Temporal Trajectories of Disease Progression Following Hypertensive Disorders of Pregnancy

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ABSTRACT

Hypertension in pregnancy leaves a lasting imprint on women's health, increasing the risk of disease across multiple organ systems long after childbirth. Yet the trajectories linking hypertensive pregnancy to long-term disease risk are not fully understood. This thesis investigates whether women with hypertensive pregnancies show early organ remodelling which predisposes them to later disease, and whether these early structural alterations set accelerated trajectories towards long-term dysfunction.

Within the current literature, there is consistent evidence of functional changes across the heart, kidneys, brain and microvasculature before, during and after hypertension during pregnancy. Despite reports of apparent recovery in renal function postpartum, the use of magnetic resonance imaging in this thesis demonstrates that women with hypertensive pregnancies show subclinical structural renal impairments at six to twelve months postpartum, which may underlie their future renal disease susceptibility.

Analyses of the retinal microvasculature at the same timepoint revealed that women with hypertensive pregnancies show increased arteriolar and venular narrowing, compared to their normotensive counterparts; with the degree of narrowing being equivalent to values typically seen in women two decades older, indicating premature microvascular ageing. Additionally, increased microvascular narrowing was consistently observed at 15-25 years postpartum, suggesting a fixed microvascular phenotype over time.

Longitudinal cardiac assessments further highlighted the prevalence of early diastolic dysfunction and concentric remodelling in women with hypertensive pregnancies. These phenotypes remained consistent across two decades postpartum, highlighting pregnancy as a critical inflection point that sets long-term cardiovascular trajectories. Furthermore,

there was evidence of an accelerated increase in left ventricular mass in women with hypertensive pregnancies, underscoring the need for increased cardiovascular monitoring in this population.

Collectively, these findings suggest that hypertensive pregnancy disorders induce early, fixed phenotypes across multiple organs, providing a mechanistic substrate for the elevated lifetime risk of disease. As such, they highlight the need for early postpartum screening, targeted prevention, and pre-pregnancy risk stratification. Future research should prioritise integrating imaging biomarkers with clinical data to develop risk stratification models and to explore artificial intelligence-driven tools for automated, scalable organ phenotyping in maternal health.

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To my family -

past, present and future.

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LIST OF ABBREVIATIONS

BMI	Body Mass Index
BSA	Body Surface Area
CAREFOL-HT	Clinical Antenatal Randomised study to Characterise key roles of Tetrahydrofolate in Hypertensive Pregnancies
CCRF	Cardiovascular Clinical Research Facility
CI	Confidence Interval
CRAE	Central Retinal Arteriolar Equivalent
CRVE	Central Retinal Venular Equivalent
DBP	Diastolic Blood Pressure
E/A	Early to Late Ventricular Filling Velocities
EGFR	Estimated glomerular filtration rate
GH	Gestational Hypertension
HR	Hazard Ratio
HELPFUL	Hypertension Explored in Long-term Postpartum Follow-up in Later Life
HELLP	Haemolysis, Elevated Liver Enzymes and Low Platelets
HT	Hypertensive
IQR	Interquartile Range
LV	Left Ventricle
LVOT	Left Ventricular Outflow tract
MRI	Magnetic Resonance Imaging
NICE	National Institute for Health and Care Excellence
NHS	National Health Service

NT	Normotensive
OR	Odds Ratio
PECOS	Population, Exposure, Comparator, Outcome, and Study Design
PET	Preeclampsia
POP-HT	Physician Optimized Postpartum-Hypertension Treatment Trial
PP	Postpartum
PVS	Preeclampsia Vascular Study
RV	Right Ventricle
SBP	Systolic Blood Pressure
SD	Standard Deviation
SNAP-HT	The Self-Management of Postnatal Antihypertensive Treatment Trial
TAPSE	Tricuspid Annular Plane Systolic Excursion
UK	United Kingdom

1 INTRODUCTION

I. Prevalence, Risks and Classifications of Hypertensive Disorders of Pregnancy

An estimated 140 million births occur each year worldwide[1]. Of these, approximately 18 million are affected by hypertensive disorders of pregnancy[2]. These hypertensive disorders are known for their deleterious effects on both the mother and foetus during pregnancy, resulting in both short and long-term target organ damage and early mortality[3]. Hypertensive disorders are first diagnosed during pregnancy when a woman's systolic or diastolic blood pressure rises above 140 or 90 mmHg, respectively[4]. If blood pressure is already raised by 20 weeks of gestation, it is likely to be worsening of existing hypertension. If it occurs after 20 weeks of gestation, it is typically indicative of pregnancy-induced hypertension[5].

In some women, the increase in blood pressure after 20 weeks of gestation arises with minimal evidence of target organ damage, this is termed gestational hypertension[5]. These women then go on to develop preeclampsia. Preeclampsia complicates approximately 2-8% of pregnancies worldwide and has diverse clinical presentation[6]. The symptoms

include proteinuria, microalbuminuria, oedema, visual disturbances and nausea/vomiting during pregnancy[4]. Preeclampsia is commonly classified as early- or late-onset, depending on whether symptoms develop before or after 34 weeks of gestation, and as mild or severe based on blood pressure levels, clinical presentation, and the extent of proteinuria[7].

Early-onset preeclampsia is often associated with placental issues such as poor implantation or placental insufficiency, and it is generally increased in disease severity compared to late-onset preeclampsia[8]. Contrarily, late-onset preeclampsia typically has milder symptoms and is thought to derive mostly from maternal risk factors in combination with placental senescence[9]. In women with preeclampsia, 0.1 - 0.2% develop eclampsia in which they present with symptoms such as seizures, confusion, altered mental state and unconsciousness during pregnancy[4]. Another 0.1 - 0.6% of women incur Haemolysis, Elevated Liver Enzymes and Low Platelets (HELLP) syndrome, which can result in hepatic rupture, haemorrhaging, renal dysfunction and pulmonary oedema[4].

Hypertensive disorders of pregnancy have life-threatening risks for both the mother and foetus during birth, as well as prolonging the need for inpatient care in hospital before, during and after delivery[10]. Furthermore, the risk of neonatal intensive care unit admission is significantly higher for infants born to hypertensive pregnancies contrasted with those born to mothers without hypertension[11]. This is due to the increased risk of premature delivery, intrauterine growth restriction, stillbirth, sepsis, low birthweight, respiratory distress syndrome and severe congenital defects affecting infants born to hypertensive pregnancies[11-12].

Although the symptoms of hypertensive pregnancy disorders mostly resolve after delivery, affected women are at increased long-term risk of disease compared to mothers

without hypertension (*Table 1*). Most notably, women with histories of hypertensive pregnancy have nearly twice the risk of experiencing a subsequent cardiovascular event[13-14] and a two-fold greater risk of heart failure than women with normotensive pregnancies[14]. Hypertensive pregnancies are also associated with a higher incidence of chronic kidney disease[18-19] and end-stage renal disease[15-18,20] later in life. Specifically, the risk of end-stage renal disease is approximately 4.9 times higher in women with preeclampsia and 3.6 times higher in women with gestational hypertension compared with normotensive counterparts[19]. Additionally, women with hypertensive pregnancies are reported to have a two-fold increased risk of hypertensive retinopathy[21], as well as dysfunction in other organs systems such as the brain and liver too[22-23].

Table 1: Long-term increased disease risk in women with hypertensive pregnancies compared to women with normotensive pregnancies.

Disease	Increased risk following PET	Increased risk following GH	Source
Chronic hypertension	2-5 fold	2-4 fold	NICE (2019, updated 2023)[4]
Cardiovascular events	1.5-3 fold	1.5-3 fold	NICE (2019, updated 2023)[4]
Stroke	2-3 fold	2-3 fold	NICE (2019, updated 2023)[4]
Vascular dementia	2-3 fold	2-3 fold	Schliep et al., 2023[24]
Chronic kidney disease	1.9- fold	1.5 fold	Barrett et al., 2020[19]
End-stage renal disease	4.9-fold	3.6-fold	Barrett et al., 2019[20]
Hypertensive retinopathy	>2 fold	>2 fold	Lee et al., 2023[21]

*NICE = National Institute for Health and Care Excellence, PET = Preeclampsia, GH = Gestational Hypertension

As women with hypertensive pregnancies are at heightened risk of widespread multi-organ dysfunction, identifying which women are on accelerated trajectories to later disease is important for developing better interventions and treatments. Early lifestyle changes, medications, and closer monitoring may delay the onset of long-term disease, particularly in women who only experience symptoms when disease development is more advanced. Not all women with hypertensive pregnancies will develop chronic conditions and the rate and severity of progression will vary among individuals. By identifying those at highest risk of rapid progression, clinicians can stratify risk more accurately and tailor preventive care to individual needs, overall improving long-term health outcomes and reducing mortality rates[25].

Knowing which women are on accelerated trajectories towards disease would also allow NHS and private healthcare teams to allocate resources more efficiently. By targeting women at greatest risk with more frequent follow-ups, screenings and lifestyle interventions; healthcare systems would not only improve patient outcomes but reduce long-term healthcare costs. Understanding disease trajectories could also inform broader public health strategies and guide future research.

If accelerated disease progression is a common pattern, guidelines could be refined allowing follow-up, preventive care and education for women about their future disease risk. This in turn would allow women to make more informed decisions about their health such as lifestyle changes, medication adherence, and family planning. At present, no well-established prognostic tests exist for hypertensive disorders of pregnancy[26]. Developing methods to predict risk of maternal complications would be highly beneficial to guide management in care facilities.

II. Is Long-term Disease Risk After Hypertensive Disorders of Pregnancy a Result of Pre-existing Dysfunction or Due to Pregnancy Related Distress?

While some evidence has begun to illuminate the role of hypertensive pregnancy in long-term disease progression, it remains unclear whether these outcomes are primarily driven by pregnancy-related factors or pre-existing conditions. The research group that I have been working with propose three potential pathways through which hypertensive pregnancy may impact long-term health (*Figure 1*). Firstly, women may have pre-existing dysfunction such as cardiovascular, renal impairments or essential hypertension prior to pregnancy which accelerate their trajectory towards long-term disease progression (*Figure 1A*). For these women, it is crucial to focus on early intervention before pregnancy, as well as increasing awareness of the associated risks when planning for pregnancy.

Conversely, it is possible that these women have no pre-existing disease but, for reasons unknown, their bodies do not adapt properly to the demands of pregnancy (*Figure 1B*). In this scenario, the physiological stress of pregnancy triggers a distinct pattern of hypertensive disease, leading to the development of a unique hypertensive phenotype. The increased strain during pregnancy could initiate a cascade of events including elevated blood pressure, endothelial dysfunction, and oxidative stress, which damage organs and accelerate progression toward disease. For these women, interventions would need to be focused on managing hypertension during pregnancy and the early postpartum period.

Alternatively, it may be that these women are born with a unique hypertensive phenotype that predisposes them to later disease and is unmasked during the pregnancy (*Figure 1C)*.

It may be that these women have unknown underlying risk, and the body does not correctly adapt to pregnancy, resulting in high blood pressure and subsequent dysfunction. This dysfunction then causes further damage, amplifying the situation further and leading to a feedback loop that intensifies hypertension and the pre-existing dysfunction. Both pre-existing dysfunction and pregnancy therefore increase the speed of the trajectory to future disease. The type of disease that develops will depend on which organs are damaged before and during pregnancy and the individual circumstances of each woman. It may be that disease trajectories can be altered through early intervention and medication, but further research is needed to fully establish this.

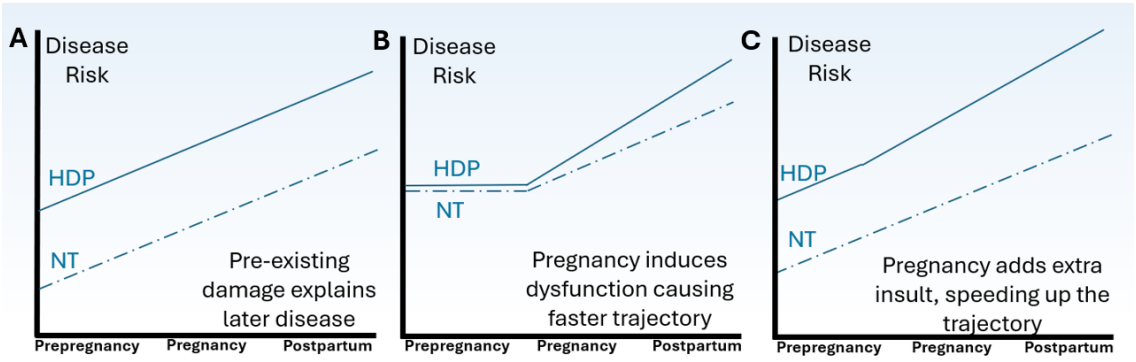


Figure 1. Hypotheses of potential disease trajectories. Three hypotheses of potential trajectories that women with hypertensive disorders of pregnancy may be on to later disease (bold line) compared to women with normotensive pregnancies (dashed line).

The evidence presented in the following sections evaluates which of these hypotheses is most likely. This is important as failing to address target organ damage early may accelerate the progression toward long-term disease. If maladaptation's are occurring during pregnancy and the early postpartum period, then interventions may be introduced to reverse target organ damage early and slow or even prevent this

accelerated trajectory. This will ultimately reduce mortality rates in women and offspring affected by hypertensive pregnancy.

III. The Complex Pathophysiology Behind Hypertensive Disorders of Pregnancy and The Current Confliction in Evidence

Certain risk factors are known to predispose women to hypertensive pregnancy disorders (*Table 2*). Nonetheless, some seemingly healthy women without prior risk develop hypertension during their pregnancy. It is unclear why these women develop hypertension during pregnancy, whilst others do not. Additionally, given the complex spectrum of disorders and varying levels of symptoms that hypertensive pregnancy disorders present, it can be difficult to disentangle the root cause of the complications. It is important to understand how potential mechanisms vary across different types of hypertensive pregnancy disorders so that individualised trajectories can be established. Currently, the etiopathogenesis behind hypertensive pregnancy is not completely elucidated. Many contributory mechanisms have been suggested but it appears that the process is complex and multifactorial.

Table 2. Clinical risk factors for hypertensive disorders of pregnancy.

High Risk Factors	Moderate Risk Factors
Previous hypertensive disorder of pregnancy	Nulliparity
Multifetal gestation	Obesity
Chronic hypertension	Family history of hypertensive disorder of pregnancy
Pregestational diabetes	African-American race
Renal disease	Low socioeconomic status
Autoimmune disease	Age ≥ 35 years or older

*Adapted from LeFevre ML, U.S. Preventative Services Task Force. Low-dose aspirin use for the prevention of morbidity and mortality from preeclampsia: U.S. Preventative Services Task Force recommendation statement. Ann Intern Med 2014;161(11):821.[27]

Firstly, women presenting with chronic hypertension display traits homogenous to those with essential hypertension. In these women, hypertension likely stems from a combination of genetic, environmental and lifestyle factors and it may be that these women simply have essential hypertension that happens to occur during pregnancy[28]. In some cases, there may be underlying conditions which cause secondary hypertension such as renal/cardiac disease, hormonal disorders or medications[28]. Alternatively, it may be that the physiological stress of pregnancy itself shifts the pattern of hypertensive disease development pushing women into a unique phenotype of hypertension. Although given the early onset of chronic hypertension, this is less likely to be the case.

In terms of pregnancy-induced hypertensive disorders such as gestational hypertension, preeclampsia, HELLP syndrome and eclampsia; the mechanisms behind them are likely interrelated but differ in severity and the specific factors involved. For instance, gestational hypertension does not have target organ involvement, whereas preeclampsia, eclampsia and HELLP syndrome do[4]. HELLP syndrome typically leads to a more exaggerated activation of the complement system and greater hepatic inflammation than preeclampsia[29]. Meanwhile, eclamptic patients develop seizures and other neurological symptoms that are not typically diagnosed in women with preeclampsia[30].

Nonetheless, both HELLP syndrome and eclampsia arise in women who are first diagnosed with preeclampsia, indicating that these disorders are advanced progressions of preeclampsia[4]. Cerebral imaging studies showing areas of vasospasm in eclamptic women supports this idea[31-32]. These studies suggest that eclamptic seizures result from increased blood pressure leading to cerebral perfusion pressure elevations and the cerebral circulation being in an 'overautoregulatory' state[30]. The formation of neurological symptoms and oedema in eclampsia may be due to rapid rises in blood

pressure overcoming the myogenic vasoconstriction of cerebral vessels, resulting in a loss of autoregulatory capacity and blood-brain barrier disruption[30]. However, this notion has been challenged as preceding hypertension and proteinuria are absent in many patients with HELLP syndrome and eclampsia, suggesting that not all hypertensive pregnancy disorders develop from the same inceptions[33-34].

With regards to the origins of hypertensive pregnancy, it is widely agreed that the placenta has a central role[35]. Placental ischemia and impaired placentation are thought to be pivotal in the development of most pregnancy-induced hypertensive disorders[35]. Impaired placentation derives from early defects in trophoblastic cells[36]. Normally, these cells invade uterine spiral arteries during pregnancy, remodelling them to increase placental blood flow[36]. However, in women with hypertensive pregnancy, inadequate remodelling leads to poor placental perfusion, resulting in placental hypoxia, oxidative stress and subsequently elevated blood pressure[37]. The failure of trophoblasts to adequately invade the uterine artery wall is thought to be partly due to immune system dysregulation and the immune rejection of trophoblastic cells[36].

Following inadequate remodelling, the distressed placenta secretes anti-angiogenic factors into the maternal circulation, causing widespread endothelial dysfunction and inflammation[38-42]. Concentrations of anti-angiogenic factors like the placental soluble fms-like tyrosine kinase one and endoglin increase[39-40]. Whilst angiogenic factors such as vascular endothelial growth factor and placental growth factor decrease[36]. The increase in anti-angiogenic factors and decrease in angiogenic factors disrupts the balance of pro-angiogenic and anti-angiogenic signals, leading to endothelial dysfunction. This results in increased vascular resistance and elevated blood pressure[35]. The increased concentrations of anti-angiogenic factors also impede the binding of vascular endothelial

growth factor and placental growth factor to their receptors, reducing nitric oxide production. This results in vasoconstriction and inadequate vascular remodelling, thus exacerbating hypertension further[42].

A recent systematic review and meta-analysis assessed the effects of vaginal progesterone on hypertensive pregnancy risk. After initiating vaginal progesterone treatment during the first trimester, there was a 29% overall reduction in hypertensive pregnancy rates and a 39% reduction in preeclampsia[43]. Progesterone is essential for uterine spiral artery remodelling and placental implantation as it regulates endothelial and smooth muscle function, immune responses and promotes vascular growth[44]. Given that increasing progesterone levels was associated with reductions in hypertensive pregnancy rates, these findings underscore the importance of the placenta in the development of hypertension during pregnancy and a key target for preventative interventions.

Despite abundant evidence supporting the role of the placenta in hypertensive pregnancy, its exact responsibility remains a subject of debate, owing to inconsistencies in the overall patterns of evidence. For instance, the placental theory fails to explain why some women develop hypertension during pregnancy despite having a normally functioning placenta, whilst others with placental abnormalities do not experience hypertension during pregnancy[45]. Additionally, if the placenta is the primary source of hypertension during pregnancy, all foetuses would be affected by foetal growth restriction, but this is not the case[26]. Instead, some researchers believe that placental damage is a downstream effect of hypertension[26,46].

IV. Is Cardiovascular Maladaptation the Cause of Placental Hypoperfusion rather than an Immune-mediated Response?

If failed trophoblastic invasion with incomplete spiral artery remodelling and uteroplacental hypoperfusion are a downstream effect of hypertension, they may not be immune-mediated, but a result of suboptimal cardiovascular adaptation to pregnancy[26,46-47]. Pre-existing cardiovascular disease is the strongest risk factor associated with developing hypertension during pregnancy, and both disorders share the same risk factors and underlying mechanisms[26]. Moreover, growing evidence suggests that cardiovascular maladaptation clinically precedes the onset of hypertension during pregnancy and that these changes persist postpartum[26,48], highlighting a key role of the cardiovascular system in hypertensive pregnancy disorders. Some researchers even suggest that the cardiovascular system is the sole initiator of hypertension during pregnancy. However, this is unlikely the case as decades of evidence endorses the notion that the placenta is a vital component[49].

Nevertheless, theories proposing that the cardiovascular system is partly responsible for placental dysfunction are plausible as cardiovascular adaptations during early pregnancy occur concomitantly with placental development to support the growing foetus[50]. As the placenta develops, the cardiovascular system increases blood volume, improves blood flow and reduces vascular resistance, allowing adequate blood flow to the placenta and foetus[51-52]. As such, abnormalities in maternal cardiovascular function such as high blood pressure or poor vascular remodelling may disrupt normal placental development and result in subsequent dysfunction.

Maternal blood pressure directly influences uterine perfusion pressure so if blood pressure is too high or too low, placental blood flow can be compromised[52]. Elevated

blood pressure increases the velocity of blood flow into the intervillous space of the placenta, damaging structures such as villous architecture[52-53]. This can result in villous collapse, rupture of anchoring villi, reduced diffusion time, and ischemia-reperfusion injury[52,54]. Meanwhile, inadequate perfusion can lead to placental insufficiency where a short supply of nutrients is delivered to the foetus. Both structural damage and placental insufficiency are thought to contribute to the development of preeclampsia and foetal growth restriction[55].

This notion is supported by a significant evidence base[48,56-61]. For example, a study in asymptomatic pregnant women at 21-23 weeks' gestation found that women with high uterine artery doppler resistance indices were significantly more likely to have functionally significant underlying cardiac defects[56]. High uterine artery doppler indices are linked to poor placental perfusion during pregnancy. Thus, this study suggests that suboptimal cardiac performance is linked with placental insufficiency.

Similarly, another study compared uterine doppler flow indices in 209 pregnant women with coronary heart disease to 70 healthy pregnant women at 20 and 32 weeks' gestation[57]. Cardiac dysfunction was associated with abnormal uteroplacental doppler flow and dysfunction prior to pregnancy. This supports the hypothesis that in some women, elevated uterine artery resistance during pregnancy may reflect pre-existing cardiac dysfunction and a reduced capacity to adapt to the demands of pregnancy.

Furthermore, Langen et al., (2024) reviewed the clinical records of 708 Norwegian women with cardiovascular disease, finding that hypertensive pregnancy incidence in women with congenital and acquired heart disease was over double that of the general Norwegian population[58]. Additionally, women at high cardiovascular risk had more than

three times the odds of developing hypertension during pregnancy to those classified as low risk[58]. These results align with previous studies reporting hypertensive pregnancy rates of 15.4% and 10.3%-11.8% in women with congenital heart disease[59-60]. As such, women with cardiovascular risk should be closely monitored for signs of placental dysfunction and hypertension during pregnancy.

Furthermore, a systematic review by Kalafat et al., (2018) evaluated the accuracy of ophthalmic artery doppler indices for predicting preeclampsia development[61]. Overall, a relationship was found between ophthalmic doppler indices and preeclampsia. Ophthalmic and uterine arteries are two completely unrelated vessels, thus given that doppler assessment of both ophthalmic and uterine arteries can be used to predict preeclampsia, it is unlikely that preeclampsia is directly a result of the placenta, but instead a general haemodynamic adaptation to pregnancy.

More recently, a review by Sonaglioni et al., (2024) reported the findings of eight studies that assessed left atrial mechanics in 566 women during hypertensive pregnancy and 420 women during normotensive pregnancy[48]. They demonstrated that women with hypertensive pregnancies showed mild left ventricular remodelling characterised by increased left ventricular mass index and mild increases in left ventricular filling pressures, expressed by an increase in the average E/e' ratio[48]. This was despite normal systolic function. The women also exhibited mild impairment in left atrial reservoir function and compliance, even with them having normal left atrial size[48]. These findings support the idea that women with hypertensive pregnancies may experience subclinical myocardial dysfunction during pregnancy. However, the findings do not prove whether cardiac impairment worsened during pregnancy or whether it was a result of pre-existing disease.

A more recent population-based prospective study by Kvalvik et al., (2023) combined several Norwegian registries to investigate how the number of hypertensive pregnancies that a woman has affects their cardiovascular disease mortality risk[62]. The risk of cardiovascular disease mortality rose with the increasing number of births complicated with either preeclampsia, placental abruption, low birth weight, stillbirth, or preterm birth[62]. These results illustrate that women with multiple instances of preeclampsia in the first and second pregnancies have more adverse cardiovascular outcomes to women with only one occurrence. Thus, it may be that each pregnancy adds an acute cardiovascular deterioration to an already impaired vascular insult, corroborating the idea that the physiological stress of pregnancy intensifies hypertension further, pushing women into a unique phenotype of hypertension.

Nonetheless, Kvalvik et al., (2023) did not examine the sequence of affected pregnancies or consider the various hypertensive pregnancy subtypes[62]. To address this gap, Wyatt et al., (2024) conducted a study exploring how different patterns of hypertensive pregnancy across the life course impact women's future risk of cardiovascular disease mortality before the age of 70[64]. They used data from multiple Norwegian birth and death registers to identify 987,378 mothers who gave birth between 1967 and 2020[64].

Wyatt et al., (2024) reported that the long-term risk of cardiovascular mortality among mothers who experienced high blood pressure only during their first, full-term pregnancy and not in subsequent pregnancies, was not higher than women who never had hypertension during pregnancy (HR 1.12, CI 0.95-1.32)[64]. However, for all other women who had hypertension during pregnancy, their risk of cardiovascular disease mortality was 1.5 to four times higher than women who had never had hypertension during pregnancy. This indicates that the first birth may sometimes only have transient

effects on the cardiovascular system with less long-term impacts. Thus, merging all cases of hypertensive pregnancy into one condition may incorrectly predict risk for some mothers.

Whilst both the cardiovascular system and placenta play key roles in the pathogenesis of hypertension during pregnancy, their involvement is not uniform across all women and all types of hypertensive pregnancy. In some cases, impaired placental function or abnormal cardiovascular responses to pregnancy may be central to the development of hypertensive conditions. However, in other instances, these mechanisms may not be as pronounced, and other factors may be involved. The interaction between maternal physiology and the specific characteristics of each pregnancy can lead to different disease trajectories and outcomes. As such, it is important to develop personalised preventative strategies for women based on their own individual timelines.

V. Can the Cause and Effect Relationship between the Kidneys and Hypertension during Pregnancy be Deciphered?

Another key organ implicated in the pathogenesis of hypertensive pregnancy disorders is the kidney. Physiological alterations in the kidney occur during normal pregnancy to support blood flow to the foetus. These include changes in kidney size as well as increases in renal plasma flow and glomerular filtration rate[63]. When renal adaptations to pregnancy do not occur correctly, dysfunction arises. In women with hypertensive pregnancy, there are increased levels of vasoconstriction and decreased levels of renal perfusion[42]. The increased force on vessel walls and hypoxic conditions can result in glomeruli damage, inflammation, endothelial dysfunction, renin-angiotensin-aldosterone

system overactivity and renal ischemia[65]. This leads to increased sodium and water retention, oedema, and heightened permeability of the glomerular filtration barrier; overall resulting in elevated biomarkers of renal dysfunction[65].

Although renal dysfunction is not present in all cases of hypertensive pregnancy disorders, the development of new-onset proteinuria is a key hallmark of preeclampsia[4], reflecting its significant role in the condition's pathophysiology. Women with preeclampsia also show additional signs of renal impairment, including elevated serum creatinine, increased blood urea nitrogen, microalbuminuria, reduced glomerular filtration rates, and oliguria[65]. These disruptions in renal function may mirror those seen in the cardiovascular system, compromising placental perfusion and triggering a cascade of endothelial dysfunction and vascular distress.

Proteinuria and microalbuminuria typically resolve within weeks to months after delivery of a pregnancy complicated by hypertension[66]. However, these women remain at increased future risk of developing kidney diseases such as albuminuria[67-68], chronic kidney disease[18-19] and end-stage renal disease[15-18,20] compared to women with normotensive pregnancies.

It is unclear why women with hypertensive pregnancies are at higher risk of kidney disease postpartum when functional symptoms seem to resolve[66]. It may be that there is underlying structural damage which causes later disease. However, no imaging studies to date have compared renal structure in women with hypertensive pregnancies postpartum to women with normotensive pregnancies to see whether there are any structural maladaptation's despite functional improvements. This is important for understanding the link to later disease.

Establishing the cause-and-effect relationship between renal alterations and hypertensive pregnancies remains difficult. Women may have undiagnosed kidney disease at the time of conception, leading to an increased risk of preeclampsia and renal disease in the future[69]. Instead, preeclampsia may directly damage the kidneys, increasing the future risk of disease[70]. It may even be a combination of factors in which preeclampsia further accelerates the loss of renal function in patients who already have renal dysfunction, speeding up their trajectory to later disease. As women do not routinely seek medical care prior to pregnancy and renal bloods are not a typical screening test in the general population or in women with uncomplicated pregnancies; it is difficult to know whether kidney disease or hypertension in women are present before their first prenatal visit[70].

Nonetheless, there is plentiful evidence supporting the hypothesis that women with hypertensive pregnancies have underlying renal disease before pregnancy[71-73]. Population-based studies show that women with chronic kidney disease and previous episodes of resolved acute kidney injury are at higher risk of developing preeclampsia[71-72]. A meta-analysis by Zhang et al., (2015) evaluated maternal complications of pregnancy in 23 studies with a total of 504,826 pregnancies[73]. Of these, 1514 pregnancies were complicated by chronic kidney disease and overall women with chronic kidney disease were at increased odds of developing preeclampsia compared to those without chronic kidney disease (OR 10.36, 95% CI 6.28-17.09)[73]. Additionally, no significant differences were observed in renal outcomes in pregnant and non-pregnant women with chronic kidney disease, suggesting that renal impairment associated with hypertensive pregnancy reflects pre-existing disease, rather than additional insult during pregnancy.

Not only are women with chronic kidney disease at higher risk of developing preeclampsia but women with other renal impairments are at higher risk too, for example women who donate or receive kidneys through transplantation[74-75]. Results from a Norwegian birth registry study by Reisaeter et al., (2009) demonstrated that women were more likely to have preeclampsia after kidney donation compared to before (5.7% to 2.6%, $p = 0.026$)[74]. This occurred even though none of the kidney donors with preeclampsia after delivery had a diagnosis of chronic hypertension[74], suggesting that preeclampsia is primarily influenced by renal dysfunction rather than generalised hemodynamic disturbances.

Similarly, a study conducted in Canada assessed pregnancy outcomes in 85 kidney donors and 510 healthy non-donors, finding that women who had donated a kidney were at significantly higher risk of preeclampsia compared to matched controls (11% vs. 5%, OR 2.7, 95% CI 1.2-5.0)[75]. Comparable findings have been reported among women with a single kidney due to unilateral renal agenesis (OR, 2.41; 95% CI, 1.23-4.72)[76]. Thus, at least some of the observed risk of future kidney disease in women with hypertensive pregnancies likely reflects pre-pregnancy dysfunction.

More recently, Li et al., (2025) conducted a study of 103 pregnant women with preeclampsia superimposed on chronic kidney disease and 103 matched chronic kidney disease patients without preeclampsia, following them for at least one year postpartum[77]. This was the first study to investigate whether superimposed preeclampsia exacerbates functional renal decline in chronic kidney disease patients. The researchers found that preeclampsia accelerates renal functional decline, particularly in patients with chronic kidney disease stages three and four, who already have higher levels of compromised renal function[77]. These findings suggest that women with poorer baseline renal function

may be more susceptible to the detrimental effects of hypertension during pregnancy. The study also revealed that the rate of renal decline was significantly faster in patients with early-onset preeclampsia compared to those with late-onset preeclampsia[77], highlighting distinct trajectories of renal dysfunction among different subtypes of hypertensive pregnancy disorders.

While causality cannot be definitively established, at least some of the observed risk of future kidney disease in women with hypertensive pregnancies likely reflects pre-existing renal dysfunction. Hypertension during pregnancy may then exacerbate underlying renal risk. Understanding the extent of this interaction is essential for identifying women at highest risk of developing kidney disease later in life and for designing effective preventive strategies. To achieve this, further research is needed to pinpoint the critical time windows during which structural and functional renal changes occur. This would enable targeted interventions before irreversible impairments develop.

VI. Insights from Blood Pressure Trajectories Regarding Long-term Disease Progression Post Hypertensive Disorders of Pregnancy

Blood pressure trajectories provide important information about the burden of vascular damage and future disease risk for women with hypertensive pregnancies. By analysing blood pressure variations over time, distinct patterns can be identified which tell us more about the onset, risk factors and pathophysiological mechanisms involved, potentially guiding preventative strategies. Early monitoring of blood pressure may allow abnormal trajectories to be detected which indicate increased risk for mothers and their offspring.

This will ultimately enhance our ability to predict, manage, and mitigate hypertensive pregnancies, contributing to better maternal and neonatal outcomes.

In healthy pregnancy, blood pressure typically follows a predictable pattern[78]. It generally decreases in the first trimester due to hormonal changes and generalised vasodilation. It then stabilises or slightly increases in the second and third trimesters as the body adapts to pregnancy. Then blood pressure returns to pre-pregnancy levels within a few weeks postpartum[78].

