matter after preeclamptic

pregnancy and also to include diffusion tensor imaging, a sensitive technique for characterising white matter microstructural integrity.

Clinical relevance

Grey matter volumes reduce with increasing age and are associated with impaired cognition in older well individuals (260). In fact reduced cognition has been shown in women with a history of hypertensive pregnancy (152). Alzheimer's, known to be closely associated with vascular risk (261, 262), is more common in women than in men (263), though a link with preeclampsia has not been reported. In addition, structural damage to white matter, highlighted as white matter hyperintensities on T2 weighted MRI imaging, predict an increased risk of stroke, dementia and death (264). Preeclampsia is a risk factor for stroke and this risk remains elevated after controlling for traditional risk factors (84). This suggests that there may be additional relevant mechanisms common to both preeclampsia and stroke which have not yet been characterised. Indeed understanding mechanisms associated with stroke risk is of particular relevance to women as it is associated with a greater proportion of deaths (9%) than in men (6%) (265). Cerebral structural changes typically precede clinical cerebrovascular disease allowing identification of populations at increased risk (150). Progression of these structural changes can be significantly reduced by aggressive treatment of high blood pressure (266).

Explanation of findings

The pattern of reductions in grey matter volumes with ageing is hypothesised to relate to reduced perfusion (267) and neuronal loss (268) . Both age and blood pressure are important influences, with several studies having shown that mid-life hypertension is associated with reduced cortical thickness in later life (269, 270). DeCarli et al found

that blood pressure in the 5th decade was strongly associated with total brain volume in the 8th decade (271). Grey matter volumes in both men and women have a strong inverse correlation with age, whereas this is not the case with white matter volumes (272).

White matter hyperintensities are thought to represent local areas of ischaemia which develop after plasma proteins leak through damaged endothelium, leading to fibrosis, wall thickening and reduced local perfusion (150, 273). The process is thought to be a continuous one with gradual development over time, rather than caused by acute ischaemic events (274, 275). Deterioration of white matter architecture has been shown to be strongly associated with ageing; however vascular risk factors, such as hypertension, have an added effect (190). Microalbuminuria has also been shown to be an independent predictor of white matter lesions and is a key diagnostic feature of preeclampsia, thereby potentially explaining its association with white matter disease (276).

Fractional anisotropy and diffusivity characterise the direction and degree of water permeability in white matter tracts (277). Risk factors for the development of white matter hyperintensities also promote adverse alterations in white matter diffusion. However changes in measures of diffusion are typically detectable prior to white matter hyperintensities (189). A large cohort of adults from the Framingham Heart Study, with a similar age to the study described in this chapter, demonstrated strong associations between age and both lower fractional anisotropy and higher mean diffusivity, with blood pressure also a relevant factor (278).

As shown in the regression analyses, differences seen in this cohort 5-10 years after preeclamptic episode, cannot entirely be explained by differences in vascular risk

factors, such as blood pressure. The mechanisms responsible for this elevated structural damage and risk of stroke above that expected by traditional risk factors remain to be described, however it is possible that endothelial dysfunction and neurovascular autonomic dysregulation at the time of preeclampsia lead to structural changes which fail to resolve after delivery.

Comparison with previous studies

Studies exploring structural brain differences in the years after preeclampsia are few and limited to differences in total brain volumes and white matter hyperintensities. Recently Mielke et al reported on a cohort of women, aged 58±10 years, who had a history of hypertensive disorder of pregnancy, showing they had evidence of reduced total brain volumes and increased white matter lesion volume compared to a control group; specifically grey matter volumes or white matter diffusion parameters were not explored (152). Aukes et al explored two similar cohorts to the one described in this chapter and found a significant difference in the prevalence of white matter lesions between women with a history of preeclampsia and parous control women (37% vs 21%, respectively) (151) and between women with a history of eclampsia and parous control women (41% vs 17%, respectively) (188). The prevalences reported in these 2 studies were much lower than found in our study which might relate to greater MRI slice thickness (5mm in these previous reports compared to 3mm in the current study) leading to reduced sensitivity for identifying smaller lesions. In addition the methodology employed by Aukes et al differed from conventional approaches. They reported only counting a white matter hyperintensity if it was hyperintense on FLAIR, proton density-weighted and T2-weighted images and not hypointense on T1-weighted images. This is out of keeping with literature which suggests that white matter lesions can be hypointense on T1-weighted imaging (279) and may therefore have detected fewer lesions. For the analysis

described in this chapter lesions were identified if they were hyperintense on FLAIR images alone, the most sensitive sequence for white matter lesion identification (279).

The prevalence of white matter hyperintensities was high in our population. White matter hyperintensities are known to be highly prevalent in older populations (280). However they are also common in younger individuals; Wen et al showed that half of a large cohort of well individuals aged 44-48 had white matter hyperintensities (281). This is lower than I found in this cohort, however Wen et al excluded mild lesions such as “periventricular rims” and “caps”, which I did not.

To my knowledge, no study has explored grey matter volumes after pregnancy complicated by preeclampsia or hypertension. However, there are several studies showing grey matter volumes are inversely associated with hypertension and other vascular risk factors (278, 282, 283), which are common risk factors for preeclampsia (284). Similarly, white matter diffusion is unexplored in this population, but also shares common risk factors with preeclampsia (190).

Aukes et al who studied the prevalence of white matter hyperintensities after preeclampsia found that the frontal lobe was the region most commonly affected, but analysis of lobes was not carried out between groups (285). Our study also found the greatest burden of white matter hyperintensities in the frontal lobe, however differences between groups were found in the burden of lesions within the temporal lobes. This was further supported by consistent differences in diffusivity and fractional anisotropy.

These patterns of white matter damage may relate to complimentary pathophysiological processes. Previous studies exploring predictors of white matter damage have shown that frontal regions of the brain are particularly sensitive to ageing, whereas temporal and occipital regions demonstrate increased sensitivity to raised blood pressure (190).

Although our analysis shows that temporal lobe differences are not entirely explained by blood pressure, there may be other related vascular mechanisms responsible either at the time of pregnancy or persisting long term.

Strengths of the study

This is the first study of grey matter volume and white matter diffusion in women after preeclampsia. The use of diffusion imaging techniques and advanced voxel-based volumetric analysis allowed detailed assessment of white matter architecture. Viewed in conjunction with the applied technique of precise quantification of volumes of cortical and subcortical brain structures allowed for an encompassing characterisation of cerebral architecture in this cohort and generated important insights on potential disease related patterns. Detailed characterisation of the study population using maternity records allowed robust analysis of association between structural brain changes and cardiovascular profile.

Limitations of the study

However, this study still leaves some unanswered questions. The cross-sectional nature of this study did not allow conclusions to be made as to when the changes occurred over time. However this study forms the basis from which a longitudinal study could be planned to explore differences in the puerperium and again years later to answer this. While my analysis of associations between brain structural changes and cardiovascular risk factors revealed some predictors such as blood pressure and HDL, results of multivariable analyses indicated that other factors, yet to be elucidated, might contribute to the pathology. These possible contributors will need to be explored in future studies. In addition differences in diffusion parameters between lobes of the brain may represent diffuse changes across the whole lobe or more focal changes, within susceptible areas of

the brain. Previously it has been demonstrated in a Framingham cohort the relative weight of effect of blood pressure compared to age on the structural integrity of individual tracts (278), future studies using these advanced analysis techniques will be valuable. Explorative subgroup analysis of early and late onset preeclampsia separately was undertaken as it has been highlighted that there may be a spectrum of cardiovascular risk associated with preeclampsia (78) and also that there might be distinct pathophysiological differences according to gestational onset (162), however this study was not powered for this analysis. The findings of these subgroups will need to be confirmed in larger studies powered to detect these differences, however these results will be valuable in determining the number of subjects required to carry out such a study.

6.6 Conclusions

This is the first demonstration of both reduced grey matter volumes and white matter microstructural damage in women with a history of preeclampsia. These findings are not fully explained by conventional risk factors and this suggests that there may be novel disease-specific mechanisms responsible which are yet to be defined. These findings are relevant to future risk of cerebrovascular disease and cognitive impairment in this population and warrant further investigation. Clinical management at the time of pregnancy and ongoing risk factor modification after delivery are both likely to be important in reducing cerebrovascular disease in later life.

Hypertensive Disorders of Pregnancy and Maternal Microvascular Phenotype

Chapter 7

7.1 Abstract

Introduction – Capillary rarefaction and angiogenic factors have been implicated in the pathogenesis of hypertensive disorders of pregnancy. Reduced capillary density is also an important factor in the development of hypertension in non-pregnant individuals. I therefore set out to determine if there were differences in capillary density and angiogenic factors long term after hypertensive pregnancy.

Methods – A cohort of women with a history of hypertensive (n=164) and normotensive pregnancy (n=104) were recruited 5-10 years after affected pregnancy. All had fasting blood collected and sFLT-1, VEGF-A and sEndoglin were measured. A subgroup (n=180) underwent intravital capillaroscopy to measure functional and structural dermal capillary density.

Results – There were no differences in levels of angiogenic factors (sFLT-1, VEGF-A and sEndoglin) between women with a history of hypertensive pregnancy and those with a history of normotensive pregnancy 5-10 years after delivery. Capillary density was lower in women with a history of hypertensive pregnancy (functional capillary density: 106.3±21.4 capillaries/mm^2 vs 116.3±19.7 capillaries/mm^2 respectively, $p<0.001$ and structural capillary density: 114.9±22.5 capillaries/mm^2 vs 123.3±20.7 capillaries/mm^2 respectively, $p=0.002$). However capillary density was not significantly associated with blood pressure or markers of angiogenesis.

Conclusions – Maternal dermal capillary density is reduced after hypertensive pregnancy, however this does not explain differences in blood pressure.

7.2 Introduction

Reduced capillary density is associated with increased peripheral vascular resistance, hypertension and increased cardiovascular risk (286, 287). Capillary density increases during normotensive pregnancy and mirrors the fall in blood pressure seen (58). Capillary rarefaction and altered levels of angiogenic markers have been shown to develop prior to the onset of hypertension in pregnancy and have been implicated in its pathogenesis (118).

Hypertensive disorders of pregnancy have been shown to be associated with an increased risk of maternal cardiovascular disease after affected pregnancy (78, 86). Soluble FMS-like tyrosine kinase (sFLT-1) has been shown to be higher years after preeclampsia (154), but this has not been replicated and it is not known if differences exist in other markers of angiogenesis or capillary structure. Long term differences in

angiogenic factors and microvascular structure may explain a proportion of the increased cardiovascular risk seen in women with a history of hypertensive pregnancy.

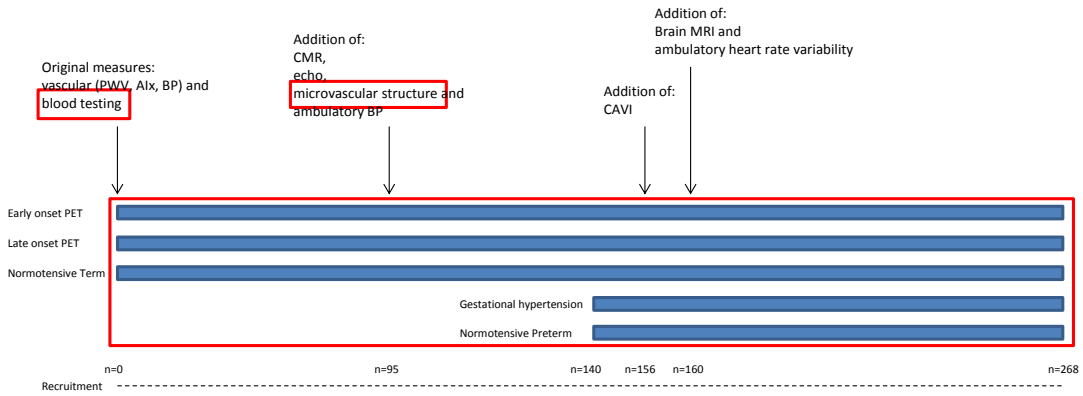
I set out to explore if markers of angiogenesis and capillary density were reduced in women with a history of hypertensive pregnancy and if this explained differences in blood pressure.

7.3 Methods

7.3.1 Study population

Levels of angiogenic markers were measured in the entire study cohort (n=268). Intravital capillaroscopy was carried out in a subgroup (n=180). Details of these cohorts and how they were recruited are set out in Study population (Chapter 2).

Figure 39. Flow chart detailing the study population and the measures employed in chapter 7.



7.3.2 Study visit

All participants attended the research department after overnight fast and had blood collected. All had the following assessments and analysis, which are described in detail in Methods (chapter 3).

- Biomarker collection and analysis (page 51)
    - Biochemistry analysis
    - ELISAs of markers of angiogenesis
- Intravital capillaroscopy (page 60)
- Brachial blood pressure measurements (office) (page 54)
- Medical notes data extraction (page 79)
- Medical history and lifestyle questionnaire (page 79)
- Anthropometry (page 51)
- CMR (page 61)
- Brain MRI (page 70)
- Carotid ultrasound (page 58)
- Brachial ultrasound (page 57)

7.3.3 Statistics

For full description of statistical methods please see statistics section in General Methods (Chapter 3, page 79). In brief statistical analysis was carried out using SPSS version 22 (IBM, NY, United States). A p-value <0.05 was used to identify significant differences.

Previously a difference of 10mmHg in systolic blood pressure between women with a history of hypertensive pregnancy and women with a history of normotensive pregnancy has been shown [unpublished data]. A study by Antonios et al (288) found a difference of 29 capillaries/mm^2 for a 10mmHg difference in systolic blood pressure. Thirteen individuals in each group provided 95% power at p=0.01 to identify differences of this size between groups.

Retrospective analysis of sample sizes of the subgroups was undertaken to ensure that groups were appropriately powered to detect differences in capillary density. Seventy three individuals in the normotensive group and 107 in the hypertensive group provided 80% power at p=0.05 to identify 0.43 standard deviation differences between groups, equivalent to a difference of 7.3 capillaries/mm^2.