In hypertensive pregnancies, blood pressure trajectories are more variable and abnormal, usually rising in the first trimester, peaking in the second and third trimesters and remaining high postpartum. In the postpartum period, the blood pressure of women post hypertensive pregnancies may decrease slightly but it then remains higher than women with normotensive pregnancies, appearing to put these women on a faster trajectory to chronic hypertension in later life *(Figure 2).*

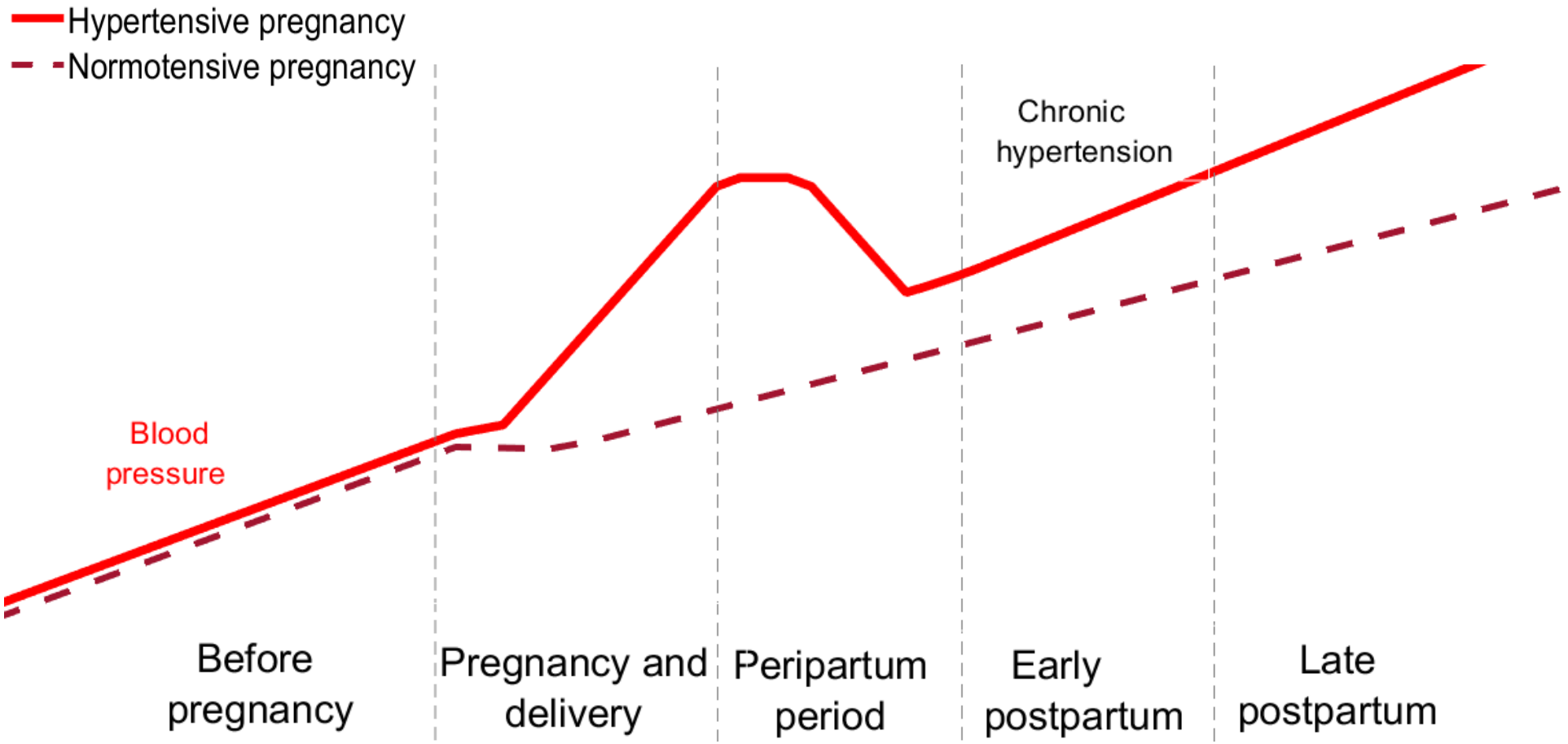


Figure 2: Established blood pressure trajectory based on the current literature (Cutler et al., 2023)[79].

Hauspurg et al., (2019)[80] characterised blood pressure trajectories during the early phases of pregnancies complicated by hypertension. Their study focused on women who developed hypertension during pregnancy but who had no history of pre-pregnancy hypertension. Trajectories were defined based on blood pressure changes between the first two trimesters, and first trimester blood pressure was categorised using the American College of Cardiology and the American Heart Association categories. First trimester blood pressure categories were significantly associated with preeclampsia and gestational hypertension, whilst increasing blood pressure trajectories were associated with higher risk for all hypertensive pregnancy disorders[80].

Importantly, both systolic and diastolic blood pressure trajectories were independently associated with hypertensive pregnancy risk, even after accounting for initial blood pressure category[80]. Women whose blood pressure was categorised as normal in the first trimester but showed an upward systolic trajectory had a 41% increased risk of developing hypertension during pregnancy compared to those with a downward systolic blood pressure trajectory[80]. These findings highlight the effectiveness of monitoring blood pressure trajectories to predict women who are at most risk of disease.

Similarly, a systematic review by Moes et al., (2024) examined the associations between three blood pressure determinants and adverse pregnancy outcomes[81]. Borderline hypertension and overt hypertension in the first trimester were significantly associated with an increased risk of developing preeclampsia. Additionally, elevated trajectories throughout pregnancy were linked to a higher likelihood of developing preeclampsia. These findings indicate that women with elevated blood pressure in their first trimester have a greater risk of adverse outcomes, highlighting an opportunity for early

management. Determining whether this risk is driven by cardiovascular, renal or other pathophysiological mechanisms could help guide targeted preventative interventions.

Furthermore, some researchers have used non-parametric approaches to simultaneously cluster longitudinal blood pressure trajectories among hypertensive pregnancy cases, yielding insights into the predictors and clinical correlates of preeclamptic subtypes[82]. These studies show that a few early pregnancy and demographic factors such as pre-pregnancy body mass index are significantly associated with blood pressure trajectory clusters[82]. These studies highlight the possibility of sub phenotypes within hypertensive pregnancy disorders that are associated with different patient characteristics and related to various pregnancy outcomes.

Genetic drivers of blood pressure patterns should also be considered as blood pressure is affected by polygenic inheritance[83-84]. Studies show that genetic predisposition to elevated systolic blood pressure, diastolic blood pressure, and body mass index are significantly associated with preeclampsia and gestational hypertension[83-84]. These results imply that these cardiometabolic risk pathways are causal in the development of hypertensive pregnancy.

Postpartum, women with pregnancies complicated by hypertension often present with unpredictable and rapid changes in blood pressure for up to six weeks after birth[85]. If this blood pressure remains high during the six-week postpartum period, women are more likely to have hypertension five to ten years later[86]. This elevated blood pressure is more frequent among women with abnormal cardiac remodelling, supporting the theory that cardiac maladaptation's and the inability to adapt to pregnancy are the primary cause of hypertension during pregnancy. Thus, even if functional symptoms recede after six weeks

postpartum, structural remodelling may still be present that acts as a substrate to later disease.

Moreover, Behrens et al., (2017) conducted a nationwide cohort study in 1,508,090 women in Denmark to determine how soon after delivery the risk of post pregnancy hypertension increases in women with hypertensive pregnancy disorders and how this rise evolves over time. In the year after delivery, women with hypertensive pregnancy disorders had 12-fold higher rates of hypertension compared to women with normotensive pregnancies[87]. Rates were also three to ten-fold higher at one to ten years postpartum and they remained twice as high more than 20 years later.

Similarly, Benshop et al., (2018) examined 200 women with a prior history of severe preeclampsia[88]. They used ambulatory blood pressure monitoring at one year postpartum and detected masked hypertension in 17.5% of the women, white coat hypertension in 9.5%, and dipping pattern hypertension in 45.5%. These findings indicate that women with prior severe preeclampsia remain at increased risk of later hypertension even in the early postpartum period.

Some researchers have also investigated blood pressure patterns longer term postpartum in women post pregnancies complicated by hypertension[89]. Bokslag et al., (2017) investigated hypertension incidence in women with histories of early-onset preeclampsia, finding that 38.2% of women develop hypertension between 9-16 years postpartum[89].

Together, these studies highlight the increased risk of chronic hypertension in women with hypertensive pregnancy disorders. Thus emphasising the importance of intervening early to prevent later disease. However, these studies do not show whether blood

pressure trajectories are altered by significant pregnancy events or whether pregnancy simply unmasks pre-existing vascular susceptibility.

VII. The Impact of Interventions During the Early Postpartum Period on Long-term Disease Risk

As the haemodynamic demands of pregnancy diminish in the puerperium and blood pressure returns to pre-pregnancy levels, less stress is placed on the renal and cardiovascular systems. This period may allow for the reversal of maladaptive remodelling that occurred during pregnancy[85], particularly with the aid of interventions that reduce afterload and promote normalisation of ventricular geometry and endothelial function.

Supporting this concept, Ghossein-Doha et al., (2017) examined the association between preeclampsia and heart failure[90]. They found that although hypertension is a recognised risk factor for heart failure, it was not significantly associated with heart failure in their cohort, whereas pre-hypertension was. Many of the women in the hypertensive cohort were already receiving antihypertensive treatment such as angiotensin-converting enzyme inhibitors and beta-blockers, which are known to have an inhibitory effect on adverse cardiac remodelling[91]. Thus, in women with a history of preeclampsia, early treatment of prehypertension with blood pressure lowering medication may have prevented the progressive increase in left ventricular mass index and relative wall thickness, thereby reducing the risk of heart failure[90]. However, whether secondary prevention strategies translate into lower rates of heart failure remains to be determined.

As such, researchers have been developing interventions to attempt to reverse concentric remodelling that may have occurred during pregnancy[92-97]. Some of these interventions have focused on tightly controlling blood pressure in the first six weeks postpartum to counteract remodelling as this is when rapid, uncontrollable blood pressure changes can occur[92]. The first effective trial to be run was the Self-Management of Postnatal Antihypertensive Treatment Trial (SNAP-HT). This randomised control pilot trial evaluated whether the accelerated trajectory to chronic hypertension following hypertensive pregnancies could be prevented by intervening in the early postpartum period[92].

The SNAP-HT trial compared tighter control of blood pressure to usual NHS care shortly after birth in 91 women with either gestational hypertension or preeclampsia, who also required antihypertensive treatment at the time of discharge[92]. Of the women, 45 were randomised to self-management and 46 to usual care. In the self-management group, women underwent daily home blood pressure monitoring and automated medication reduction via telemonitoring. At six weeks postpartum, blood pressure was significantly lower in the intervention group compared to the control group[92], with average systolic and diastolic blood pressure measurements being −5.2 and −5.8 mm Hg lower in the intervention group compared to the control group, respectively.

Eighty-two women were subsequently followed up at six months postpartum[92]. At six months, this difference in diastolic blood pressure decreased but remained significantly lower, with a −4.5 mmHg difference even after cessation of antihypertensive medication[92]. The difference may partly be due to the lower sample size at six months. Nonetheless, these results indicate that blood pressure self-management after pregnancy results in better blood pressure control than usual care.

Following the initial intervention, Kitt et al., (2021) conducted a follow-up of the same women at approximately 3.6 years postpartum[93]. The researchers retained 61 participants in their follow-up and found that ambulatory diastolic blood pressure was significantly lower by -7.0 mmHg in the intervention group compared to the control group[93]. This difference persisted even after adjusting for blood pressure at delivery and pre-pregnancy blood pressure, with the difference even slightly increasing between the two groups[94].

These findings suggest that short-term postpartum interventions can lead to sustained long-term improvements in blood pressure. However, the limited sample size used in the study constrains the strength and generalisability of the findings[93]. Consequently, the Physician Optimized Postpartum-Hypertension Treatment Trial (POP-HT), was designed to identify whether physician guided self-management of antihypertensive medication could result in consistently lower blood pressure in a larger population[94-97]. The POP-HT study compared tightly controlled blood pressure during the early postpartum period to usual care in 220 women with prior hypertensive pregnancies.

Consistent with the previous findings, they found significantly lower ambulatory blood pressure at six to twelve months postpartum in the intervention group compared to the control group[94]. The difference in diastolic blood pressure was -5.8 mmHg and the difference in systolic blood pressure was -6.5 mmHg. This difference was found despite most participants no longer taking antihypertensive treatment[94], highlighting the effectiveness of the intervention even in larger sample sizes.

The POP-HT study later examined cardiovascular indices in the same cohort of women, finding more favourable remodelling of the left and right ventricles and left atrium in the intervention group compared to the usual care group[95]. Specifically, relative wall

thickness was 0.06 cm lower in the intervention group compared to the control group. Left ventricular mass was -6.37 g/m^2 lower and end-diastolic and end-systolic volumes were -3.87 mL/m^2 and -3.25 mL/m^2 lower, respectively. Left atrial volumes were also reduced by -4.33 mL/m^2 between baseline and follow-up in the intervention group[95]. Diastolic function demonstrated a mean difference in average E/E' of 0.52 between baseline and follow-up in the intervention group and left and right ejection fraction were higher by 2.6% and 2.8%, respectively[95].

Similarly in another analysis, the POP-HT study reported no baseline differences in aortic blood pressure or pulse wave velocity between the intervention and control groups. However, by six to twelve months postpartum, women in the intervention arm had significantly lower aortic diastolic blood pressure (-5.2 mm Hg, 95% CI, -8.0 to -2.2; *P*<0.001), and pulse wave velocity (-0.71 m/s, 95% CI, -1.42 to -0.006; *P*=0.048) compared with those receiving usual care. This was accompanied by greater aortic distensibility in the intervention group in the intervention group (0.78×10^{-3} mm Hg^{-1}, 95% CI, -0.01 to 1.55; *P*=0.046)[97]. Collectively, these findings suggest that maladaptive cardiovascular remodelling may be at least partially reversible through early postpartum interventions and reduce future cardiovascular disease risk.

Nonetheless, these interventional studies have focused exclusively on cardiovascular outcomes, leaving uncertainty about whether other organ systems follow similar trajectories or whether maladaptive changes in those systems can also be reversed. It would be valuable to determine if comparable patterns occur in the kidneys, brain, and microvasculature. Furthermore, the absence of a normotensive control group limits the interpretation of these findings, as the true magnitude of blood pressure differences and the extent of the beneficial effects of tighter control of antihypertensive medication

remains undetermined. The follow-up period in these studies was also limited to a maximum of 12 months postpartum, making it difficult to assess the persistence of any improvements over the long term. At present, only a few studies have followed-up women longitudinally, so the optimal timing for intervention to achieve sustained benefits is yet to be established.

VIII. Summary of Evidence and Future Directions

The evidence presented above supports the hypothesis that pre-existing cardiovascular disease and renal dysfunction precede the development of hypertension during pregnancy, while pregnancy related factors further excaberate hypertension and target organ damage, amplifying long-term disease risk. The early puerperium has emerged as a critical window during which adverse remodelling in target organs may be reversible and interventions could be most effective. Implementing targeted interventions during this period may help slow or even halt accelerated trajectories towards chronic disease (*Figure 3)*, thereby improving long-term outcomes for women. Accordingly, future research efforts should prioritise developing peripartum interventions aimed at promoting recovery from end-organ injury and improving long-term health.

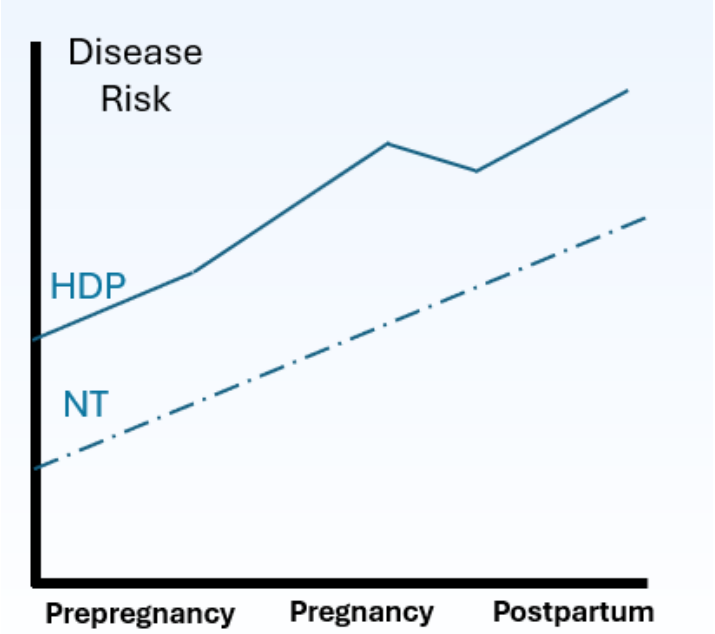


Figure 3. A schematic representation of disease trajectories showing how early interventions may alter the accelerated progression to later disease in women with hypertensive pregnancy disorders (HDP) (bold line) compared to women with normotensive pregnancies (NT) (dashed line).

To optimise the timing and effectiveness of interventions, a greater understanding of the organ-specific pathophysiological changes that occur at distinct stages after hypertensive pregnancy is important. Therefore, the overarching goal of this work is to characterise the temporal patterns of structural and functional alterations in target organs following hypertensive pregnancies with an emphasis on elucidating the mechanistic links between pregnancy-related hypertension and long-term disease. Identifying key timepoints at which maladaptive changes emerge, or progress will facilitate earlier recognition of women at greatest risk and facilitate targeted intervention before the onset of hypertensive disorders or the development of end-organ disease. Additionally, determining which women are on accelerated disease trajectories will support the development of personalised monitoring and management strategies, ultimately improving long-term outcomes and reducing the burden on global healthcare systems.

IX. Thesis Aims and Hypotheses

This thesis is guided by two overarching hypotheses. First, I hypothesise that the higher long-term disease risk in women with hypertensive pregnancies can be demonstrated earlier in life through the presence of early structural changes across multiple organ systems during the puerperium and six to twelve months postpartum. This hypothesis will be addressed in chapters two, three and four. Second, I hypothesise that the early target organ changes observed shortly after pregnancy will place women with hypertensive pregnancy disorders on a faster trajectory towards later disease. This hypothesis will be explored in chapters four and five.

As well as the two overarching hypotheses, this thesis is broken down into six chapters with four main sections. The first section will be addressed in chapter two which will involve a systematic literature review aimed at answering three key objectives. The first aim is to identify whether there is existing evidence of changes across the brain, vasculature, heart, and renal systems during hypertensive pregnancy, referred to as target organ damage. The second aim is to explore the persistence of these changes in the immediate, early, and later postpartum periods. Finally, the third aim is to assess whether these patterns of target organ damage correlate with blood pressure variations or whether they follow distinct trajectories.

The second part of this thesis will be outlined in chapter three. This will involve a cross-sectional analysis of previously unexamined imaging data from the POP-HT study and the recruitment of a new normotensive cohort from the CAREFOL-HT study (Clinical Antenatal Randomised study to Characterise key roles of Tetrahydrofolate in Hypertensive Pregnancies) at six to twelve months postpartum as a comparison group. This work will focus on comparing structural and functional renal differences in women

six to twelve months postpartum who have previously had a hypertensive or normotensive pregnancy.

The third part of this research will be detailed in chapter four. This chapter focuses on the analysis of retinal imaging data collected during the POP-HT and CAREFOL-HT studies at six to twelve months postpartum, as well as from an observational study called HELPFUL (Hypertension Explored in Long-term Postpartum Follow-up in Later Life) which was conducted at 15-25 years postpartum. This chapter aims to assess whether a fixed microvascular phenotype persists from early postpartum to later life in women with histories of hypertensive pregnancy, compared with those with normotensive pregnancy.

Finally, in chapter five, cross-sectional and longitudinal modelling techniques will be used to determine whether women who have experienced hypertensive pregnancy disorders show accelerated rates of cardiac decline compared to women with normotensive pregnancies. This will be completed by following up women from the Preeclampsia Vascular Study (PVS), a study conducted at five to ten years postpartum to the HELPFUL study, which was conducted at 15-25 years postpartum, in the same cohort of women.

SYSTEMATIC REVIEW 2

I. Overview and Aims

In this chapter, I present a systematic literature review which explores the temporal trajectories of target organ changes during and after hypertensive pregnancies. This chapter provides a more detailed and updated analysis of work that has been published in the European Journal of Preventative Cardiology. This article can be found at the following reference: *Cutler. H.R., et al., 2023. Temporal Patterns of Pre- and Post-Natal Target Organ Damage Associated With Hypertensive Pregnancy: A Systematic Review. European Journal of Preventative Cardiology, doi.org/10.1093*[79]*.* By systematically reviewing the literature, this chapter provides an evidence-based foundation for the subsequent chapters of this thesis, offering insights which inform the design and execution of research at later stages.

The primary objective of this chapter is to assess whether evidence supports the hypothesis that women with hypertensive pregnancy disorders exhibit structural changes across multiple organ systems during the puerperium and later postpartum. In the previous chapter of this thesis, I explored long-term disease risk for women with hypertensive pregnancies and whether this risk is driven by pregnancy related factors or

pre-existing conditions. However, it was not clear whether generalised haemodynamic adaptations, cardiovascular alterations, renal inadequacy or other underlying target organ modifications were the primary drivers. Examining multiorgan disease patterns during pregnancy and postpartum may help to establish which organs are most affected and which require priority for targeted interventions.

To test my hypothesis, I structured the literature review around three primary aims. First, I aimed to identify evidence of changes across the neural, renal, cardiac and vascular systems during a hypertensive pregnancy, which could be considered hypertensive target organ damage. Second, I aimed to understand to what extent these changes continue to be reported in the immediate, early and later postpartum periods. Third, I aimed to examine whether these patterns of target organ changes parallel patterns of blood pressure variation or follow distinct trajectories.

Through conducting a systematic literature review, I have ensured that the selection and analysis of literature is rigorous and transparent, minimising bias and maximising the reproducibility of the findings. Additionally, the methodology adopted for the review follows the Synthesis Without Meta-Analysis In Systematic Reviews guidelines[98] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[99] to ensure a robust and systematic approach to evidence gathering.

For this chapter, I have organised the review into several key sections. First, I outline the methodology adopted to select relevant studies and synthesise the findings. Then I discuss the characteristics of the studies and what evidence there is in the literature of target organ changes across multiple organ systems at various timepoints during pregnancy and postpartum. Finally, I discuss the implications of these findings, whether multiorgan patterns parallel blood pressure trajectories and how these findings have

shaped ideas for research that I conduct in the subsequent chapters of this thesis.

II. Methodology - Search Strategy and Selection Criteria

I performed a systematic search in Ovid/MEDLINE and EMBASE to provide a comprehensive coverage of the medical literature. I also conducted a manual search using Google Scholar, Cliniclatrials.gov and reference lists from retrieved reports and relevant reviews. The search was originally conducted to cover any articles published up until February 2023. I have since undertaken an additional manual search to include reports up until 1st October 2025. The search strategy and selection criteria were structured according to the PECOS (Population, Exposure, Comparator, Outcome, and Study Design) framework[100]. Each of these components helped to define the scope of the studies included and they ensured that the review addressed the research question comprehensively.

The population was defined as women during pregnancy or with a pregnancy history. Exposures included hypertensive pregnancy disorders such as superimposed hypertension, gestational hypertension, preeclampsia, pregnancy-induced hypertension, eclampsia and HELLP syndrome. For comparison, women with normotensive pregnancies were also included. The study outcomes comprised target organ changes and temporal patterns of blood pressure associated with hypertensive pregnancy. Target organ changes were defined as structural and functional alterations in organs based on clinical thresholds established in the literature.

Temporal blood pressure patterns referred to variations in blood pressure measurements during pregnancy and postpartum. These blood pressure patterns were then compared to target organ changes to determine whether they exhibited similar

trends. Morbidity and mortality risk were not classified as target organ changes as they were considered outcomes that result from underlying alterations. Additionally, as several reviews have previously highlighted the long-term impact of hypertensive pregnancy on disease risk, this topic was established enough to warrant inclusion in the current review [18, 23, 101-102].

Among the target organ changes, cardiac alterations were selected as the primary outcome as they are widely known to be affected by hypertensive pregnancies, and they may play a role in its aetiology. These included: cardiac remodelling, ventricular hypertrophy, atrioventricular morphology indices, cardiac dysfunction and fibrosis. Vascular, renal, neural, and haemodynamic alterations were considered secondary outcomes. Vascular changes included vasoconstriction, vasodilation, atherosclerosis, arteriosclerosis, angiogenesis, calcification and microvascular dysfunction. Renal changes encompassed functional biomarkers and structural alterations such as serum creatinine, cystatin C, proteinuria, microalbuminuria, estimated glomerular filtration rate, urinary albumin-to-creatinine ratio, corticomedullary differentiation and renal volume. Meanwhile, neural changes included white matter lesions, volume deterioration, vasogenic oedema, blood-brain barrier disruptions and cognitive impairments.

Any studies with a primary focus on maternal conditions that were not specifically related to hypertension were excluded. Additionally, if the study timeframe was not stated or if the women were not investigated during pregnancy or postpartum, the studies were omitted. Abstracts, oral communications, guides, guidelines, reviews, systematic reviews, meta-analyses, case reports, opinion papers, and posters of congress were also excluded. Whereas original research studies, human studies, trend studies, cohort studies, panel designs, and time-series designs were included.

To conduct the search, the following search terms were used: (((preeclampsia OR pre-eclampsia OR eclampsia) OR ((hypertension OR hypertensive) AND (pregnancy OR gestational))) OR 'Hypertension, Pregnancy-Induced') AND (((phenotype* OR imaging OR modelling OR remodelling OR 'machine learning' OR 'disease progression*' OR 'disease exacerbation*' OR 'life course*') OR (modelling OR remodelling)) OR ('Multimodal Imaging'[Mesh])). Filters were applied to include only studies written in the English language involving human participants and with full text availability. This search strategy ensured the maximum inclusion of relevant studies, and it was adapted to fit each database.

III. Study Selection Process

The original search was performed by myself using the search strategy stated above. This search strategy was developed with the help of a Bodleian librarian, and it was approved by my supervisors. I then imported all of the records that were identified into Rayyan software[103]. Using this software, duplicate studies were removed, and titles and abstracts of articles were screened by myself and one other reviewer to identify studies that fitted the PECOS criteria. If agreement was reached that the abstract of an article fulfilled the criteria, then the article was selected for full-text review.

Some abstracts with selection discrepancies were discussed and then a mutually agreed decision was made based on the discussions. If no consensus was reached, then a third reviewer made the final decision. During the full text review, the articles were read thoroughly by myself and the second reviewer. If the articles did not fit the PECOS criteria or were deemed irrelevant to the review they were excluded. This decision was approved by both reviewers.

IV. Data Extraction and Synthesis

After selecting the articles with full-text availability, data were extracted from the studies by myself and the second reviewer using a standardised form in Microsoft excel. I extracted information from half of the studies, whilst the second reviewer extracted data from the remaining half. Both reviewers then cross-checked each other's extracted data for accuracy and to identify any missing information. If disagreements arose regarding the extracted data, discussions were held until consensus was reached. If consensus was not achieved, a third reviewer made the final decision.

The extracted data included each study's: authors, publication year, country, study design, time period, inclusion criteria, number of women and pregnancies, outcomes per group, subgroup analysis, conclusions, strengths and limitations. Additionally, for each outcome, data were collected regarding measurement tools and units, effect sizes, and corresponding p-values. In cases where findings were repeated across multiple articles, only unique data were extracted, and these studies were treated as a single study. For studies involving mixed populations, such as women with gestational diabetes, only data related to hypertensive disorders of pregnancy were extracted. Moreover, for studies with multiple measures, only the primary outcomes most relevant to the review were included.

V. Quality Assessment and Risk of Bias

The data was quality checked for risk of bias using the Newcastle-Ottawa scale[104] by myself and the third reviewer. This scale was used as it can assess both cohort and case control studies which meant that it was useful with the variety of studies included in the review. The Newcastle-Ottawa scale evaluates risk of bias based on three domains and

assigns a maximum of nine points depending on whether a study fits the criteria of the domains[104]. Nine points is the highest score that can be given. This score indicates higher methodological quality and lower risk of bias that could distort the findings of the study.

The first domain evaluates how participants were selected for the study. This includes whether the sample used is representative of the general population as well as: the validity of the external control group selection, the ascertainment of the exposure, and whether the outcome of interest was present at the start of the study[104]. Four points is the maximum number that can be assigned to a study based on this domain. The second domain assigns a maximum of two points and evaluates the comparability of the groups and whether the studies controlled for important confounding variables that might distort the relationship between the exposures and the outcomes. Finally, the third domain assigns a maximum of three points and focuses on the outcome measurement and whether the results were reliably assessed[104].

Using the Newcastle-Ottawa scale, studies which scored between zero and three points were classified as being at high risk of bias and were excluded from the final analysis. Meanwhile, studies which scored four to six points, or seven to nine points were classified as moderate or low risk of bias, respectively. These studies were subsequently included in the final analysis. The third reviewer and I scored each article based on the criteria within each domain. We then compared our scores, and any disagreements were addressed by re-evaluating and discussing the article at fault until a consensus was reached. A qualitative sensitivity analysis was subsequently conducted by myself to assess whether the exclusion of high risk of bias articles influenced the findings of the review.

VI. Synthesis of Results

A qualitative synthesis was employed to organise the findings of the review. Due to the heterogeneity of the outcomes, a quantitative meta-analysis was not conducted, and no attempts were made to aggregate the data into a single outcome measure. Sources of heterogeneity were explored qualitatively by comparing study designs, exposures, disease outcomes, and statistical methodologies. Effect sizes and corresponding p-values as reported by the original authors of each study were tabulated. Risk of bias scores for each study were also documented alongside the effect sizes, outcomes and study timeframes. Finally, the evidence was synthesised using a vote-counting approach based on the direction of effect of target organ alterations. For each study, the direction of effect on outcomes was categorised as ‘favourable’, ‘non-favourable’, or ‘no difference’.

Non-favourable outcomes were assigned when hypertensive pregnancies were associated with worsening effects on target organs. Favourable outcomes were allocated when hypertensive pregnancies were linked to improved target organ outcomes and 'no difference' was ascribed if there were no effects of hypertensive pregnancy disorders on target organ outcomes. To be classified as either ‘non-favourable’ or ‘favourable’ in case-control studies, more than 70% of the outcomes in a study had to show a clear directional difference between the normotensive and hypertensive groups. For classification as 'no difference,' more than 70% of the outcomes had to demonstrate similar trends between the two groups. Non-favourable outcomes were reported even when studies demonstrated postpartum improvements, as these findings still indicated the presence of adverse organ involvement associated with hypertensive pregnancy. In cohort studies, the direction of effect was based on the presence or absence of significant negative target

organ alterations. The results were then presented visually using harvest plots.

VII. Results - Study Characteristics

Through database searching and manual searching, 7653 articles were obtained, these were then screened down to 203 articles which were read in full (*Figure 4*). Studies reporting on blood pressure patterns were included in the original published review but not for this chapter as they were already addressed in chapter one, so they were excluded.

This resulted in 78 studies that were accepted for the review as they reported target organ changes during pregnancy and postpartum. Two studies were then removed due to being deemed as high risk of bias leaving 76 studies classified as low to moderate risk of bias. The removal of these studies did not affect the conclusions of the review.

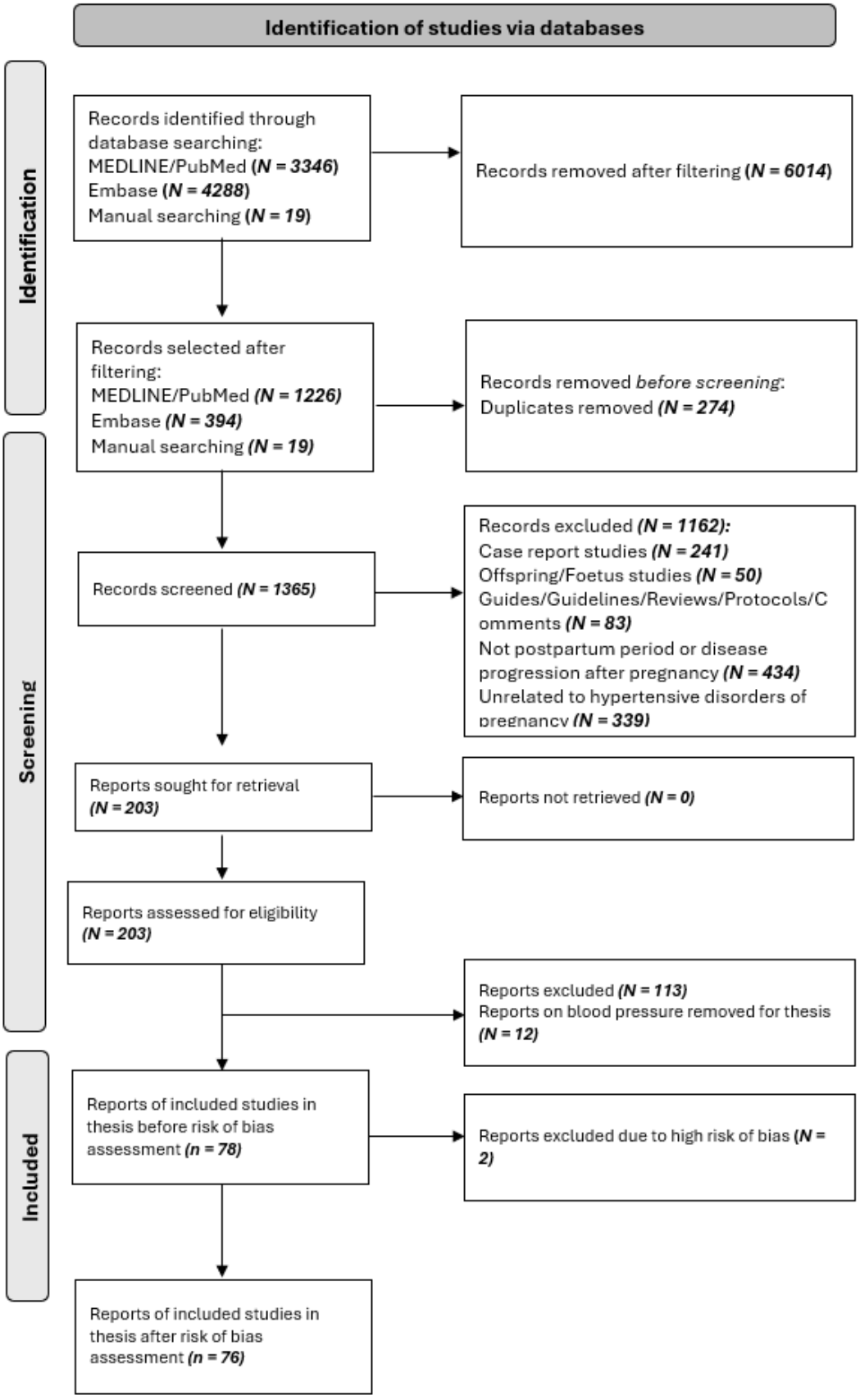


Figure 4. Flowchart of the study selection process. The flowchart is adapted from the original published review to fit the thesis chapter[79] and is based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[99].

Of the 76 articles included in this chapter, 30 articles (39.5%) reported cardiac outcomes[90,105-123,125-134], 20 (26.3%) reported vascular outcomes[135-154], 9 (11.8%) reported renal outcomes[155-165] and 17 (22.4%) reported cerebral outcomes[166-167,169-174,176-184], see *Figure 5*. Preeclampsia was the predominant hypertensive disorder examined across the studies. However, the studies also assessed women with eclampsia, gestational hypertension, pregnancy-induced hypertension and HELLP syndrome.

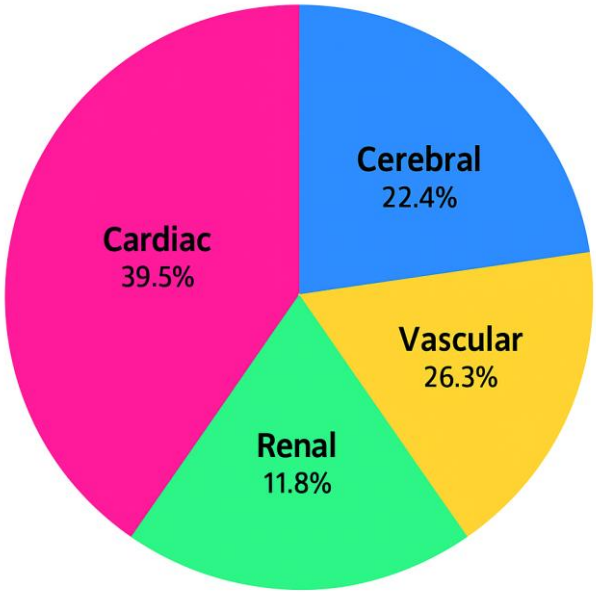


Figure 5. Distribution of reported outcomes among articles included in the review. This pie chart illustrates the proportion of outcome categories reported across the 76 studies included in this chapter. Cardiac outcomes are shown in pink, cerebral outcomes in blue, vascular in yellow, and renal in green.

Out of the articles examining target organ changes, 'non-favourable' outcomes in women with hypertensive pregnancy disorders were reported in 72 studies (94.7%) and 'no differences' were reported in four studies (5.26%) (*Figure 6*). No studies reported 'favourable outcomes in women with hypertensive pregnancies. In total, 24 (33.3%) of the studies reporting non-favourable outcomes were during pregnancy and 48 studies

(62.9%) reporting non-favourable outcomes were postpartum. Three studies (75%) reporting 'no differences' were conducted postpartum and one study (25%) reporting 'no differences' was during pregnancy.

In terms of cardiac variables, non-favourable outcomes were reported in 29 studies (96.67%) and 'no differences' were reported in one study (3.4%). Of the non-favourable cardiac outcomes 12 (41.4%) were reported during pregnancy and 17 (58.6%) were reported postpartum. Non-favourable vascular outcomes were reported in 18 studies (90%) and 'no differences' in two studies (10%). Of the non-favourable outcomes, 12 (63.2%) were reported during pregnancy and seven were reported postpartum (36.8%).

Non-favourable renal alterations were reported in eight studies (88.9%) and 'no differences' were reported in one study (11.1%). All the non-favourable outcomes were reported postpartum (100%). Non-favourable neurological alterations were reported in 16 studies (94.1%) and 'no differences' in one study (5.9%). Of the non-favourable outcomes, one study reported the outcomes during pregnancy (6.3%), and 15 studies (93.8%) reported the outcomes postpartum.

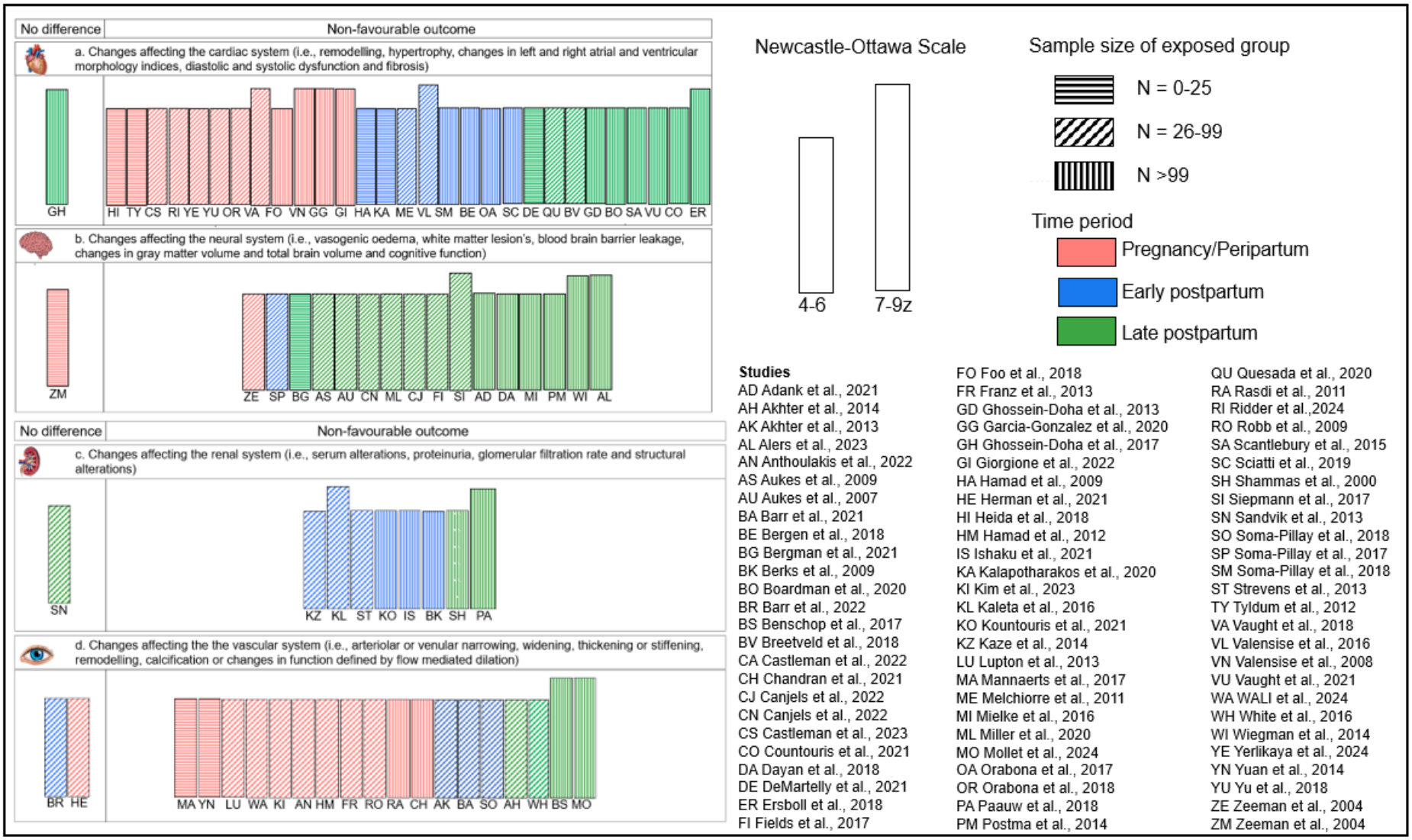


Figure 6. Harvest plot showing studies categorised based on a vote-counting approach. Direction of effect was defined for each outcome domain and type of effect size measure was labelled as 'non-favourable outcome', 'favourable outcome' or 'no difference'. Results

were separated by outcome domain and bars are colour coded representing the timeframe of each study. Red represented studies conducted during pregnancy and peripartum. Blue indicated studies conducted in the early postpartum period and green indicated any studies conducted in the late postpartum period. The patterns on the bars represent the sample size of the exposed group with *N* meaning the number of participants in the study. The height of the bars represents the risk of bias score. Only high and moderate risk of bias studies were included in the plot. Authors are represented by letters that match their names. The harvest plot has been updated from the original published review to include newly added studies.

VIII. Do Cardiovascular Maladaptations in Hypertensive Pregnancy Correlate with Blood Pressure Variations?

The relationship between blood pressure fluctuations during hypertensive pregnancy and target organ changes is becoming increasingly evident. In hypertensive pregnancies, blood pressure typically rises during the first trimester, peaks in the second and third trimesters, then slightly decreases postpartum. It subsequently remains higher than in women with previous normotensive pregnancies[80]. This sustained blood pressure elevation may accelerate the progression to chronic hypertension later in life. Cardiac indices are closely tied to blood pressure patterns as increased blood pressure raises afterload, which in turn impacts stroke volume and cardiac output[26]. When the heart cannot compensate effectively, maladaptive myocardial changes can occur, further exacerbating cardiovascular disease risk.

After reviewing the literature, I identified articles that examined cardiac indices across the first, second, and third trimesters of pregnancy, as well as postpartum and pre-pregnancy. Using this data, I have constructed a timeline of the changes that occur in women with hypertensive pregnancy disorders, and I have assessed the extent to which they correlate with blood pressure variations.

Few studies have comprehensively characterised the temporal progression of cardiac changes across the full course of a hypertensive pregnancy. However, Heida et al. (2018) filled this gap by evaluating cardiac alterations before, during and after pregnancy[105]. Haemodynamic and echocardiography parameters were assessed in 16 women with gestational hypertension and 25 women with normotensive pregnancies. Cardiac parameters including isovolumetric relaxation time, E/e' ratio and Tei Index were modestly impaired: before pregnancy, at four to eight weeks of gestation, at 32-36 weeks

of gestation and at six to ten weeks postpartum in the gestational hypertension group compared to the control group[105]. These findings suggest that diastolic dysfunction begins before the onset of hypertension, indicating a critical role of myocardial function in the pathogenesis of hypertension during pregnancy.

Another study by Foo et al., (2018) assessed cardiac indices in 530 healthy women intending to conceive[106]. Although no women were classified as hypertensive or diagnosed with cardiovascular disease prior to pregnancy, those who later developed preeclampsia showed lower preconception cardiac output and index, along with higher mean arterial pressure and total peripheral resistance[106]. These findings indicate that cardiac differences exist before the onset of hypertension during pregnancy, potentially even before placental dysfunction occurs. As such, they raise the possibility of identifying a distinct haemodynamic signature or cardiovascular profile in women at risk of hypertensive pregnancy disorders and related adverse outcomes.

Similarly, Ridder et al., (2023) conducted a study during early pregnancy[107]. Cardiac indices were assessed in 128 pregnant women between 11 + 0 and 13 + 6 weeks' gestation as part of a routine screening for preeclampsia. Among the participants, 57 were classified as low risk for developing preeclampsia and 71 were identified as high risk. Participants at high risk exhibited significantly higher left ventricular mass and left ventricular mass index, as well as altered left ventricular geometry to those at low risk[107].

Furthermore, Garcia-Gonzalez et al., (2020) recorded maternal characteristics and preeclampsia risk scores from a competing risks model in 1602 women with normotensive pregnancies at 35 to 37 weeks' of gestation. They also measured cardiac indices using echocardiography. They found that women with higher risk scores with E/e' ratios of more than or equal to 7.3 and left ventricular mass index more than or equal

to 63.2 g/m^2 developed late-onset preeclampsia [108], supporting the concept of concentric remodelling prior to hypertension.

In essential hypertension, alterations in left ventricular mass are typically driven by increased cardiac workload[124]. Therefore, if such changes are observed prior to pregnancy, this suggests that cardiac remodelling may contribute to the development of hypertension rather than being solely a consequence of it. Nonetheless, compensatory remodelling can also occur during pregnancy as an adaptive mechanism to preserve cardiac output under conditions of increased volume and pressure load.

Several other studies have demonstrated the persistence of left ventricular remodelling in women with hypertensive pregnancies in the third trimester of pregnancy[109-110]. Vaught et al., (2018) observed increased left ventricular wall thickness, higher filling pressures, and worsened diastolic dysfunction in women with severe preeclampsia compared to normotensive controls in the third trimester of pregnancy[109]. Similarly, Tyldum et al., (2012) reported impaired left ventricular function and concentric hypertrophy in women with preeclampsia to women with normotensive pregnancies[110]. These findings consistently demonstrate an altered cardiac phenotype in women with hypertensive pregnancy disorders.

Furthermore, some studies have compared cardiac indices before and after delivery to assess changes in cardiac function and morphology[111]. Giorgione et al., (2021) evaluated echocardiography parameters in 30 women with hypertensive pregnancies, measured two to eight days before delivery and again two to six days postpartum[111]. During pregnancy, 70% of the women showed signs of left ventricular hypertrophy and concentric remodelling. Shortly after delivery, no notable differences in their cardiac morphology or function since their previous scans was observed. These findings were

found despite significant shifts in maternal cardiovascular volume and resistance at birth. Thus, suggesting that due to chronic changes in cardiovascular load during pregnancy or pre-existing cardiac dysfunction, cardiac indices may take a long time to resolve postpartum, potentially altering long-term cardiovascular health.

Nevertheless, Tyldum et al., (2012) observed a normalisation in left ventricular function three months postpartum in women with preeclampsia[110]. Similarly, Vaught et al., (2021) found that cardiac abnormalities returned to baseline in women with severe preeclampsia at four years postpartum[113]. These findings suggest that left ventricular hypertrophy may be a secondary physiological response to increased peripheral resistance and that vascular mechanisms, rather than persistent cardiac hypertrophy, contribute to the elevated long-term cardiovascular risk. However, the results of these studies should be interpreted with caution due to the small sample sizes involved.

Other researchers such as Hamad et al., (2009) report that cardiac dysfunction persists after three months postpartum in women with preeclampsia even with blood pressure normalisation[114]. The variation in recovery time observed across the different studies may partly be attributed to the distinct subtypes of hypertensive pregnancy disorders involved. Yu et al., (2018) found that at three months postpartum, left ventricular systolic function had recovered in women with gestational hypertension, but not in those with preeclampsia, suggesting that preeclamptic women experience more substantial cardiac damage that requires a longer recovery period[115].

Additionally, Valensise et al., (2008) demonstrated that women with early and late- onset preeclampsia present with different hemodynamic profiles prior to the clinical onset of preeclampsia, likely reflecting varying underlying pathophysiological mechanisms[116].

These aetiological differences may influence the severity of cardiac involvement and the extent to which cardiac indices recover postpartum.

Furthermore, Orabona et al., (2018) conducted a study comparing 30 women after early-onset preeclampsia, 30 women after late-onset preeclampsia, and 30 women after uncomplicated pregnancies, with the follow-up period ranging from six months to four years[117]. The early-onset preeclamptic group exhibited greater fibrosis compared to the late-onset group and the controls, indicating more adverse cardiac remodelling.

In a separate study, Orabona et al., (2017) compared 60 women with preeclampsia to 49 women with HELLP syndrome during the same postpartum period[118]. Only women with HELLP syndrome showed significant left ventricular concentric hypertrophy. However, both the HELLP syndrome and preeclamptic groups displayed left ventricular concentric remodelling, diastolic dysfunction, and reduced left ventricular ejection fraction. The overlap of echocardiographic features between women post preeclampsia and HELLP syndrome suggests shared pathophysiological mechanisms. Nonetheless, HELLP syndrome appears to result in more severe cardiovascular remodelling in the short to medium term following delivery.

Sciatti et al., (2019) also found similar echocardiography results in 60 women with preeclampsia and 49 women with HELLP syndrome in a follow-up ranging from six months to four years postpartum[119]. There was significant overlap between aortic elastic properties in both groups, again supporting the idea of a common pathophysiologic pathway. However, women who experienced HELLP syndrome on average had larger aortas, higher blood pressure and more altered ventricular-arterial coupling[119].

Several studies have also explored the long-term cardiac changes in women with hypertensive pregnancies, revealing that these changes persist for months, years, and in some cases, even decades after delivery[120-130]. Countouris et al., (2021) assessed echocardiographic parameters in 30 women following normotensive pregnancies, 21 women with a preeclampsia history, and nine women post gestational hypertension, all at eight to ten years postpartum[120]. Even after adjusting for cardiovascular risk factors such as current hypertension, women with a hypertensive pregnancy history showed increased interventricular septal wall thickness and relative wall thickness compared to those with normotensive pregnancies.

Notably, the study also conducted a subgroup analysis, identifying that woman with both a hypertensive pregnancy history and current hypertension exhibited the most severe echocardiographic alterations, including higher rates of left ventricular remodelling[120]. This finding highlights that hypertensive insults in women with a prior hypertensive pregnancy history may be more severe due to pre-existing dysfunction or inadequate reverse remodelling. Interestingly, the study found no significant structural changes in women with hypertension alone[119], suggesting that a hypertensive pregnancy history imposes an additional pathological burden beyond the effects of essential hypertension. Thus, women with a hypertensive pregnancy history may present with a distinct cardiovascular phenotype. These findings align with Quesada et al., (2020) who also reported that women with a hypertensive pregnancy history who later develop hypertension have higher left ventricular mass compared to women with a hypertensive pregnancy history alone[121].

Boardman et al., (2020) similarly examined whether phenotypical changes in cardiac characteristics were still present five to ten years postpartum in women with prior

hypertensive pregnancies, and whether they could be explained by underlying hypertension[122]. They used multimodality imaging and computational modelling in 73 women with histories of a normotensive pregnancy and 100 women with previous preeclampsia or gestational hypertension. After adjusting for blood pressure, women who previously had a hypertensive pregnancy showed increased left ventricular mass index, higher left atrial volume, reduced capillary density and reduced aortic compliance[122]. A distinct pattern of left ventricular mass distribution was also found specifically related to a hypertensive pregnancy history[122]. These findings highlight the presence of disease specific mechanisms involved in hypertensive disorders of pregnancy which are distinct to those involved in essential hypertension.

These findings are supported by other studies which demonstrate that concentric remodelling is increased, and diastolic dysfunction is mildly impaired postpartum[123,125-133]. Scantlebury et al., (2015) found that women with a history of hypertensive pregnancy disorders have greater left ventricular hypertrophy, left atrial size and lower mitral E/A ratio[123]. Similarly, Demartelly et al., (2021) found that women with histories of preeclampsia have worse global longitudinal strain, left ventricular posterior wall thickness and intraventricular septal wall thickness ten years postpartum[125]. The high prevalence of these cardiac abnormalities following preeclampsia is not likely to be a temporary phase of recovery from cardiac remodelling, but rather a defining feature of hypertensive pregnancy.

Overall, cardiac indices appear to mirror blood pressure variations during hypertensive pregnancies, particularly in terms of left ventricular mass, geometry, and function. These alterations are evident before, during, and after pregnancy, with lasting effects on cardiovascular health. While some cardiac changes may resolve postpartum, others, such

as left ventricular hypertrophy and diastolic dysfunction persist, especially in women with histories of preeclampsia. Additionally, a hypertensive pregnancy history seems to impose an extra cardiovascular burden beyond essential hypertension, leading to more pronounced cardiac changes and a distinct cardiovascular profile. Early detection of these changes, possibly through preconception cardiac evaluation, could facilitate the development of targeted preventive strategies to reduce long-term cardiovascular risks in these women.

IX. The Role of Vascular Mechanisms in Long-Term Disease Progression Following Hypertensive Pregnancy.

Hypertensive pregnancies significantly impact vascular health throughout the course of pregnancy, with notable changes in both arterial and venous function. These changes include increased systemic vascular resistance during the first trimester, along with elevated peripheral resistance, endothelial dysfunction, vasoconstriction, arterial stiffening and higher blood pressure in the second and third trimesters[80].

As a result, women with hypertensive pregnancies are at a greater risk of developing cardiovascular disease and chronic hypertension later in life compared to those with uncomplicated pregnancies[25]. Nonetheless, it remains uncertain whether cardiac hypertrophy or vascular mechanisms are the primary factors driving the development of cardiovascular disease later in life. A deeper exploration of the literature could provide valuable insights into this question.

Several studies have demonstrated evidence of vascular dysfunction during and after hypertensive pregnancy using pulse wave analysis and pulse wave velocity measures[135-

[137]. Robb et al., (2009) found increased arterial stiffness in women with preeclampsia during pregnancy and postpartum[135]. Even with postpartum blood pressure normalisation, this elevated arterial stiffness remained seven weeks postpartum. Increased arterial stiffness therefore seems to be a feature of preeclampsia that extends beyond pregnancy and a contributor to adverse cardiovascular outcomes[135].

Similarly, Franz et al., (2013) compared 53 healthy pregnant women to 21 patients with preeclampsia between 11 + 6 and 13 + 6 gestational weeks[136]. Women with both early and late- onset preeclampsia displayed significantly elevated augmentation indexes during pregnancy. However, pulse wave velocity was only significantly elevated in the early- onset preeclamptic group. Additionally, augmentation indexes only remained elevated in the early-onset preeclamptic group six months postpartum. These results suggest early-onset preeclampsia has a profound effect on vasoconstriction and arterial stiffness, but not late-onset preeclampsia.

Moreover, Yuan et al., (2014) compared cardiovascular indices in 23 preeclamptic women and 40 matched normotensive controls[138]. Whilst both left ventricular and carotid remodelling were observed during preeclampsia; cardiac parameters returned to the levels of their normotensive counterparts one to two years postpartum, whereas carotid vascular remodelling persisted. Thus, it is likely that peripheral vascular remodelling plays a critical role in long-term cardiovascular disease development in women with hypertensive pregnancies, and in at least some subsets of women with hypertensive pregnancies, vascular changes rather than cardiac alterations, are the primary contributors to the heightened risk of cardiovascular disease later in life.

Akhter et al., (2013) further found that common carotid artery intima thickness and intima-media thickness were significantly higher in preeclamptic women at the time of

diagnosis compared to women with normotensive pregnancies[139]. Approximately one year postpartum, these measurements significantly decreased in both groups. However, women with a preeclampsia history still showed significantly thicker arterial walls. These findings suggest that despite some improvements occurring at delivery, significant cardiac remodelling is occurring in the preeclamptic groups that does not completely normalise after delivery.

Another study by the same authors, at 11 years postpartum, found that both common carotid artery intima and media thicknesses remain significantly higher in women with histories of severe preeclampsia to women with normotensive pregnancies[140]. The relative differences in intima and intima-media thickness between women with and without histories of severe preeclampsia were more pronounced in this study than in the previous one. This may be a result of the disease severity increasing. Alternatively, the differences may reflect deterioration of arterial walls with age as well as the long-term and potentially progressive effects of preeclampsia on vascular components.

Hypertensive pregnancies also significantly impact microvascular function. Reduced flow-mediated dilation is commonly found in women with prior hypertensive pregnancies compared to women with normotensive pregnancies[110,129,141]. Additionally, heightened endothelium-dependent and independent microvascular reactivity in preeclamptic women, has been shown using laser speckle contrast imaging combined with iontophoresis, six months to five years after pregnancy[143]. Although the precise mechanisms remain unclear, endothelial dysfunction is known to involve leukocyte adhesion, oxidative stress, and reductions in nitric oxide bioavailability. These processes may contribute to nascent atherosclerotic changes that induce permanent alterations in vascular elasticity, like the effects seen in established atherosclerosis. In certain

hypertensive pregnancy subtypes, this may represent a primary pathway to cardiovascular disease development.

The microvascular changes induced by hypertensive pregnancies also manifest in the eye, affecting both the choroidal and retinal circulation[144-150]. These changes increase the risk of serous retinal detachment and retinal lesions, compromising long-term visual acuity[145]. For example, a recent study by Wali et al., (2024), examined 94 women diagnosed with either preeclampsia or eclampsia and found that 23.4% were presenting with headaches and 20.2% with blurring of vision. Additionally, 56.3% of the women had hypertensive retinopathy. Interestingly, the magnitude of the hypertensive retinopathy was significantly correlated to the severity of the hypertensive pregnancy disorder.

Furthermore, several studies show narrowing of the retinal microvasculature in women with hypertensive pregnancies compared to those who remained normotensive[146-149]. Lupton et al., (2013) examined microvascular calibre in 92 women at 13, 19, 29, and 38 weeks of gestation[146]. The central retinal arteriolar equivalent, adjusted for mean arterial pressure, was significantly reduced at 13 (P=0.03), 19 (P=0.007), and 38 (P=0.03) weeks of gestation in the preeclamptic group. Likewise, central retinal venular equivalents, adjusted for mean arterial pressure were also significantly lower at 13 (P=0.04) and 19 (P=0.001) weeks of gestation in the women who progressed to preeclampsia. These findings suggest that microvascular narrowing precedes the clinical onset of preeclampsia, reflecting early systemic endothelial dysfunction and impaired vascular adaptation to pregnancy.

Similarly, Chandran et al., (2020) observed retinal narrowing in 150 patients with preeclampsia or eclampsia between 26-39 weeks of gestation[147] and Soma-Pillay et al., (2018) found that the corrected central retinal arteriolar equivalent and corrected central retinal venular equivalent were significantly lower in the preeclamptic group compared to the control group both at delivery and one year postpartum ($p < 0.001$)[148].

Benschop et al., (2017) further assessed retinal microvascular structure in 145 women with gestational hypertension, 63 women with preeclampsia, and 3183 women with normotensive pregnancies at an average of 6.2 years postpartum[149]. Both hypertensive groups showed narrower retinal arteriolar calibres to normotensive women, with the greatest narrowing observed in the gestational hypertension group. Women with gestational hypertension also showed larger venular calibres to normotensive women. These findings show that pregnancy-related hypertension leads to persistent microvascular alterations, with distinct patterns between gestational hypertension and preeclampsia.

Although there is consistent evidence of arteriolar narrowing across these studies, findings regarding venular calibre remain inconsistent. Some studies report venular narrowing, whilst others report venular dilation. This likely reflects differences in disease stage, severity, or underlying vascular adaptation. However, longitudinal studies tracking these microvascular changes from pregnancy through to later life are lacking, limiting our understanding of the progression of retinal changes and long-term clinical impact. This is an area which could be examined in future research.

Overall, hypertensive pregnancy disorders significantly impact vascular health, resulting in increased vascular resistance, endothelial dysfunction, vasoconstriction, and higher blood pressure. These alterations contribute to a heightened risk of cardiovascular

disease and chronic hypertension. Although the relative contributions of these mechanisms to later cardiovascular disease remain unclear, accumulating evidence suggests that vascular changes, such as arterial stiffness and carotid wall thickening are key mediators. Beyond macrovascular function, hypertensive pregnancies also impair microvascular integrity, with endothelial dysfunction and oxidative stress potentially accelerating atherosclerotic processes. Persistent postpartum microvascular arteriolar narrowing persists postpartum and may link hypertensive pregnancies to future cardiovascular disease, underscoring the need for longitudinal studies to clarify these trajectories.

X. Renal Complications Following Hypertensive Pregnancy: Pathways to Long-Term Disease Development

It is well established that women with histories of hypertensive pregnancy are at significantly increased risk of developing chronic kidney disease and end-stage renal failure later in life[15-20]. One study by Barrett et al., (2020) analysed data from Swedish medical birth and renal registers which followed 1,924,409 women for an average of 20.7 years postpartum[19]. Both women with gestational hypertension and preeclampsia were at significantly higher risk of developing chronic kidney disease later in life, with a diagnosis occurring 2.7 years earlier in women with preeclampsia than those without. Given this higher risk of disease, it is likely that the renal dysfunction occurring during pregnancy does not fully resolve postpartum.

Evidence regarding the persistence of renal dysfunction postpartum currently remains mixed. Some studies show persistence of renal dysfunction, whilst others do not. Kountouris et al., (2021) found that 32% of 740 women with hypertensive pregnancies

experienced renal dysfunction at six to eight weeks postpartum[155]. They defined renal dysfunction as a low estimated glomerular filtration rate, proteinuria or a urinary albumin-to-creatinine ratio more than 3 mg/mmol. Notably, renal dysfunction was more frequent among women with preeclampsia (46%) than among those with gestational or chronic hypertension (22%).

Kaze et al., (2014) found a high prevalence of persisting proteinuria at six weeks postpartum in 54 women with severe preeclampsia or eclampsia. However, all but one patient showed full recovery at six months postpartum[156]. Meanwhile, Ishaku et al., (2021) found that women with hypertensive pregnancies were more likely to have a reduced estimated glomerular filtration rate compared to normotensive women at six months postpartum[157]. Additionally, Kaleta et al., (2016) examined 44 women who had preeclampsia during a previous pregnancy, finding that most showed persistent proteinuria six months to one year after delivery[158]. However, the observed reduction in estimated glomerular filtration rate only persisted up to six months postpartum.

Other studies report substantial postpartum resolution of renal abnormalities. Berks et al., (2009) followed 205 women with preeclampsia at 1.5, 3, 6, 12, 18, and 24 months after delivery[159]. At three months postpartum, 14% of the women had proteinuria which decreased to 2% of the women at two years postpartum. Also, longer recovery times were associated with higher initial proteinuria levels. Similarly, Sandvik et al., (2013) assessed renal biomarkers in 89 women with prior preeclampsia and 69 women with prior normotensive pregnancies at nine to eleven years postpartum. They found that women with prior preeclamptic pregnancies did not have higher estimated glomerular filtration rate than women without[160]. These findings suggest that functional renal parameters recover postpartum.

Paauw et al., (2018) also observed minimal long-term differences in renal biomarkers between women with and without prior hypertensive pregnancy disorders. They followed women up to eleven years postpartum and evaluated renal biomarkers over time in 1805 women without patient-reported hypertensive pregnancies and 977 with patient-reported hypertensive pregnancies[161]. During follow-up, they observed no differences in albuminuria. However, evaluation of the course of estimated glomerular filtration rates showed minimal, but significant differences between groups with slightly lower estimated glomerular filtration rates at all visits and a slightly steeper estimated glomerular filtration rate decline in women with a hypertensive pregnancy history.

Others such as Shammas et al., (2000) have shown that renal impairment lasts long into the postpartum period. These researchers assessed renal function ten years postpartum in 54 women with histories of pregnancy induced hypertension, 47 women with histories of preeclampsia and 46 women with prior normotensive pregnancies[162]. Women with histories of pregnancy induced hypertension or preeclampsia showed increased risk of microalbuminuria later in life compared to women with normotensive pregnancies. As microalbuminuria appeared in both the pregnancy induced hypertension and preeclamptic groups, it seems to be a blood pressure dependent factor and not preeclampsia specific.

Overall, women with a history of hypertensive pregnancy, particularly those with preeclampsia, are at an increased risk of developing kidney disease in the postpartum period. While some studies report persistent renal dysfunction after delivery, others suggest that renal function gradually normalises over time. However, there is a notable lack of research examining structural renal impairments postpartum in women with hypertensive pregnancies. Such structural alterations may persist even when functional

measures appear improved. This gap in understanding will be explored in greater detail in the following chapters of this thesis.

XI. Neural Complications of Hypertensive Pregnancy: Timing and Manifestation

Of all the clinical complications that result from hypertensive pregnancies, the neurovascular outcomes which carry significant maternal risk are the most unique and well known. Unlike, essential hypertension, hypertensive pregnancies can result in the sudden development of maternal tonic clonic seizures, despite the absence of underlying health risks. These seizures are rare in developed countries at 1.6 per 10,000 deliveries but rise to 50-151 per 10,000 deliveries in developing countries[164].

Hypertensive pregnancy can also lead to the development of posterior reversible encephalopathy syndrome, which is characterised by headaches, cognitive impairments, convulsions, visual disturbances and cerebral oedema during pregnancy[165]. The occurrence of these neurological complications highlights a unique aspect of hypertensive pregnancies compared with essential hypertension. However, these symptoms are not present in all women affected, and it is unclear why. Furthermore, the precise physiological mechanisms underlying eclamptic seizures and posterior reversible encephalopathy syndrome are still poorly understood.

To help answer these questions, Bergman et al., (2021) compared the cerebrospinal fluid from women with eclampsia to that of women with normotensive pregnancies at delivery. They found elevated inflammatory markers and blood brain barrier injury in those with preeclampsia compared to those with normotensive pregnancies[166]. These findings suggest that the disruption of cerebrovascular autoregulation, which is normally

responsible for maintaining stable cerebral blood flow and solute transport across the blood brain barrier, becomes altered in preeclampsia, leading to neurotoxicity and seizures[166].

Zeeman et al., (2004) further found that blood flow in the large cerebral artery was significantly higher in women with preeclampsia compared to normotensive women during pregnancy, suggesting that increased cerebral blood flow caused by elevated blood pressure precedes the onset of convulsions[167]. Nevertheless, given that maternal seizures do not develop in all women with hypertensive pregnancies, the stimulus of hypertension alone, whether pre-existing or gestationally developed, is clearly not the primary cause.

This has led to the hypothesis that pro-inflammatory, anti-angiogenic circulating factors play a role in seizure development, rather than hypertension alone[167]. In severe, placental subtypes of the disorder, elevated circulating levels of soluble vascular endothelial growth factor receptor and decreased placental growth factor could increase cerebrovascular permeability and raise the risk of seizures. Supporting this hypothesis, Torres-Vergara et al., (2022) used human endothelial cells as an in vitro model of the blood brain barrier to show how plasma from women with preeclampsia increases cell apoptosis and permeability by the phosphorylation of the vascular endothelial growth factor receptor two[168].

Moreover, Canjels et al., (2022) investigated the blood brain barrier integrity five to eight years postpartum in 55 women with prior preeclampsia and 17 normotensive controls using ultra-high field magnetic resonance imaging[169]. Leakage rate and fractional leakage volume were significantly higher in the white and gray matter of women with previous preeclampsia. Regionally, the frontal and parietal cortical gray matter, and the frontal,

temporal and occipital white matter showed higher leakage rates. Whether the higher blood brain barrier leakage observed was causally related to the impact of preeclampsia on the brain or to the risk profile of the women with preeclampsia cannot be concluded as they did not adjust for other factors. Nonetheless, the results suggest that blood brain barrier impairment is likely involved.