7.4 Results

7.4.1 Study cohort characteristics

There were no significant differences in age, years since index pregnancy or smokers (Table 54). Women with a history hypertensive pregnancy were more likely to be currently taking antihypertensive medication. They also had lower delivery gestations and offspring birthweight, gave birth to fewer live offspring but had more complicated pregnancies. Height was lower in the cohort of women with a history of hypertensive pregnancy but body mass index was higher. Women with a history of hypertensive pregnancy also had higher blood pressure, heart rate and exhibited adverse metabolic differences in high density lipoprotein cholesterol, total cholesterol: high density lipoprotein cholesterol, triglyceride and homeostatic model assessment of insulin resistance.

Table 54. Entire study population characteristics

	NTN (n=104)	HTN (n=164)	p-value
Demographics			
Age (years)	40.8±4.6	41.1±5.2	0.48
Yrs. since index pregnancy	9.0±1.9	9.3±2.0	0.66
Smokers, n (%)	11 (11)	11 (7)	0.31
Taking antihypertensive medications, n (%)	0 (0)	12 (7)	**0.005**
Pregnancy Characteristics			
Gestation (weeks)	37.2±4.8	35.9±3.2	**<0.001**
Offspring birthweight (g)	3042±1038	2513±895	**<0.001**
Index twin pregnancies, n (%)	7 (7)	10 (6)	0.75
Total number of live births	2.4±1.0	2.0±0.8	**<0.001**
Total number of complicated pregnancies	0.3±0.6	1.4±0.7	**<0.001**
Anthropometrics			
Height (m)	1.65±0.06	1.63±0.06	**0.005**
Weight (kg)	70.1±14.8	72.7±15.4	0.11
BSA (m^2)	1.79±0.19	1.81±0.19	0.30
BMI (Kg/m^2)	25.6±5.5	27.2±6.0	**0.01**
Waist: Hip	0.858±0.076	0.849±0.091	0.23
Haemodynamics			
SBP (mmHg)	114.0±13.0	121.5±13.9	**<0.001**
DBP (mmHg)	73.7±8.3	79.3±10.1	**<0.001**
MAP (mmHg)	87.2±9.4	93.4±10.7	**<0.001**
PP (mmHg)	40.3±8.0	42.2±8.9	**0.02**
Heart rate (bpm)	59.5±8.5	61.9±9.6	**0.02**
Biochemistry			
Total cholesterol (mmol/L)	4.84±0.87	4.87±0.93	0.94
LDL cholesterol (mmol/L)	2.82±0.77	2.89±0.80	0.49
HDL cholesterol (mmol/L)	1.64±0.37	1.50±0.34	**0.004**
Total cholesterol: HDL cholesterol	3.09±0.85	3.38±0.84	**0.003**
Triglyceride (mmol/L)	0.87±0.41	1.02±0.59	**0.01**
HsCRP (mg/L)	2.13±4.01	2.53±4.08	0.14
HOMA IR	1.71±1.31	2.16±1.54	**0.007**

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at $p<0.05$. BSA: body surface area; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; HOMA IR: homeostatic model assessment of insulin resistance; HsCRP: highly sensitive C reactive protein.

7.4.2 Markers of angiogenesis and history of hypertensive pregnancy

Women with a history of hypertensive pregnancy had no significant differences in sEndoglin compared to women with a history of normotensive pregnancy (3.92±0.89

ng/ml vs 4.02±0.92 ng/ml, p=0.27) (Table 55 and figure 40). There were also no differences in sFLT (77.4±20.9 pg/ml vs 76.6±18.8 pg/ml, p=0.99) or VEGF (63.3±38.5 pg/ml vs 55.1±27.6 pg/ml, p=0.20). In addition there were no differences in sFLT: VEGF (1.74±1.27 vs 1.88±1.37, p=0.27).

Figure 40. Bar graphs showing no difference in markers of angiogenesis in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (soluble fms-like tyrosine kinase-1 (sFLT-1): 77.4±20.9 ng/ml vs 76.6±18.8 ng/ml, p=0.99; vascular endothelial growth factor (VEGF): 63.3±38.5 pg/ml vs 55.1±27.6 pg/ml, p=0.20; sFLT-1:VEGF: 1.74±1.27 vs 1.88±1.37, p=0.27; soluble endoglin (sEndoglin): 3.92±0.89 ng/ml vs 4.02±0.92 ng/ml, p=0.27).

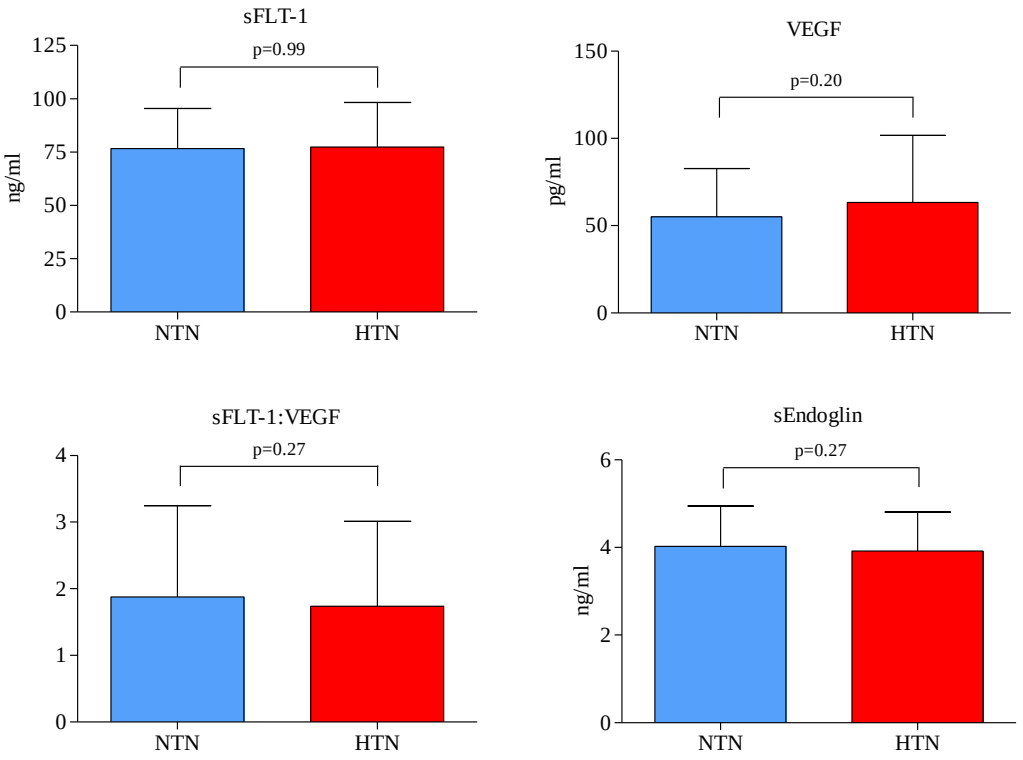


Table 55. Markers of angiogenesis

	NTN (n=97)	**HTN (n=154)**	**p-value**
sEndoglin (ng/ml)	4.02±0.92	3.92±0.89	0.27
sFLT1 (pg/ml)	76.6±18.8	77.4±20.9	0.99
VEGF (pg/ml)	55.1±27.6	63.3±38.5	0.20
sFLT: VEGF	1.88±1.37	1.74±1.27	0.27

Values as mean ± standard deviation. Bold text indicates significant group differences at p<0.05

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Further analysis of the subgroups of women with a history of hypertensive pregnancy according to the severity of hypertensive disorder at the time of pregnancy demonstrated no significant differences in any markers of angiogenesis between subgroups of hypertensive disorders of pregnancy and those with a history of normotensive pregnancy.

7.4.2 Microvascular imaging

A subgroup of individuals (n=180) underwent microvascular imaging to assess dermal capillary density. The groups of women with a history of normotensive and hypertensive pregnancy were well matched in age, years since index pregnancy and proportion of current smokers (Table 56). Women with a history of hypertensive pregnancy were more likely to be taking antihypertensive medication and also have lower delivery gestation and birthweight of index offspring. In addition they were more likely to have fewer live offspring but a greater number of complicated pregnancies, higher blood pressure, heart rate and HOMA-IR.

I also compared the characteristics of the subgroup of women who had intravital capillaroscopy with the characteristics of the whole study population to assess whether this microvascular subgroup were representative of the whole study population (Table 25). The microvascular subgroup characteristics were representative of the entire cohort, with no significant differences in any of the characteristics between the subgroup of individuals who had microvascular imaging and the entire study population.

Table 56. Characteristics of subgroup with microvascular imaging

	NTN (n=73)	HTN (n=107)	p-value
Demographics			
Age (yrs.)	41.0±5.0	42.3±5.0	0.07
Yrs. since index pregnancy	8.6±1.9	9.4±2.2	0.05
Smokers, n (%)	9 (12)	5 (6)	0.16
Taking antihypertensive medications, n (%)	0 (0)	9 (10)	**0.005**
Pregnancy Characteristics			
Delivery gestation (weeks)	36.8±4.8	36.1±3.3	**0.006**
Offspring birthweight (g)	2911±1079	2600±900	**0.02**
Index twin pregnancies, n (%)	6 (9)	7 (8)	0.88
Total number of live births	2.3±0.9	1.9±0.8	**<0.001**
Total number of complicated pregnancies	0.4±0.6	1.3±0.6	**<0.001**
Anthropometrics			
Height (m)	1.66±0.06	1.64±0.06	**0.04**
Weight (kg)	71.0±15.9	71.7±13.4	0.33
BSA (m^2)	1.80±0.20	1.80±0.17	0.64
BMI (Kg/m^2)	25.9±5.8	26.8±5.0	**0.07**
Waist: Hip circumference	0.878±0.072	0.862±0.082	0.18
Haemodynamics			
SBP (mmHg)	115.3±14.1	122.1±14.2	**<0.001**
DBP (mmHg)	74.5±8.2	81.2±10.5	**<0.001**
MAP (mmHg)	88.1±9.6	94.9±11.1	**<0.001**
PP (mmHg)	40.8±9.1	40.9±9.1	0.55
Heart rate (bpm)	58.2±8.2	61.2±9.6	**0.02**
Biochemistry			
Total cholesterol (mmol/L)	4.87±0.89	4.85±0.82	0.64
LDL cholesterol (mmol/L)	2.85±0.78	2.87±0.70	0.87
HDL cholesterol (mmol/L)	1.62±0.37	1.52±0.34	0.06
Total cholesterol: HDL cholesterol	3.13±0.86	3.33±0.82	0.10
Triglyceride (mmol/L)	0.90±0.42	1.00±0.65	0.48
HsCRP (mg/L)	2.25±4.41	1.81±2.47	0.41
HOMA IR	1.60±1.30	1.99±1.45	**0.02**

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at $p<0.05$. BSA: body surface area; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; HsCRP: highly sensitive C reactive protein;.HOMA IR: homeostatic model assessment of insulin resistance.

7.4.2.1 Capillary rarefaction in women with a history of hypertensive pregnancy

Women with a history of hypertensive pregnancy demonstrated capillary rarefaction compared to women with a history of normotensive pregnancy (Table 57 and figure 41). This was found in both functional capillary density (106.3±21.4 capillaries/mm^2 vs 116.3±19.7 capillaries/mm^2, $p<0.001$) and structural capillary density (114.9±22.5

capillaries/mm^2 vs 123.3±20.7 capillaries/mm^2, p=0.002). Capillary recruitment was not different between women with a history of hypertensive and normotensive pregnancy (8.4±14.6 % vs 7.1±14.4 % respectively, p=0.20).

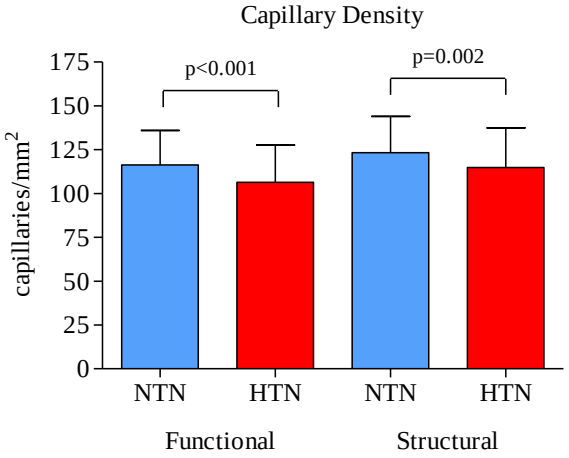
Sensitivity analysis showed that differences in both functional and structural capillary density were still present between groups after excluding women with a history of normotensive preterm delivery (p<0.001 and p=0.002 respectively) and separately excluding those individuals currently taking antihypertensive medication (p<0.001 and p=0.001 respectively).

Table 57. Capillary density

	NTN (n=73)	**HTN (n=107)**	**p-value**
Functional capillary density (capillaries/mm^2)	116.3±19.7	106.3±21.4	**<0.001**
Structural capillary density (capillaries/mm^2)	123.3±20.7	114.9±22.5	**0.002**
Capillary recruitment (%)	7.1±14.4	8.4±14.6	0.20

Mean ± standard deviation, bold text indicates significant group differences at p<0.05

Figure 41. Bar graphs showing both lower functional and structural capillary density in women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue) (functional capillary density: 106.3±21.4 capillaries/mm2 vs 116.3±19.7 capillaries/mm2, p=<0.001; structural capillary density: 114.9±22.5 capillaries/mm2 vs 123.3±20.7 capillaries/mm2, p=0.002).