Supporting these findings, Zeeman et al., (2024) reported magnetic resonance imaging evidence of cerebral oedema in 93% of 27 nulliparous women with eclampsia, a sign of compromised blood-brain barrier integrity[170]. The high prevalence of reversible vasogenic oedema that they observed supports the hypothesis that cerebral oedema in eclampsia results primarily from hyperperfusion exceeding the autoregulatory capacity of cerebral capillary beds.

Historically, hypertensive pregnancy disorders have been considered to have no lasting adverse effects on neurologic function. However, growing evidence suggests that they raise the risk for neural complications long after pregnancy, underlining the need for more focused longitudinal postpartum studies. For instance, women with prior hypertensive pregnancies have cerebral white matter lesion's more often and more severely than women with normotensive pregnancies several years after pregnancy[171-173]. These lesions are not specific to the pregnancy itself[175] and have been accompanied by long-term alterations in white matter structure, cortical gray matter volume and decreased total brain volume years after pregnancy[173].

One study by Aukes et al., (2009) examined magnetic resonance imaging scans of formerly eclamptic and normotensive women at an average of 6.4 ± 5.6 years remote from the index pregnancy. They noted that women with preeclampsia demonstrated subcortical white matter lesion's more than twice as often as their normotensive

counterparts[171]. Wiegman et al., (2014) noted similar findings at seven years postpartum[172]. These findings are important as they correlate structural adaptations after preeclampsia to functional outcomes. Brain lesions that are evident remote from pregnancy appear to track with the initial anatomic lesion that was discovered in pregnancy. While more evidence is needed in this area, women who develop eclamptic seizures should be monitored closely to limit the risk of postpartum complications.

Moreover, Siepmann et al., (2017) examined 34 women at five to fifteen years post preeclampsia. Temporal lobe white matter disease was increased and cortical gray matter volume was decreased in women with previous preeclampsia compared to 49 women with previous normotensive pregnancies[173]. In the women with histories of preeclampsia, the degree of structural impairment increased with time, consistent with continued damage postpartum. This suggests that persistent neural injury occurs post hypertensive pregnancy disorders, again highlighting the long-term impact of a hypertensive pregnancy history.

Nevertheless, the cerebrovascular disease burden associated with hypertensive disorders of pregnancy may be, at least in part, modifiable. One study reported that the prevalence of white matter lesions reduced from 61.7% at delivery to 47.9% at one-year postpartum[174]. As high intensity lesions on magnetic resonance imaging reflect protein and fluid extravasation, this reduction likely represents resorption and reversibility. The capacity to restore or preserve cerebral integrity is clinically significant, as structural brain alterations are linked to cognitive impairments[176].

Women with a history of hypertensive pregnancy disorders also frequently perform worse on cognitive assessments and report emotion- and cognition-related symptoms that adversely affect their quality of life[176-184]. Mielke et al., (2016) demonstrated that

women with hypertensive pregnancy histories performed worse on all measures of processing speed. This was accompanied with lower total brain volumes compared to women with histories of normotensive pregnancies[176].

As hypertensive disorders of pregnancy are increasingly recognised as cardiovascular disease risk factors, it could be suggested that cognitive decline and neural changes are mediated solely by subsequent diagnoses of hypertension or cardiovascular disease. Nonetheless, Mielke et al., (2016) adjusted for cardiovascular disease, hypertension, and family history, finding that the association between having a hypertensive pregnancy history, processing speed and brain atrophy remained significant[176]. Again supporting, the idea that women with hypertensive pregnancy histories have a unique phenotype compared to women with essential hypertension.

More recently, associations have also been made between elevated aortic hemodynamic and cognitive functional decline in later in life. Miller et al., (2020) performed cognitive tests and applanation tonometry in 34 postmenopausal women with a history of preeclampsia and 30 women with a history of normotensive pregnancy. Aortic haemodynamics were associated with the summary cognitive index in women with preeclampsia, supporting the idea that a history of preeclampsia leads to long-term effects on brain health[177].

Dayan et al., (2018) further assessed cognitive function in 193 women with histories of preeclampsia and 375 women with histories of normotensive pregnancy, approximately 18 years postpartum[178]. Women with preeclampsia scored significantly lower on the Digit Symbol Substitution Test and on the third trial of the Stroop Test than women with normotensive pregnancies, but there were no differences in the Rey Auditory Verbal Learning Test. These results were still significant even after adjustment for age,

education, depression, and metabolic risk factors which are known to be more prevalent in formerly preeclamptic women. The results were also unchanged when women with gestational hypertension were added to the exposed group, supporting the idea that most hypertensive pregnancy disorders lead to some form of neurocognitive decline later in life.

The psychological and cognitive complaints commonly reported by women with a history of preeclampsia align with differences in functional connectivity in the limbic regions and the prefrontal cortex. Canjels et al., (2022) explored whether formerly preeclamptic women exhibit differences in functional brain organisation. They particularly explored the regions that are related to the commonly reported emotional symptoms and cognitive complaints in women years after pregnancy[179]. Network properties were examined using ultra-high field magnetic resonance imaging and graph theoretical measures. Overall, compared with the controls, the women with a history of preeclampsia had higher local efficiency in the prefrontal cortex and anterior cingulate cortex but lower local efficiency and local clustering coefficient in the amygdala and parahippocampal cortex[179].

Essential hypertension similarly has been associated with cognitive impairment and changes in the functional brain networks[185]. Vicario et al., (2005) demonstrated decreased executive function, attention and alterations in functional connectivity in individuals with essential hypertension compared to those without hypertension[185]. Although this study includes both sexes and mostly older participants, the results suggest that functional network alterations in formerly preeclamptic women are comparable with those seen in hypertensive patients and they may just represent accelerated progression of end-organ changes typical of essential hypertension.

Overall, a cascade of events in hypertensive pregnancy appears to contribute to neurotoxicity, seizures, and long-term neurological complications, particularly in women with preeclampsia, eclampsia, and HELLP syndrome. Seizures in these conditions seem to be linked to pregnancy-related changes rather than the mother's underlying cardiovascular health. Cerebrovascular and structural changes are consistently reported postpartum, as well as long-term cognitive and emotional impairments. More research is needed to understand the underlying mechanisms and why some women experience these long-term effects but not others. In addition, more research is needed to explore the potential treatments for protecting and improving cerebral integrity.

XII. Strengths and Limitations

This review was conducted using established systematic methods, ensuring a comprehensive and inclusive collection of all relevant studies published to date. As a result, the findings offer the most current and detailed summary of publicly available evidence on hypertensive target organ changes during and after hypertensive pregnancy. The review makes a significant contribution to the field by incorporating a large number of studies and a substantial sample size. However, several important limitations should be noted.

Firstly, due to the heterogeneity of the data, a meta-analysis was not performed to estimate the effect sizes for various variables. While this limits the conclusions that can be drawn, the discussion provides a qualitative comparison of effect sizes at different time points for measures with sufficient available evidence. Secondly, due to the observational design of most of the included studies, along with the lack of consideration

for potential confounding factors such as maternal age, lifestyle, and environmental influences, a clear cause and effect relationship cannot always be established.

Finally, the findings may only be generalisable based on factors such as geographic location, socioeconomic status, and access to healthcare. All of which require further discussion and consideration. This review did not address potential confounding factors, such as maternal age, lifestyle and underlying diseases that could influence the development of hypertensive target organ damage during pregnancy and postpartum.

XIII. Discussion and Concluding Remarks

This review highlights evidence of target organ damage, typical of hypertensive disease, affecting the cardiac, vascular, renal, and cerebral systems both during pregnancy and postpartum in women with hypertensive pregnancies *(Figure 7).* While there appears to be some resolution of target organ alterations postpartum, significant differences persist. These changes occur despite considerable variability in blood pressure.

The high prevalence of target organ alterations during the peripartum period underscores the need for further research to understand their role in disease progression and long-term disease risk. Identifying patterns of target organ changes during this time could help pinpoint women at highest risk of future disease, and interventions aimed at reversing these changes should be tested to determine if clinical outcomes can be improved.

Additionally, despite the large body of evidence on target organ alterations during and after hypertensive pregnancy, several key questions remain. One critical question is whether women with hypertensive pregnancies follow accelerated disease trajectories compared to those with normotensive pregnancies. Establishing this is challenging due

to the limitations of observational studies in determining cause and effect. A longitudinal cohort study is needed to address this question further. Moreover, another key question that remains is whether structural impairments in the kidneys persist postpartum despite functional improvements.

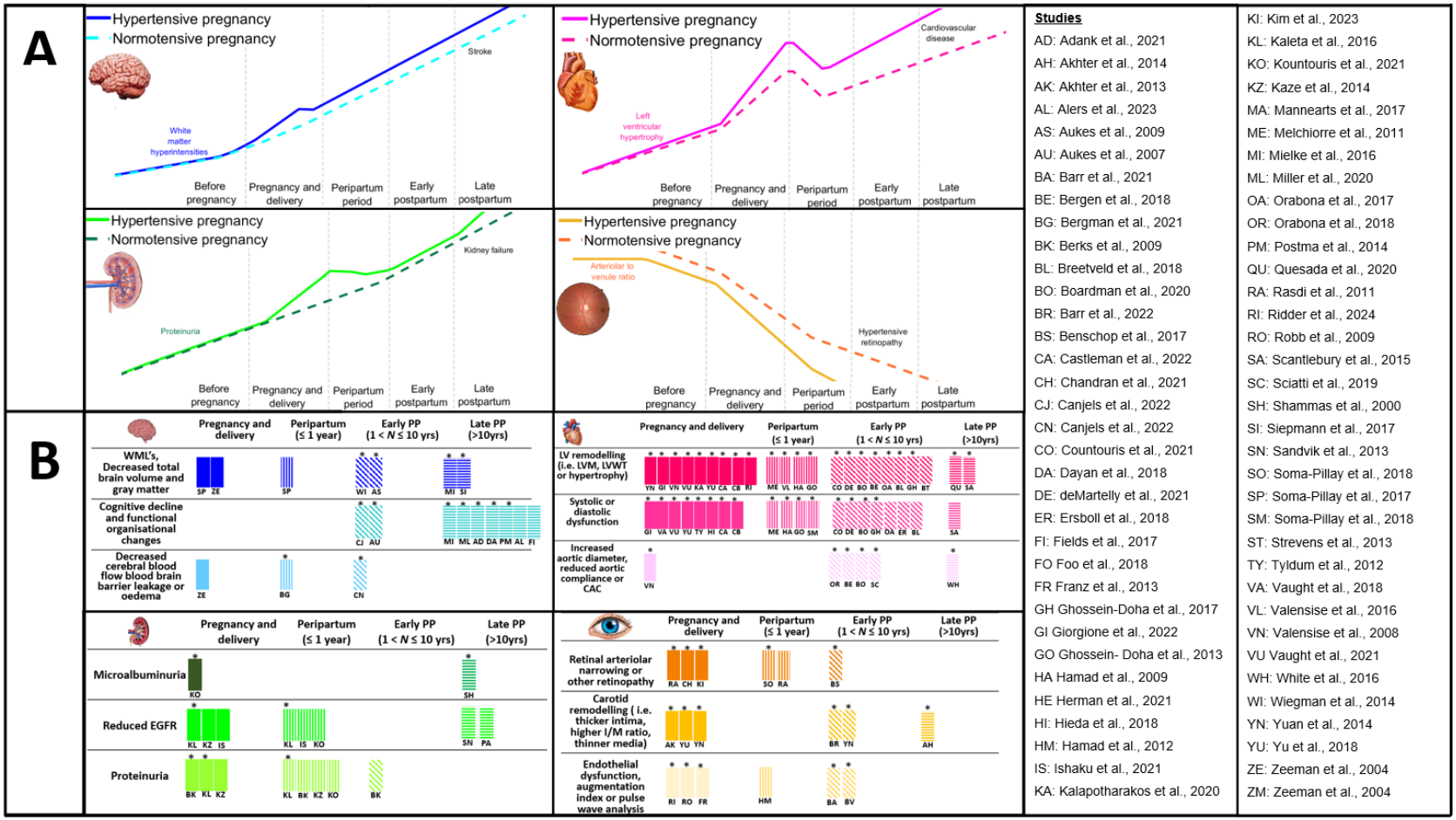


Figure 7. (*A*) Theoretical trajectories of target organ differences across hypertensive and healthy pregnancy based on the evidence provided in this review. (*B*) An evidence-based plot of the types of target organ damage caused by hypertensive pregnancies. Significant differences are represented by an asterix. Different time periods are represented by different patterned bars and different colours represent the different types of target organ alterations. Papers corresponding to each result are represented by the letters relating to the name of the first author. The figure been updated from the original published review to represent the papers included in this chapter.

CROSS-SECTIONAL RENAL DIFFERENCES AT 6-12 MONTHS POSTPARTUM

3

I. Overview and Aims

This chapter presents a more comprehensive assessment of the short-term postpartum impact of pregnancy hypertension, with a particular focus on renal structure. The early postpartum period represents a critical window for detecting subclinical signs of end-organ dysfunction, which may underlie the increased risk of chronic kidney disease[15-20], cardiovascular disease[4], and other health complications observed in this population. A cross-sectional approach at the standardised timepoint of 6-12 months postpartum offers a unique opportunity to identify early subclinical phenotypes of end-organ damage. Identifying these changes early can enhance risk stratification and the development of interventions in this at-risk population.

In chapter two, I observed that functional kidney parameters improve postpartum after hypertensive pregnancy. Yet, these women remain at elevated lifetime risk of developing chronic kidney disease and end-stage renal failure later in life[15-20]. The POP-HT trial previously investigated the impact of tightly controlled blood pressure in the early puerperium compared to standard NHS care in women with hypertensive pregnancies, finding beneficial effects on cardiovascular remodelling at 6-12 months postpartum[95].

Whilst cardiovascular changes are well-documented, and interventions have been aimed at reversing early signs of cardiovascular remodelling[92-97]; the renal system remains relatively underexplored.

I hypothesise that subclinical structural changes in the kidneys persist even when functional parameters such as proteinuria and estimated glomerular filtrate rates improve. As such, the primary aim of this chapter is to evaluate whether women with a hypertensive pregnancy history show persistent renal structural changes compared to women with previous normotensive pregnancies, despite the absence of overt functional symptoms. A secondary aim of this chapter is to observe whether there are any structural renal differences between those who had tightly controlled blood pressure and those who did not.

To investigate this, I conducted a secondary analysis of two clinical trials in women with hypertensive disorders of pregnancy that involved multi-organ imaging, including kidney magnetic resonance imaging at 6-12 months postpartum. In this chapter, I present an overview of the cohorts, methodology used, and the results of the analysis used to investigate the renal system postpartum. This is a more detailed analysis of work that is published in Hypertension, which can be found at the following reference: *Cutler. H.R., et al., 2025, Subclinical Postpartum Renal Structure After Hypertensive Pregnancy Disorders. Hypertension, doi/10.1161/HYPERTENSIO NAHA.125.25130*[186]*.*

II. Study Design and Population

The hypertensive cohort for this analysis were recruited from the POP-HT study. During the trial, these women were randomised 1:1 to either telemonitored home blood pressure management with physician-assisted self-management or standard NHS-led care.

Detailed descriptions of recruitment, patient characteristics, methodology, and prespecified outcomes from this trial have been published previously[93-97].

The POP-HT study did not recruit a normotensive comparator group. Therefore, the normotensive cohort for this specific analysis were recruited from the CAREFOL-HT study which investigated the effects of tetrahydrofolate supplementation on women with hypertensive pregnancies. The normotensive cohort included in this study were not given any additional interventions beyond standard postpartum care, so they were able to be used as a comparison group.

Recruitment for the POP-HT study occurred between January 2020 and November 2021, whilst recruitment for the CAREFOL-HT study started in July 2023 and is still ongoing. The recruitment strategies for both studies were similar, with recruitment being carried out through several different channels. Some women were actively recruited at the Women's Centre of the John Radcliffe Hospital. Others were identified from hospital records, birth registers and previous studies. Meanwhile, some women were invited through their local hypertension clinics.

Eligible individuals who were willing to participate were provided with a participant information leaflet, either in-person, by email or through post, outlining the study information in advance of consent. After at least 72 hours to review the information, the women attended a study visit where informed consent was taken. Research governance and ethical approval were obtained for both CAREFOL-HT (21/WA/1069) and POP-HT (19/LO/1901) before commencing the studies.

Data collection for POP-HT ended in November 2021 and recruitment for the CAREFOL-HT study started in October 2021. I commenced my DPhil in October 2021 so for the first few months of my DPhil, I helped with the CAREFOL-HT data collection. I then submitted

an amendment for the follow-up of the CAREFOL-HT cohort in January 2022. The aim was to have a follow-up visit at 6-12 months postpartum with consistent methodology to the POP-HT 6-12 month postpartum follow-up. The amendment was approved in December 2022, so I started recruiting for the follow-up in January 2023. I then analysed the unexamined renal imaging data from the hypertensive POP-HT cohort and compared it to the data that I had collected from the normotensive CAREFOL-HT cohort.

III. Inclusion and Exclusion Criteria

Participants were deemed eligible for the follow-up visit if they were 6-12 months postpartum, provided informed consent and were not pregnant again at the time of consent. The women were also required to have no known history of cardiac impairment, pre-existing chronic renal disease, pre-pregnancy hypertension, contraindications to magnetic resonance imaging, or any other condition that in the judgement of either myself or the other investigator's, could pose a risk or influence their participation or the study outcomes.

For inclusion specifically in the hypertensive cohort, participants were required to have previously taken part in the POP-HT study and received a clinician-confirmed diagnosis of gestational hypertension or preeclampsia at birth, based on the UK National Institute for Health and Care Excellence guidelines[4]. They must have also still been requiring antihypertensive medication at hospital discharge, and they must have had no known history of antihypertensive use before pregnancy.

Based on the National Institute of Health and Care Excellence guidelines, gestational

hypertension was defined as blood pressure readings of more than or equal to 140/90 mmHg on more than two occasions after 20 weeks of gestation, without significant proteinuria or organ dysfunction[4]. Meanwhile, preeclampsia was diagnosed as new-onset hypertension with blood pressure readings of more than or equal to 140/90 mmHg during pregnancy, with the addition of one or more signs of organ dysfunction[4].

Organ dysfunction included a proteinuria reading of more than or equal to 300 mg in a 24-hour urine collection, a serum creatinine reading of more than or equal to 90 μmol/L (or 1.02 mg/dL) and elevated liver transaminases (ALT or AST) exceeding 40 IU/L[4]. Any neurological complications such as eclamptic seizures, altered mental status, cortical blindness, stroke, clonus, severe headaches or persistent visual scotomata, as well as haematological complications such as thrombocytopenia or evidence of disseminated intravascular coagulation were also considered indicators of end-organ involvement[4]. Utero-placental dysfunction including foetal growth restriction or abnormal doppler findings were also recognised as end-organ dysfunction[5].

Preeclampsia diagnoses were further classified into early-onset if the symptoms occurred before 34 weeks of gestation, and late-onset if the symptoms occurred at or after 34 weeks of gestation[4]. Information on sex, race, and ethnicity were self-reported in line with the National Institute for Health Research categories[187]. Birth and family history data were obtained through a combination of prospective collection, access to medical notes, and by questionnaire.

Meanwhile, participants in the normotensive cohort were required to have taken part in the CAREFOL-HT study and they must have maintained blood pressure readings below

140/90 mmHg throughout their entire pregnancy, with fewer than two moderate risk factors for hypertensive disorders in pregnancy as defined by the National Institute for Health and Care Excellence guidelines[4]. These risk factors included nulliparity, age over 40 years, a body mass index greater than 30 kg/m^2, a history of hypertensive pregnancy or a family history of preeclampsia, multifetal gestation or diabetes. The women also needed to have a soluble fms-like tyrosine kinase-one to placental growth factor ratio below 23 throughout the entire pregnancy.

IV. Overview of the Follow-Up Visit

Each follow-up visit lasted approximately two to four hours and was conducted at the Cardiovascular Clinical Research Facility in the John Radcliffe Hospital, UK by myself and a team of researchers. All measurements were taken using standardised calibrated equipment and the same equipment was used across both studies. As the women were between 6-12 months postpartum, childcare facilities were available during their visits. However, due to the demands of caring for an infant and the time constraints that come with it, some participants were not able to complete all measurements during the visit.

On arrival, the women were given the opportunity to ask questions and subsequently written informed consent was documented with dated signatures from both the participant and myself or another investigator. A copy of the signed informed consent was given to the participant, and the original version was stored in a locked room. The informed consent process was taken in accordance with the Declaration of Helsinki principles[188].

During the visit, the women underwent several measurements *(Figure 8)* including: anthropometry, blood sampling, peripheral blood pressures, microvascular imaging of

the retina and auricle, echocardiography, electrocardiography, magnetic resonance imaging, brachial and aortic blood pressure measurements, body composition, detailed lifestyle/medical history questionnaire and physical activity monitoring. In the following sections, I will outline the main study measurements that were used for this specific analysis in more detail.

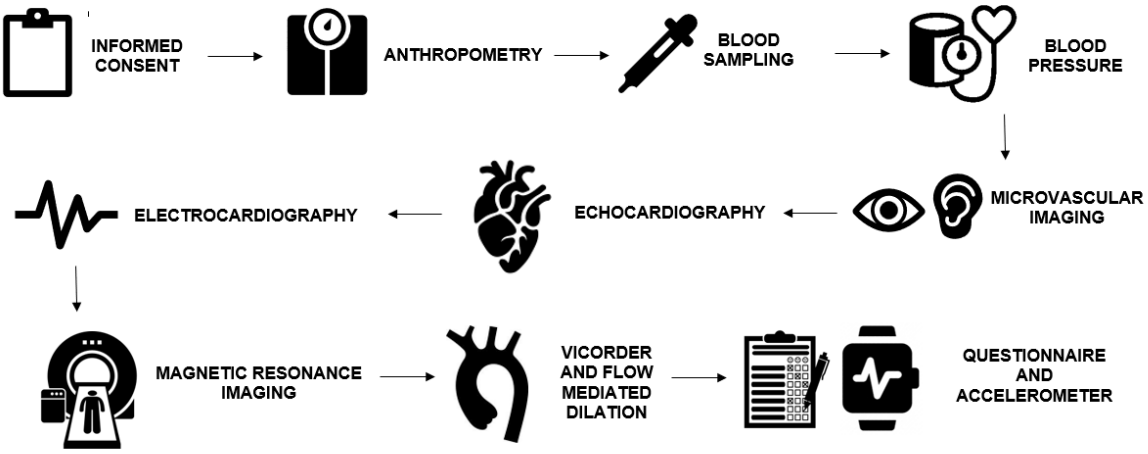


Figure 8. Overview of the study methodology used in the CAREFOL-HT and POP-HT studies. This figure illustrates the key stages of the study design for the CAREFOL-HT and POP-HT studies including informed consent, data collection and the clinical assessments performed.

V. Anthropometric Measurements

Anthropometric measurements including height, weight, hip, waist, and mid-left arm circumference were collected following standardised procedures as follows:

Participants were instructed to remove shoes and heavy clothing prior to measurement. Height was then measured using a calibrated free-standing stadiometer (SECA, UK) to the nearest centimetre, with the participant standing upright and with their head positioned in the Frankfort horizontal plane. Weight was recorded using precision digital scales

(SECA, UK) to the nearest 0.1 kg after participants were instructed to stand still and evenly distribute their weight on both feet.

Waist circumference was measured at the midpoint between the lower rib and the iliac crest, just above the umbilicus, using a flexible, non-stretchable measuring tape, ensuring that it was snug but not compressing the skin. Hip circumference was measured at the widest point of the gluteal region. Both circumferences were measured to the nearest centimetre for consistency.

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

$$BMI\ \left(\frac{kg}{m^2}\right) = \frac{weight\ (kg)}{height\ (m^2)}$$

Waist-to-hip ratio was calculated by dividing the waist circumference by the hip circumference.

Body surface area (BSA) was calculated using the Mosteller formula:

$$BSA\ (m^2) = \sqrt{\frac{[height\ (m) x\ weight\ (kg)]}{3600}}$$

VI. Blood Sampling and Biochemistry Analysis

Fasting venous blood samples were collected to assess angiogenic, metabolic, and vascular biomarkers associated with renal function. Blood samples were drawn by myself and other researchers. Once drawn, they were centrifuged at 2500 × g for 15 minutes at 4°C to separate the plasma from the cellular components. The plasma was then isolated and aliquoted into cryovials which were stored at -80°C to preserve the integrity of the samples until analysis could be performed.

Subsequent sample analysis was conducted at the Oxford University Hospitals NHS Foundation Trust Clinical Biochemistry Laboratory using validated, quality-controlled clinical assays. Estimated glomerular filtration rate was calculated using the 2021 chronic kidney disease-Epidemiology Collaboration equation[189]. This equation estimated kidney function based on serum creatinine levels, age and sex.

VII. Clinical Blood Pressures

Blood pressure was measured using a validated automated monitor (GE Dinamap Carescape V100, UK). The protocol used was based on the guidelines of the British Heart Foundation and the British and Irish Hypertension Society[190]. Measurements were taken whilst the participants were seated with their arm supported at the level of the heart, feet flat on the floor, legs uncrossed, and they were instructed not to speak during the measurements. Measurements were taken on the left arm at the level of the brachial artery, unless contraindicated. After resting for five minutes, five consecutive readings were taken at one-minute intervals. Monitors were appropriately calibrated and validated for both pregnant and non-pregnant populations.

The mean of the final two blood pressure readings was used for analysis, in accordance with the latest European Society of Hypertension guidelines[191]. Then mean arterial pressure (MAP) was calculated using the formula:

$$MAP = Diastolic\ Pressure + \frac{1}{3}\ (Systolic\ Pressure - Diastolic\ Pressure)$$

VIII. Magnetic Resonance Imaging Overview

Magnetic resonance imaging was conducted at the Oxford Centre for Magnetic Resonance Imaging at the John Radcliffe Hospital. A 3.0 Tesla PRISMA scanner (Siemens Medical Solutions, Erlangen, Germany) was used, equipped with an 18-channel phased array surface coil. Magnetic resonance imaging was chosen due to its gold-standard status for multi-organ assessments[192]. Specifically, it provides high-resolution, multiplanar imaging which allows for both regional and global analysis of renal structure, providing detailed insight into kidney morphology without requiring geometric assumptions[193].

Established protocols were followed during the scans, which were conducted by myself and another researcher. Images were acquired without contrast agents to minimise potential risks for the participants and to maximise the number of participants that would be willing to have an imaging scan. Prior to scanning, I completed the facility's safety screening forms with each participant which reviewed the potential risks of the magnetic resonance imaging scan. Participants were then asked to change into hospital scrubs and remove any metal objects before entering the control room.

Each participant was carefully introduced to the scanner, and they were informed that they could withdraw at any time. Once positioned supine on the scanner, participants were fitted with hearing protection and an emergency buzzer to alert myself or the other operators if needed. For participants with claustrophobia, the imaging scan was offered, but they were excluded from the magnetic resonance imaging component only if they opted out.

IX. Data Management

Data were recorded using electronic case report forms on Castor Electronic Data Capture, a secure, general data protection regulation-compliant platform widely used in clinical and academic research. Real-time data entry was performed on an encrypted study laptop during the visit. All data were anonymised using unique study-specific identification codes to ensure confidentiality and facilitate blinded analysis.

X. Primary and Secondary Outcomes

The primary outcome for the renal investigation was the difference in total kidney volume indexed to body surface area across hypertensive and normotensive groups. Secondary outcomes included group differences in right and left kidney volumes indexed to body surface area (accounting for their anatomical position), corticomedullary differentiation on T1 mapping and renal blood biomarkers particularly estimated glomerular filtration rate as well as creatinine, urea, sodium and potassium levels.

Total kidney volume was chosen as the primary outcome due to its clinical importance. Renal mass provides insight into renal functional reserve, and reduced renal volumes are commonly observed in patients diagnosed with chronic kidney disease and end-stage renal failure[194]. Corticomedullary differentiation was included as a secondary outcome as a loss of distinction between the cortex and medulla can reflect renal filtration barrier damage, impaired filtration and disruptions in fluid balance[195]. These together over time can worsen and lead to more severe renal disease.

XI. Renal Magnetic Resonance Imaging Procedures

The complete magnetic resonance imaging protocol consisted of multiple components to provide a comprehensive anatomical and functional understanding of the kidneys. Axial, coronal and sagittal localiser scans were initially performed to properly orient the imaging slices relative to the kidney anatomy.

Then unenhanced sagittal three-dimensional T2-weighted volumetric interpolated breath-hold images were acquired to assess renal volumes. This sequence provided high resolution images with excellent tissue contrast, particularly useful for delineating the boundaries of the kidney. The volumetric nature of the T2 images allowed for the calculation of kidney volumes with minimal motion artifacts due to the breath-hold acquisition technique.

The T_1 Modified Look-Locker Inversion map was then captured to evaluate corticomedullary differentiation. This sequence is sensitive to T1 relaxation times, meaning that the distinction between the cortex, which has a shorter T1 relaxation time, and the medulla, which has a longer T1 relaxation time, can be distinguished[195]. The T1 map provides valuable information on tissue composition and integrity.

The volumetric T2-weighted images were acquired using the following parameters to ensure optimal image quality and resolution: a slice thickness of 3.0 mm, matrix size of 320 × 256, field of view 380 mm, acquired voxel size 1.2 × 1.2 × 3 mm, echo time 1.26 ms, repetition time 3.17 ms and flip angle 7°. These parameters provided high spatial resolution, allowing for accurate delineation of the kidney boundaries. The slice thickness and scan time also ensured sufficient detail for volume calculation whilst maintaining patient comfort during the scan.

The T1 mapping was performed using a 5-3 s - 3 sampling scheme, with the following specific settings for optimal T1 mapping: oblique coronal orientation, repetition

time/echo time of 2.7 / 1.15 ms, a flip angle of 35°, matrix size of 194 × 192, field of view of 320 × 320 mm^2, slice thickness of 5.5 mm, simulated heart rate of 1s, inversion times (TI1/TI2) 100 ms / 180 ms.

XII. Kidney Volume Analysis

Total kidney volumes were calculated using the voxel count approach in CVI42 Version 5.12.1 (Circle Cardiovascular Imaging Inc, Calgary, Canada). This method involves quantification of kidney volume by counting the number of voxels within the contoured boundaries of the kidney, with each voxel corresponding to a small unit of tissue volume. The voxel count approach is highly accurate, as it involves contouring multiple consecutive two-dimensional slices that cover the full three-dimensional volume of the kidneys[196]. After the regions of interests are contoured, the total volume is derived by summing all the voxels contained within the kidney's boundaries across all slices. (*Figure 9A)*.

Before contouring the kidneys, the images were quality-checked to ensure that all slices were properly acquired and that no sections were missing due to scanning errors. Any scans that had incomplete or missing slices were excluded from the analysis to maintain the accuracy and integrity of the volume measurements. The kidneys were then segmented manually through contouring the perimeter of the kidneys on two-dimensional sagittal slices. I performed the manual contouring by carefully tracing the perimeter of the kidneys, slice by slice. This was done to ensure an accurate representation of the kidney's shape and to capture subtle anatomical variations, especially in the presence of anatomical asymmetry or subtle renal changes.

Once the contours were complete, they were then reviewed and validated by a trained blinded investigator to minimise potential bias and to ensure accuracy. The CVI42 program then automatically interpolated between traced slices to create a continuous three-dimensional representation of the kidneys. The software subsequently summed the voxel volumes enclosed within the contoured regions, providing a precise calculation of kidney volume. Kidney volumes were calculated for right and left kidneys individually and then the total of these was summed to give total kidney volume.

To account for individual differences in body size, the calculated kidney volumes were indexed to body surface area using the Mosteller formula[197], which is commonly used to adjust for variations in body size and provide a more comparable measure of kidney volume across a range of participants.

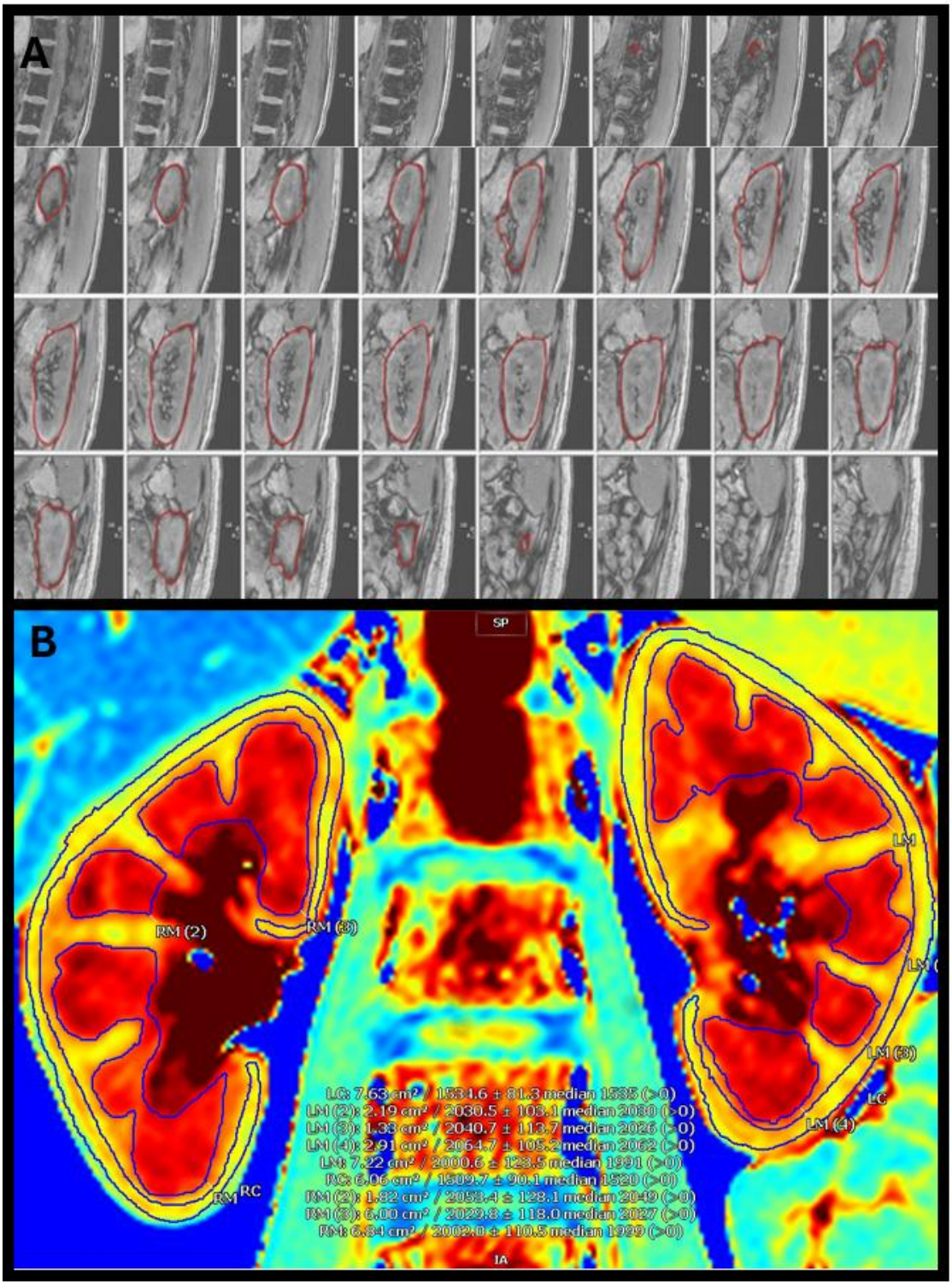


Figure 9. Analysis of renal structure using magnetic resonance imaging in CVI42. Panel A - Kidney volume calculations using the total voxel approach. Panel B - Corticomedullary differentiation contours.

XIII. Inter and Intra-Observer Reliability Assessments

To evaluate inter-observer reliability of the kidney volume measurements, a third blinded investigator contoured 20 randomly selected kidney volumes (both left and right) from ten patients in each of the three groups: normotensive, gestational hypertension, and preeclampsia. This selection ensured that a diverse range of kidney volumes were included.

A Bland-Altman analysis was then conducted with the contoured volumes to assess the degree of consistency between the observers. Bland-Altman analysis involves calculating the mean difference between the two measurements and determining the limits of agreement. If the limits of agreement are narrow and centred around zero, it indicates good agreement between the measurements. Conversely, wider limits may suggest discrepancies between the observers.

For intra-observer reliability, I independently recontoured 20 randomly selected kidney volumes from ten patient's a few months after the initial contouring to assess the degree of consistency over time by the same observer. Again, Bland-Altman analysis was applied to evaluate the degree of agreement between the measurements taken at different time points by the same observer.

XIV. Corticomedullary Differentiation Analysis

T1 mapping were performed using data acquired through the MOLLI technique, following methods adapted from previously validated studies[198]. T1 values were estimated using a three-parameter curve model in the form:

$$T1(x) = A - B \cdot \exp\left(-\frac{x}{T1}\right)$$

Curve fitting was implemented using the Levenberg-Marquardt algorithm, a non-linear least squares optimisation method. Alongside the T1 maps, R^2 goodness-of-fit and residual error maps were generated for each scan to aid in quality control. To ensure anatomical accuracy, T2-weighted images were used to guide the segmentation of the kidneys. These images provided high contrast between the renal parenchyma and surrounding structures, enabling clear identification of kidney boundaries.

Once segmented, regions of interest masks for the renal cortex and medulla were defined based on histogram analysis of the T1 values within each kidney. To increase robustness and reduce sensitivity to outliers, the upper and lower semi-quartiles of voxel intensities were excluded from each region's histogram. This step removed extreme values caused by artifacts or partial volume effects and also ensured that peripheral areas of the kidney subject to partial volume averaging, particularly at the outer cortex near perirenal fat or the central medulla adjacent to renal sinus fat were excluded[198].

These areas are especially prone to distortion from residual registration errors or signal contamination, which can falsely elevate or reduce T1 values. In addition, care was taken to avoid banding artifacts that can occur in MOLLI acquisitions due to off-resonance effects, especially at higher field strengths[191]. Even small frequency offsets can cause

substantial error in T1 quantification, so the exclusion of them was essential to maintain accuracy.

A pseudo colour scale was applied to all T1 maps to visually enhance corticomedullary contrast and assist with the placement of regions of interest. The scale ranged from 50 to 2500 arbitrary brightness units (a.b.u.), with 2000 a.b.u represented in red to indicate the medulla and 1500 a.b.u. in yellow, which indicated the cortex (*Figure 9B)*. This colour scale standardised region of interest selection across participants.

I then placed ellipsoid regions of interest within the cortex and medulla in both the left and right kidneys, avoiding large vessels and artifact-prone regions. The mean T1 values for the regions of interest in the left and right cortex and medulla were computed separately. From these values, corticomedullary differentiation was calculated for each kidney as the ratio of the cortical to medullary T1 values using the following equation:

$$CMD = \frac{T1cortex}{T1medulla}$$

A higher corticomedullary differentiation ratio typically reflects preserved corticomedullary differentiation contrast and structural integrity. Meanwhile, a lower corticomedullary differentiation ratio suggests that there is some form of underlying alterations to the structural integrity of the kidneys[195].

As part of the quality control process, each T1 map was rated according to a predefined artifact severity scale ranging from zero to three. If there were no artefacts on either kidney, a quality score of zero was given. If there was minor artefact affecting one kidney, a score of one was given. If there was major artefact affecting one kidney or minor artefact

affecting both kidneys, a score of two was given and if there were major artefacts on both kidneys, a score of three was given.

Scans receiving a score of three were excluded from any further analysis due to significant degradation of image quality. Scans which scored either one or two were re-examined in detail. If the artifact was found to compromise T1 accuracy in either kidney, those individual kidneys were excluded from the analysis. Importantly, all quality control checks and exclusions of renal images were completed prior to conducting any statistical testing to prevent bias in the final results. Quality checks were completed by myself and two other investigators.

XV. Statistical Analysis

Statistical analysis was conducted using R (version 4.4.2) and R Studio (version 2024.12.0 + 467). Normality was tested using the Shapiro-Wilks test for continuous variables where the number of samples was less than or equal to 50 and for larger samples, visual observation using Q-Q plots and histograms were used. Normally distributed continuous variables were reported as means with standard deviations. Whereas continuous variables with skewed distributions were reported as medians with interquartile ranges. Categorical data were presented as counts and percentages.

The specific comparative analyses presented here were exploratory and they were not pre-registered in any dedicated statistical analysis plan. Nevertheless, the comparisons of primary and secondary outcomes across the hypertensive and normotensive groups were planned before any analysis was begun. Post-hoc tests were then conducted to further investigate statistical differences following the initial statistical tests.

Differences across group means of normally distributed continuous variables were tested using an analysis of covariance adjusting for time postpartum and age as covariates to assess for any confounding effects. Levene's test was carried out to check the homogeneity of variance. For multiple comparisons, between group differences were tested using the Tukey's Honest Significant Difference test.

For variables with skewed distributions, differences across group medians were tested using the Kruskal-Wallis test, with between group differences tested using the Dunn's post-hoc test and Bonferroni correction for multiple comparisons. Differences across categorical variables were tested using the chi-square test and post-hoc pairwise Z tests.

Pearson's correlation coefficient was used to look for associations between normally distributed variables and the Spearman's Rank Correlation coefficient test was used to examine any associations between non-parametric variables.

A sensitivity analysis was conducted to investigate the effect of telemonitored home blood pressure monitoring postpartum compared to standard NHS care on renal outcomes in the hypertensive group. This involved using a Welch's two-sample t-test to assess differences in mean total kidney volume indexed to body surface area between participants who received tightly controlled blood pressure to those who received standard care. The Mann-Whitney U test was employed to compare corticomedullary differentiation across the two groups.

A post hoc power analysis was conducted using G*Power to assess whether the sample size was adequate to detect a significant effect using a one-way analysis of variance[199]. The analysis assumed a medium effect size (Cohen's f = 0.30) at an alpha level of 0.05.

XVI. Results - Study Population

Renal imaging data were available for 125 women at 6-12 months postpartum. This dataset included 61 women with preeclampsia (13 early-onset, 48 late-onset) and 33 women with gestational hypertension, as well as 31 women with normotensive pregnancies.

Of the preeclamptic group, 36 out of 61 women (59%) were randomised to telemonitored home blood pressure management postpartum and 25 out of 61 women (41%) were given standard NHS care. Of the gestational hypertension group, 19 out of 33 women (57.6%) were randomised to telemonitored home blood pressure management postpartum and 14 out of 33 women (42.4%) were given standard NHS care.

Full demographic, anthropometry and pregnancy characteristics of the renal imaging subgroup are reported in *Table 3*. Time of postpartum follow-up in days was equivalent in each group as was participant age but those with normotensive pregnancies had lower body mass index, low-density lipoprotein levels, triglyceride levels and blood pressure at 6-12 months postpartum compared to those with either preeclampsia or gestational hypertension during pregnancy ($p < 0.05$).

Table 3. A summary of the participant anthropometry, demographic data, clinical blood pressure readings, blood test results and pregnancy characteristics.

Variable	NT (N = 31)	PET (N = 61)	GH (N = 33)	χ^2 or F	Post Hoc
Anthropometry and demographic variables at 6-12 months postpartum					
Age, years	31.1 ± 4.8[a]	32.9 ± 4.7[a]	33.6 ± 5.4[b]	*0.27*	
Time postpartum, days	259.1 ± 52.7[a]	256 ± 13[b]	257.4 ± 17.7[a]	*1.96*	
Indices of Multiple Deprivation	8 ± 4[b]	8.5 ± 3[b]	8 ± 3[b]	*1.91*	
Height, m	1.6 ± 0.06[a]	1.6 ± 0.07[a]	1.7 ± 0.06[a]	*2.93*	
Weight, kg	69 ± 14.9[b]	75.1 ± 22[b]	84.8 ± 20.5[b]	*9.38**	△▲
BMI, kg/m^2	25.9 ± 6.9[b]	28.5 ± 10.3[b]	30.6 ± 6.5[b]	*9.39**	△▲
Waist: hip ratio (cm:cm)	0.9 ± 0.09[b]	0.9 ± 0.05[a]	0.9 ± 0.06[a]	8.91*	△▲
Mid left arm circumference (cm)	28 ± 4[b]	32.1 ± 4.4[a]	32 ± 6[b]	15.06*	△▲
Systolic Blood Pressure, mmHG,	111.1 ± 12[a]	127.1 ± 11[a]	129.2 ± 8.5[a]	*28.44**	△▲
Diastolic Blood Pressure, mmHG,	68.6 ± 7.5[a]	84 ± 8.1[a]	85.9 ± 7.7[a]	*49.15**	△▲
Breastfeeding, No., (%)	23 (74.2)	23 (37.7)	11 (33.3)	*7.53**	△▲

Ethnicity					
Asian, No., (%)	4 (12.9)	3 (4.9)	3 (9.1)	0.94	
Hispanic or Latino, No., (%)	0 (0)	1 (1.6)	3 (9.1)	5.03	
Carribbean, African or other background, No., (%)	4 (12.9)	4 (6.6)	1 (3)	2.23	
Non-Hispanic White and Eastern European, No., (%)	23 (74.2)	53 (86.9)	26 (78.8)	0.40	
Antenatal measurements at 12 week booking appointment					
Groups	**NT**	**PET *(N=61)***	**GH**	**χ^2 or F**	**Post**
	(N = 31)		***(N =33)***		**Hoc**
Antenatal booking height, m, Mean ± SD	1.7 ± 0.06[a]	1.6 ± 0.07[a]	1.7 ± 0.07[a]	3.31*	▧
Antenatal booking weight, kg, Median ± IQR	66 ± 10.9[b]	73 ± 18.5[b]	75 ± 17.5[b]	8.01*	▲
Antenatal booking BMI, kg/m2, Median ± IQR	24.3 ± 4.6[b]	26.6 ± 8.12[b]	27.4 ± 5.1[b]	8.85*	△▲
Antenatal booking SBP, mmHg, Mean ± SD	112.7 ± 9.7[a]	116.7 ± 10.48[a]	121.5 ± 11.3[a]	5.62*	▲
Antenatal booking DBP, mmHg, Mean ± SD	67.4 ± 10.1[a]	71.8 ± 8.5[a]	75.1 ± 9.2[a]	5.68*	▲
Pregnancy Characteristics					
Previous hypertensive pregnancy No., (%)	0 (0)	8 (13.1)	8 (24.2)	9.24*	△▲

Nulliparous, No., (%)	17 (54.8)	46 (75.4)	20 (60.6)	5.21	
Neonatal unit admission, No., (%)	1 (3.2)	16 (26.2)	7 (21.2)	5.88	
Pre-pregnancy smoking reported, No., (%)	2 (6.5)	15 (24.6)	12 (36.4)	7.60*	△▲
Gestational diabetes mellitus, No., (%)	0 (0)	6 (9.8)	3 (9.1)	3.21	
Delivery Method					
Spontaneous Vaginal Delivery, No., (%)	19 (61.3)	18 (29.5)	16 (48.5)	8.59*	△
Instrumental, No., (%)	1 (3.2)	12 (19.7)	6 (18.2)	4.62	
Emergency caesarean-section, No., (%)	4 (12.9)	30 (49.2)	9 (27.3)	12.99	
Elective caesarean-section, No., (%)	7 (22.6)	1 (1.6)	2 (6.1)	12.47*	△
Birth characteristics					
Birthweight, kg	3.6 ± 0.6[a]	2.9 ± 0.9[a]	3.3 ± 0.6[a]	9.28*	△▧
Gestation at delivery, weeks	39.6 ± 1.1[a]	37.3 ± 3.8[a]	39.8 ± 1.7[a]	10.05*	△▧

Results are represented as Mean ± SD[a], Median ± IQR[b] or counts and percentages. BMI = Body Mass Index, cm = centimetres, DBP = Diastolic Blood Pressure, GH = Gestational Hypertension, IQR = Interquartile range, kg = Kilograms, m^2 = meters squared, mmHg = millimetres of mercury, NT = Normotensive, N/No = Number, PET = Preeclampsia, SBP = Systolic Blood Pressure, SD = Standard deviation, △ Differences between normotensive and pre-eclamptic ▲ Differences between normotensive and gestational hypertension ▧ Differences between preeclamptic and gestational hypertension. *Represents significant results.

XVII. Data availability and power analysis

Of the 125 renal imaging datasets, comprising 250 kidneys, 122 right kidney volumes and 114 left kidney volumes were of sufficient quality for analysis. As a result, total kidney volume indexed to body surface area was compared across 114 participants (91.2%). Additionally, 115 out of 125 women (92%) had sufficient quality T1 maps for analysis of corticomedullary differentiation. The post hoc power analysis indicated that a sample of 114 participants provided 80% power at $p = 0.05$ to identify a medium effect size, suggesting that the sample size was sufficient. The renal characteristics of the participants at birth and at 6-12 months postpartum are presented in *Table 4.*

Table 4. Renal characteristics at birth and 6-12 months postpartum including magnetic resonance imaging variables, blood biomarker levels one day after delivery and 6-12 months postpartum.

Variable	NT *(N = 31)*†‡	PET *(N = 61)* ‡	GH *(N = 33)* ‡	χ^2 or F	Post Hoc
Renal Magnetic Resonance Imaging Characteristics at 6-12 months postpartum					
BSA-adjusted Total Kidney Volume (ml/m^2)	206.3 ± 24.6[a]	190.8 ± 26.9[a]	203 ± 24.7[a]	4.63*	△
BSA-adjusted Right Kidney Volume (ml/m^2)	100.1 ± 13.9[a]	93 ± 13.2[a]	100.6 ± 13.2[a]	3.87*	△▨
BSA-adjusted Left Kidney Volume (ml/m^2)	105.2 ± 13.6[a]	97.9 ± 14.7[a]	102.15 ± 13.03	2.44	
Left cortex (cm^2)	5.74 ± 1.12[a]	5.38 ± 1.47[a]	4.90 ± 1.61	2.04	
Right cortex (cm^2)	6.37 ± 1.10[a]	6.20 ± 1.45[a]	6.26 ± 1.76[b]	0.29	
Left medulla (cm^2)	11.03 ± 3.35[b]	10.23 ± 3.17[b]	11.10 ± 2.77[b]	2.41	
Right medulla (cm^2)	10.94 ± 2.47[a]	10.61 ± 3.17[a]	12.10 ± 2.93[a]	2.52	
Mean T1 Cortex (cm^2)	11.67 ± 2.47[a]	10.91 ± 3.11[a]	10.73 ± 3.28[a]	0.79	
Mean T1 Medulla (cm^2)	21.19 ± 6.26[a]	19.57 ± 6.53[a]	22.09 ± 5.6[a]	1.76	
Corticomedullary differentiation	0.58 ± 0.164[a]	0.59 ± 0.179[b]	0.51 ± 0.17[b]	6.47*	▲▨
Bloods one day after Delivery					

Creatinine (umol/L)	53 ± 16[b]	64 ± 16[b]	57.69 ±11.86[a]	16.08*	△▨
Urea (mmol/L)	3.25 ± 1.38[b]	3.9 ± 1.45[b]	3.1 ± 1.7[b]	5.85	
EGFR ml/min/1.73 m^2	121 ± 7[b]	111 ± 26[b]	120 ± 17[b]	13.57*	△▨
Potassium (mmol/L)	3.8 ± 0.2[b]	4 ± 0.5[b]	4 ± 0.2[b]	3.64	
Sodium (mmol/L)	137 ± 2[b]	135.80 ± 2.46[a]	137 ± 3[b]	2.07	
Bloods at 6-12 months Postpartum					
Total cholesterol (mmol/L)	4.56 ± 0.63[a]	5.04 ± 1.01[a]	4.8 ± 0.68[a]	2.87	
HDL Cholesterol (mmol/L)	1.5 ± 0.23[a]	1.42 ± 0.42[a]	1.35 ± 0.31[a]	1.37	
LDL Cholesterol (mmol/L)	2.68 ± 0.55[a]	3.64 ± 1.05[a]	3.45 ± 0.67[a]	10.79*	△▲
Triglycerides (mmol/L)	0.81 ± 0.39[a]	1.41 ± 0.81[a]	1.39 ± 0.9[a]	5.96*	△▲
Creatinine (umol/L)	61.05 ± 8.11[a]	62.97 ± 8.49[a]	62.21 ± 8.5[a]	0.46	
Urea (mmol/L)	4.89 ± 1.37[a]	4.549 ± 1[a]	4.18 ± 0.92[a]	3.05	
EGFR ml/min/1.73 m^2	114 ± 9.67[a]	111.49 ± 11.61[a]	111.59 ± 11.54[a]	0.48	
Potassium (mmol/L)	4.24 ± 0.46[a]	3.86 ± 0.34[a]	3.85 ± 0.28[a]	10.49*	△▲
Sodium (mmol/L)	139.96 ± 1.66[a]	140 ± 1.59[a]	140.34 ± 1.49[a]	0.59	

Glucose (mmol/L)	4.78 ± 0.64[a]	4.85 ± 0.6[a]	4.55 ± 0.71[a]	2.34	
Insulin (pmol/L)	61.09 ± 34.05[a]	110.78 ± 81.19[a]	111.19 ± 89.55[a]	4.04*	△▲

Results are presented as Mean ± SD[a], Median ± IQR[b] . EGFR = Estimated glomerular filtration rate, GH = Gestational Hypertension, HDL = high-density lipoprotein, IQR = Interquartile range, LDL = Low-density lipoprotein, umol/L = micromoles per litre, mmol/L = millimoles per litre, NT = Normotensive, N = Number, PET = Preeclampsia, pmol/L = picomoles per litre, SD = Standard deviation. †Only 17 normotensive women had bloods at delivery. ‡11 kidneys were excluded from volume analysis and 11 renal datasets for corticomedullary different due to image quality. △ Differences between normotensive and pre-eclamptic ▲ Differences between normotensive and gestational hypertension ▧ Differences between preeclamptic and gestational hypertension. *Represents significant results.

XVIII. Kidney volumes Indexed to Body Surface Area

Total kidney volumes indexed to body surface area varied across the three groups (F = 4.63, p = 0.012). After adjusting for age and time postpartum, the group differences remained significant (p = 0.027) and neither age nor time were significant covariates in the model (p = 0.46 and p = 0.22 respectively). The post hoc analysis subsequently revealed that the preeclamptic group had lower total kidney volume than the normotensive group (mean difference: -15.42 ml/m^2, 95% CI: [-30.84, -0.005] p = 0.049), with no significant differences between the normotensive group and the gestational hypertension group (mean difference: -3.3 ml/m^2, 95% CI: [-20.70, 14.10] p = 0.89), (*Figure 10).*

There was a significant difference in right kidney volume indexed to body surface area across the groups [F = 3.87, p = 0.02], and a trend towards reduced left kidney volume (F = 2.44, p = 0.09). After adjusting for age and time postpartum, the difference in right kidney volumes remained significant (p = 0.011). The preeclamptic group had lower right kidney volumes than the normotensive group (mean difference: -7.11 ml/m^2, 95% CI: [-14.13, -0.09], p = 0.046) and the gestational hypertension group (mean difference: 7.66 ml/m^2, 95% CI: [0.56, 14.76] p = 0.03), with no significant differences between the normotensive and gestational hypertension group (mean difference: 0.55 ml/m^2, 95% CI: [-7.60, 8.70], p = 0.99).

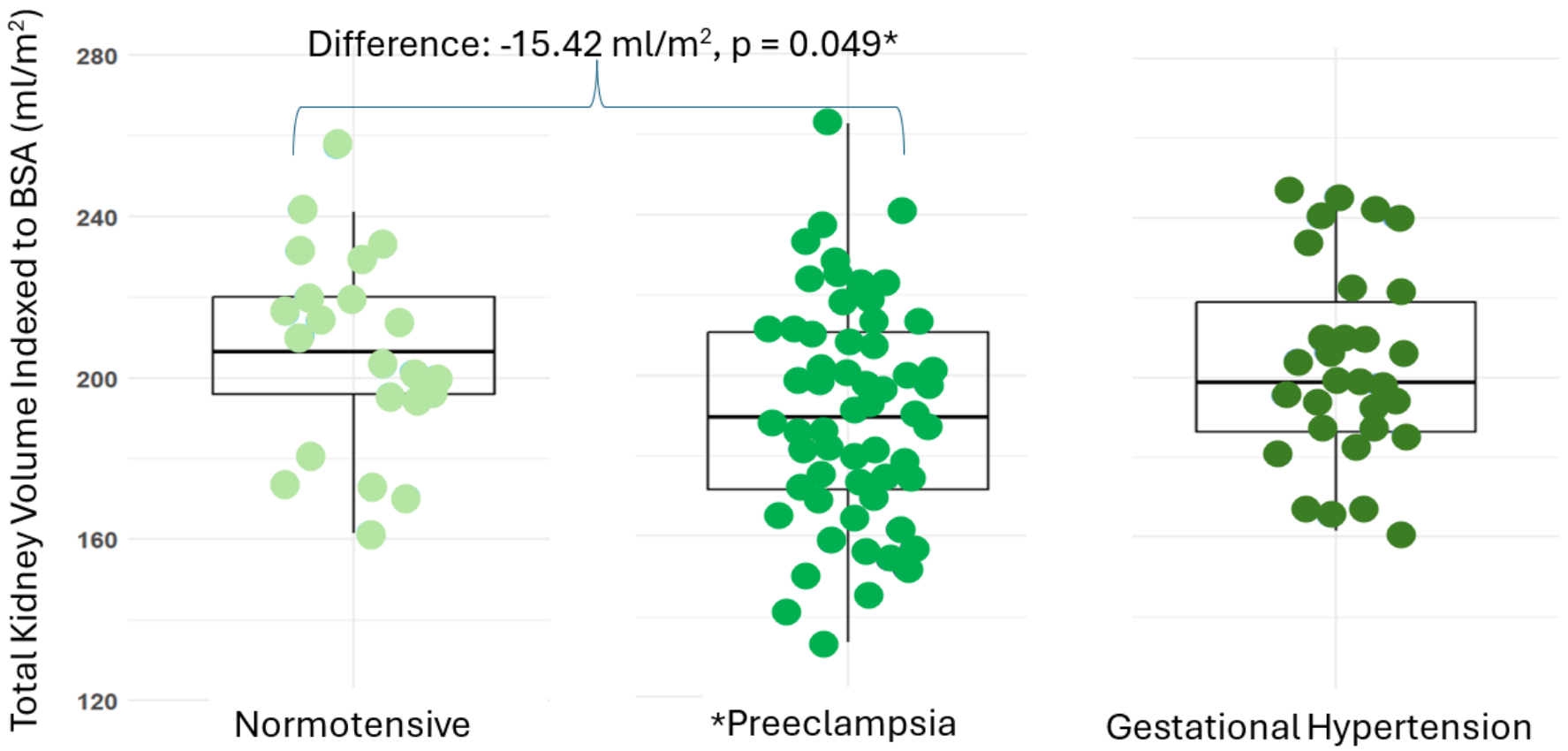


Figure 10. Boxplots comparing kidney structure across groups. Total kidney volume indexed to body surface area across normotensive (light green), preeclampsia (medium green) and gestational hypertension (dark green) groups.

XIX. Corticomedullary differentiation

There were no significant differences in mean T1 cortex or medulla values between the groups (*Table 4)*. However, there was an overall significant difference in corticomedullary differentiation across the three groups (χ^2 = 6.47, df = 2, p = 0.04) (*Figure 11)* due to lower corticomedullary differentiation in the gestational hypertension group compared to the preeclamptic (β: 2.42, p = 0.02) and the normotensive group (β: 2.01, p = 0.007). There was no significant effect of age (p = 0.61) or time postpartum (p = 0.32) on corticomedullary differentiation.

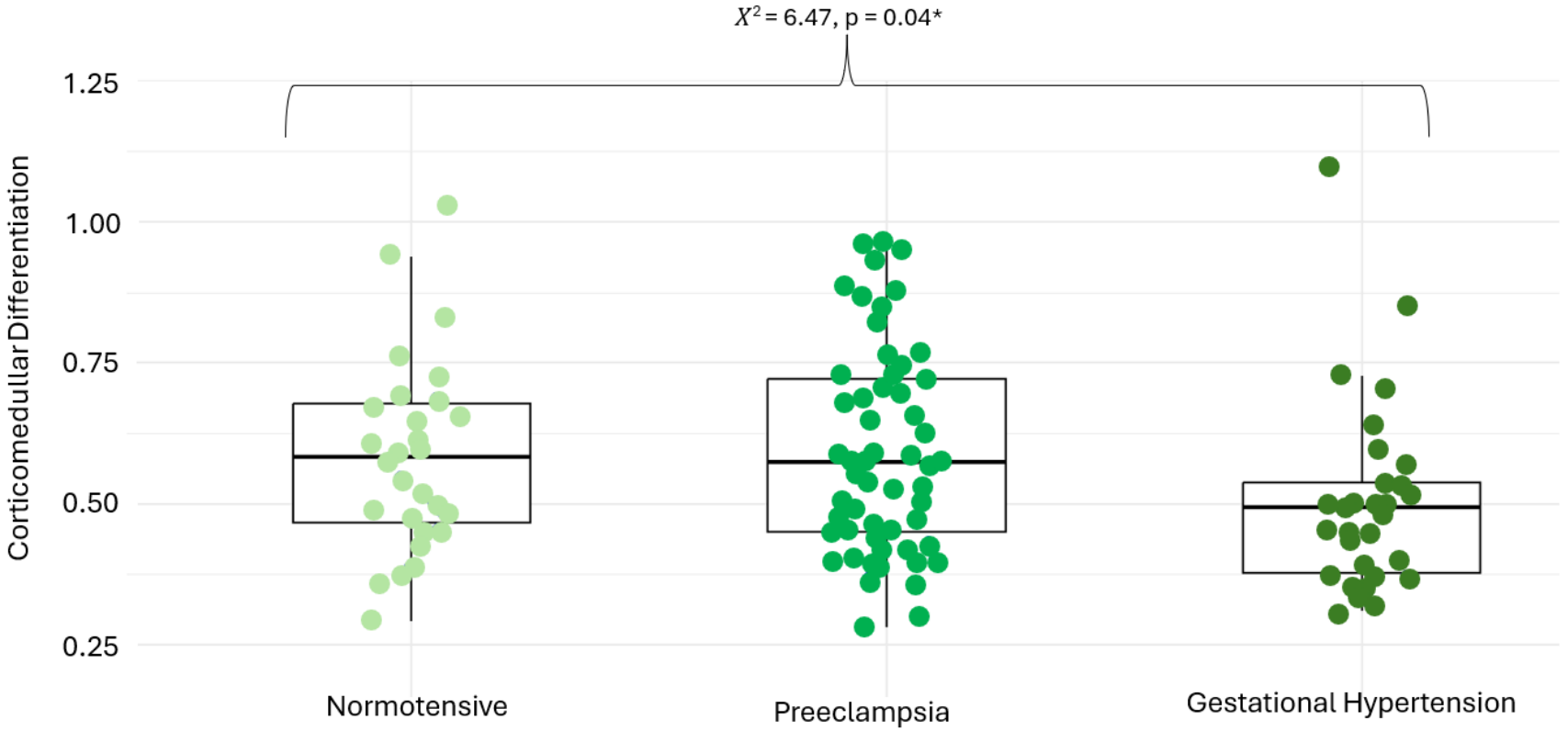


Figure 11. Corticomedullary differentiation across groups. Boxplots with overlaid dots showing corticomedullary scores for each participant, with each participants score represented by a dot. The scores of women with normotensive pregnancies are shown in light green, the women with preeclampsia are represented in medium green and the women with gestational hypertension are represented by dark green dots. *Represents significant results.

XX. Structural Renal Differences in Early vs Late-Onset Preeclampsia

Out of the 61 preeclamptic women, 13 women were classed as early-onset (≤ 33 + 6 weeks gestation) and 48 women were classed as late-onset (> 34 weeks gestation). The early-onset group had on average lower renal volumes but there were no significant differences between groups (median difference: 13.3 ml/m^2, p = 0.896). Additionally, on average the early onset preeclamptic group had lower corticomedullary differentiation (median difference: 0.077), but this also did not reach statistical significance (p = 0.37).

XXI. Renal Function

At the time of delivery, 14 out of 61 (23%) women with preeclampsia had estimated glomerular filtration rates ≤ 90 mL/min/1.73 m^2, compared to three out of 33 (9%) women with gestational hypertension and one out of 17 (5%) women who had normotensive pregnancies. The absolute levels of estimated glomerular filtration rates or creatinine were significantly different between groups at the time of delivery *(Table 4).* By the time of postpartum follow-up estimated glomerular filtration rates had returned to > 90 mL/min/1.73 m^2 for 113 out of 119 (94.9%) women.

Of the women with estimated glomerular filtration rates of ≤ 90mL/min/1.73 m^2, five women had preeclampsia, and one woman had gestational hypertension during their pregnancy. However, there were no between-group differences in absolute levels of estimated glomerular filtration rates or creatinine at the time of postpartum follow-up (*Table 4*).

There was a positive correlation between total kidney volume indexed to body surface area at 6-12 months postpartum and estimated glomerular filtration rate at the time of

delivery (Spearman's rho (ρ): 0.47, p < 0.001) but not with estimated glomerular filtration rates at 6-12 months postpartum (Spearman's rho (ρ): 0.13, p = 0.19) (*Figure 12)*.

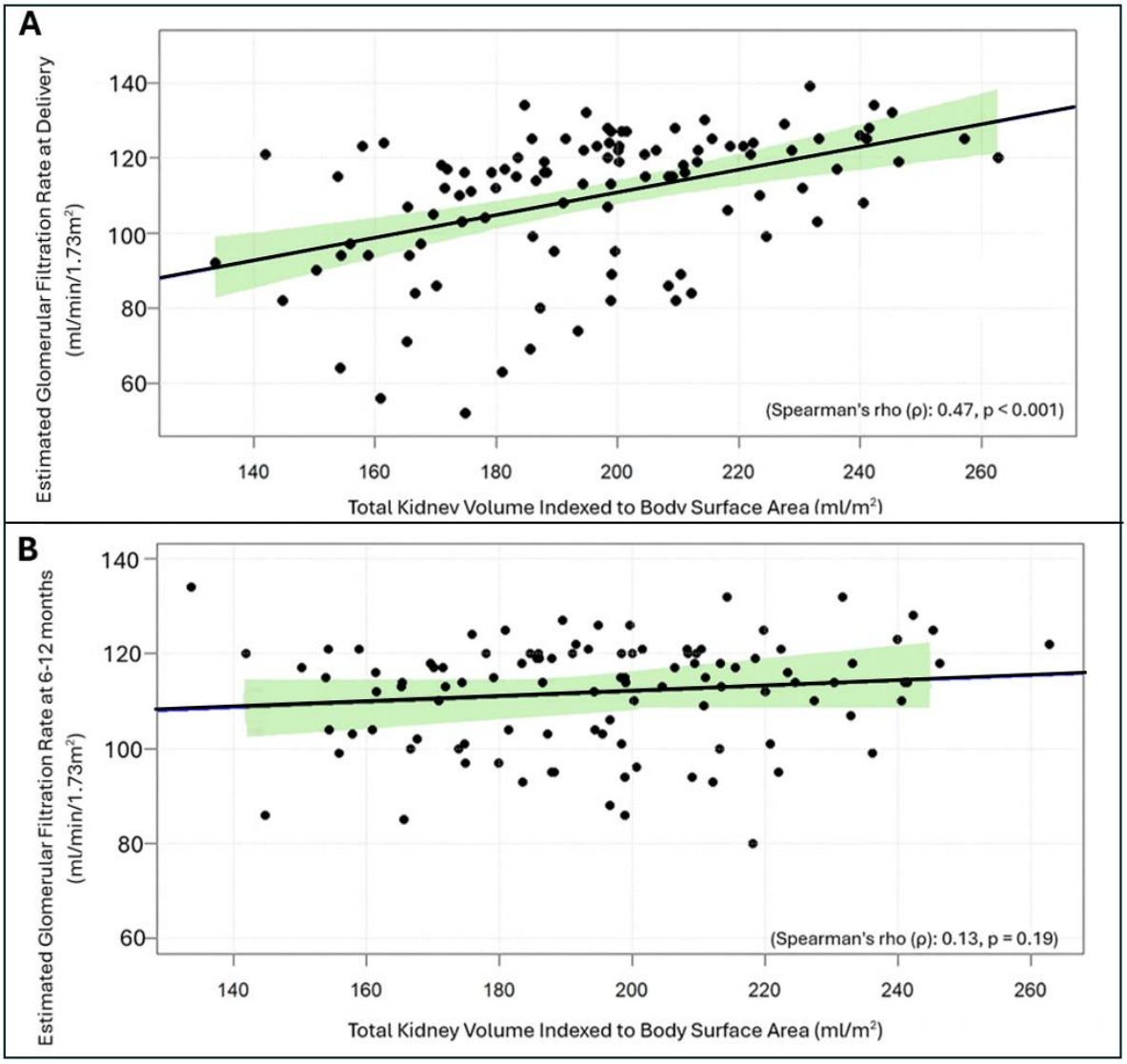


Figure 12. Linear regression models showing total kidney volume indexed vs estimated glomerular filtration rates at one day after delivery (Panel A) and 6-12 months postpartum (Panel B) for all participants. *Represents significant results.