I then explored whether capillary density was different between women with a history of normotensive pregnancy and those with a history of early onset preeclampsia, late onset preeclampsia or GH separately. Compared to women with a history of normotensive pregnancy women with a history of early onset preeclampsia had no

significant differences in functional or structural capillary density (p=0.12 and p=0.13 respectively) (Table 58). Women with a history of late onset preeclampsia had both lower functional and structural capillary density compared to women with a history of normotensive pregnancy (functional: 104.2±22.6 capillaries//mm^2 vs 116.3±19.7. capillaries/mm^2, p=0.001 and structural: 111.7±22.2 capillaries//mm^2 vs 123.3±20.7 capillaries//mm^2, p=0.002 respectively). Women with a history of GH demonstrated reduced functional capillary density but a statistically non-significant trend to reduced structural capillary density compared with women with a history of normotensive pregnancy (functional: 103.3±25.1 capillaries/mm^2 vs 116.3±19.7 capillaries/mm^2, p=0.003 and structural: 114.1±28.7 capillaries/mm^2 vs 123.3±20.7 capillaries/mm^2 respectively, p=0.05). There were no significant differences in capillary recruitment between any of the subgroups of hypertensive disorders of pregnancy and normotensive pregnancy (all p>0.05).

Table 58. Capillary density according to severity of hypertensive disorder of pregnancy

	NTN (n=73)	Early PET (n=39)	Late PET (n=49)	GH (n=19)	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)	NTN vs GH (p-value)
Functional capillary density (capillaries/mm^2)	116.3±19.7	111.2±16.6	104.2±22.6	103.3±25.1	0.12	**0.001**	**0.003**
Structural capillary density (capillaries/mm^2)	123.3±20.7	119.5±19.2	111.7±22.2	114.1±28.7	0.13	**0.002**	0.05
Capillary recruitment (%)	7.1±14.4	7.9±13.3	7.7±15.4	11.3±15.3	0.50	0.40	0.11

Mean ± standard deviation, bold text indicates significant group differences at $p<0.05$. PET: history of preeclampsia; GH: history of gestational hypertension.

I then explored which relevant factors were associated with capillary density and might explain the differences found between women with a history of hypertensive and normotensive pregnancy (Table 59). A number of relevant factors were significantly associated with functional capillary density, including: height, weight and delivery gestation of index pregnancy.

Table 59. Univariate regression of functional capillary density and relevant factors

Functional capillary density	Unstandardized β Coefficient	95% Confidence Lower limit	Upper limit	p-value
Age	-0.16	-0.796	0.477	0.62
Height	62.47	9.415	115.523	**0.02**
Weight	0.24	0.038	0.448	**0.02**
Smoking (currently)	-6.22	-17.523	5.080	0.28
Number of pregnancies	0.24	-3.281	3.763	0.89
Years since index pregnancy	0.15	-1.497	1.802	0.86
Twin pregnancies	1.16	-11.838	14.153	0.86
Preterm delivery	-1.79	-8.192	4.617	0.58
Delivery gestation (wks.)	0.85	0.060	1.643	**0.04**
Offspring birthweight (g)	0.003	0.000	0.006	0.08
Subject birthweight (g)	0.004	-0.003	0.011	0.30
SBP (mmHg)	-0.069	-0.295	0.156	0.55
DBP (mmHg)	-0.20	-0.341	0.301	0.90
MAP (mmHg)	-0.05	-0.346	0.248	0.74
PP (mmHg)	-0.16	-0.526	0.208	0.39
Central SBP* (mmHg)	-0.09	-0.307	0.120	0.39
Central DBP* (mmHg)	-0.03	-0.335	0.285	0.88
Central MAP* (mmHg)	-0.07	-0.351	0.215	0.64
Central PP* (mmHg)	-0.23	-0.589	0.131	0.21
Current antihypertensive medication usage	-1.46	-16.727	13.815	0.85
Total Cholesterol (mmol/L)	1.73	-2.029	5.484	0.37
LDL (mmol/L)	0.607	-3.692	4.906	0.78
HDL (mmol/L)	4.68	-4.830	14.193	0.33
Triglyceride (mmol/L)	4.27	-2.120	10.657	0.19
HOMA IR	-1.48	-3.862	0.894	0.22
HsCRP (mg/L)	0.48	-0.358	1.318	0.26
sEndoglin (ng/ml)	-3.06	-6.670	0.555	0.10
sFLT1 (pg/ml)	-0.11	-0.269	0.058	0.21
VEGF (pg/ml)	0.07	-0.028	0.163	0.17

Statistical significance was defined as $p<0.05$. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; *central blood pressure measured using SphygmoCor; † booking blood pressure was the blood pressure recorded at the first ante-natal appointment in the index pregnancy; LDL: low density lipoprotein; HDL: high density lipoprotein; T Chol: total cholesterol; HsCRP: highly sensitive C reactive protein; BMI: body mass index.

I tested a model of relevant factors, which included a history of hypertensive disorder of pregnancy, height, weight, delivery gestation and offspring birthweight. Both weight

and a history of hypertensive pregnancy were independent predictors of functional capillary density (Table 60).

Table 60. Model of functional capillary density

Functional Capillary Density Model	Standardized β Coefficient	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
Weight (kg)	0.20	0.27	0.06	0.48	**0.01**
History of HDP	-0.25	-10.73	-17.20	-4.26	**0.001**

Bold text indicates statistical significance at p<0.05. HDP: hypertensive disorder of pregnancy.

7.4.2.2 Skin capillary density is associated with adverse cardiac structural changes

Analysis of the relationships between functional capillary density and end organ structure revealed a significant association with cardiac but not cerebral structural differences (Table 61). Both left and right ventricular mass index were significantly and inversely associated with functional capillary density (β=-0.07, p=0.03 and β=-0.04, p=0.01 respectively), as was left atrial volume index (β=-0.09, p=0.01 and β=-0.07, p<0.05 respectively).There were no significant differences with cerebral structural differences.

Table 61. Univariate regression of functional capillary density and relevant markers of cardiac and cerebral end organ disease

	Functional capillary density (capillaries/mm^2) β	p-value
Cardiac		
LV mass index (g/m^2)	-0.07	**0.03**
RV mass index (g/m^2)	-0.04	**0.01**
Left atrial volume index (ml/m^2)	-0.09	**0.01**
Cerebral		
Total brain volume (ml^3)	-0.39	0.34
Grey matter volume (ml^3)	0.14	0.58
Temporal lobe white matter lesion volume (μl)	-0.01	0.94
Temporal lobe radial diffusivity (x10-3 mm2/sec)	<-0.01	0.36

Bold text indicates statistical significance at p<0.05. LV: left ventricle; RV: right ventricle; total brain volume was calculated from the sum of grey matter, white matter and cerebrospinal fluid.

7.5 Discussion

The results presented in this chapter provide the first evidence that a history of hypertensive pregnancy is associated with capillary rarefaction several years after delivery. The additional finding of a significant association with the number of hypertensive pregnancies suggests there may be a dose effect. Dermal capillary density was not associated with blood pressure. However capillary density was shown to be an independent predictor of left ventricular mass index. Markers of angiogenesis were not different between women with a history of hypertensive or normotensive pregnancy and did not explain differences in capillary density after affected pregnancy.

Dermal capillary density is readily measured non-invasively and has been shown to be representative of systemic capillary density (289). Capillary rarefaction is seen with increasing age and is considered to play an important role in increased peripheral vascular resistance found in patients with hypertension (290-292). Indeed there is some evidence that it might play a causative role in hypertension as reduced capillary density is seen in normotensive individuals at increased risk of hypertension (293). Other cardiovascular diseases also exhibit reduced capillary density, including several phenotypes of heart failure (294, 295). Indeed microvascular coronary disease has been shown to be more common in women and hence alterations in capillary structure may be of particular relevance in a female population (296, 297). Furthermore rat models of induced myocardial hypertrophy have also shown an association with reduced myocardial capillary density (298).

Microvascular rarefaction is not irreversible, commonly prescribed antihypertensive agents, such as calcium channel antagonists, ACE inhibitors and angiotensin receptor blockers have all been shown to increase capillary density (299). These drugs are already first line agents for treating hypertension (173). However in women with a

history of hypertensive pregnancy and reduced capillary density, these pharmacological agents may be particularly effective in controlling blood pressure and may have vascular benefits above their blood pressure lowering effects.

Raised blood pressure is known to be a risk factor for hypertensive disorders of pregnancy (90) and this raises the question of whether capillary density is reduced before conception in women who go onto develop hypertension during pregnancy. However there is data to suggest this is not the case. Nama et al studied women through pregnancy until 3 months postpartum, showing that during the 1st and 2nd trimesters of preeclamptic pregnancy maximal (structural) capillary density was not different from women during normotensive pregnancy (118). The diagnosis of preeclampsia in this cohort was typically late in the 3rd trimester, however early in the 3rd trimester maximal capillary density was reduced compared to normotensive pregnancy and these differences were still present several weeks postpartum.

Another group showed similar effects by measuring resting peripheral blood flow, a measure of microvascular function, through pregnancy. Women who went on to develop preeclampsia showed no difference in peripheral blood flow at the beginning of pregnancy from women who had normotensive pregnancies. However in the preeclamptic group peripheral blood flow reduced prior to preeclampsia being diagnosed (300). These studies suggest that although capillary changes likely precede clinical presentation of preeclampsia they are not different at the start of pregnancy.

The finding of no differences in angiogenic markers several years after hypertensive pregnancy may relate to generally low levels of production in these factors outside of affected pregnancy. VEGF, sFLT and soluble Endoglin are all highly expressed by the placenta, both in normotensive and hypertensive pregnancy (116, 301). Removal of the

poorly implanted ischaemic placenta and resolution of haemodynamic upset may explain comparable levels in the early years after delivery. Although angiogenic markers are known to be associated with cardiovascular disease (302-305) our study population was well and free from overt disease. This study recruited women between 5 and 10 years after affected pregnancy, it could well be that these populations will demonstrate separation in angiogenic levels at a later time point when cardiovascular disease is more advanced.

Capillary density has not, to my knowledge, been previously reported years after hypertensive pregnancy. However indirect measures of total peripheral resistance, calculated using mean arterial pressure and cardiac output (measured using echo), have shown values 20% higher in women 23 years after preeclampsia compared to normotensive pregnancy (306).

Most studies measuring sFLT-1 and VEGF years after preeclamptic and normotensive pregnancy have been small. However VEGF levels have been shown not to differ according to a history of preeclampsia (153, 307-309). Those which have measured sFLT levels in the first 8 years after pregnancy have had mixed results (78, 153, 154, 307, 308). However the largest study, carried out by Sandvik et al, in 168 women 9 years after pregnancy reported no differences in sFLT levels between women with or without preeclampsia (310). Soluble endoglin has been shown to be increased in women with preeclampsia, at the time of diagnosis, compared to normotensive pregnancy (311). However at 1 and 12 weeks postpartum levels of soluble endoglin do not differ between groups (149, 311)

A major strength of this study was the comprehensive set of related measures detailing capillary density and markers of angiogenesis in a large well described cohort. Paired

data from cardiac, brain and macrovascular imaging as well allowed analysis of relevant relationships between these organs and microvascular structure. In addition access to maternity records allowed a high level of confidence in the diagnosis of hypertension during pregnancy and the disease characteristics during pregnancy and the puerperium.

However there are some limitations of this study. As the microvascular measures were added as an amendment to the study protocol, not all the individuals recruited to the study underwent these investigations. However the characteristics of these individuals were similar to the study population as a whole. Also, the analysis of capillary density was undertaken by 3 different analysers which may have introduced added variance between subjects measured by different analysers, though inter-operator coefficient of variance was low (Methods, chapter 3). The participants recruited to this study were studied at a single time point and we were therefore not able to collect data on capillary density and angiogenic markers during pregnancy which would allow us to draw conclusions on whether there were differences present in capillary density and angiogenic markers during pregnancy and whether they have changed since delivery.

7.6 Conclusions

In summary, this work demonstrates for the first time that women with a history of hypertension during pregnancy have reduced capillary density 5-10 years after affected pregnancy. This may, in part, explain cardiac structural differences seen in these individuals.

Although capillary density was not found to be significantly associated with blood pressure we cannot say if capillary density in other organs which more directly affect blood pressure such as the heart or kidneys are not associated. Indeed capillary density

may be more relevant to blood pressure response to stress, such as pregnancy or exercise, when individuals are more vasodilated and maximal capillary density may be a limiting factor in haemodynamic adaptation.

Certain antihypertensive agents are known to improve capillary density and these may be of particular benefit in women with a history of hypertensive pregnancy. Prospective studies comparing different antihypertensive agents during pregnancy would be of value in exploring whether targeted early antihypertensive treatment with agents which modify microvascular structure are able to attenuate long term capillary rarefaction.

Hypertensive Disorders of Pregnancy and Maternal Autonomic Function

Chapter 8

8.1 Abstract

Introduction – Hypertensive pregnancy is associated with autonomic dysfunction characterised by altered blood pressure dipping and reduced heart rate variability. Autonomic dysfunction is also associated with a wide variety of cardiovascular disease and may explain the increased cardiovascular risk seen in women with a history of hypertensive pregnancy.

Methods – Autonomic function was assessed in healthy women free from cardiovascular disease and diabetes 5-10 years after hypertensive (n=97) and normotensive pregnancy (n=65). Ambulatory monitors worn for 24 hours recorded blood pressure and heart rate variability.

Results – Nocturnal blood pressure was higher in women with a history of hypertensive compared to normotensive pregnancy (systolic blood pressure: 106.6±12.5 mmHg vs

102.0±9.7 mmHg, p<0.05; diastolic blood pressure: 63.4±7.3 mmHg vs 59.9±7.7 mmHg, p=0.006). However daytime blood pressure was not different (systolic blood pressure: p=0.20, diastolic blood pressure: p=0.17) and the proportion of women with non-dipping nocturnal blood pressure was not significantly different (systolic blood pressure: p=0.56, diastolic blood pressure: p=0.31). Furthermore heart rate variability was not significantly different between women with history of hypertensive pregnancy compared to those with a history of normotensive pregnancy.

Conclusions – Nocturnal blood pressure is higher several years after hypertensive pregnancy. However blood pressure dipping status and heart rate variability do not reveal significant autonomic differences.

8.2 Introduction

Hypertensive pregnancy is associated with maternal autonomic dysfunction at the time of pregnancy, characterised by altered blood pressure dipping and reduced heart rate variability (312). Later in life women with a history of hypertensive pregnancy are at increased risk of cardiovascular morbidity and mortality (78, 79). Distinct from pregnancy, alterations in heart rate variability and blood pressure dipping are associated with coronary heart disease (313, 314), heart failure (315-317), left ventricular mass (318, 319), renal disease (320, 321), cerebrovascular disease (322, 323), risk of hypertension (324) and death (325). Long term alterations in heart rate variability and blunted circadian blood pressure dipping after hypertensive pregnancy may contribute to cardiovascular risk. If autonomic dysfunction is associated with hypertensive pregnancy then it may be a relevant way of identifying individuals at greatest

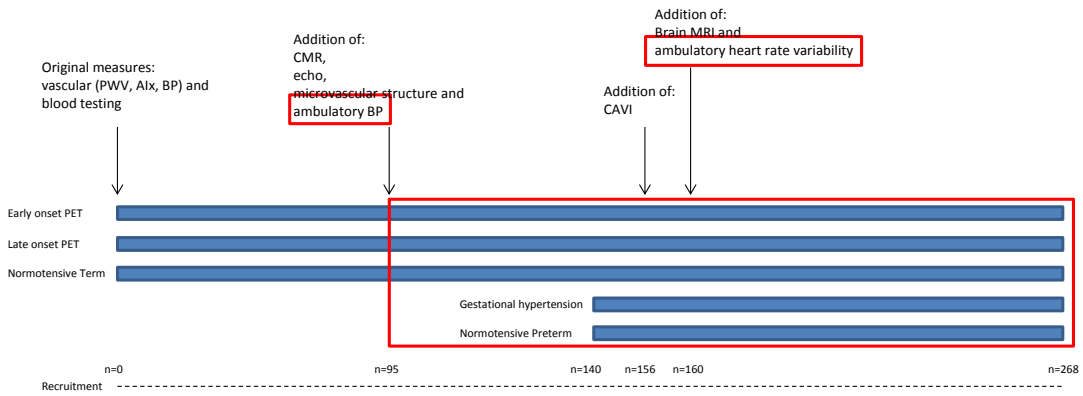
cardiovascular risk after affected pregnancy and also represent an effective therapeutic target (326).

8.3 Methods

8.3.1 Study population

This chapter includes 162 participants from the cohort who underwent investigation of autonomic function. Details of this cohort and how they were recruited are set out in Study population (Chapter 2).

Figure 42. Flow chart detailing the study population and the measures employed in chapter 8.



8.3.2 Study visit

Participants attended the research department in the morning after overnight fast and had blood collected. Ambulatory blood pressure (n=162) and heart rhythm monitoring (n=96) was undertaken. These investigations are described in detail in Methods (Chapter 3, page 75).

8.3.3 Statistics

For full description of statistical methods please see statistics section in General Methods (Chapter 3, page 79). In brief statistical analysis was carried out using SPSS version 22 (IBM, NY, United States). A p-value <0.05 was used to identify significant differences.

Previously reports of heart rate variability 7 months after preeclamptic and uncomplicated pregnancies have shown differences of 30% in low frequency: high frequency (158). If differences were maintained 5-10 years after affected pregnancy then 32 individuals in each group provided 80% power at p=0.01 to identify differences of this size between groups.

8.4 Results

8.4.1 Study cohort characteristics

The cohorts of women were not significantly different in terms of age or proportion of current smokers. However women with a history of hypertensive pregnancy were more likely to be studied later after affected pregnancy and be taking antihypertensive medications (Table 63). They were also more likely to have fewer live births but more likely to have had pregnancies complicated by hypertension or preterm delivery. Office blood pressure and heart rates were higher in women with a history of hypertensive pregnancy and they also exhibited greater insulin resistance.

The characteristics of the subgroup who had ambulatory monitoring (Table 62) were, in the main, representative of the entire cohort (Table 25). However there were a few notable exceptions; the normotensive group who had ambulatory monitoring were studied earlier after the index pregnancy compared to the entire normotensive group

(8.3±1.8 years vs 9.0±1.9 years, p=0.02) and also had a higher waist: hip ratio (0.883±0.075 vs 0.858±0.076 p=0.04).

Table 62. Baseline characteristics of subgroup with ambulatory monitoring.

	NTN (n=65)	HTN (n=97)	p-value
Demographics			
Age (yrs.)	41.0±5.2	42.3±4.7	0.12
Yrs. since index pregnancy	8.3±1.8	9.3±2.2	**0.01**
Smokers, n (%)	9 (14)	7 (7)	0.18
Taking antihypertensive medications, n (%)	0 (0)	9 (9)	**0.01**
Born preterm or to PET pregnancy, n (%)	8 (13)	17 (22)	0.19
Pregnancy Characteristics			
Gestation (weeks)	35.7±5.2	35.8±3.4	0.26
Offspring birthweight (g)	2717±1136	2521±965	0.24
Index twin pregnancies, n (%)	7 (12)	6 (7)	0.25
Total number of live births	2.3±1.0	1.9±0.9	**0.007**
Total number of complicated pregnancies	0.4±0.6	1.4±0.6	**<0.001**
Anthropometrics			
Height (m)	1.65±0.06	1.64±0.06	0.17
Weight (kg)	69.8±14.2	71.9±13.6	0.18
BSA (m^2)	1.78±0.19	1.80±0.18	0.31
BMI (Kg/m^2)	25.6±5.1	26.7±5.2	0.09
Waist: Hip	0.883±0.075	0.870±0.098	0.23
Haemodynamics			
SBP (mmHg)	115.7±14.0	123.4±14.5	**<0.001**
DBP (mmHg)	74.1±8.4	80.5±10.2	**<0.001**
MAP (mmHg)	88.0±9.8	94.7±10.9	**<0.001**
Heart rate (bpm)	58.7±8.8	61.5±9.2	**0.03**
Biochemistry			
Total Cholesterol (mmol/L)	4.93±0.82	4.86±0.82	0.53
LDL (mmol/L)	2.91±0.77	2.91±0.66	0.94
HDL (mmol/L)	1.64±0.39	1.52±0.32	0.07
Total cholesterol: HDL	3.16±0.85	3.30±0.72	0.21
Triglyceride (mmol/L)	0.82±0.32	0.92±0.44	0.24
Highly sensitive CRP (mg/L)	2.20±3.98	2.02±3.54	0.63
HOMA IR	1.58±1.45	2.05±1.63	**0.01**

Values as mean ± standard deviation unless otherwise stated. Bold text indicates significant group differences at p<0.05. BSA: body surface area; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; HOMA IR: homeostatic model assessment of insulin resistance; HsCRP: highly sensitive C reactive protein.

8.4.2 Higher blood pressure in women with a history of hypertensive pregnancy

Women with a history of hypertensive pregnancy did not have significant differences in mean ambulatory systolic blood pressure compared to women with a history of normotensive pregnancy (120.7±10.9 mmHg vs 117.2±11.2 mmHg, p=0.10) (Table 63) (figure 43). However they did have higher mean ambulatory diastolic blood pressure (74.3±7.0 mmHg vs 72.0±7.3 mmHg, p<0.05 and mean arterial blood pressure (89.4±7.9 mmHg vs 86.8±8.2 mmHg, p=0.04).

Ambulatory blood pressures during the awake periods were not significantly different between groups for systolic, diastolic or mean arterial pressure (p=0.20, p=0.17 and p=0.11 respectively). However ambulatory blood pressures during sleep periods were significantly different; women with a history of hypertensive pregnancy had higher systolic blood pressure (106.6±12.5 mmHg vs 102.0±9.7 mmHg, p<0.05), diastolic blood pressure (63.4±7.3 mmHg vs 59.9±7.7 mmHg, p=0.006) and mean arterial pressure (77.5±8.6 mmHg vs 73.5±8.1 mmHg, p=0.006). The proportion of individuals whose blood pressure did not dip more than 10 mmHg was not significantly different between cohorts (systolic blood pressure: p=0.56; diastolic blood pressure: p=0.31).

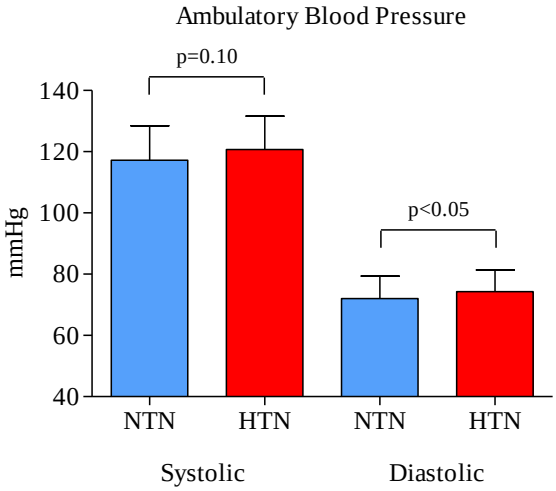
Sensitivity analysis showed that differences in nocturnal systolic blood pressure were still present between groups after excluding women with a history of normotensive preterm delivery (p=0.04) but not significant after excluding women currently taking antihypertensive medication (p=0.10). Furthermore differences remained in both nocturnal diastolic and mean arterial pressure after excluding women with a history of normotensive preterm delivery (p=0.007 and p=0.009 respectively) and separately after excluding women currently taking antihypertensive medication (p=0.01 and p=0.01 respectively).

Table 63. Ambulatory blood pressure in women with a history of normotensive and hypertensive pregnancy.

	NTN (n=65)	HTN (n=97)	p-value
Systolic blood pressure (mmHg)			
Total recording	117.2±11.2	120.7±10.9	0.10
Awake	121.4±12.4	124.2±10.8	0.20
Asleep	102.0±9.7	106.6±12.5	**<0.05**
Awake - sleep difference (mmHg)	18.9±11.2	16.5±10.0	0.19
Nocturnal dipping ≤10mmHg, n (%)	16 (28)	21 (23)	0.56
Diastolic blood pressure (mmHg)			
Total recording	72.0±7.3	74.3±7.0	**<0.05**
Awake	75.4±7.6	77.1±7.4	0.17
Asleep	59.9±7.7	63.4±7.3	**0.006**
Awake - sleep difference (mmHg)	15.2±7.1	13.5±6.7	0.15
Nocturnal dipping ≤10mmHg, n (%)	13 (22)	27 (30)	0.31
Mean arterial pressure (mmHg)			
Total recording	86.8±8.2	89.4±7.9	**0.04**
Awake	90.3±8.6	92.5±7.9	0.11
Asleep	73.5±8.1	77.5±8.6	**0.006**
Awake - sleep difference (mmHg)	16.4±8.1	14.5±7.1	0.13
Nocturnal dipping ≤10mmHg, n (%)	14 (24)	21 (23)	0.91

Mean ± standard deviation; n=number, % = percentage; bold text indicates significant group differences at p<0.05.

Figure 43. Bar graphs showing ambulatory blood pressure over the total recording period between women with a history of hypertensive disorder of pregnancy (HTN - red) compared to women with a history of normotensive pregnancy (NTN – blue). Systolic blood pressure was not significantly different between women with a history of hypertensive pregnancy and women with a history of normotensive pregnancy (120.7±10.9 mmHg vs 117.2±11.2 mmHg, p=0.10). However diastolic blood pressure was higher in women with a history of hypertensive disorder of pregnancy (74.3±7.0 mmHg vs 72.0±7.3 mmHg, p<0.05).



Furthermore I explored whether ambulatory blood pressures were different between women with a history of normotensive pregnancy and those with a history of early onset preeclampsia, late onset preeclampsia or GH separately. Compared to women with a history of normotensive pregnancy women with a history of early onset preeclampsia had higher nocturnal systolic, diastolic and mean arterial pressure (p=0.02, p=0.009 and p=0.009 respectively) (Table 64). Women with a history of late onset preeclampsia had no significant differences in ambulatory blood pressure. Women with a history of GH had higher mean ambulatory systolic, diastolic and mean arterial pressure (p=0.02, p=0.03 and p=0.02 respectively).

Table 64. Ambulatory blood pressure monitor readings for women with a history of normotensive, early and late onset preeclampsia and gestational hypertension.

	NTN (n=65)	Early PET (n=36)	Late PET (n=41)	GH (n=20)	NTN vs Early PET (p-value)	NTN vs Late PET (p-value)	NTN vs GH (p-value)
SBP							
Total recording (mmHg)	117.2±11.2	120.8±11.5	119.1±10.1	124.0±11.4	0.33	0.40	**0.02**
Awake (mmHg)	121.4±12.4	124.0±11.2	123.2±9.9	126.6±11.9	0.30	0.42	0.10
Asleep (mmHg)	102.0±9.7	107.6±13.3	105.6±13.0	107.4±10.2	**0.02**	0.29	0.05
Nocturnal dipping ≤10mmHg, n (%)	16 (28)	8 (25)	7 (17)	6 (35)	0.79	0.22	0.54
DBP							
Total recording (mmHg)	72.0±7.3	74.1±6.0	73.5±7.0	76.5±8.7	0.16	0.31	**0.03**
Awake (mmHg)	75.4±7.6	76.6±6.2	76.7±7.6	78.7±8.8	0.44	0.39	0.11
Asleep (mmHg)	59.9±7.7	64.3±6.9	62.6±7.3	63.8±8.0	**0.009**	0.09	0.07
Nocturnal dipping ≤10mmHg, n (%)	13 (22)	11 (34)	13 (32)	3 (18)	0.22	0.30	0.67
MAP							
Total recording (mmHg)	86.8±8.2	89.4±7.3	88.3±7.7	91.9±9.1	0.12	0.34	**0.02**
Awake (mmHg)	90.3±8.6	92.1±7.1	91.9±8.0	94.2±9.2	0.30	0.35	0.09
Asleep (mmHg)	73.5±8.1	78.4±8.5	76.6±8.8	78.0±8.4	**0.009**	0.08	0.05

Mean ± standard deviation, bold text indicates significant group differences at $p<0.05$. NTN: normotensive pregnancy, Early PET: early onset preeclampsia, Late PET: late onset preeclampsia, GH: gestational hypertension, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure.