XXII. Associations between Renal Outcomes, Blood Pressure, Body Mass Index and Blood Pressure Management Postpartum

At 6-12 months postpartum, total kidney volumes indexed to body surface area were not significantly associated with either systolic (r = 0.0108, t(111) = 0.114, 95% CI, [-0.174 to 0.195], p = 0.91) or diastolic blood pressure (r = -0.073, t(111) = -0.77, 95% CI [-0.2539631 to 0.1136132], p = 0.44) (*Figure 13)*, nor measures of body mass index at the postpartum visit (p > 0.05). Similarly, there were no associations between corticomedullary differentiation, renal function, blood pressure or body mass index (p > 0.05).

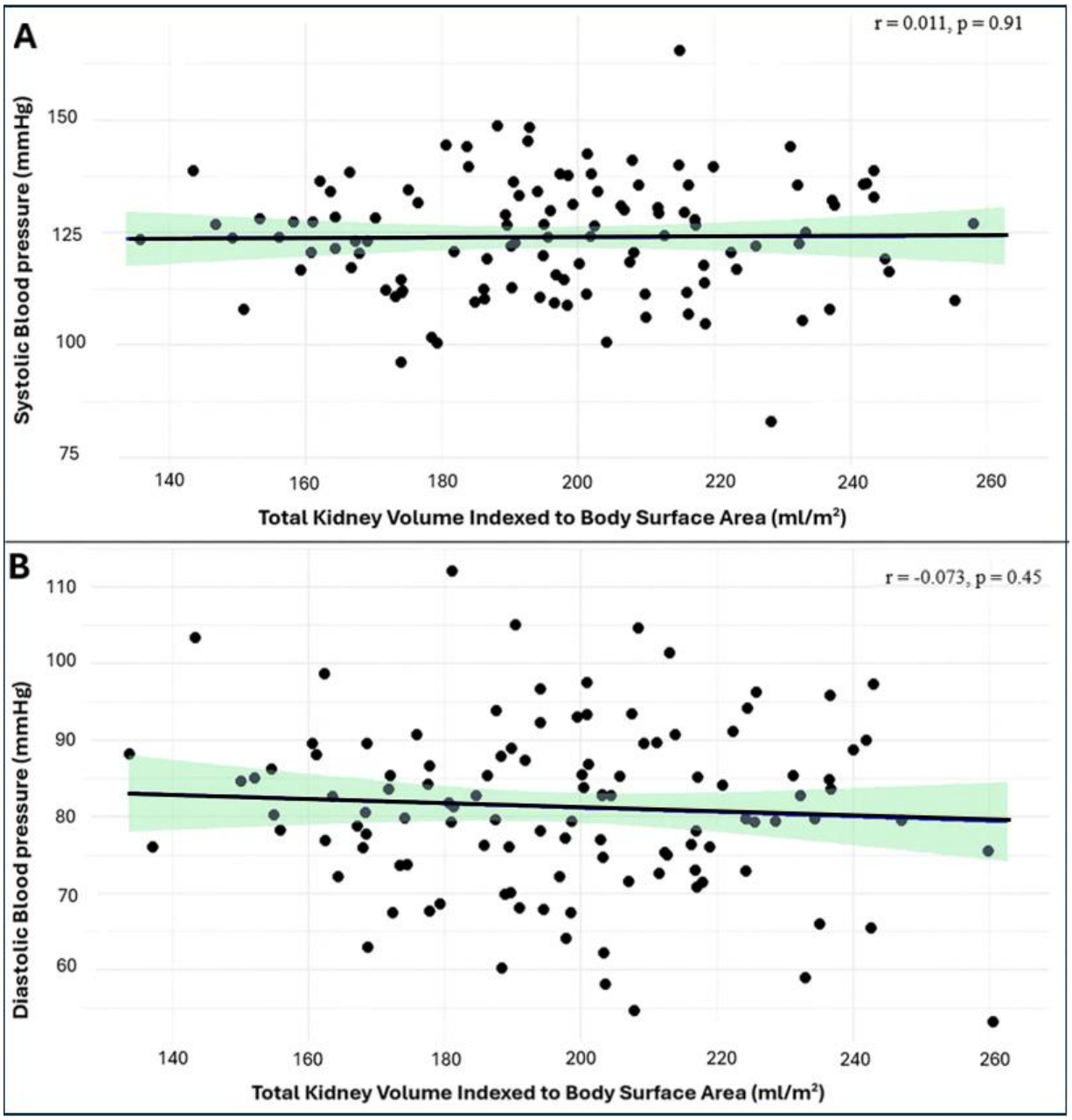


Figure 13. Linear regression models showing systolic **(Panel A)** and diastolic blood pressure 6-12 months postpartum **(Panel B)** vs total kidney volumes indexed to body surface area.

Furthermore, when comparing the effect of postpartum blood pressure management, there were no significant differences between those who received telemonitored home blood pressure management and those who received standard NHS care postpartum.

Specifically, there were no group differences in total kidney volume, corticomedullary differentiation or renal function (all $p > 0.05$).

XXIII. Inter-observer and Intra-observer Variability Results

Inter-observer agreement was high, with a mean difference of -3.02 ml/m^2 (95% limits of agreement: -11.72 to 5.69), indicating minimal measurement bias between observers and tight agreement across the datasets *(Figure 14).* This result reflects the robustness of the manual contouring and voxel count approach, suggesting that different trained researchers reliably performed these measurements.

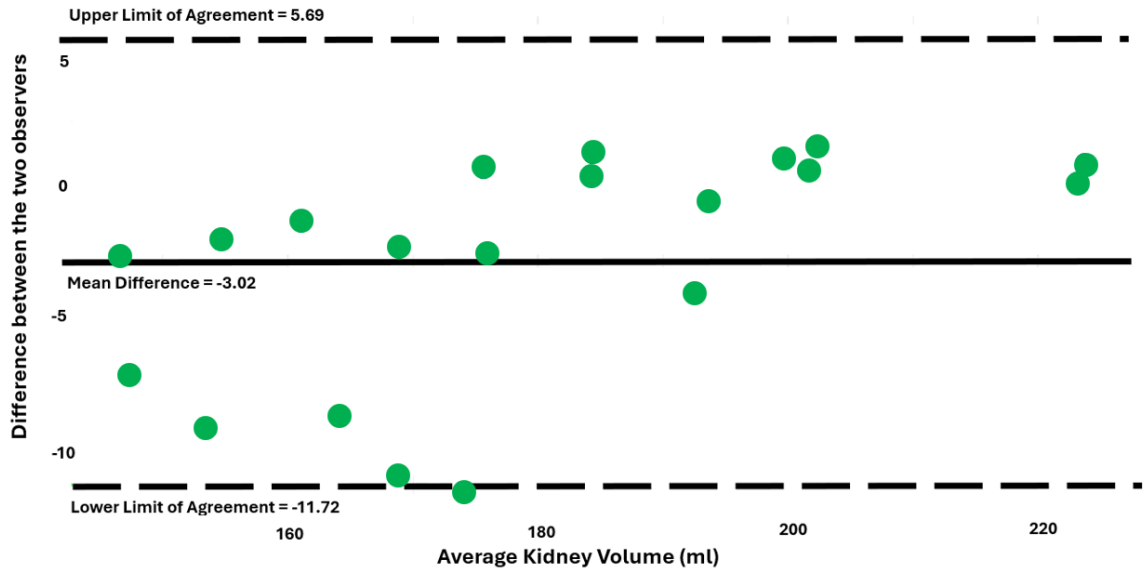


Figure 14. Inter-observer variability limits of agreement. The difference in kidney volumes measured by two different observers, tested using Bland-Altman Analysis.

Furthermore, intra-observer variability was even lower, with a mean difference of just 0.56 ml/m^2 (95% limits of agreement: -3.03 to 4.16), confirming excellent within-

observer consistency over time *(Figure 15).* This demonstrates that the contouring protocol was not only accurate but also highly repeatable, supporting the validity of the methodology used for renal volume quantification.

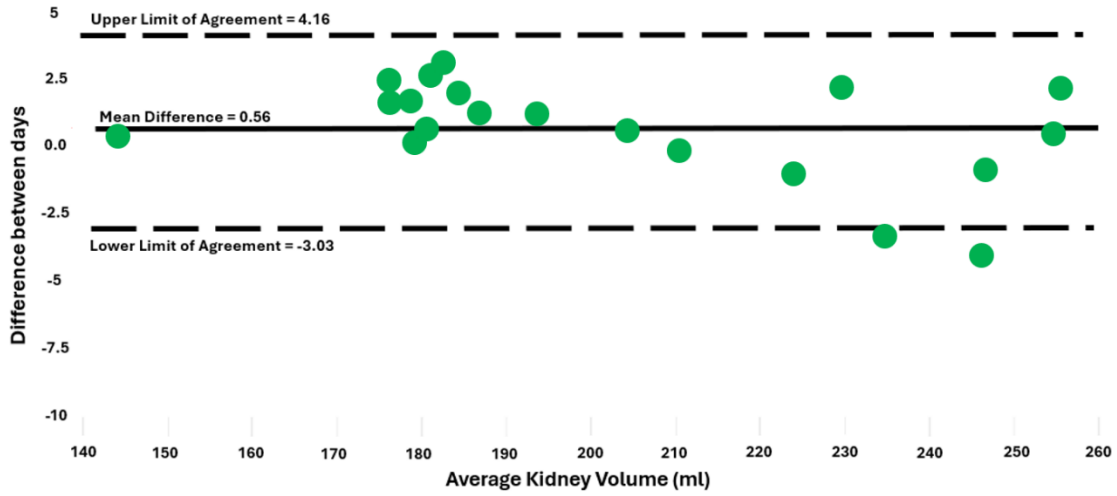


Figure 15. Intra-observer variability agreement. The difference in kidney volumes over multiple days by the same observer, tested using Bland-Altman analysis.

XXIV. Discussion

The findings presented in this chapter demonstrate persistent postpartum differences in kidney morphology among women who have experienced hypertensive disorders of pregnancy compared to their normotensive counterparts. At 6-12 months postpartum, women with a history of preeclampsia showed significantly lower total kidney volume which correlated with estimated glomerular filtration rates at delivery, but not postpartum. Importantly, this difference in total kidney volume was not explained by altered corticomedullary differentiation, which only differed in women with gestational hypertension compared to women with normotensive pregnancies, indicating distinct pathophysiological mechanisms between the two hypertensive subtypes.

A reduction in total kidney volume of just ten millilitres has previously been associated with a six to ten percent higher risk of chronic kidney disease later in life[200]. The difference in kidney volume that I observed in women with prior preeclampsia equates to a 24-millilitre reduction in absolute volume, therefore appearing clinically meaningful. These structural changes are consistent with the known five-fold increased risk of end-stage renal failure that is observed in preeclamptic women compared to women with normotensive pregnancies[20].

Furthermore, the correlation observed between kidney volume and renal filtration capacity at delivery reinforces the prognostic relevance of these structural differences. This correlation may reflect a link between renal size and the kidney's capacity to maintain homeostasis during pregnancy. Smaller kidney volumes often reflect reduced nephron numbers or functional reserves, limiting the kidney's adaptive response to increase circulatory volume[201]. With fewer functioning nephrons, smaller kidneys experience greater hemodynamic loading per glomerulus, predisposing to filtration dysfunction and proteinuria[202].

Notably, a high proportion of women with preeclampsia had an estimated glomerular filtrate rate ≤ 90mL/min/1.73m^2 at this time, despite the known rise in estimated glomerular filtration rate during healthy pregnancy[203]. This rise is normally driven by systemic vasodilation, plasma volume expansion and increased renal perfusion. Thus, the low estimated glomerular filtration rates indicate subclinical renal dysfunction and impaired renal adaptive capacity.

By 6-12 months postpartum, renal function was similar across the groups and there was no association with blood pressure at booking, during pregnancy or at follow-up. This could be explained by the physiological reversal of pregnancy-induced hemodynamic

changes which place a functional load on the kidneys[204]. After delivery, the expanded circulatory volume gradually returns to pre-pregnancy levels, reducing renal perfusion pressure and alleviating the strain on renal function. Despite this improvement in function, these findings suggest that the subclinical reductions in kidney size remain.

Given these findings, structured postpartum follow-up is important**.** Currently, the National Institute for Health and Care Excellence guidelines advise urine protein measurement six to eight weeks after delivery in individuals with preeclampsia and further review with primary care or a specialist at three months after birth to assess kidney function in those with ongoing proteinuria[4]. However, there are presently no recommendations for follow-up assessment of kidney structure. Future guidelines may need to incorporate structural and functional markers to better identify women at risk of long-term kidney disease.

Moreover, the volume differences found here may signify underlying microvascular injury. Such injury can predispose women to a range of cardiovascular, neural and metabolic disorders[205]. Conditions like hypertension, heart failure, atherosclerosis and stroke have been associated with microvascular damage, as it compromises the organ's ability to regulate blood flow and manage fluid balance efficiently[205]. In women with hypertensive pregnancies, these early renal changes might act as subtle indicators of broader vascular dysfunction, which could potentially accelerate the onset of these diseases. This hypothesis is likely given that preeclampsia is characterised by endothelial dysfunction, vasoconstriction, and abnormal placentation, all of which could contribute to microvascular injury within the kidney[36]. These changes may reduce kidney perfusion and impair the glomerular filtration barrier, leading to nephron damage and subsequent reductions in renal volume[206].

Notably, women with a history of preeclampsia were shown to have lower kidney volumes without evidence of impaired corticomedullary differentiation. Corticomedullary differentiation refers to the visible distinction between the renal cortex and medulla[195]. This structural contrast reflects the normal organisation of kidney tissue, with the cortex housing glomeruli and the medulla containing the tubular system. Loss or blurring of corticomedullary differentiation is often seen in pathological states such as chronic kidney disease, acute tubular necrosis, or advanced hypertensive nephropathy, typically reflecting tissue damage, inflammation, fibrosis, or loss of normal nephron architecture[195].

The preservation of corticomedullary differentiation in these women, despite reduced kidney volume, suggests that the kidneys may not have undergone overt structural injury during pregnancy. Instead, this pattern points towards a pre-existing structural predisposition, such as a lower nephron endowment or smaller baseline kidney size. This finding is supported by the fact that I did not find any differences between those who had tighter control of blood pressure to usual care. Together, these findings challenge the notion that pregnancy-induced hypertension is the primary driver of structural renal changes, raising the possibility that women who develop preeclampsia may already have underlying renal or vascular differences that predispose them to both preeclampsia and their long-term renal risk.

In contrast to women with preeclampsia, women with gestational hypertension did not show any differences in renal volume compared to the normotensive pregnancy group. However, they did demonstrate a loss of corticomedullary distinction. This diminished distinction between the cortex and medulla may indicate ongoing damage due to sustained high blood pressure for prolonged time periods, for example during

pregnancy[207]. Supporting this, the women with gestational hypertension delivered at a later gestational age than those with preeclampsia and the mean antenatal blood pressure at the 12 week booking appointment in the gestational hypertensive group was significantly higher compared to the normotensive group, being 121+/-11 mmHg compared to 113+/-10 mmHg, with this difference not being evident in those who developed preeclampsia *(Table 3)*. These findings demonstrate that the women with gestational hypertension were potentially exposed to elevated pressures for a longer period. The women with gestational hypertension were also more likely to have had a previous history of hypertensive pregnancy and to smoke, both of which may influence corticomedullary differentiation and warrant consideration as potential contributing factors.

These findings raise important questions about the utility of early postpartum imaging, for identifying women at higher risk of future renal disease or recurrent hypertensive pregnancies. Kidney volume could serve as non-invasive biomarker of long-term risk, guiding personalised intervention strategies and screening procedures could be implemented to target populations at the highest risk such as women with a family history of hypertensive pregnancy.

Whilst magnetic resonance imaging is unlikely to be feasible for routine screening in the general population, renal ultrasound could offer a practical alternative for high-risk groups such as women with a history of preeclampsia. Also, combining imaging with emerging plasma and urine biomarker panels may enhance risk stratification and disease monitoring in women with hypertensive pregnancy disorders[201].

Additionally, preconception interventions aimed at optimising renal function and morphology through blood pressure optimization, lifestyle modification, and

pharmacological strategies to improve estimated glomerular filtration rate or restore kidney volume may reduce susceptibility to recurrent preeclampsia and attenuate the trajectory toward chronic kidney disease.

Future research should aim to determine which interventions are most effective for improving long-term renal outcomes in women with different hypertensive pregnancy phenotypes. Longitudinal studies could be useful to pinpoint the exact timepoints at which structural changes are occurring in women with hypertensive disorders of pregnancy. Pre-pregnancy imaging scans may also be of value to understand whether renal changes are useful markers of preeclampsia risk and postpartum scans at 6-12 months may be useful to identify women who are at most risk of future disease.

XXV. Strengths and Limitations

A key strength of this work is the use of high-resolution magnetic resonance imaging, which offers superior anatomical detail and is less susceptible to operator-dependent variability and artefacts compared to ultrasound[192]. In addition, another strength is the inclusion of a large sample size of 125 women, all of whom had detailed demographic and pregnancy-related data, adding significant depth and detailed interpretation to the analysis. Moreover, the imaging analysis techniques that I used demonstrated strong inter- and intra-observer reliability, ensuring that the findings of this work could be reproduced and reinforcing the robustness of the methodology.

However, there are some limitations of the work that must be noted. One limitation is the generalisability of the study. Participants were recruited from a single tertiary care hospital in the UK, and so were predominantly of white European ancestry and living in

low deprivation areas which may limit the applicability of these findings to broader populations. Health disparities in kidney disease are well documented, with individuals of Black and Hispanic descent experiencing a disproportionately higher lifetime risk of developing end-stage renal disease[208]. This risk is 3.4-fold and 1.3-fold higher respectively, compared to their white counterparts. As such the structural renal associations that I observed in this cohort may not fully capture the spectrum of changes in more ethnically diverse populations. Future large-scale, multi-centre studies with representative sampling across different ethnic groups would be useful to validate and extend these findings.

Second, women with known pre-existing hypertension or diagnosed renal disease were excluded from this study to isolate the effects of hypertensive disorders of pregnancy on renal structure. While this approach enhances internal validity, it limits the extrapolation of findings to populations with underlying cardiometabolic and cardiorenal comorbidities. These groups may be at even greater risk of postpartum renal sequelae. Further research is needed to explore whether similar structural renal changes occur in women with pre-existing comorbidities and whether these conditions interact synergistically with hypertensive pregnancy disorders to accelerate renal decline.

Third, there were limitations related to the collection of blood samples. Blood samples at delivery were not available for all normotensive participants, as this is not part of routine clinical care for this group. Additionally, six women declined venepuncture at the 6-12 months postpartum follow-up visit. However, a qualitative comparison of demographic and clinical characteristics between those who provided blood samples and those who did not showed that there was no evidence of selection bias based on the characteristics of those who provided blood samples.

Fourth, not all women enrolled in the POP-HT study underwent renal imaging. The renal imaging component was introduced by a protocol amendment implemented partway through the trial. Nonetheless, participants were recruited sequentially into this sub-study after the amendment, reducing the likelihood of systematic bias. The imaging protocol was also standardised, and all scans were performed and interpreted by trained assessors blinded to clinical group assignment to reduce observer bias.

Additionally, 4.4% of the renal scans were omitted due to poor image quality that precluded reliable corticomedullary differentiation calculation or volume estimation. However, these exclusions were necessary to maintain validity and ensure data quality. Also, a post-hoc power analysis confirmed that the remaining sample size retained sufficient statistical power to detect meaningful differences in renal outcomes between groups.

Another important limitation of the study is that renal imaging was only conducted at a single time point. As a result, I was unable to assess baseline kidney status prior to conception or early in pregnancy, nor could I monitor potential changes in renal structure as the pregnancy progressed. This lack of longitudinal data limits the ability to determine whether any observed renal findings were pre-existing, pregnancy-induced, or transient versus persistent changes. Nonetheless, the finding that kidney volume correlated with estimated glomerular filtration rate at birth and not postpartum, suggests that the structural differences were likely present before or early in pregnancy and not an impact of the pregnancy.

While these limitations should be considered when interpreting the findings, they do not diminish the observed associations between hypertensive pregnancy disorders and postpartum structural renal changes. Rather, they underscore the need for future studies

employing more comprehensive sampling frameworks, pre-pregnancy baseline imaging and extended follow-up to further understand risk factors for long-term renal disease and improve interventions for women affected by hypertensive pregnancies.

XXVI. Conclusions

This chapter provides evidence of persistent postpartum structural differences in the kidneys in women with hypertensive disorders of pregnancy, despite largely recovered renal function. These findings support my hypotheses that women with hypertensive pregnancies experience subclinical structural damage during pregnancy which then puts them on an accelerated trajectory towards disease progression later in life.

These findings show that at 6-12 months postpartum, preeclamptic women have significantly lower total kidney volume which correlates with estimated glomerular filtration rates at delivery but not postpartum. Importantly, this difference in volume was independent of corticomedullary differentiation differences, which were only lower in women with gestational hypertension. The persistence of these structural changes, particularly in the absence of ongoing functional impairment may reflect subclinical microvascular injury, rather than acute damage acquired during pregnancy.

These structural findings could serve as early markers of long-term renal or cardiovascular risk and may support a more detailed risk stratification approach based on hypertensive pregnancy subtype. Further research should investigate whether structural kidney differences are present before pregnancy and to explore which interventions are most effective to prevent later disease. Notably, we found no effect of the intervention on renal measures, indicating that while some cardiovascular changes during pregnancy may be reversible, renal alterations may be more resistant.

CROSS-SECTIONAL RETINAL DIFFERENCES AT 6-12 MONTHS AND 15-25 YEARS POSTPARTUM

4

I. Overview and Aims

The preceding chapters of this thesis demonstrate that there are short-term structural differences across multiple organs postpartum in women with hypertensive disorders of pregnancy, including the heart and the kidneys. Some of these structural maladaptation's occur despite overt functional recovery[186], suggesting the presence of early subclinical remodelling that may predispose these women to long-term disease. These findings offer a plausible mechanistic link between hypertensive pregnancy and the well-documented elevated lifetime risk of cardiovascular disease, stroke and chronic kidney disease observed in these women[4, 15-20].

A central pathophysiological mechanism underlying these observations is microvascular dysfunction. Organs such as the heart and kidneys are highly dependent on an intact microvascular network for normal function, and microvascular damage is increasingly recognised as a key contributor to chronic organ injury[205]. Microvascular changes may contribute to structural remodelling through two principal pathways: directly via trophic effects on the vasculature and indirectly by increasing vascular resistance and afterload, thereby inducing organ alterations such as left ventricular hypertrophy[209].

In the kidneys, microvascular dysfunction can lead to glomerular ischemia, reduced nephron perfusion, and progressive loss of renal mass[210]. In the heart, microvascular impairment contributes to increased afterload and structural changes such as left ventricular hypertrophy, ultimately increasing cardiovascular risk[209]. These processes may occur before or during pregnancy but remain clinically silent for years.

Importantly, the retinal microvasculature offers a unique non-invasive window into systemic vascular health[211]. Alterations in retinal vessel calibre and morphology are thought to reflect broader microvascular alterations elsewhere in the body including in the heart and kidneys[209-210]. Retinal imaging may therefore serve as a valuable surrogate marker for assessing systemic vascular health and associated structural adaptations, such as increased left ventricular mass[211].

Whilst much of the existing evidence on the retinal microvasculature that I reviewed during my systematic literature review in chapter two focuses on the short-term postpartum outcomes; fewer studies have addressed the long-term health implications that emerge in midlife. Gaining insight into the sustained impact of hypertensive pregnancy on the retinal microvasculature during this stage is valuable for informing healthcare strategies and improving early interventions to prevent long-term disease progression. Additionally, to date, no studies have compared the retinal microvascular structure over two postpartum timepoints limiting our understanding of whether such changes represent transient pregnancy-related phenomena or fixed lasting phenotypes.

This chapter bridges the gap between short and long-term postpartum changes in women with hypertensive pregnancy disorders, investigating whether the structural retinal changes observed in the early postpartum period persist, worsen or resolve by midlife. I hypothesise that there will be a fixed retinal microvascular phenotype present at both

early and late postpartum timepoints. As such, the primary aim of this chapter is to compare retinal microvascular calibre across women with normotensive and hypertensive pregnancies at two postpartum timepoints.

Given that blood pressure is a primary determinant of microvascular integrity, both chronic pre-pregnancy blood pressure and its management during pregnancy and postpartum are likely to influence the degree of microvascular adaptation or injury. Therefore, as a secondary aim, I assessed the impact of pre-pregnancy blood pressure and postpartum blood pressure management on microvascular calibre.

II. Analysis Design and Population

Population Cohorts: This analysis involved a pooled cohort where participants were recruited from three larger studies. Ethical and research governance approval were obtained for all three studies (POP-HT: 19/LO/1901, CAREFOL-HT: 21/WA/1069, HELPFUL: 22/LO/0781).

For the 6-12 months postpartum analysis, I utilised data from the POP-HT and CAREFOL-HT cohorts. Detailed information on study designs, recruitment and population characteristics for these cohorts is provided in chapter three. For the 15-25 years postpartum analysis, I employed data from the HELPFUL Study (Hypertension Explored in Long-Term Postpartum Follow-up of Later Life). HELPFUL is a study which I initiated with the support of my colleagues to investigate the long-term impact of hypertensive pregnancies.

Participants for the HELPFUL study were recruited between June 2023 and June 2024, with ethical approval granted in May 2023 (22/LO/0781). The study cohort is a

continuation of the Preeclampsia Vascular Study (PVS) (08/H0604/127), which previously followed women at approximately five to ten years postpartum after experiencing hypertensive or normotensive pregnancies[86,122]. The study was initially set-up with the aim of exploring the effects of different hypertensive pregnancy disorders on the long-term cardiovascular disease risk. The HELPFUL study revisits the same participants at approximately 15-25 years postpartum.

III. Inclusion and Exclusion Criteria

To be eligible for the CAREFOL-HT, POP-HT or HELPFUL studies, participants were required to provide informed consent and have adequate understanding of spoken and written English. Women were excluded if they were currently pregnant or lactating, had contraindications to magnetic resonance imaging, a known history of congenital heart disease, chronic renal disease, pre-existing hypertension, or any other significant medical condition, that in the opinion of the investigators could pose a risk or influence participation or study outcomes.

During pregnancy, the hypertensive cohort required a clinician confirmed diagnosis of gestational hypertension or preeclampsia based on the National Institute for Health and Care Excellence guidelines[4]. This included new-onset hypertension after 20 weeks of gestation in at least one pregnancy, with diastolic blood pressure greater than 90 mmHg or systolic blood pressure greater than 140 mmHg on two or more separate occasions during the pregnancy, within a 24-hour period. Further criteria for preeclampsia or hypertension with superimposed preeclampsia included elevated blood pressure as defined above, and the presence of new-onset proteinuria or end-organ dysfunction. Proteinuria was defined as 300 mg or more of protein in a 24- hour urine collection, more

than 30 mg of protein per mmol of creatinine in a single urine sample, or at least two consecutive dipstick tests showing protein levels of two or higher.

To be included as part of the normotensive pregnancy cohort, eligibility included a normal obstetric history in all pregnancies, including pregnancies that took place after PVS. This meant no history of still birth or neonatal death, placental abruption, recurrent miscarriages or hypertensive pregnancy disorder. Additionally, diastolic blood pressure needed to be consistently below 90 mmHg and systolic blood pressure consistently under 140 mmHg during pregnancy, with no more than a trace of proteinuria.

For inclusion in the early postpartum follow-up, the women were required to be 6-12 months postpartum, aged over 18 years, and they must have previously taken part in the CAREFOL-HT or POP-HT studies. To be eligible for the long-term follow-up, the women needed to be aged between 30 and 70 years old at the time of consent, with an index pregnancy 15-25 years prior to the study. The women also needed to be able to comply with all study requirements, as determined by myself and the study team.

IV. HELPFUL, CAREFOL and POP-HT Study Methodology

The HELPFUL study visit procedures were largely consistent to those used for the CAREFOL-HT and POP-HT studies, detailed in *Chapter 3*, with a few additional measures introduced to gather a more comprehensive dataset *(Figure 16).* Each study visit took place at the Cardiovascular Clinical Research Facility at the John Radcliffe Hospital, Oxford, UK. All measurements were taken by myself and a team of researchers. During the visits, participants underwent a series of clinical and imaging assessments.

Each visit began with the process of informed consent, during which participants were given full details about the study procedures and their rights to withdraw. This was followed by anthropometric measurements including height, weight and hip and waist circumference, which were used to calculate body mass index and the hip to waist ratio.

Blood pressure was then measured using a validated automated monitor (GE Dinamap Carescape V100, UK), following the guidelines of the British Heart Foundation and the British and Irish Hypertension Society[190]. Additionally, fasting blood samples were collected and analysed to look for blood biomarkers related to renal, cardiovascular and metabolic function.

Subsequently, body composition was measured using the InBody 720 bioelectrical impedance analysis machine to provide detailed assessments of body fat, lean body mass, and total body water. Measurements were conducted in accordance with the standard operating procedures, with participants instructed to fast for at least six hours prior to the measurements being taken. During the measurements, participants stood barefoot on the device's foot electrodes and held the hand electrodes while maintaining an upright posture. The InBody 720 analysis machine then generated comprehensive body composition data, which was recorded for subsequent analysis.

Following initial measurements, participants then underwent a cardiopulmonary exercise test with spirometry to assess aerobic fitness, ventilatory efficiency, and respiratory function under controlled exertion. Participants also underwent stress and resting echocardiography to evaluate cardiac structure and function under different physiological states as well as microvascular imaging of the retina and the auricle. They also underwent an additional assessment with a 12-lead electrocardiogram monitoring to measure baseline cardiac rhythm.

Participants then underwent a magnetic resonance imaging scan (3.0 Tesla, PRISMA, Siemens Medical Solutions, Erlangen, Germany) of the heart, liver, brain, and kidneys to explore organ-specific structural and functional changes. During the scan, arterial stiffness was evaluated through pulse wave velocity measurement using a vicorder device, offering further insight into vascular ageing and cardiovascular risk.

At the end of the visit, participants were provided with a detailed self-report lifestyle and medical history questionnaire (*Appendix A*), which included questions on diet, physical activity and medication usage. The questionnaire also included information on their smoking habits, salt and alcohol intake, physical activity levels, and family history of diseases.

Participants were also fitted with a wrist-worn accelerometer (GeneActiv Axivity AX3®) on their non-dominant wrist. They were asked to wear this continuously for seven days, 24 hours a day. Participants were instructed to return both the completed questionnaire and the accelerometer by post using pre-paid packaging and the data was processed using GeneActiv software to quantify the amount time of time they spent sleeping, being sedentary and completing light or moderate-to-vigorous physical activity. The data was then extracted using custom-made R scripts. All clinical, imaging, and questionnaire data were securely captured and managed using CASTOR Electronic Data Capture.

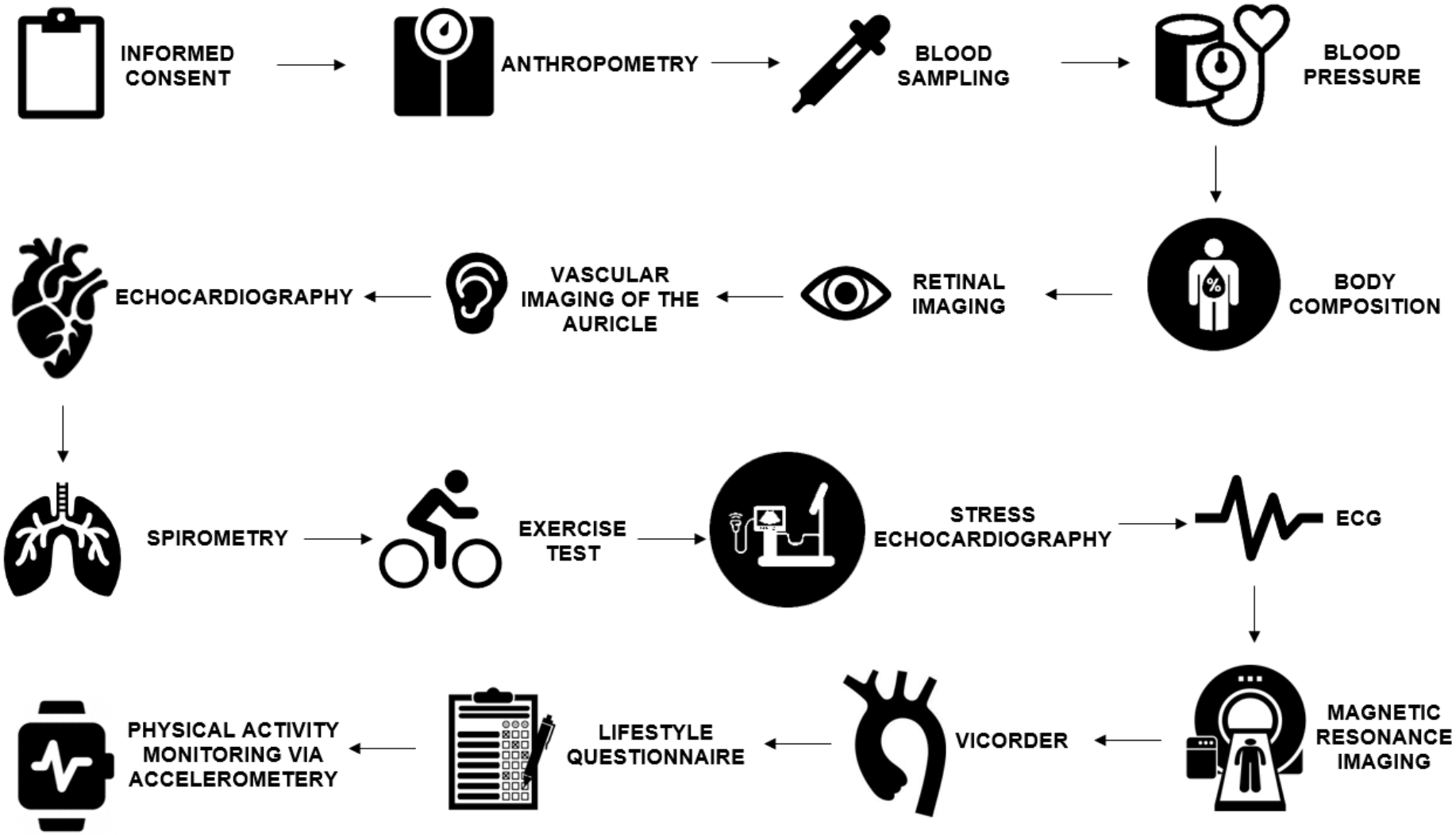


Figure 16. Overview of the study methodology for the HELPFUL study. This figure illustrates the key stages of the study design for the HELPFUL including informed consent, data collection and the clinical assessments performed.

V. Postpartum Blood Pressure Status

As part of the analysis, participants were stratified based on their blood pressure status at 6-12 months postpartum. Hypertension 6-12 months postpartum was defined as a mean systolic blood pressure reading of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg. This definition was used to categorise women into those with normalised blood pressure postpartum and those without to compare the arteriovenous ratio across groups.

VI. Primary and Secondary Outcomes for the Microvascular Investigation

The primary outcome for the microvasculature investigation was the arteriovenous ratio. This was defined as the ratio of the diameter of the central retinal artery to the central retinal vein. This metric provides a composite measure of retinal vascular calibre and is widely used as a non-invasive indicator of microvascular health[212]. It reflects the balance between arteriolar narrowing and venular dilation, with lower values indicative of microvascular compromise.

Secondary outcomes included the central retinal arteriolar equivalent and central retinal venular equivalent, both calculated using the Parr-Hubbard formula[213-214]. These are validated measures for quantifying retinal vessel diameters and are used to show the extent of arteriolar and venular narrowing in the retina.

These outcomes were chosen as alterations in the retinal microvasculature serve as non-invasive markers of hypertension-related pathogenic processes and end-organ alterations. These changes are thought to mirror cerebrovascular disturbances and reflect broader systemic vascular responses[215]. Additionally, narrowing of the retinal

vessels is commonly observed in women with pregnancy hypertension and is associated with increased risks of chronic hypertension, chronic kidney disease, diabetes cardiovascular disease, stroke and all-cause mortality[216-220].

VII. Retinal Imaging Processing

Retinal imaging was performed using a calibrated three-dimensional digital non-mydriatic fundus retinal imaging camera (NIDEK TRC Co., Japan). Participants were rested in a dark room for a few minutes before images were taken to achieve natural pupil dilatation without pharmacological mydriasis. Optic disc-centred digital photographs of both fundi were captured, with the right eye chosen for analysis due to comparable retinal vessel characteristics between the right and left eyes[221]. Additionally, by selecting the right eye, it standardised comparisons across participants.

Retinal images were securely transferred to the Department of Sport, Exercise and Health at the University of Basel where they were analysed using semi-automated Vesselmap 2® analysis software (IMEDOS Systems; GmbH; Jena; Germany). This software facilitates precise measurement of retinal vessel diameters based on a validated algorithm[222]. The retinal analysis adhered to standardised protocols that have been published previously[223] to ensure reproducibility and consistency across assessments *(Figure 17).* Where possible, three high-quality images of the right eye were selected per participant and averaged.

The central retinal arteriolar and central retinal venular equivalents were calculated by measuring the diameter of the largest arterioles and venules within a standard zone around the optic disc and averaged using the revised Parr-Hubbard formula[213-214]. To adjust for physiological influences on vessel calibre such as blood pressure at the time of

assessment, central retinal arteriolar and venular equivalents were corrected for mean arterial pressure which was calculated using this formula:

$$MAP = Diastolic\ Pressure + \frac{1}{3}\ (Systolic\ Pressure - Diastolic\ Pressure)$$

The arteriovenous ratio was then calculated by dividing the corrected central retinal arteriolar equivalent by the central retinal venular equivalent. All image analyses and quality control procedures were performed by trained and experienced graders blinded to the participants clinical data, including pregnancy history and blood pressure status.

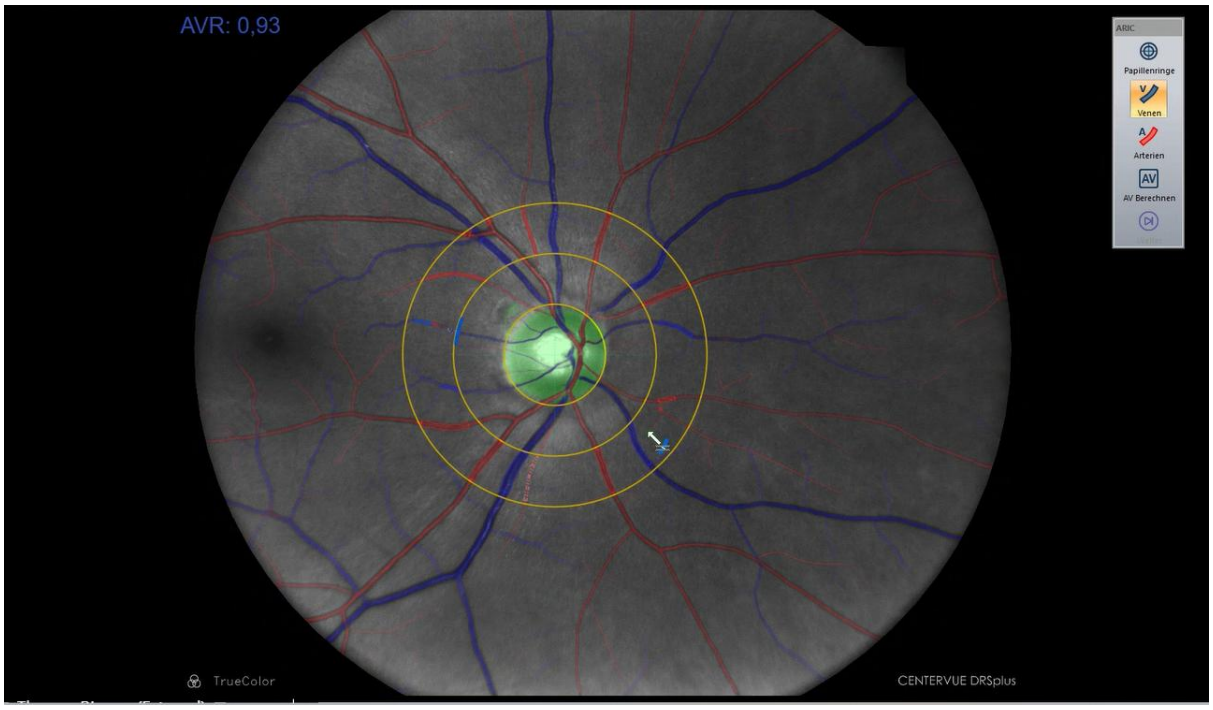


Figure 17. Measurement of microvascular calibre using fundus photography. Fundus photograph centred on the optic disc, acquired using a non-mydriatic retinal camera (NIDEK TRC) and analysed using Imedos software. Retinal vessel analysis was performed on this image to measure the central retinal arteriolar equivalent, central retinal venular equivalent, and the arteriovenous ratio. Measurements were taken within a standardised zone surrounding the optic disc, following established protocols for vessel calibre assessment.

VIII. Statistical Analysis

Statistical analysis was conducted using R (version 4.4.2) and R Studio (version 2024.12.0 + 467). Datasets were tested for normality using the Shapiro-Wilks test for continuous variables where $n \leq 50$ and visual observation using Q-Q plots and histograms for larger samples. Normally distributed continuous variables were reported as means and standard deviations. Skewed continuous variables were reported as medians and interquartile ranges. Categorical data were presented as counts and percentages.

When comparing demographic, anthropometric, and pregnancy data, independent two-samples t-tests were used to compare the differences between the means across groups for parametric continuous variables. Whilst the Mann-Whitney U test was used to compare the medians across groups for non-parametric variables. Chi-square tests were used to compare the percentages in each group of categorical variables.

To compare retinal characteristics across women with normotensive and hypertensive pregnancies, *t*-tests and Mann-Whitney U tests were performed, and the corresponding statistics were reported in the tables. Following this, multiple linear regression models, adjusted for body mass index and time postpartum, were used to compare across the hypertensive and normotensive groups at both timepoints. They were also used to examine any differences between women with preeclampsia and gestational hypertension in the 6-12 months cohort.

A sensitivity analysis was conducted to investigate the effect of telemonitored home blood pressure monitoring versus standard NHS care postpartum on retinal outcomes within the hypertensive group. This involved using a Welch's two-sample t-test to compare retinal characteristics across intervention and control groups.

A one-way analysis of variance, adjusted for body mass index and time postpartum, was used to compare women with hypertensive pregnancies at 6-12 months postpartum, with and without blood pressure normalisation, to women with normotensive pregnancies. For multiple comparisons, the post-hoc Tukey's Honest Significant Difference test was applied.

To compare the effect sizes between the 6-12 month and 15-25 year postpartum cohorts, Z-tests were conducted to determine whether the differences in the effect sizes of the regression models were statistically significant. The standard errors for the coefficients were used to calculate the Z-scores for each comparison.

The formula for the z-test was:

$$z = \frac{Effect\ size1 - Effect\ Size2}{\sqrt{SE1^2 + SE2^2}}$$

Effect Size 1 and Effect Size 2 are the respective effect sizes of the 6-12 month postpartum cohort and the 15-25 year cohort for each retinal variable. SE1 and SE2 are the standard errors of the effect sizes for the 6-12 month and 15-25 year cohorts. The p-value was determined based on the z-distribution to assess statistical significance. In addition, 95% confidence intervals were calculated for each effect size estimate.

Finally, to evaluate whether antenatal blood pressure measurements predict retinal calibre, simple linear regression models were used with systolic and diastolic antenatal blood pressures modelled as the predictors and retinal calibre as the dependent variable.

IX. Results - Study Cohort

A total of 301 women with high quality retinal imaging datasets were included in the final analysis. Among them, 231 women had hypertensive pregnancies, and 70 women had normotensive pregnancies. Retinal images at 6-12 months postpartum were captured in 196 women and at 15-25 years postpartum in 105 women.

Figure 18 presents a detailed recruitment flowchart, outlining the numbers within each group, with additional information provided in the subsequent results sections. In total, 28 datasets (11.2%) were excluded due to poor image quality. Reasons for exclusion included improper centering of the optic nerve head, suboptimal image angles and data extraction errors.

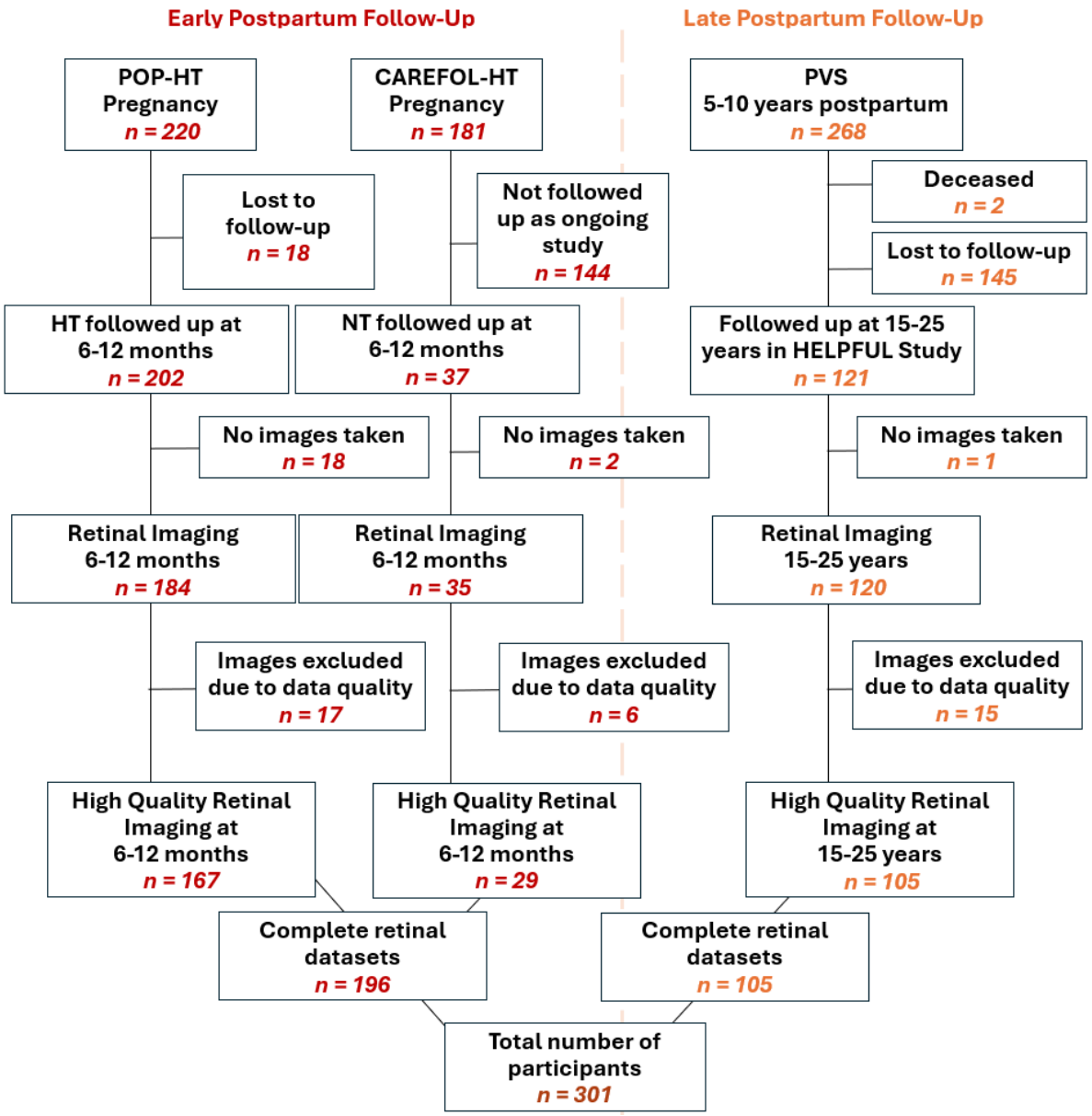


Figure 18: ***Participant Flow Diagram.*** This diagram illustrates the participant selection process and the number of individuals included at each stage of the study. The study involved recruitment from three original cohorts: POP-HT (n = 220), CAREFOL-HT (n = 181), and PVS (n = 268). The total number of participants with retinal imaging data included in the final analysis was 301.

X. Data Availability and Completeness

Data availability for the POP-HT study has been previously published[93-97] and the CAREFOL-HT study is still ongoing, and therefore it was not included in this data completeness summary. In terms of the HELPFUL study, high data integrity was achieved, with most participants having complete demographic and clinical data *(Table 5).*

Across all measurements, data collection rates exceeded 94%, and the datasets used for analysis were greater than 86% complete for all core study variables. Anthropometry, blood pressure, body composition, and echocardiography data were complete for all participants (100%). High completion rates were also achieved for spirometry (99.2%), cardiopulmonary exercise testing (99.2%), and microvascular ear imaging (96.7%).

Retinal imaging and magnetic resonance imaging had slightly lower completeness, with 86.8% analysed. This number reflects the impact of image quality exclusions and a small number of participants declining or being contraindicated for magnetic resonance imaging procedures. Blood sampling data were available for 96.7% of participants, while questionnaire data was complete for 97.5% of the participants.

The reasons for missing data primarily resulted from participants being unable or unwilling to undertake certain measurements, non-return of mailed questionnaires, or the occasional loss of study materials in transit. Importantly, the pattern of missingness appeared random and unrelated to hypertensive status or major demographic variables, reducing the likelihood of bias.

Table 5. Data availability for measurements in the HELPFUL Study. The first column shows the type of measurement, the second column shows the total number of datasets collected for each measurement, along with the percentage of participants. The third column indicates the number of datasets used in the final analysis, with corresponding percentages.

Measurement	Datasets collected	Datasets used for analysis
Anthropometry, No., (%)	121 (100)	121 (100)
Blood pressures, No., (%)	121 (100)	121 (100)
Body composition, No., (%)	121 (100)	121 (100)
Retinal Imaging, No., (%)	120 (99.2)	105 (86.8)
Microvascular Ear Imaging, No., (%)	117 (96.7)	117 (96.7)
Magnetic Resonance Imaging, No., (%)	114 (94.2)	105 (86.8)
Echocardiography, No., (%)	121 (100)	121 (100)
Spirometry, No., (%)	120 (99.2)	120 (99.2)
Cardiopulmonary Exercise Test, No., (%)	120 (99.2)	120 (99.2)
Blood Sampling, No., (%)	119 (98.3)	117 (96.7)
Lifestyle Questionnaire, No., (%)	118 (97.5)	118 (97.5)

Data are presented as counts and percentages.

XI. 6-12 Months Postpartum Cohort

Altogether, 219 women had retinal imaging at 6-12 months postpartum. Of these, 23 datasets were excluded due to image quality (10.5%). As a result, 196 women with complete imaging data were included in the analysis. This included 167 women (85.2%) with previous hypertensive pregnancies and 29 women (14.8%) with prior normotensive pregnancies. Demographic, anthropometric and pregnancy characteristics of the study population are summarised in *Table 6.*

Within the hypertensive cohort, 103 women had a history of preeclampsia (25 early-onset, 78 late-onset), and 64 women had a history of gestational hypertension. Of the hypertensive women, 87 (52.1%) were randomised to receive physician-optimized home blood pressure management with 52 of these women having had preeclampsia and 35 having had gestational hypertension. The remaining 80 women (47.9%), including 51 women with preeclampsia and 29 women with gestational hypertension received standard NHS care postpartum.

The average age of the whole cohort was 33.41 ± 5.13 years and 83.2% were Non-Hispanic White or Eastern European, with an average multiple deprivation index score of 8 indicating living in areas of low deprivation. There were no differences in age, time postpartum, height, ethnicity or indices of multiple deprivation across the cohorts ($p > 0.05$). However, women in both hypertensive cohorts had higher weight, body mass index and blood pressure postpartum compared to the normotensive cohort ($p < 0.05$).

Table 6. Participant characteristics at the 6-12 month postpartum follow-up visit, including anthropometry, clinical blood pressure readings, pregnancy and demographic characteristics.

Variable	NT (N = 29)	PET (N = 103)	GH (N = 64)
Anthropometry and participant characteristics			
Age at 6-12 months postpartum, years, Mean ± SD	33.4 ± 5.2	33.6 ± 5.2	33.1 ± 5
Time postpartum, days, Median ± IQR	242 ± 32	252 ± 20.5	251.5 ± 20.5
Height, m, Mean ± SD	1.6 ± 0.06	1.6 ± 6.45	1.7 ± 0.06
Weight, kg, Median ± IQR	69 ± 13.1	76.5 ± 23.3	77.5 ± 27.8
BMI, kg/m^2, Median ± IQR	25.8 ± 5.5	27.9 ± 10	28.6 ± 8.4
Waist: hip ratio (cm:cm), Median ± IQR	0.9 ± 0.09	0.9 ± 0.08	0.9 ± 0.09
Mid left arm circumference (cm), Median ± IQR	28.3 ± 4	31 ± 5.8	31 ± 6
Systolic Blood Pressure, mmHG, Mean ± SD	110.3 ± 8.8	127.6 ± 12.3	129.6 ± 9.8
Diastolic Blood Pressure, mmHG, Mean ± SD	67.6 ± 5.5	83.4 ± 8.6	84.7 ± 8.1
Mean Arterial Pressure, Mean ± SD	81.8 ± 5.9	98.1 ± 9	99.7 ± 8
Indices of Multiple Deprivation, Median ± IQR	8 ± 4	9 ± 3	8 ± 3

Breastfeeding at 6-12 months postpartum, No., (%)	21 (72.4)	51 (49.5)	34 (53.1)
Ethnicity			
Asian, No., (%)	1 (3.5)	8 (7.8)	5 (7.8)
Hispanic or Latino, No., (%)	0 (0)	3 (2.9)	4 (6.3)
Carribbean, African or other background, No., (%)	3 (10.3)	6 (5.8)	2 (3.1)
Non-Hispanic White and Eastern European, No., (%)	25 (86.2)	86 (83.5)	53 (82.8)
Contraception type			
Progesterone only pill, No., (%)	0 (0)	22 (21.4)	13 (20.3)
Combined oral contraceptive pill, No., (%)	1 (3.5)	5 (4.9)	1 (1.6)
Contraceptive Implant, No., (%)	1 (3.5)	2 (1.9)	5 (7.8)
Contraceptive Coil, No., (%)	2 (6.9)	9 (8.7)	4 (6.3)
Depot injection, No., (%)	0 (0)	1 (1)	3 (4.7)
None, No., (%)	25 (86.2)	64 (62.1)	38 (59.4)
Antenatal measurements			
Antenatal booking height, m, Mean ± SD	1.7 ± 0.07	1.6 ± 0.07	1.7 ± 0.07

Antenatal booking weight, kg, Median ± IQR	66 ± 11.2	74 ± 23.5	72.5 ± 22.5
Antenatal booking BMI, kg/m2, Median ± IQR	23.6 ± 5.2	27.2 ± 8.7	27.2 ± 6.8
Antenatal booking SBP, mmHg, Mean ± SD	110.2 ± 9.6	116.7 ± 10.9	119.8 ± 9.8
Antenatal booking DBP, mmHg, Mean ± SD	66.2 ± 10.5	72.1 ± 9.1	74.6 ± 8.6
Pregnancy characteristics			
Previous hypertensive pregnancy No., (%)	0 (0)	17 (16.51)	16 (25)
Nulliparous, No., (%)	19 (65.5)	73 (70.9)	41 (64.1)
Neonatal unit admission, No., (%)	2 (6.9)	33 (32)	7 (10.9)
Gestational diabetes mellitus, No., (%)	0 (0)	10 (9.7)	5 (7.8)
Birthweight, kg, Mean ± SD	3.6 ± 0.6	2.9 ± 0.9	3.35 ± 0.5
Gestation at delivery, weeks, Median ± IQR	39.6 ± 1.2	37.9 ± 3.6	40 ± 1.3
Delivery Method			
Spontaneous Vaginal Delivery, No., (%)	19 (65.5)	30 (29.1)	27 (42.2)
Instrumental, No., (%)	1 (3.5)	17 (16.5)	18 (28.1)
Emergency caesarean-section, No., (%)	4 (13.8)	54 (52.4)	12 (18.8)

Elective caesarean-section, No., (%)	5 (17.24)	2 (1.94)	7 (10.94)

cm = centimetres, GH = Gestational Hypertension, IQR = Interquartile range, kg = Kilograms, m^2 = meters squared, mmHg = millimetres of mercury, Number, NT = Normotensive, PET = Preeclampsia, SD = Standard deviation,

XII. 15-25 Years Postpartum Cohort

Altogether, 120 women had retinal imaging taken at 15-25 years postpartum. The participant characteristics of this cohort are summarised in *Table 7.* Of these, 15 datasets (12.5%) were excluded due to suboptimal image quality, resulting in a final sample of 105 women (64 hypertensive, 41 normotensive). Within the hypertensive cohort, 54 women (84.38%) were diagnosed with preeclampsia (31 late-onset, 23 early-onset) and 10 women (15.62%) were diagnosed with gestational hypertension during pregnancy. Due to the small number of women with gestational hypertension, these cases were combined with the preeclamptic group for the purposes of this analysis, forming a single hypertensive cohort.

The average age of this cohort was 52.9 ± 4.8 years and 98.1% of the women were Non-Hispanic White or Eastern European, with an average multiple deprivation index score of 9 indicating that the women were living in areas of low deprivation. There were no differences in age, time postpartum, height, weight, ethnicity, diastolic blood pressure or indices of multiple deprivation across the cohorts ($p > 0.05$). However, women in the hypertensive pregnancy cohorts had on average higher systolic blood pressure postpartum compared to the normotensive cohort ($W = 859$, $p = 0.003$).

Table 7. Participant characteristics at the 15-25 years postpartum follow-up visit, including anthropometry, clinical blood pressure readings, pregnancy and demographic characteristics. cm = centimetres, GH = Gestational Hypertension, IQR = Interquartile range, kg = Kilograms, m^2 = meters squared, mmHg = millimetres of mercury, Number, NT = Normotensive, PET = Preeclampsia, SD = Standard deviation,

Variable	NT (N = 41)	HT (N = 64)
Anthropometry and participant characteristics at 15-25 years postpartum		
Age, years	52.2 ± 5.1[a]	53.2 ± 4.6[a]
Time postpartum, years	22.6 ± 3[b]	22 ± 3[b]
Height, m	1.7 ± 0.1[a]	1.6 ± 0.1[a]
Weight, kg	69 ± 23.2[b]	73.8 ± 20.9[b]
BMI, kg/m^2	24.9 ± 6.9[b]	27.4 ± 7.1[b]
Waist: hip ratio (cm: cm)	0.8 ± 0.1[b]	0.9 ± 0.1[b]
Mid left Arm circumference (cm),	28.7 ± 4.5[a]	29.6 ± 3.8[a]
Clinical SBP, mmHG,	118.9 ± 13.5[a]	127.2 ± 15.3[a]
Clinical DBP, mmHG,	71.1 ± 7.4[a]	73.8 ± 8.6[a]
Mean Arterial Pressure	89.5 ± 9[a]	94.5 ± 11[a]

Multiple Deprivation Indices	9 ± 3[b]	9 ± 3[b]
Ethnicity		
Asian, No., (%)	0 (0)	1 (1.6)
Hispanic or Latino, No., (%)	40 (97.6)	62 (96.9)
Caribbean, African or other, No., (%)	0 (0)	1 (1.6)
Non-Hispanic White/Eastern European, No., (%)	1 (2.4)	0 (0)
Antenatal measurements at 12-week booking appointment		
Height, m	1.7 ± 0.1[a]	1.6 ± 0.1[a]
Weight, kg,	63.5 ± 16.5[b]	69 ± 22.3[b]
BMI, kg/m2,	23 ± 8[b]	25 ± 10.6[b]
Clinical SBP, mmHg,	109.6 ± 10.2[a]	114 ± 12[a]
Clinical DBP, mmHg,	64.9 ± 9.02[a]	68.5 ± 7.6[a]
Pregnancy characteristics		
Age at first pregnancy, years,	29.6 ± 4.8[a]	31.3 ± 4.6[a]
Total number of pregnancies,	3 ± 1.5[a]	2.6 ± 1.1[a]

Total complete pregnancies	2.4 ± 0.8[a]	2 ± 0.8[a]
Total number of complete hypertensive pregnancies	0 ± 0[a]	1.4 ± 0.6[a]
Total number of complete normotensive pregnancies	2.4 ± 0.8[a]	0.6 ± 0.8[a]
Primiparous, no., (%)	4 (9.8)	6 (12.5)

Data are presented as Mean ± SD[a], Median ± IQR[b] and counts and percentages.

XIII. Retinal Calibre at 6-12 months Postpartum

A detailed summary of the retinal calibre characteristics at 6-12 months postpartum, including corrected and uncorrected central retinal arteriolar equivalents, central retinal venular equivalents, and arteriovenous ratio for both the hypertensive and normotensive cohorts is presented in *Table 8.*

Table 8. Retinal calibre characteristics of the 6-12 months postpartum cohort. Independent samples t-tests were used to compare the groups.

6-12 months Postpartum Retinal Characteristics				
Variable	**NT (N = 29)**	**HT (N = 167)**	**Statistic**	**P value**
CRAE, µm,	186.9 ± 14.7[a]	171.6 ± 15.2[a]	T = 5.16	< 0.001*
CRVE, µm,	219 ± 15.9[a]	214.4 ± 15.8[a]	T = 1.44	0.15
Corrected CRAE, µm/mmHg,	2.3 ± 0.3[a]	1.8 ± 0.2[a]	W = 1711.5	< 0.001*
Corrected CRVE, µm/mmHg,	2.7 ± 0.3[a]	2.2 ± 0.2[a]	T = 2.76	< 0.001*
Arteriovenous Ratio,	0.9 ± 0.06[a]	0.8 ± 0.06[a]	T = 4.16	< 0.001*

Data are presented as Mean ± SD[a], CRAE = Central Retinal Arteriolar Equivalent, CRVE = Central Retinal Venular Equivalent

After adjustment for time postpartum and body mass index, corrected central retinal arteriolar equivalents were lower in women with hypertensive pregnancies compared to normotensive women (mean difference = 0.5 µm/mmHg, adjusted R^2 = 0.39, $p < 0.001$), with no effect of body mass index ($p = 0.051$) or time postpartum ($p = 0.98$) *(Figure 19).*

Similarly, corrected central retinal venular equivalents adjusted for time postpartum and body mass index were lower in women with hypertensive pregnancies compared to normotensive women (mean difference = 0.5 µm/mmHg, adjusted R^2 = 0.35, $p < 0.001$), with no effects of body mass index ($p = 0.37$) or time postpartum ($p = 0.75$) *(Figure 19)*. The same was found for arteriovenous ratio (mean difference: 0.1, adjusted R^2 = 0.09, $p < 0.001$), with no effect of time postpartum (p = 0.66) but a small effect of body mass index ($p = 0.04$) *(Figure 19)*.

There were no significant differences in corrected central retinal venular equivalent (mean difference = 0.02 µm/mmHg, adjusted R^2 = -0.014, $p = 0.88$) or arteriovenous ratio (mean difference = 0.02, adjusted R^2 = 0.03, $p = 0.06$) between women with gestational hypertension and preeclampsia. However, the preeclamptic group showed slightly higher corrected central retinal arteriolar equivalents to the gestational hypertension group (mean difference = 0.07 µm/mmHg, adjusted R^2 = 0.03, $p = 0.04$) *(Figure 19).* This measure was negatively associated with body mass index ($p = 0.05$), but not time postpartum ($p = 0.51$).

When comparing just women with preeclampsia to women with normotensive pregnancies, the differences were still apparent with women with preeclampsia having lower mean arteriovenous ratios (mean difference: 0.05, adjusted $R^2 = 0.07$, $p < 0.01$), corrected central retinal arteriolar equivalents (mean difference = 0.52 μm/mmHg, adjusted $R^2 = 0.42$, $p < 0.001$) and corrected central retinal venular equivalents (mean difference: 0.5 μm/mmHg, adjusted $R^2 = 0.39$, $p < 0.001$), with no effects of body mass and time postpartum ($p > 0.05$).

Additionally, the differences were still apparent when comparing only women with gestational hypertension to women with normotensive pregnancies. Women with gestational hypertension showed lower arteriovenous ratios (mean difference: 0.07, adjusted $R^2 = 0.23$, $p < 0.001$), with each unit increase in body mass index associated with a 0.03 decrease in the arteriovenous ratio ($p < 0.01$) but with no effect of time postpartum ($p = 0.81$). Women with gestational hypertension also showed lower corrected central retinal arteriolar equivalents (mean difference: 0.59 μm/mmHg, adjusted $R^2 = 0.56$, $p < 0.001$) and central retinal venular equivalents (mean difference: 0.52 μm/mmHg, adjusted $R^2 = 0.51$, $p < 0.001$), with no effects of body mass index or time postpartum ($p > 0.05$).