8.4.3 Heart rate variability in women with a history of normotensive and hypertensive pregnancy

There were no significant differences in heart rate recording length, mean heart rate, or range of heart rate during the recording between women with a history of normotensive pregnancy compared to women with a history of hypertensive pregnancy (Table 65). There were no significant differences in any time domain or frequency domain measures of heart rate variability between women with a history of hypertensive pregnancy and women with a history of normotensive pregnancy. There were however non-significant trends to reduced RMSSD and SDSD in women with a hypertensive pregnancy compared to those with a history of normotensive pregnancy (RMSSD: 35.0±13.4 vs 41.1±14.9, p=0.06 and SDSD: 26.3±11.0 vs 31.0±12.7, p=0.07 respectively).

Table 65. Ambulatory heart rate variability in women with a history of normotensive and hypertensive pregnancy.

	NTN (n=49)	**HTN (n=49)**	**p-value**
Recording characteristics			
Recording length (hours)	22.3±6.4	21.0±7.0	0.99
Mean heart rate (beats/minute)	76.9±9.7	79.8±9.8	0.20
Minimum heart rate (beats/minute)	53.5±7.4	55.0±7.0	0.34
Maximum heart rate (beats/minute)	122.0±23.0	128.5±21.8	0.15
Heart rate variability			
SDNN Index (msec)	62.4±17.9	61.2±15.5	0.86
RMSSD (msec)	41.1±14.9	35.0±13.4	0.06
SDSD (msec)	31.0±12.7	26.3±11.0	0.07
pNN50 (%)	13.6±8.8	10.5±7.5	0.10
Triangular Index	42.1±12.2	42.2±11.6	0.75
VLF ($msec^2$)	1209±1016	998±755	0.20
LF ($msec^2$)	992±926	892±776	0.33
HF ($msec^2$)	652±494	564±592	0.14
VHF ($msec^2$)	121±286	74±60	0.94
LF: HF	1.97±1.35	2.24±1.53	0.44

Mean ± standard deviation, bold text indicates significant group differences at $p<0.05$; SDNN Index: standard deviation of NN intervals index; RMSSD: square root of the mean of the sum of the squares of differences between adjacent NN intervals; SDSD: standard deviation of differences between adjacent NN intervals; pNN50: proportion of adjacent NN intervals varying by 50 msec or more; VLF: very low frequency; LF: low frequency; HF: high frequency; VHF: very high frequency.

Similarly no differences existed between groups in RMSSD after excluding participants with a history of normotensive preterm delivery (p=0.08), however borderline differences were present when women currently taking antihypertensive medication were excluded from the analysis (p<0.05). Furthermore analysis of pNN50 revealed no significant differences remained after exclusion of women with a history of normotensive preterm delivery, or those currently taking antihypertensive medication (both p=0.08).

I also explored if differences were present when comparing sleeping (10 minute periods at 2am), as opposed to mean ambulatory (24 hour) recordings. There were no significant differences in any heart rate variability parameters, including RMSSD and SDSD (p=0.52 and p=0.22 respectively) (Table 66).

Table 66. Nocturnal heart rate variability over 10 minutes in women with a history of normotensive and hypertensive pregnancy.

	NTN (n=49)	**HTN (n=49)**	**p-value**
SDNN Index (msec)	63.7±28.5	57.3±25.3	0.24
RMSSD (msec)	45.4±25.9	40.1±23.5	0.52
SDSD (msec)	28.7±17.1	24.8±14.1	0.22
pNN50 (%)	21.9±19.3	17.7±20.2	0.67
Triangular Index	15.0±5.6	14.3±5.6	0.41
VLF ($msec^2$)	1132±974	1170±1778	0.30
LF ($msec^2$)	1119±965	1114±1088	0.91
HF ($msec^2$)	953±1054	843±1230	0.83
VHF ($msec^2$)	109±305	69±69	0.38
LF: HF	2.34±3.02	3.05±4.29	0.65

Mean ± standard deviation, bold text indicates significant group differences at p<0.05; SDNN Index: standard deviation of NN intervals index; RMSSD: square root of the mean of the sum of the squares of differences between adjacent NN intervals; SDSD: standard deviation of differences between adjacent NN intervals; pNN50: proportion of adjacent NN intervals varying by 50 msec or more; VLF: very low frequency; LF: low frequency; HF: high frequency; VHF: very high frequency.

To further understand if there were significant associations between heart rate variability and pregnancy characteristics I carried out univariate regression of ambulatory RMSSD and relevant characteristics of pregnancy. There were significant associations between ambulatory RMSSD and age (β=-0.68, p=0.02) and also diastolic

blood pressure (β=-0.30, p<0.05) but not with any characteristics of pregnancy (Table 67).

Table 67. Univariate regression associations with ambulatory RMSSD

RMSSD (24hr)	Unstandardized β Coefficient	95% Confidence Lower limit	95% Confidence Upper limit	p-value
Age	-0.68	-1.26	-0.01	**0.02**
Smoking	-7.62	-18.08	2.84	0.15
Currently taking antidepressants	-7.76	-22.54	7.01	0.30
Currently taking antihypertensive medication	2.85	-11.39	17.09	0.69
Number of pregnancies	1.39	-1.88	4.67	0.40
Number of hypertensive pregnancies	-2.50	-6.05	1.04	0.16
Years since index pregnancy	-0.16	-1.72	1.39	0.84
Twin pregnancies	-2.23	-14.55	10.08	0.72
Delivery gestation (wks.)	-0.33	-1.04	0.37	0.35
Offspring birthweight (g)	<-0.01	<-0.01	<0.01	0.52
Subject birthweight (g)	<0.01	<-0.01	0.01	0.43
SBP (mmHg)	-0.05	-0.26	0.16	0.62
DBP (mmHg)	-0.30	-0.59	<-0.01	**<0.05**
MAP (mmHg)	-0.20	-0.47	0.08	0.15
PP (mmHg)	0.25	-0.09	0.59	0.15

Statistical significance was defined as p<0.05. RMSSD: square root of the mean of the sum of the squares of differences between adjacent NN intervals; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure.

8.5 Discussion

This chapter reports differences in ambulatory blood pressure after hypertensive pregnancy. Several years after affected pregnancy nocturnal blood pressure was higher, however no significant difference in daytime blood pressure was detected. The proportion of non-dippers (<10mmHg difference between awake and asleep mean blood pressures) was not different according to history of hypertensive pregnancy and there were no significant differences in heart rate variability between groups or significant associations with pregnancy characteristics

Blood pressure

High blood pressure is the leading risk factor for global cardiovascular disease, with two thirds of stroke and half of ischaemic heart disease attributable to raised levels (327). The problem is very common, with the Framingham Heart Study showing 90% of individuals who reach middle age developing hypertension and 60% receiving antihypertensive medication (328). Ambulatory blood pressure measurements are predictive of cardiovascular risk over and above conventional measures of blood pressure (329). In fact nocturnal blood pressure is a stronger predictor than daytime blood pressure of both all-cause and cardiovascular mortality (330, 331).

Guidelines for diagnosing hypertension suggest the use of ambulatory blood pressure monitors for confirming hypertension and highlight the greater reliability of ambulatory readings over clinic (office) readings (173). Studies which rely on clinic readings may overestimate the prevalence of hypertension in this population. This in turn could lead to an overestimation of the contribution of blood pressure to this population's future cardiovascular risk at the expense of other causes, as highlighted in previous chapters.

The amplification of differences between groups in office blood pressure compared to ambulatory monitored blood pressure also suggests an element of white coat hypertension. This condition, characterised by high clinic readings but normal ambulatory or home readings, was for many years thought to have little prognostic importance. However it is now apparent that it is not a benign diagnosis and is associated with an intermediate level of cardiovascular risk compared to sustained hypertension (332). Individuals with white coat hypertension have evidence of subclinical cardiac and renal organ damage, as well as increased risk of cardiovascular events compared to normotensive individuals (332-334).

Blood pressure is related to cardiac output and peripheral resistance however there are a multitude of physiological mechanisms which influence this relationship. Cardiac output does not differ between groups in the cohort I have described (chapter 4), however reduced aortic compliance and augmentation index illustrate increased arterial stiffness (chapter 5). The finding of capillary rarefaction (chapter 7) further supports a widespread difference in peripheral resistance throughout the vascular tree in women with a history of hypertensive disorder of pregnancy. However we should also consider the roles of metabolic, hormonal and renal factors. Women with a history of hypertensive disorder of pregnancy compared to women with a history of normotensive pregnancy exhibit an adverse metabolic phenotype, with higher BMI, greater insulin resistance and adverse lipid profiles (full cohort described in table 25). Nocturnal norepinephrine release has been shown to be higher in women with preeclampsia in a small study and it is not known if this trend is continued after delivery (335). Renal function may well contribute to differences in blood pressure in this population, however the effect size is likely small. I found no significant differences in creatinine. It was not possible to measure other markers of renal function such as renin, angiotensin and aldosterone in this study, however it has previously been reported that there are no differences when measured several years after affected pregnancy (336).

In agreement with my findings several other studies have also reported an increased risk of raised blood pressure after hypertensive pregnancy (80, 337). The ALSPAC study, one of the largest studies to explore blood pressure several years after complicated pregnancy, showed comparable differences in office blood pressure to the results reported in this chapter, however ambulatory monitoring was not performed (338).

Mangos et al measured ambulatory blood pressure in women 3 years after hypertensive pregnancy and found higher blood pressures during both during awake and sleep

periods after both preeclamptic pregnancy and gestational hypertension compared to women with a history of normotensive pregnancy, however similar to my findings there were no significant differences in dipping (336). The study was smaller than the cohort I describe however both groups of women with a history of preeclampsia and gestational hypertension had higher BMI (25 kg/m^2 and 27 kg/m^2) compared to the control group (23 kg/m^2), which may explain the greater differences in blood pressure.

Collen et al measured ambulatory blood pressure 40 years after hypertensive pregnancy but found no differences compared to women with a history of normotensive pregnancy (339). However this study included less than 30 individuals, and reported comparable blood pressure readings to this study, despite the population being more than 20 years older. Survival bias may have also affected these results and therefore it may be that the results for this population cannot be easily compared to a population in their fourth and fifth decades.

It is also relevant that the cohort of women with a history of normotensive pregnancy did not only consist of uncomplicated pregnancies but also women who had delivered preterm though uncomplicated by hypertension. It has been shown that women with a history of preterm delivery, independent of hypertension, have higher blood pressure compared to women with a history of uncomplicated pregnancy (340) though the mechanisms are not yet fully understood.

The explanation for higher blood pressures several years after hypertensive pregnancy is likely multifactorial, with differences in vascular structure and function, as well metabolic differences likely explaining a proportion of the differences seen. However, although vascular changes can lead to increased blood pressure, higher blood pressure can also lead to vascular changes and understanding the temporal relationship of

individual factors can be complex. Furthermore we cannot determine from this study when these differences occur in relation to the affected pregnancy, though previous literature suggests that a proportion is present before pregnancy (99).

There were significant differences in nocturnal blood pressure, however it is perhaps surprisingly there was no significant difference in blood pressure during awake periods. This highlights the subtle nature of differences in ambulatory blood pressure compared to clinic measurements. Ambulatory blood pressure is more closely associated with cardiovascular morbidity and mortality than clinic measured blood pressure (341), it is therefore unlikely that these subtle differences in blood pressure solely explain the higher rates of mortality and morbidity associated with a history of hypertensive pregnancy (78, 79) and supports the findings reported in earlier chapters of this thesis of adverse cardiovascular and cerebral phenotypic changes which are independent of differences in blood pressure.

Heart rate variability

There were only non-significant trends to differences between groups in ambulatory heart rate variability. It might be expected that true differences in heart rate variability would be more apparent during sleep periods when the variance associated with confounding factors, such as activity and environmental stimulus would be expected to be lower. This might indicate that the trend to reduced RMSSD and SDSD seen in ambulatory recordings was due to confounding factors during the day that we are not able to quantify. The effect of known potential confounders such as depression and smoking (342, 343) was assessed, however these factors did not significantly affect the results.

This lack of differences between groups may be explained by the participants I studied being well individuals with no overt cardiovascular disease or diabetes, which were exclusion criteria of the study. Thus any differences in heart rate variability were minimal and the study underpowered to detect them.

Alternatively, it may be that differences in autonomic function are driven by alterations in sympathetic activity which is known to be poorly correlated with heart rate variability, which is much more specific for assessing parasympathetic function (344). Greenwood et al and Schobel et al both measured muscle sympathetic nerve activity during hypertensive and normotensive pregnancy and showed increased sympathetic nerve activity during pregnancy but by a few months postpartum these differences were no longer present (213, 345). Similarly Mangos et al reported no difference in cold pressor test response between women with a history of hypertensive compared to normotensive pregnancy, 3-4 years after affected pregnancy (336) and Sverrisdóttir et al found no differences in muscle sympathetic nerve activity in similar populations 20 years after affected pregnancy (339).

However we must consider the possibility that ambulatory recording, whether over 24 hours or by analysing shorter durations at night, is not as sensitive for detecting differences in heart rate variability as testing for shorter periods in an observed and controlled environment. There are no large scale studies of heart rate variability comparing recordings over short observed periods with longer ambulatory recordings. I collected data on exercise and sleeping periods during the recording period. However I was not able to monitor and control for every factor which may have influence heart rate variability such as intake of caffeine (346) and alcohol (347).

Scholten et al measured heart rate variability in a small number of women with a history preeclampsia and women after normotensive pregnancy and reported differences in low frequency: high frequency 6-12 months after pregnancy (158). It may be that differences in heart rate variability found during pregnancy are still detectable in early postpartum, however by 9 years postpartum these differences are attenuated. However the findings of Scholten et al may be due to selection bias as individuals with a history of preeclampsia were recruited from a specialist preeclampsia clinic, whereas the control population were recruited using newspaper advertisement, which may have attracted a healthier population.

The study reported in this chapter is novel and has a number of strengths. It is the first study to measure both ambulatory blood pressure and heart rate variability after hypertensive pregnancy. In addition access to individual maternity records allowed a high level of confidence in the diagnosis of hypertensive disorder of pregnancy. The time point of several years after affected pregnancy allows us to explore differences in blood pressure and heart rate variability which are likely relevant to future cardiovascular risk and distinct from transient alterations relating to the pregnancy.

However there are some limitations with this study. There were few individuals with a history of hypertensive pregnancy who were hypertensive and fewer than have been reported in some previously published studies (140, 348), this might suggest that the population studied were not representative of a general population of women with a history of hypertensive pregnancy as they are healthier with lower rates of hypertension. If this was the case it would lead to underestimating phenotypic differences.

Measurement of heart rate variability was not observed and participants undertook normal daytime activities which may have introduced variation in behaviour and

environment between individuals and groups. I carried out exploratory subgroup analysis to assess if there were differences in ambulatory blood pressure between women with a history of normotensive pregnancy and those with early onset and late onset preeclampsia, as well as gestational hypertension separately. However the study was not powered to detect differences in these subgroups and differences may exist which this study was unable to identify.

8.6 Conclusions

Nocturnal, but not daytime, blood pressure was increased 9 years after hypertensive pregnancy. However there were no significant differences in ambulatory heart rate variability, which suggests that parasympathetic dysfunction does not explain a significant proportion of the increased cardiovascular risk seen in women with a history of hypertensive pregnancy. Further studies exploring heart rate and blood pressure beat to beat variability in a controlled environment with orthostatic stress and assessment of skeletal muscle sympathetic nerve activity would give greater confidence in autonomic dysfunction not explaining a large degree of the increased cardiovascular risk seen years after hypertensive pregnancy.

Conclusions

Chapter 9

In conclusion this body of work has provided a comprehensive understanding of cardiovascular phenotypic differences several years after pregnancy complicated by hypertension. This has covered a spectrum of both structural and functional characteristics from the heart and large arteries, through to capillaries and angiogenic biomarkers. It has also included cerebral structure, in both white and grey matter regions, as this is an organ known to be at risk both during, and remote to, preeclamptic pregnancies.

These insights were achieved through recruitment of women with both hypertensive and normotensive pregnancies, using detailed maternal records, and performing detailed cardiovascular and cerebral phenotyping 5-10 years after affected pregnancy. This time point was specifically selected to avoid transient changes associated with pregnancy and allow confidence in the findings representing long-term associations relevant to cardiovascular disease in later life.

The first objective (chapter 4) was to determine if cardiac structure and function differed after hypertensive pregnancy. Multi-modality assessment, utilising the strengths of both cardiac magnetic resonance and echocardiography, allowed detailed

assessment of both ventricles. These techniques showed for the first time an increase in left ventricular mass independent of differences in blood pressure in women with a history of hypertensive disorder of pregnancy but related to the number of hypertensive pregnancies. In addition smaller left ventricular volumes, larger left atrial volumes and reduced diastolic function were demonstrated in this group.

The second objective (chapter 5) was to assess vascular function and again I used a variety of complimentary techniques to measure this. I demonstrated for the first time in this population using MRI, that despite no differences in aortic cross-sectional area, global aortic compliance and distensibility were lower in women with a history of hypertensive pregnancy. This difference was proportional to the variance in blood pressure between groups. Furthermore pulse wave velocity, a technique closely related to blood pressure, was not different between groups.

The third objective (chapter 6) was to identify if cerebral structural differences were present between women with or without a history of preeclampsia. Cerebral MRI using both T1 and T2 weighted sequences assessed anatomy and structure, to characterise lower grey matter volumes, increased temporal lobe white matter lesion volumes and increased white matter diffusivity. These findings are the most comprehensive description to date, of both grey and white matter structural differences, in women with a history of preeclampsia. Furthermore differences seen in white matter structure were independent of age and blood pressure.

The fourth objective (chapter 7) was to assess if capillary density and markers of angiogenesis were different years after hypertensive pregnancy. No differences in angiogenic markers existed between groups, however women with a history of hypertensive pregnancy demonstrated capillary rarefaction, which was independent of

differences in blood pressure. Further analysis revealed it was significantly associated with left ventricular mass.

In the final results chapter (chapter 8), I described the results of testing my fifth hypothesis, that autonomic function, would be impaired after hypertensive pregnancy. Using ambulatory blood pressure and holter monitoring I showed that although nocturnal blood pressure was higher, there were no significant differences in heart rate variability.

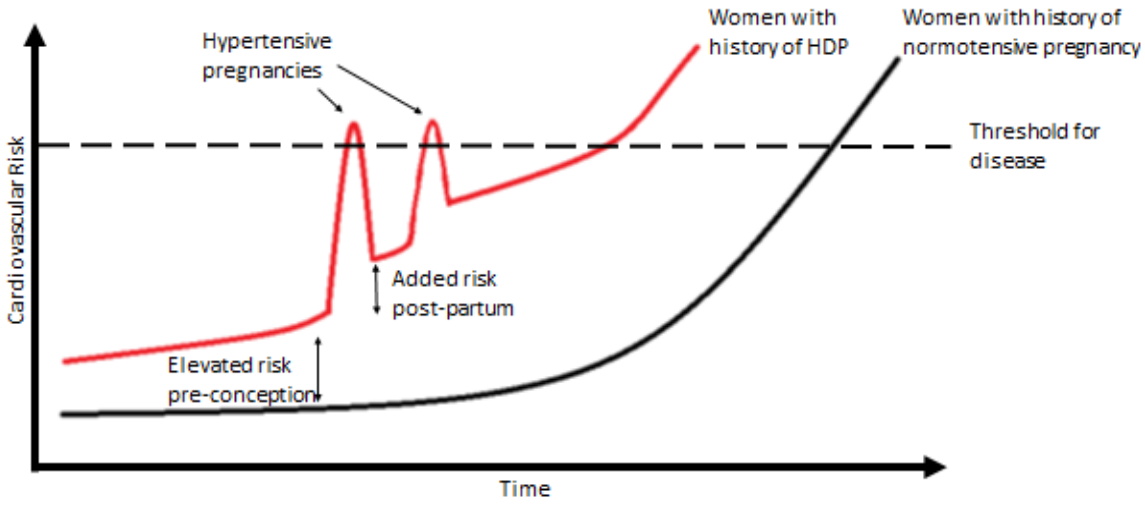
These results paint an encompassing picture of a distinct cardiovascular phenotype with increased cardiovascular risk in individuals several years after hypertensive pregnancy. It is not possible from this study to determine how long these differences have been present, however based on the significant overlap of risk factors for both hypertensive disorders of pregnancy and cardiovascular disease (90, 93) and the limited literature examining the contribution of pre-pregnancy cardiovascular risk factors to cardiovascular disease after hypertensive pregnancies (99) it is possible that a significant proportion existed before affected pregnancies. In support of this the results of this study demonstrate that many of the cardiovascular phenotypic differences found are significantly associated with risk factors for hypertensive disorders of pregnancy. However the results also suggest that there is an additive effect of the hypertensive pregnancy, notably in cardiac, cerebral and capillary structure. In this study capillary rarefaction was shown to be significantly associated with hypertensive pregnancy; given that hypertensive pregnancies are characterised by increased systemic vascular resistance and increased activation of coagulation factors (161), we can speculate that the affected pregnancy may be associated with a permanent decruitment of capillaries, possibly induced by vasoconstriction and coagulopathy. Capillary rarefaction is an established mechanism of hypertension and increased peripheral vascular resistance

which might contribute to increased aortic stiffness and cardiac afterload which over time promotes left ventricular remodelling. Capillary rarefaction is also a potential mechanism whereby increased cellular to capillary diffusion distance leads to reduced cerebral perfusion and accelerates age-related changes of grey matter atrophy and white matter architecture disruption.

We can only speculate on the trajectory of the phenotypic differences demonstrated in this study, though the finding of significant associations with the number of hypertensive pregnancies suggests a cumulative dose effect for cardiac, arterial and cerebral differences. There is limited literature on cerebral structural changes in the months and years after hypertensive pregnancy, however reports of cardiovascular differences in pregnancy suggest these are partially attenuated postpartum (140). These data in conjunction with the findings reported in this thesis support the theory that women with a history of hypertensive pregnancy experience accelerated vascular ageing, with additional hypertensive pregnancies leading to an increment in cardiovascular risk (see Figure 44 for illustration).

Throughout my testing of these hypotheses, I also performed exploratory analysis to assess if there were differences in subgroups of hypertensive pregnancy. It has been previously described that there is additional future cardiovascular risk associated with preeclampsia diagnosed at early gestation, or in conjunction with preterm delivery (79, 87, 88). Similarly it has been suggested that there are distinct pathophysiological differences between early and late onset preeclampsia (162). Previous reports of the cardiovascular associations of hypertensive pregnancies have variously combined preeclampsia with gestational hypertension (143) or reported them separately (79). This

Figure 44. Potential trajectory of cardiovascular risk according to pregnancy characteristics



study showed that classifying according to the gestation of preeclampsia diagnosis or gestational hypertension was not helpful in refining the spectrum of cardiovascular and cerebral changes and highlights that cardiovascular risk is increased across the range of hypertensive disorders of pregnancy. Future larger studies should explore which clinical features of affected pregnancy predict future cardiovascular and cerebral phenotypic differences.

As has been reported in the preceding chapters a wide range of measures were performed in the women recruited to this study. This was by design to allow a detailed understanding of the range of cardiovascular differences associated with a history of hypertensive pregnancy, rather than focus on an isolated organ or part of the vascular tree. However it should be acknowledged that in attempting to form an encompassing characterisation there is an increased risk of both type 1 and 2 errors due to multiple testing. Replication of the results presented in this thesis in other cohorts, such as the UK biobank, would give greater confidence in the findings."

9.1 Future questions

This work is important in identifying key pathophysiological mechanisms by which women with a history of hypertensive pregnancy might be at increased risk of cardiovascular disease in later life. However the findings also lead onto other important research questions which are yet to be answered. The four key questions in the author's opinion are:

1. What therapeutic strategies during hypertensive pregnancy or early postpartum can mitigate cardiovascular and cerebral phenotypic differences described in this thesis?

Currently treatment strategies for hypertensive disorders of pregnancy focus on reducing acute risk for both the mother and fetus. They consist of controlling blood pressure with medications known to be of negligible harm to the fetus, reducing risk of eclamptic fit and delivery of fetus when these treatment strategies are insufficient or if the fetus is close enough to term gestation for the risk of delivery and ex-utero exposure to be minimal.

Effective treatments already in use for other diseases with similar patterns of organ dysfunction, such as antihypertensive medication for left ventricular hypertrophy and aortic stiffness, may allow expedited translation of likely therapeutic strategies.

Currently the mainstays of antihypertensive treatment during pregnancy are beta-blockers (such as labetalol) and calcium channel blockers (such as nifedipine). After delivery if pharmacological treatment is still required ACE-inhibitors are sometimes prescribed. It has been reported that calcium channel blockers and ACE-inhibitors increase capillary density (299). Preferentially choosing these agents during the acute illness, when vasculature might be most susceptible to modification, over alternatives

such as beta-blockers, not known to increase capillary density, might have long term benefits in vascular structure. In addition maximising ACE-inhibitor dose in the early post-partum period might be of particular value in individuals after hypertensive pregnancy. Early postpartum left ventricular hypertrophy regresses after both normotensive and hypertensive pregnancy and ACE-inhibitors, which have been shown to be beneficial in left ventricular hypertrophy and heart failure (349, 350), may be particularly effective at this time point.

2. Which cardiovascular and cerebral phenotypic characteristics most accurately predict future cardiovascular morbidity and mortality?

In this thesis I have measured cardiovascular structure and function in order to better understand mechanisms which might explain future cardiovascular disease. However I did not measure hard endpoints of cardiovascular morbidity or mortality. Having identified key differences in women with a history of hypertensive pregnancy several years after affected pregnancy the next important step would be to understand how these phenotypic differences associate with future cardiovascular disease. Some features, or combination of features, might be associated with certain cardiovascular diseases or outcomes. The vast majority of women recruited to this study gave permission to be contacted for future related studies. Validated questionnaires and access to medical records in several years' time would be cost-effective in linking detailed phenotypic data with clinical endpoints.

Effective treatments already in use for other diseases with similar patterns of organ dysfunction, such antihypertensive medication for left ventricular hypertrophy and aortic stiffness, may allow expedited translation of likely therapeutic strategies.

In addition much larger datasets are now becoming available. The UK biobank which consists of 500 000 individuals, recruited from 2006 to 2010, aged between 40 and 69 at the time of recruitment. Biobank subjects provided a wide range of data including pregnancy characteristics. Furthermore an imaging sub-study is underway to perform detailed cardiac and cerebral MRI, using similar sequences to this study, in 100 000 biobank participants. I have submitted a request for data to the UK biobank for the initial 5000 participants included in the imaging sub-study with details of pregnancy history and cardiac and cerebral MRI data. If the analysis of the larger and older population from the UK biobank confirm those demonstrated in this thesis then it will allow a high level of confidence that these findings are persistent long term. Furthermore long term follow-up of the UK biobank population over time will allow linkage of imaging biomarkers to clinical endpoints.

3. What pre-conception cardiovascular differences predispose women to develop hypertension during pregnancy?

and

4. What are the key markers in hypertensive pregnancy which predict cardiovascular risk in later life?

In order to better understand when phenotypic differences occur and therefore target or prevent them first occurring in women who experience a hypertensive pregnancy we need to better understand whether differences are already present before conception. It is possible that differences exist before pregnancy as many cardiovascular risk factors, such as hypertension and diabetes, are risk factors for developing hypertension during pregnancy. In addition, although many important factors have been identified in the

pathophysiology of hypertensive disorders of pregnancy the predictors of future maternal cardiovascular risk are still to be clearly elucidated. Many studies have recruited women after they have been diagnosed with hypertension during pregnancy, however very few studies have studied nulliparous women from pre-conception with the aim of characterising differences before conception. Understandably studies powered to detect differences between women who develop hypertension during pregnancy and those who don't would be enormously costly in both time and money. However there are no easy shortcuts in answering these important research questions.

In collaboration with other researchers from the University of Oxford, from the departments of Cardiovascular Medicine and the Nuffield Department of Obstetrics and Gynaecology, I am collaborating on a pilot study to address some of these questions. The Oxfordshire Women and Their Children's Health: A Feasibility Study (OxWATCH study, Ethics Ref: 12/SC/0492), led by Dr Ingrid Granne, aims to recruit up to 300 nulliparous women from Oxfordshire and carry out detailed cardiovascular phenotyping, which will include measurement of blood pressure, arterial stiffness, cardiac structure and function, carotid intima media thickness, metabolic characteristics, blood and urine biomarkers (351). Women who become pregnant after enrolment and wish to continue in the study will undergo 5 further assessments during pregnancy and a final visit at 3 months postpartum. The study has so far recruited 215 women of whom more than 50 have attended during pregnancy. It is hoped that if feasibility of this pilot study can be demonstrated then the data collected can guide planning of a larger multicentre study which would be powered to detect differences in cardiovascular measures in hypertensive disorders of pregnancy.

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Appendices

Participant information leaflet and invitation

Ambulatory blood pressure monitor instructions and diary

Participant questionnaire

Explanation of study population included in each chapter

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05 December 2016

Pregnancy Complications Vascular Study (08/H0604/127)

Pregnancy Complication

Dear ,

We are conducting a research study at the John Radcliffe Hospital looking at raised blood pressure in pregnancy and early delivery and would like to invite you to take part. We are contacting all patients who had pre-eclampsia, pregnancy induced hypertension or delivery before 37 weeks gestation, and I enclose an information sheet which explains the study. You are under no obligation to take part and this study will not affect your treatment at the hospital in any way, but the results may help us treat patients with heart disease in the future.

Many thanks for your consideration of this. If you would like to take part or have any questions, please do contact me at the above address/phone number/e-mail address. Or complete the reply slip below with your details and return in the enclosed SAE.

Yours sincerely,

Professor Chris Redman
Professor of Obstetric Medicine

Dr Brenda Kelly
Consultant Obstetrician

Prof Paul Leeson
Consultant Cardiologist

Re: **Pregnancy Complications Vascular Study (08/H0604/127)**
I would like to be considered for the above research study and would prefer to be contacted by phone / letter / e-mail / any method (delete as appropriate)

My contact details are:

Name: _____

Address: _____

_____

Telephone: _____ E-mail: _____

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PARTICIPANT INFORMATION SHEET

Study name: Pregnancy Complications Vascular Study (08/H0604/127)

Pregnancy Complication

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Thank you for reading this.

PART 1

What is the purpose of the study?

To find why having high blood pressure during pregnancy or delivering early during pregnancy can increase the risk of cardiovascular problems in later life.

Raised blood pressure during pregnancy (pre-eclampsia and pregnancy induced hypertension) affects 3-5% of pregnancies. Mothers who have elevated blood pressure during pregnancy are more likely to develop cardiovascular disease and risk factors, such as high blood pressure, in later life. Mothers who have raised blood pressure during pregnancy are also more likely to deliver early. However, mothers who deliver early whether or not they have raised blood pressure are also more likely to develop cardiovascular disease in later life.

Therefore we want to find out what is different about the cardiovascular system (heart and blood vessels) and brains of mothers who have raised blood pressure in pregnancy or who delivered at less than 37 weeks gestation so that we can understand why they are at increased risk. We hope to find new ways to reduce the risk of mothers developing cardiovascular disease in the future.

Why have I been invited?

You are being asked to take part because you were cared for by the Oxford Maternity Services around 5 to 10 years ago. Your records show you had high blood pressure during pregnancy and / or delivered before 37 weeks gestation. We want to find out what is different about the cardiovascular system (heart and blood vessels) and brains of mothers who have raised blood pressure in pregnancy or who delivered at less than 37 weeks gestation so that we can understand why they are at increased risk.

Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part, you are free to withdraw consent at any time without giving a reason. This would not affect the standard of care you receive. If you decide that you no longer wish to continue with the study, we would still retain any data already obtained from you unless you request otherwise.

What would happen to me if I take part?
You would attend 1 visit at the John Radcliffe Hospital. You would undergo scans of your heart, brain, blood vessels in your neck and limbs and have a blood test, the visit in total would last approximately 4 hours. Finally, we will ask whether you are happy for us to contact you again about future research studies.

1. **Urine Sample**
   We will ask you to provide a urine sample when attending the department. This will be stored and analysed for markers of blood vessel function.
2. **Height and Weight** (5 minutes)
   We shall measure your body size and weight using skinfold callipers, tape measures and scales.
3. ***MRI scan*** *(60-70 mins)*
   You will have an MRI scan of your heart, main artery (aorta) and brain. MRI scans are painless but involve the use of a strong magnetic field, so if you have any of the following, you would not be suitable for a scan, and would not be able to take part in this trial:

   - a permanent pacemaker
   - metal clips in blood vessels of the brain
   - an injury to the eye involving fragments of metal
   - shrapnel injuries
   - other metal or electronic implants affected by the magnetic field

   The MRI scanner is shaped like a polo mint, the hole inside measuring about 60 centimetres wide. You will be asked to lie still on your back while your heart and brain are scanned. You will be asked to breathe in and out and hold your breath for several seconds for some of the scans.
4. ***ECHO***. *(30 mins)*

We will perform an ultrasound (Echocardiogram or ECHO) of your heart. This is a safe and painless procedure that takes 30 minutes. You will be asked to lie on a couch on your left side. A probe is placed on your chest and lubricating jelly is used so the probe makes good contact with the skin. Ultrasound waves then create images of your heart on the scanner monitor. It normally takes 30 minutes to acquire these images.

5. **Ultrasound of your arm blood vessels** (30 mins):
   We will carry out a short ultrasound scan of the blood vessel in your arm. This involves you lying still for around 30 minutes while we take pictures of the artery in your arm using a scanner.
   a. *GTN spray (5 mins)*
   To assess how the artery works, we will blow up a blood pressure cuff on the arm for a few minutes and give you a spray of GTN under your tongue which helps to relax the artery in your arm (you may already use this medication if you suffer from angina).
6. **Ultrasound of neck blood vessels** (15 minutes)
   We will carry out a short ultrasound on an artery in the neck, to measure the thickness of the vessel wall.
7. **Pulse wave assessment** (20 minutes)
   We shall look at how well your blood vessels are working by placing a small probe against the pulse in your neck, arm and leg and separately another machine which tests the blood pressure in all 4 limbs
8. **Microcirculation assessment** (20 minutes)
   You will undergo scans of the small vessels in your skin, using a microscope.
9. **Blood test** (5 minutes)
   We will ask you to donate a sample of blood (up to 40 ml – 8 teaspoons) for storage and scientific analysis of substances and genes involved in blood vessel function and cardiovascular disease development. The detailed information about the blood sample is given in Part 2.
10. **Ambulatory blood pressure monitor** (5 minutes)
    You will be fitted with blood pressure cuff and a small recorder that measures blood pressure regularly over a 24 hour period at the end of the study. The unit is a small walkman-type box that can be clipped to the belt or waistband and is connected to the cuff by a rubber tube. You will notice the blood pressure cuff inflating every 30 minutes during the day and hourly at night. This provides a more robust way of assessing blood pressure changes throughout the day. The unit can be switched off under certain circumstances. Ambulatory blood pressure monitoring should not interrupt your daily activities although avoiding contact with water would be recommended. We will ask you to post this back to us in a pre-paid envelope.
11. **Heart rhythm monitor** (5 minutes)
    You will be fitted with a 24 hour heart rhythm monitor which involves 3 stickers on your torso connected to a recorder (similar to the ambulatory blood pressure monitor). It should not interrupt your daily activities although avoiding contact with water would be recommended. After 24 hours it can be removed and posted back in a pre-paid envelope.
12. **Questionnaire**
    We will ask you to complete a questionnaire about your lifestyle, diet and medical history relevant to cardiovascular disease.

13. If you have children, we will also ask whether you are happy to receive information about a related study that compares your cardiovascular system to that of your child. We are interested to understand whether pregnancy has any long term effects on children.
14. We will also ask your permission to contact you again in the future in case we plan further studies relevant to cardiovascular health.

Expenses

We will reimburse travel expenses to and from the hospital if you send us receipts. Light refreshments will be available.

What will I have to do?

1. Attend 1 visit to John Radcliffe Hospital
2. Consent to taking part in this study by signing a form
3. Avoid any caffeine (tea, coffee, chocolate, soft drinks etc) for 24 hours before your MRI scans and fast from midnight before the scan (ie eat normally the night before and avoid any breakfast on the morning of the scan)."
4. Undergo the procedures as described above.

What is being tested?

We are examining the differences in hearts, brain and blood vessels between women who had complications in pregnancy and those who did not.

Are there any side effects of participating?

One of the scans involves seeing how your arteries respond to different stimuli. One stimuli is a GTN spray under the tongue, commonly used to treat angina. GTN spray may make some people feel dizzy, light headed or flushed for a few minutes.

Are there any other possible risks from taking part?

Magnetic resonance imaging is very safe and there are no known significant side effects from the types of scanner that we use. The scan is noisy and we provide headphones to protect your ears. The scan also involves lying flat in a slightly confined space and a small number of people find this too claustrophobic. If you are pregnant or breast-feeding you should not take part.

Some people find having a blood test uncomfortable and there can be bruising at the site of needle entry. Our staff are highly trained and we will make sure you are as comfortable as possible.

What are the possible benefits?

We cannot promise the study will help you but the information we get from this study will help improve the treatment of people with raised blood pressure during pregnancy and those who give birth prematurely.

What happens when the research study stops?

You will officially end participation in the study. Copies of any publications connected to this study are available on request from Dr H Boardman, (contact details above).

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

What if relevant new information becomes available?
Sometimes we (the study investigators) get new information about the procedures being studied. If this happens, one of us will tell you and discuss whether you should continue in the study. If there is sufficient evidence to suggest you may be harmed from participating in this study, the study could be stopped.

Unexpected findings on your scan
In the unlikely event of us seeing any structural abnormalities on your MRI scan, a designated clinical specialist will discuss the implications with you and may arrange for further investigations as necessary. However, it is important to note that we do not carry out scans for diagnostic purposes, and therefore these scans are not a substitute for a clinical appointment. Rather, our scans are intended for research purposes only. So if we find anything unusual, it would be appropriate for us to contact your GP so that they can arrange ongoing clinical care for you. But we would only do this after we and the specialist had discussed your options and gained your permission.

What will happen if I don't want to carry on with the study?
You are free to withdraw from the study at any time. If you wish, we can then just make use of the information we already have about you. Alternatively, we can ensure if your samples and information are used for future research it is entirely anonymously, or we can destroy any identifiable samples or information we hold about you.

What if something goes wrong or I have a complaint?
If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Paul Leeson on 01865 572846, email paul.leeson@cardiov.ox.ac.uk or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office, email ctrg@admin.ox.ac.uk

Harm:
The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which you are provided.

Will my taking part in the study be kept confidential?
If you take part in the study and if you give permission, your GP would be informed. Some parts of your medical records and the data collected from the study would be looked at by authorised persons from the University of Oxford, to check that the study is being carried out correctly. They may also be looked at by representatives of the regulatory authority or authorised persons from the NHS Trust. All investigators have a duty of confidentiality to you as a research participant and nothing that could reveal your identity would be disclosed outside the research site. The data collected from the study will be recorded anonymously and you would not be identifiable from this.

Participation in future research (patients)
We will ask if we can contact you about future studies. This is optional ie you can take part in this study but decline to be contacted again. If you consent, we will keep your contact details separately from research data you have provided. Both your details and data will carry the same unique ID. This means your data is anonymised but that we can "link" details to data. In

this way we can approach patients about studies relevant to their particular healthcare status. You can withdraw your consent for future contact at any time.

Involvement of the general practitioner
If you give permission your general practitioner (GP) will be informed of your participation in the study. No extra visits to your GP will be required.

What will happen to any samples I give?
All samples will be retained securely for future analysis and will be stored anonymously at the University of Oxford under the custodianship of the Department of Cardiovascular Medicine. The samples will be stored and may be used in future research as our understanding of blood vessel function grows. Future research may include genetic research (see below). Samples will be destroyed by the research team when ethical favourable opinion lapses. Or, if you consent they may be used in other future studies which have been ethically approved.

Will any genetic tests be done?
It is possible that some samples will be used for genetic research. This research may be conducted by the study research team or collaborating research teams. The samples will be stored anonymously but a record of who donated the samples will be kept. This is so that we can relate any findings to your medical history. This is also useful if you decide to withdraw your consent for us to keep your data, as we could then destroy your samples. The genetic tests would involve looking at common variations in genes that affect how blood vessels work. We do not propose to test for inherited genetic diseases, or for conditions that will involve any other members of your family. It is unlikely that the results of these tests will have significant implications for you personally.

What will happen to the results of the research study?
We anticipate that the results will be published in a scientific journal for the benefit of the wider medical community. However, individual patients will not be identified in any publication and your personal and clinical details will remain strictly confidential. Any scientific publications arising from the study will be available on request to all participants. You would have no legal right to a share of any profits that may arise from the research.

Who is organising and funding the research?
The investigators are Paul Leeson, Harry Boardman and Yvonne Kenworthy. If you wish to know more about any aspect of the study, please contact on (01865) 572832

The research is funded by National Institute for Heath Research Oxford Biomedical Research Centre, Fukuda Denshi Ltd. and the British Heart Foundation.

Who has reviewed the study?
The Oxfordshire Research Ethics Committee A has reviewed and approved the study.

Where can I find independent information about taking part in research?
You can contact local branches of the NHS Patient Advisory Liaison Service (PALS). Here is their website: **http://www.pals.nhs.uk/** (Tel for John Radcliffe site: 01865 741166). You can also to http://www.invo.org.uk/ which gives information about public involvement in clinical trials

Further information and contact details

Dr Paul Leeson
Consultant Cardiologist

Cardiovascular Clinical Research Facility
Level 1 Oxford Heart Centre
John Radcliffe Hospital
Headley Way
Headington
Oxford
OX3 9DU

01865 572846

24 Hour Blood Pressure Monitoring

PLEASE NOTE

For your safety the blood pressure monitor should not be switched on whilst driving. During the 24 hours, you should not do any vigorous sport or use machinery. You are asked not to bath or shower.

The recorder is programmed to take your blood pressure automatically half hourly during the day and hourly at night (23:00 – 7:00).

When the machine is switched off only the current time is displayed.
To switch the machine on press and hold the black button marked AUTO ON/OFF. A small A will appear in the top left hand corner.
The machine will now automatically take your blood pressure.

To switch the machine off, press and hold the black button marked AUTO ON/OFF. The small A will then disappear.

Note: Do not at any time press the red button as this will erase the recorded data.

Please switch the machine off after you have completed the 24 hours, remove the cuff and place all items (BP monitor, cuff and the ***This*** sheet filled out) in the bag provided and post it back to us.

In the unlikely event of a malfunction please switch off and remove the cuff. If you have any questions please contact me on 01865 572832 or 07747031263

Make sure to have a normal day. Do not spend the day relaxing!

Patient Details and Diary

Study Number:_____

Date of recording:_____

Please list any medications you are on and/or took during the 24 hour monitoring period in the following table:

Name	Daily dose	Time Taken	For approximately how long have you been taking this tablet?

The following questions will be useful in the analysis of your recording:

1) **What time did you go to bed?_____ What time did you get up?_____**

2) Were you awake during the night?_____ For how long?_____

At what time (s)? _____

3) Were there any times where your heart rate or blood pressure may have been elevated or dropped? (i.e. running for a bus, having just walked up a flight of stairs, carrying something heavy, taking a nap) If so, when?

PVS-1

Subject's Study Number .....

Date of Birth .....

Study Date……..

Questions about the subject's medical history

1a Have you ever been told that you have high blood pressure? No Yes

1b Have you ever been treated for high blood pressure? No Yes Don't know

1c If yes, how were you treated (including the name of any medication) .....

1d Were you pregnant when you had high blood pressure? No Yes

2a Have you ever been told that you have diabetes? No Yes

2b Have you ever been treated for diabetes? No Yes Don't know

2c If yes, how were you treated (including the name of any medication) .....

2d Were you pregnant when you developed diabetes? No Yes

3a Have you ever been told that you have high cholesterol? No Yes

3b Have you ever been treated for high cholesterol? No Yes Don't know

3c If yes, how were you treated (including the name of any medication) .....

4a Have you been diagnosed with any long term illness or been treated for any? No Yes

4b If yes, what is the illness? .....

4c Have you been treated for this condition? No Yes Don't know

4d If yes, how were you treated (including the name of any medication) .....

5a Are you taking any other medicines, tablets or pills regularly? No Yes

5b If yes,what are they (**including oral contraceptive if normally taken**)

..... for how long.....

..... for how long.....

6 Were you born prematurely? No Yes

7 Did your mother have Pre-eclampsia during her pregnancy with you? No Yes

8 What was your weight at birth…….

Questions about pregnancy

1a How many pregnancies have you had?.....

About your **FIRST** pregnancy

2a Which year and month did you become pregnant?.....

2b Were there any problems during the pregnancy? No Yes

2b If yes, what where they and at what stage of your pregnancy did you have the problem?…….

2c Was your baby alright? No Yes

2d If yes, how many months into the pregnancy were they born?.....

2e If yes, what type of delivery did you have?.....

2f Did the baby need any special care after delivery? No Yes

2g, If yes, what care did they receive…….. ……..

2h Were you told you had pre-eclampsia during your pregnancy? No Yes

2i, If yes, when was it diagnosed and what treatment did you receive?……

About your **SECOND** pregnancy

2a Which year and month did you become pregnant?.....

2b Were there any problems during the pregnancy? No Yes

2b If yes, what where they and at what stage of your pregnancy did you have the problem?……..

2c Was your baby alright? No Yes.

2d If yes, how many months into pregnancy were they born?.....

2e If yes, what type of delivery did you have?.....

2f Did the baby need any special care after delivery? No Yes

2g, If yes, what care did they receive……. ……

2h Were you told you had pre-eclampsia during your pregnancy? No Yes

2i, If yes, when was it diagnosed and what treatment did you receive?……...

About your **THIRD** pregnancy

2a Which year and month did you become pregnant?.....

2b Were there any problems during the pregnancy? No Yes

2b If yes, what where they and at what stage of your pregnancy did you have the problem?……..

2c Was your baby alright? No Yes.

2d If yes, how many months into pregnancy were they born?.....

2e If yes, what type of delivery did you have?.....

2f Did the baby need any special care after delivery? No Yes

2g, If yes, what care did they receive……. ……

2h Were you told you had pre-eclampsia during your pregnancy? No Yes

2i, If yes, when was it diagnosed and what treatment did you receive?……
(If there are further pregnancies complete the information on separate sheets)

6 What was the first day of your last mentrual period?.....

7 Are you currently pregnant No Yes

Questions about the subject's family medical history

Have any members of your family ever suffered from (if more than 2 affected siblings continue on blank paper): Please tick all that apply

	mother	m age of onset	father	f age of onset	sibling1	s1 age of onset	sibling2	s2 age of onset
1 Angina	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
2 Heart attack	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
3 High blood pressure	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
4 Stroke	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
5 Diabetes	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
6 High blood cholesterol	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
7 Pre-eclampsia	☐	☐☐			☐	☐☐	☐	☐☐
8 Restricted growth at birth	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐

9a Is there an illness which runs in your family? No Yes Don't know

9b If yes, what is the illness .....

Questions about the subject's smoking, alcohol and diet

1 What is your normal weekly intake of

1a Beer, lager or cider.....pints

1b Red Wine ..... ……wineglasses White Wine ..... …….wineglasses

1c Sherry, Port and aperitifs.....glasses

1d Spirits.....pub measures

2a Do you **currently** smoke cigarettes, cigars or a pipe regularly? (e.g. at least 1 cigarette a day for the last 6 months) No Yes

2b **If yes**, how many cigarettes do you smoke each day at present?.....cigarettes
how many cigars do you smoke each day at present? .....cigars
how many ounces of tobacco do you smoke each day at present? .....ounces

2c **If no,** Have you in the **past** smoked cigarettes, cigars or a pipe regularly? No Yes
(e.g. at least 1 cigarette per day for a period of 6 months)

2d If yes, how long did you smoke for? .....months

2e If yes, how many cigarettes did you smoke each day? .....cigarettes
how many cigars did you smoke each day?.....cigars
how many ounces of tobacco in a pipe did you smoke each day?.....ounces

2f If you are a smoker or have ever smoked, how old were you when you started smoking?..... years.....

3 How often do you eat the following foods? (tick)

	Never	Less than once a week	One or two days a week	Most days	Once a day	More than once a day
Fresh fruit in the summer	.....	.....	.....	.....	.....	.....
Fresh fruit in the winter	.....	.....	.....	.....	.....	.....
Salads in summer	.....	.....	.....	.....	.....	.....
Salads in winter	.....	.....	.....	.....	.....	.....
Green vegetables	.....	.....	.....	.....	.....	.....
Fish (all kinds)	.....	.....	.....	.....	.....	.....
Poultry (chicken, turkey)	.....	.....	.....	.....	.....	.....
Red meat (beef, lamb, pork, ham, bacon)	.....	.....	.....	.....	.....	.....
Processed meat (burgers, sausages, pies, pasties, tinned meat, pate)	.....	.....	.....	.....	.....	.....
Cheese	.....	.....	.....	.....	.....	.....

Questions about the subject's exercise

1a Do you currently participate in any regular activity or program designed to improve or maintain your physical fitness? No Yes

1b For how many hours a week do you take part in these activities? …….

Questions to ensure a balanced sociodemographic group

1 Are you currently....

Married / in a stable relationship

single, divorced, widowed, separated

2 What is the highest educational qualification/training you have obtained?

None GCSE or equivalent A-levels or equivalent Degree or higher

3 Are you ...caring for the home or children full time?
...in full-time paid employment?
...in part-time paid employment?
...a student/apprentice

4 What is/was the name of your most recent job/occupation?

.....

What job did or do you actually do?

.....

5 Are you ...a manager working for an employer?
...a foreman or supervisor working for an employer?
...working for an employer/apprentice/student?
...self employed with employees?
...self employed without employees?

Explanation of study population included in each chapter

In the chapter 4 (cardiac results) 173 participants underwent cardiac imaging and were included in the results, made up of 70 women with a history of normotensive pregnancy and 103 with a history of hypertensive pregnancy. After CMR was added to the study protocol 173 women were recruited but 20 women (7 with a history of normotensive pregnancy and 13 with a history of hypertensive pregnancy) declined CMR scans, usually due to claustrophobia. Therefore a total 153 CMR scans were included in the analysis (63 in women with a history of normotensive pregnancy and 90 in women with a history of hypertensive pregnancy).

Similarly after echocardiography was added to the study protocol 173 women were recruited but 8 different women declined a scan (4 each with a history of hypertensive and normotensive pregnancy), 4 further scans which were performed were not analysable and not included in the analysis. Therefore a total 164 echo scans were included in the analysis (67 in women with a history of normotensive pregnancy and 97 in women with a history of hypertensive pregnancy).

Right ventricular function was not assessed in the original cohort and was difficult to assess in some women with poor echo windows. Since the additional cohort was recruited and right ventricular function included as a routine part of the echo assessment 128 women were recruited (55 with a history of normotensive pregnancy and 73 with a history of hypertensive pregnancy). Four women from each group declined an echo and in 14 women who had a history of hypertensive pregnancy the images were unanalysable. Therefore a total 106 echo scans of the right ventricle were included in the analysis (51 in women with a history of normotensive pregnancy and 55 in women with a history of hypertensive pregnancy).

In chapter 5 (vascular results) the entire cohort of 268 participants were included. It was intended that all would have assessment of vascular function using SphygmoCor however this was not possible in 14 individuals (8 with a history of normotensive pregnancy and 6 with a history of hypertensive pregnancy) due to the participant declining the test (11 participants) or technical problems with the equipment (3 participants). Therefore the results of vascular function assessed using SphygmoCor (including carotid-femoral PWV) from 237 individuals were included in the analysis (92 in women with a history of normotensive pregnancy and 145 in women with a history of hypertensive pregnancy).

After Vicorder assessment was added to the study protocol 165 participants were recruited, however 4 of these individuals declined the test (1 with a history of normotensive pregnancy and 3 in women with a history of hypertensive pregnancy) and 4 individuals had results which were unanalysable (all 4 with a history of hypertensive pregnancy). Therefore the results of vascular function assessed using Vicorder (including brachial-femoral PWV) from 157 individuals were included in the analysis

(68 in women with a history of normotensive pregnancy and 89 in women with a history of hypertensive pregnancy).

After CAVI was added to the study protocol 113 participants were recruited, however 1 individual (with a history of normotensive pregnancy) declined the test and the results were unanalysable in 4 individuals (all with a history of hypertensive pregnancy). Therefore a total 108 CAVI results were included in the analysis (54 in women with a history of normotensive pregnancy and 54 in women with a history of hypertensive pregnancy).

In chapter 6 (brain results) 46 women with a history of normotensive pregnancy and 32 women with a history of preeclampsia were included with brain imaging results. However this was not including 4 women with a history of normotensive pregnancy who had brain imaging suggestive of inflammatory disease and 9 women who declined brain imaging (5 with a history of normotensive pregnancy and 4 with a history of hypertensive pregnancy).

In chapter 7 (microvascular results) the entire cohort of 268 participants are included. It was intended that all would have assessment of angiogenic markers however this was not possible in 17 individuals (7 with a history of normotensive pregnancy and 10 with a history of hypertensive pregnancy) due to the participant declining the test. Therefore the results from 251 individuals were included in the analysis (97 in women with a history of normotensive pregnancy and 154 in women with a history of hypertensive pregnancy).

After microvascular assessment was added to the study protocol 197 individuals were recruited (81 with a history of normotensive pregnancy and 116 with a history of hypertensive pregnancy). However it was not possible to include the results from 17 individuals due to 4 individuals declining the test, 8 individuals having unanalysable results and equipment failure with 5 recruits. Therefore the results from 180 individuals were included in the analysis (73 in women with a history of normotensive pregnancy and 107 in women with a history of hypertensive pregnancy).

In the 8th chapter (autonomic function results) I include the results of ambulatory blood pressure monitoring. A total of 173 individuals were recruited after this test was added to the study protocol (70 with a history of normotensive pregnancy and 103 with a history of hypertensive pregnancy). However 11 individuals could not be included as they declined the test (5 with a history of normotensive pregnancy and 6 with a history of hypertensive pregnancy). Therefore the results from 162 individuals were included in the analysis (65 in women with a history of normotensive pregnancy and 97 in women with a history of hypertensive pregnancy).

Finally, ambulatory holter monitors were added to the study protocol after which 109 individuals were recruited. However 11 individuals declined this test (6 with a history of normotensive pregnancy and 5 with a history of hypertensive pregnancy) and therefore 98 individuals were included (49 in each group).