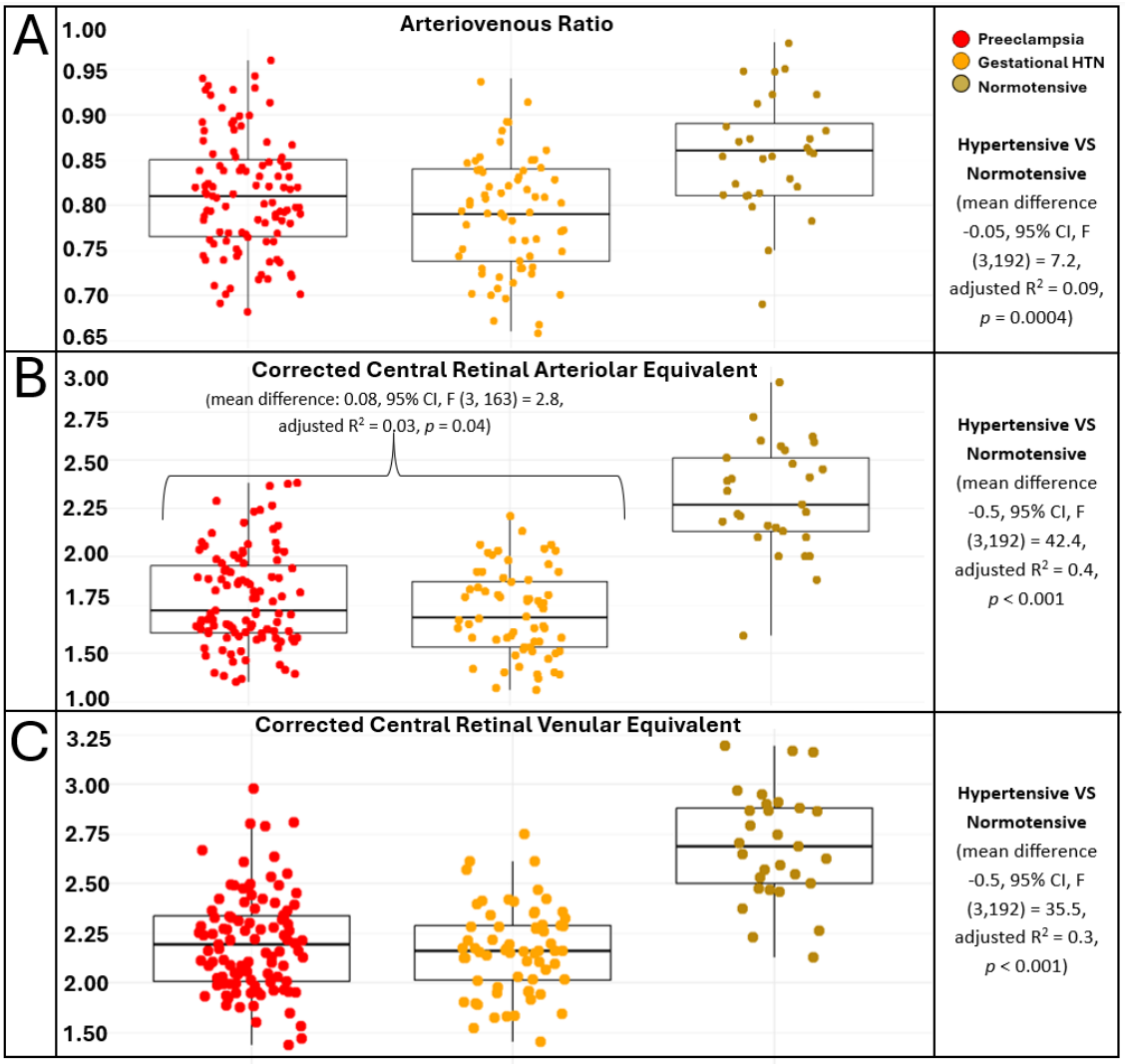


Figure 19. **Comparison of retinal vascular calibre characteristics in women with hypertensive and normotensive pregnancies at 6-12 months postpartum, stratified by hypertensive subtypes.** The figure displays boxplots comparing Arteriovenous Ratio **(Panel A),** Corrected Central Retinal Arteriolar Equivalent **(Panel B),** and Corrected Central Retinal Venular Equivalent **(Panel C)** among three groups: preeclampsia (red), gestational hypertension (yellow), and normotensive (gold). Data are presented as boxplots with individual data points overlaid, and statistical results are indicated for each comparison in the side panels.

XIV. The Impact of Postpartum Blood Pressure Control on Retinal Calibre at 6-12 Months Postpartum

When comparing women who received physician-optimized home blood pressure management and those who received standard NHS care, there were no significant differences in corrected central arteriolar equivalents (mean difference = 0.04 μm/mmHg, t = 1.06, p = 0.29), corrected central venular equivalents (mean difference = 0.02 μm/mmHg, t = 0.68, p = 0.5) or arteriovenous ratios (mean difference: 0.09, t = 0.91, p = 0.36).

However, I did find a graded difference in retinal calibre based on blood pressure normalisation status *(Figure 20)*. Women with preeclampsia and postpartum blood pressure ≥ 140/90 mmHg had the lowest arteriovenous ratios. These were significantly lower than normotensive women (mean difference: 0.09, 95% CI: [0.05, 0.13], p < 0.001). Similarly, women with gestational hypertension and postpartum blood pressure ≥ 140/90 mmHg also had significantly lower arteriovenous ratios than normotensive women (mean difference: 0.08; 95% CI: 0.039-0.13; p < 0.001).

Those with preeclampsia but normal postpartum blood pressure also had a reduced ratio but to a lesser extent (mean difference: 0.05, 95% CI: [0.02, 0.09], p < 0.01). The same was found for women with gestational hypertension who had normal postpartum blood pressure. They also had a reduced ratio, though to a lesser extent (mean difference: 0.06; 95% CI: 0.030-0.10; p < 0.001). The same results were observed when preeclampsia and gestational hypertension cohorts were grouped together. When the preeclampsia and gestational hypertension cohorts were analysed together, the same stepwise trend persisted, arteriovenous ratio

decreased progressively with hypertensive pregnancy exposure and with the persistence of elevated blood pressure postpartum.

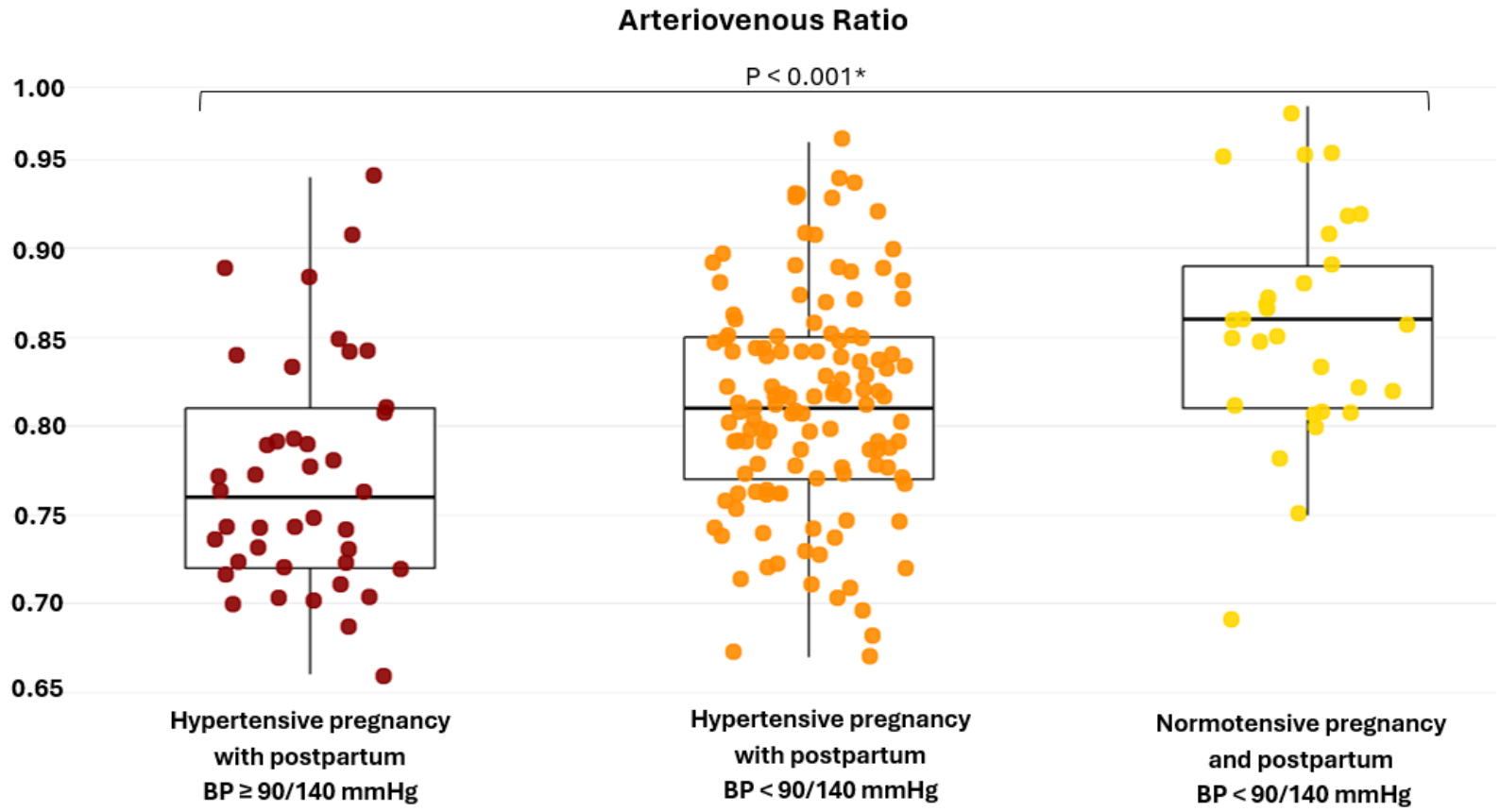


Figure 20. Boxplots with individual data points representing the arteriovenous ratio of each participant overlaid and coloured by group: dark red for women with hypertensive pregnancies with postpartum blood pressure ≥ 90/140 mmHg, dark orange for women with hypertensive pregnancies and postpartum blood pressure < 90/140mmHg, and yellow for women with normotensive pregnancies who continue to have blood pressure < 90/140mmHg measurements. *Represents significant differences.

XV. Retinal Calibre Characteristics at 15-25 Years Postpartum

A detailed summary of the retinal calibre characteristics at 15-25 year postpartum, including corrected and uncorrected central retinal arteriolar equivalents, central retinal venular equivalents, and arteriovenous ratio for both the hypertensive and normotensive cohorts is presented in *Table 9.*

Table 9. Retinal Calibre Characteristics of the 15-25 Years Postpartum Cohort.

15-25 year postpartum retinal characteristics				
Variable	**NT (N = 41)**	**HT (N = 64)**	**Test Statistic**	**P value**
CRAE, µm	177.8 ± 17[a]	171.2 ± 20.1[a]	T = 1.82	0.07
CRVE, µm	210.5 ± 20[a]	204.1 ± 19.2[a]	W = 1511	0.19
Corrected CRAE, µm/mmHg	2 ± 0.3[a]	1.9 ± 0.3[a]	T = 2.59	0.01*
Corrected CRVE, µm/mmHg	2.37 ± 0.3[a]	2.2 ± 0.3[a]	T = 2.83	0.006*
Arteriovenous Ratio	0.85 ± 0.07[a]	0.84 ± 0.07[a]	T= 0.47	0.64

Data are presented as Mean ± Standard Deviation, NT = Normotensive, HT = Hypertensive

After adjustment for time postpartum and body mass index, corrected central retinal arteriolar equivalents were lower in the hypertensive group than the normotensive group at 15-25 years postpartum (mean difference: 0.15 μm/mmHg, adjusted R^2 = 0.04, p = 0.02), with no effect of time postpartum (p = 0.84) or body mass index (p = 0.32).

Similarly, after adjustment for time postpartum and body mass index, corrected central retinal venular equivalents were lower in the hypertensive group compared to the normotensive group at 15-25 years (mean difference: 0.18 μm/mmHg, adjusted R^2 = 0.04, p = 0.008) with no effect of time postpartum (p = 0.71) or body mass index (p = 0.84).

However, there were no significant differences in arteriovenous ratio between the hypertensive and normotensive cohorts (mean difference 0.01, adjusted R^2 = 0.01, p = 0.81), with no effect of time postpartum (p = 0.41) or body mass index (p = 0.08).

XVI. Comparison of 6-12 months and 15-25 years postpartum cohort

The difference in corrected arteriolar equivalents to those with a normotensive pregnancy was narrower at 15-25 years postpartum (β = - 0.15, SE = 0.06, 95% CI, - 0.27 to - 0.03) compared to 6-12 months (β = - 0.53, SE = 0.05, 95% CI, - 0.63 to 0.43) (z = - 4.19, p < 0.001) *(Figure 21).*

The difference in corrected central venular equivalents was also narrower at 15-25 years postpartum (β = -0.18, SE = 0.06, 95% CI, - 0.3 to -0.06) than 6-12 months postpartum (β = - 0.5, SE = 0.05, 95% CI, - 0.6 to - 0.4) (z = -3.83, p < 0.001).

There was no significant difference in the arteriovenous ratio effect sizes between the 6-12 month cohort (β = -0.05, SE = 0.01, 95% CI, −0.07, −0.03) and the 15-25 year cohort (β = -0.04, SE = 0.01, 95% CI, −0.06, −0.02) (z = -0.61, p = 0.54).

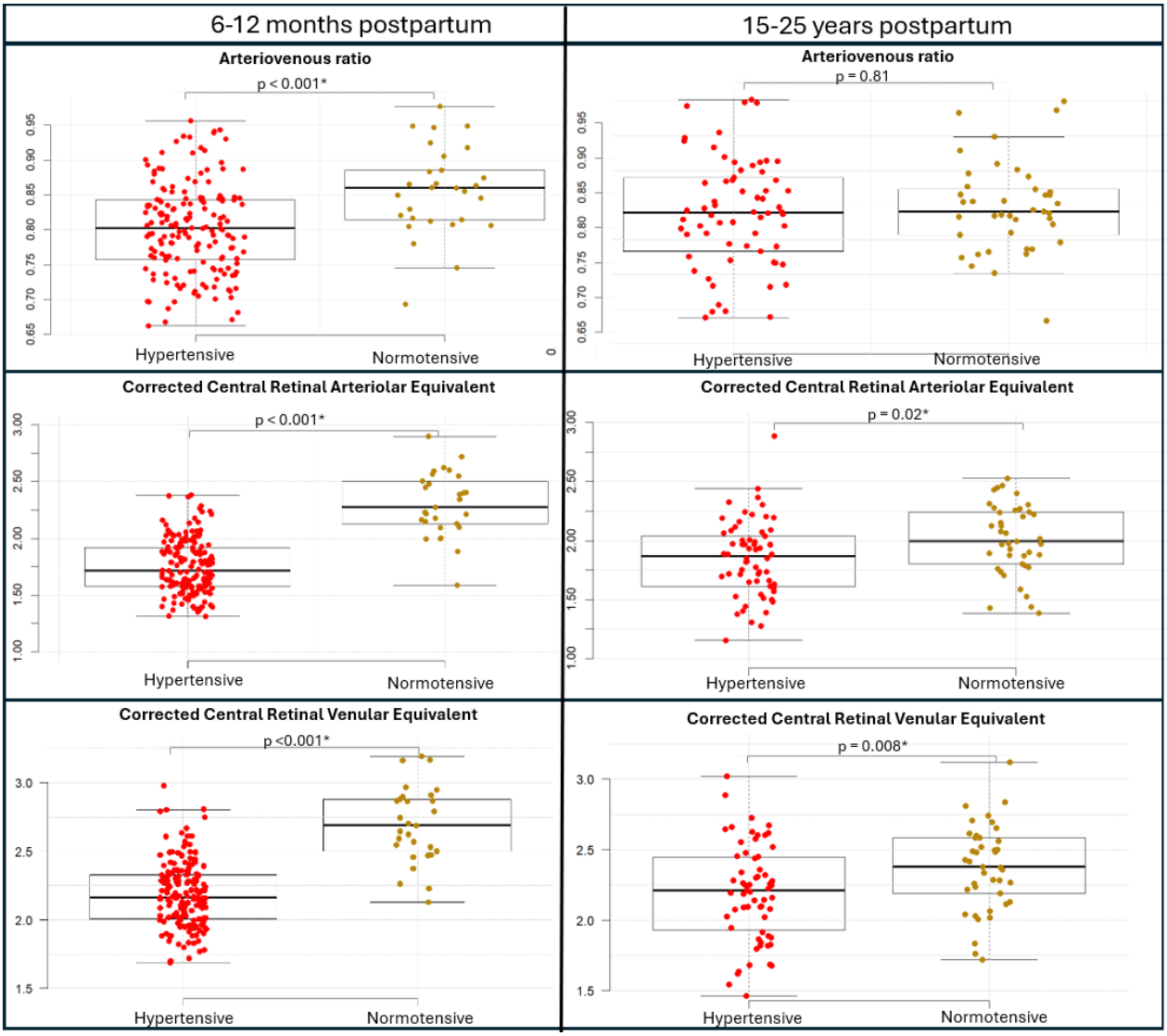


Figure 21. Comparison of retinal vascular parameters between hypertensive and normotensive pregnancy cohorts at two postpartum time points. Box plots (top to bottom) show group differences in: arteriovenous ratio, corrected central retinal arteriolar equivalent, and corrected central retinal venular equivalent at 6-12 months postpartum (left column) and 15-25 years postpartum (right column). Each plot displays individual data points, group medians, and interquartile ranges. P values are reported above each graph from the multiple linear regression results. An asterix is used to represent significant differences.

XVII. Associations between Retinal Calibre and Antenatal Blood Pressure

At 6-12 months postpartum, antenatal systolic blood pressure, measured at the 12-week standard booking appointment, were significantly correlated with corrected central retinal arteriolar ($\beta = -0.01$, $p < 0.001$) and venular equivalents ($\beta = -0.01$, $p < 0.001$), as well as arteriovenous ratio ($\beta = -0.001$, $p < 0.001$) *(Figure 22).*

However, at 15-25 years postpartum, antenatal systolic blood pressure was not significantly associated with corrected central retinal arteriolar equivalent ($\beta = -1.79$, $p = 0.65$) or corrected central retinal venular equivalent ($\beta = -1.95$, $p = 0.58$) but it was associated with the arteriovenous ratio ($\beta = -0.001$, $p = 0.043$) *(Figure 22).*

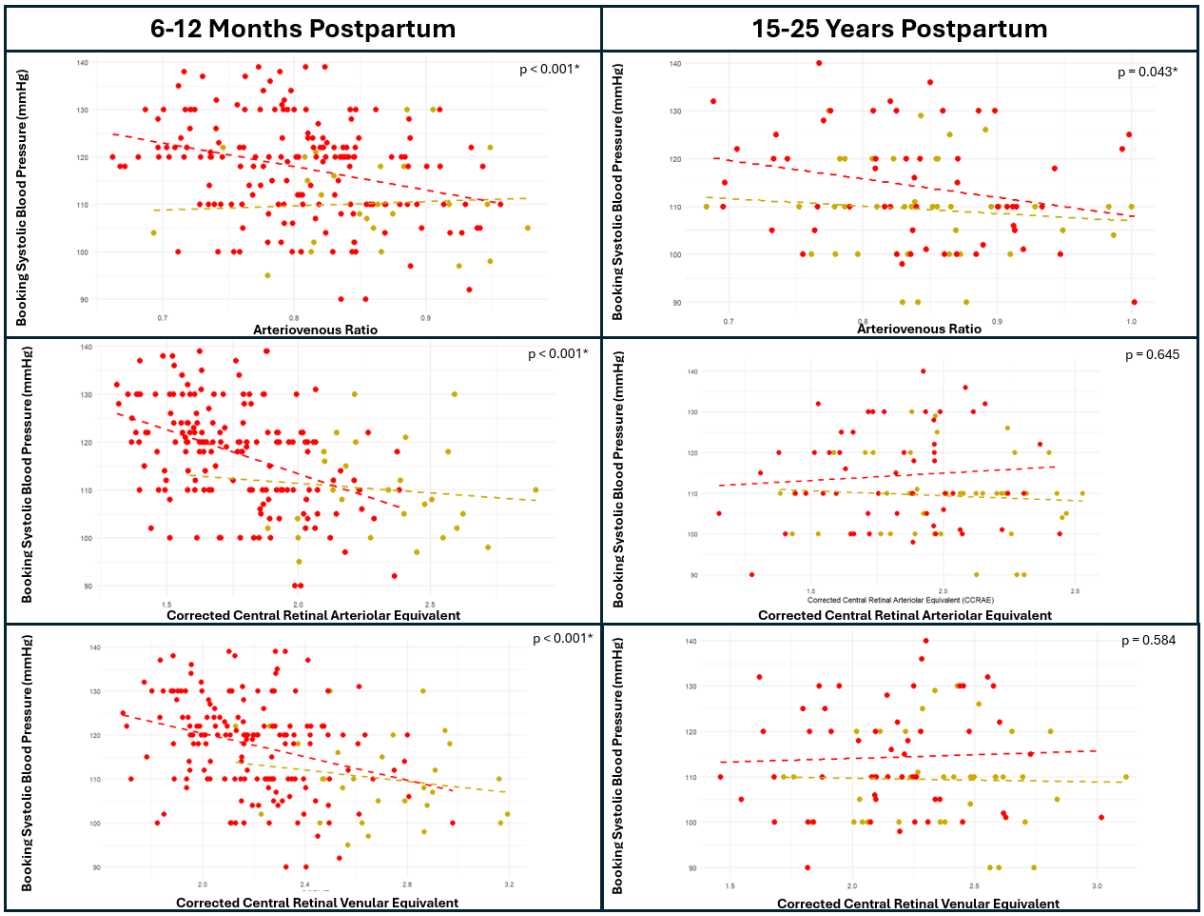


Figure 22. Relationship between retinal vascular calibre and antenatal systolic blood pressure. Scatterplots illustrate the association between antenatal systolic blood

pressure (measured at booking) and retinal microvascular parameters at 6-12 months postpartum (left column) and 15-25 years postpartum (right column). Each row represents a different retinal variable. Top row: Arteriovenous ratio. Middle row: Corrected Central Retinal Arteriolar Equivalent. Bottom row: Corrected Central Retinal Venular Equivalent. Red dots represent participants with a history of hypertensive disorders of pregnancy, and gold dots represent normotensive controls. Dashed lines represent linear regression lines with 95% confidence intervals. P values are reported above each graph from the multiple linear regression results. An asterix is used to represent significant differences.

At 6-12 months postpartum, antenatal diastolic blood pressures were also significantly correlated with corrected central retinal arteriolar ($\beta = -0.02$, $p < 0.001$) and venular equivalents ($\beta = -0.01$, $p < 0.001$) as well as arteriovenous ratio ($\beta = -0.002$, $p < 0.001$) *(Figure 23)*.

At 15-25 years postpartum, diastolic blood pressure was not significantly associated with corrected central retinal arteriolar equivalent ($\beta = -0.005$, $p = 0.2$) but it was associated with the corrected central retinal venular equivalent ($\beta = -5.3$, $p = 0.04$) and the arteriovenous ratio ($\beta = -0.0001$, $p = 0.9$) *(Figure 23)*.

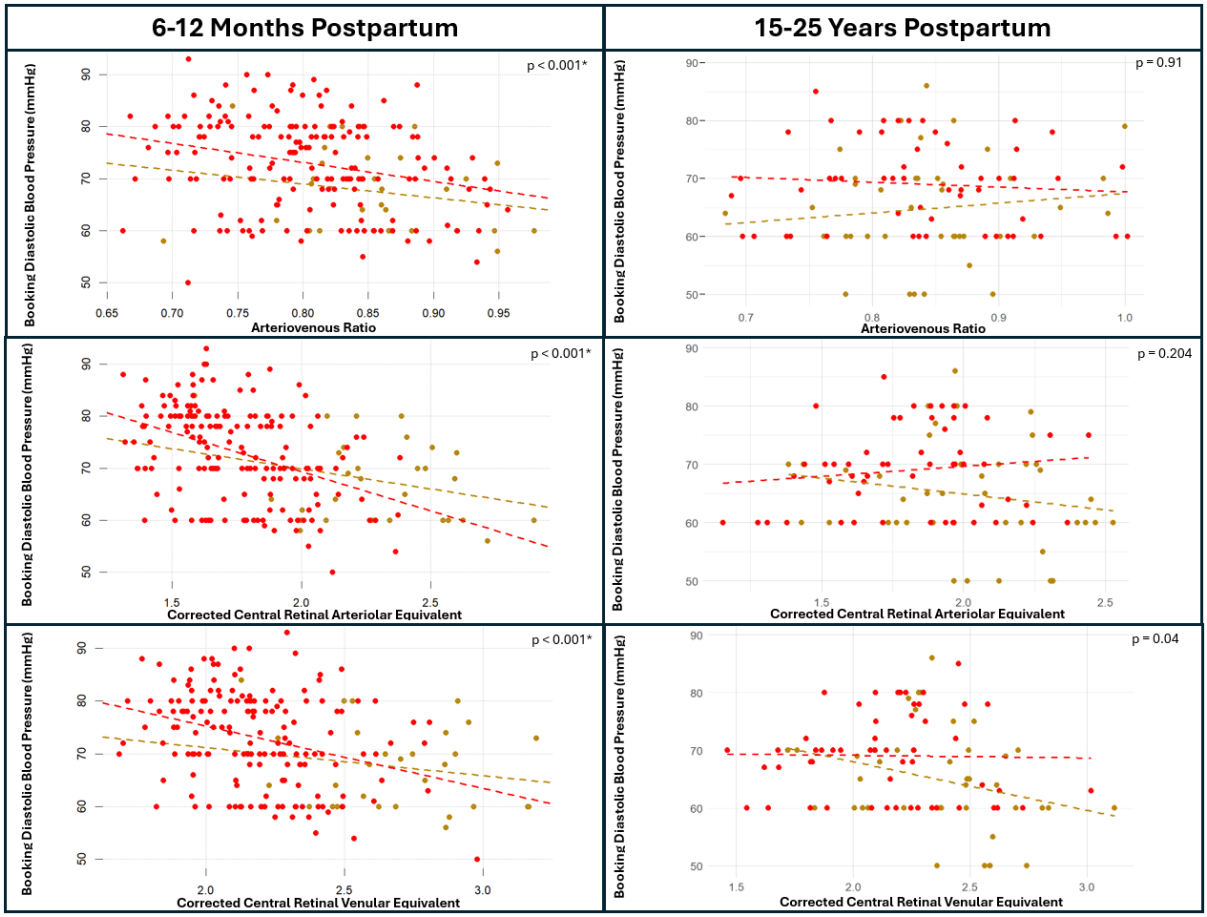


Figure 23. Relationship between retinal vascular calibre and antenatal diastolic Blood Pressure. Scatterplots illustrate the association between antenatal diastolic blood pressure (measured at booking) and retinal microvascular parameters at 6-12 months postpartum (left column) and 15-25 years postpartum (right column). Each row represents a different retinal variable. Top row: Arteriovenous ratio. Middle row: Corrected Central Retinal Arteriolar Equivalent. Bottom row: Corrected Central Retinal Venular Equivalent. Red dots represent participants with a history of hypertensive disorders of pregnancy, and gold dots represent normotensive controls. Dashed lines represent linear regression lines with 95% confidence intervals. P values are reported above each graph from the multiple linear regression results. An asterix is used to represent significant differences.

XVIII. Discussion

This chapter sought to determine whether there is a fixed microvascular phenotype across the life course in women with hypertensive pregnancy disorders, which may drive the widespread end-organ dysfunction that is well characterised in this cohort. In addition, this chapter aimed to investigate whether the retinal microvascular phenotype observed in women with hypertensive pregnancy disorders, is associated with early blood pressure measurements, and whether postpartum blood pressure control can alter this phenotype.

Retinal arteriolar and venular calibre were consistently narrower in women with hypertensive pregnancies at both 6-12 months and 15-25 years postpartum, with the most pronounced differences observed at 6-12 months. This was evident in women with both gestational hypertension and preeclampsia, with more marked arteriolar narrowing in those with gestational hypertension. There were no significant differences in retinal calibre between women receiving tighter blood pressure control to those who were given standard NHS care in the puerperium. However, suboptimal blood pressure control at 6-12 months postpartum was linked to more adverse microvascular outcomes. In addition, retinal calibre was associated with antenatal blood pressures at 6-12 months postpartum.

The finding that retinal arteriolar and venular narrowing were observable as early as 6-12 months postpartum, even after adjustment for body mass index and time since delivery, is consistent with Soma-Pillay et al's., (2018) findings that corrected central retinal arteriolar equivalent and corrected central retinal venular equivalent were significantly lower in women with preeclampsia, compared to controls both at delivery and one year postpartum ($p < 0.001$)[148].

What is striking about these findings is that the average central retinal arteriolar equivalent in the hypertensive pregnancy group at 6-12 months postpartum was 170.63 μm, which is below the normative values for women over the age of 55[224]. Considering the average age of this cohort was 33.41 years, this suggests that there may have been premature pregnancy-induced microvascular injury or a predisposition to hypertensive pregnancy.

Narrowing of the microvasculature can have widespread systemic consequences. Impaired microvascular integrity increases peripheral vascular resistance, contributing to higher afterload and promoting left ventricular hypertrophy through chronic pressure overload[209]. Over time, these microvascular alterations are thought to play a key role in the pathogenesis of heart failure with preserved ejection fraction, where increased ventricular stiffness and impaired relaxation are driven by microvascular rarefaction and endothelial dysfunction[225].

Similarly, reduced microvascular density within the cerebral circulation can compromise perfusion and autoregulation, increasing the risk of white matter lesions, cognitive decline, and vascular dementia[215]. In the renal microvasculature, structural rarefaction and capillary loss can lead to impaired filtration, glomerulosclerosis, and progressive renal dysfunction[210]. Collectively, these findings highlight how early microvascular alterations may represent a shared pathway linking cardiovascular, cerebral, and renal target organ damage in hypertension and related disorders.

Furthermore, our finding of greater arterial narrowing in the women with gestational hypertension is consistent with Benschop et al.,'s (2017) findings[149]. Given that arteriolar calibre is influenced by blood pressure and weight[226-227], this finding may reflect the higher weight of the gestational hypertension group in this cohort. Alternatively, it may

reflect a longer or more severe period of antenatal hypertension compared to the preeclamptic group. This postulation is likely given that the women with gestational hypertension in this cohort had a higher gestational age at delivery and higher antenatal blood pressures compared to the normotensive and preeclamptic groups *(Table 6),* suggesting that they were exposed to high blood pressure for longer.

Another interesting finding was that venular narrowing was also increased at 6-12 months postpartum in women with hypertensive pregnancies compared to women with normotensive pregnancies. Venular narrowing is often associated with ageing due to factors like venous stiffening, reduced elasticity and hormonal alterations[228-229]. Thus, these findings indicate the possibility of an accelerated vascular ageing process in women with hypertensive pregnancies, possibly driven by altered nitric oxide availability and other pregnancy-related hormonal disruptions[228-229].

The persistence of increased retinal microvascular narrowing in the hypertensive group compared to the normotensive group, even at 15-25 years postpartum supports the hypothesis that hypertensive pregnancies lead to lasting vascular alterations. However, it is important to note that the magnitude of difference between the normotensive and hypertensive groups was smaller at 15-25 years postpartum, compared to 6-12 months postpartum. This finding suggests that after the initial insult of pregnancy, microvascular calibre does not reduce at a faster rate than the normotensive pregnancy population.

Linking in with this, I did not find any significant differences in the arteriovenous ratio at 15-25 years postpartum between women with hypertensive and normotensive pregnancies. This is likely due to an age-related narrowing of the venules without any further decline in arteriolar narrowing. If both the venules and arterioles constrict at the

same rates, then there would be a limited ability to detect overall differences in the arteriovenous ratio at this timepoint.

Given that retinal narrowing is already present at 6-12 months postpartum in women with hypertensive pregnancies and that the gap between them and women with normotensive pregnancies does not appear to widen later postpartum, these findings suggest that it may be better to intervene during pregnancy or early postpartum to prevent organ changes and reduce long-term disease risk.

Even though I did not find any significant differences between those who had usual NHS care and those with physician optimised blood pressure control postpartum, the graded association between retinal measures and blood pressure normalisation at 6-12 months postpartum suggests that vascular changes may be, to some extent, reversible. Alternatively, there may be a subgroup of patients who for currently unknown reasons recover better from their hypertensive pregnancies and have both better blood pressure and retinal calibre.

Interestingly, retinal calibre at 6-12 months postpartum was significantly associated with antenatal blood pressures, suggesting that pre-existing microvascular changes may contribute to the development of hypertensive pregnancy disorders. The persistent venular narrowing in relation to higher antenatal blood pressures supports this notion, indicating that microvascular alterations may precede and contribute to hypertension during pregnancy. These findings highlight the need for future research to investigate how pre-existing microvascular changes contribute to the development of hypertension during pregnancy. Identifying high-risk women early, through pre-pregnancy vascular assessments, could provide new avenues for preventive care. Additionally, the use of retinal vessel measurements as a valuable adjunct to blood pressure monitoring could

improve cardiovascular risk stratification in women with hypertensive disorders of pregnancy.

XIX. Strengths and Limitations

This work provides valuable insights into the short and long-term microvascular impact of hypertensive disorders of pregnancy. A major strength of the work is the large sample size of 301 women and the use of two distinct timepoints postpartum. Additionally, a key strength of this data is that it was all collected and analysed by the same research groups using identical protocols. This methodological consistency enables a direct cross-sectional comparison of the magnitude of calibre differences across different postpartum timepoints and ensures that any observed changes are attributed to the time postpartum rather than differences in the methodology.

Nevertheless, it is important to acknowledge the limitations of this work. First, the main limitation of this study is that it is not a longitudinal study. Therefore, any direct changes in participants from early to late postpartum cannot be inferred. The data were derived from several different studies, including two intervention trials and an observational cohort, rather than being derived from a single, purpose-built study. This may have introduced some heterogeneity in participant selection and follow-up protocols. However, all studies adhered to standardised retinal and cardiac imaging protocols with rigorous quality control processes, which helped to ensure methodological consistency across the datasets.

Second, the relatively small sample size of the normotensive cohort at 6-12 months postpartum limits the statistical power to detect group differences and may reduce the generalisability of the findings. This is particularly relevant for subgroup analyses.

Further research using more balanced cohorts could be useful to validate and expand upon the findings of this chapter.

A third limitation is the exclusion of retinal data from some participants, which may have reduced the statistical power to detect more subtle associations. However, this exclusion was due to data quality rather than participant characteristics, thereby minimising systematic bias.

Fourth, the study cohort were recruited from a single site, which may limit the external validity of the findings and applicability of the results to broader populations. The cohort was relatively homogenous in terms of demographic and geographic background. Therefore, a more diverse, multi-centre study that includes women from diverse geographic regions and ethnic backgrounds would increase the generalisability of the findings and provide a better reflection of the full spectrum of microvascular risk in women following hypertensive disorders of pregnancy.

Lastly, the lack of pre-pregnancy vascular measurements makes it difficult to determine whether the observed microvascular alterations are a consequence of hypertensive pregnancy or whether they reflect pre-existing vascular abnormalities which predispose women to hypertensive pregnancies. Longitudinal data tracking retinal and cardiac measures from pre-pregnancy through to late postpartum would allow us to more precisely understand temporal trajectories of disease progression.

XX. Conclusions

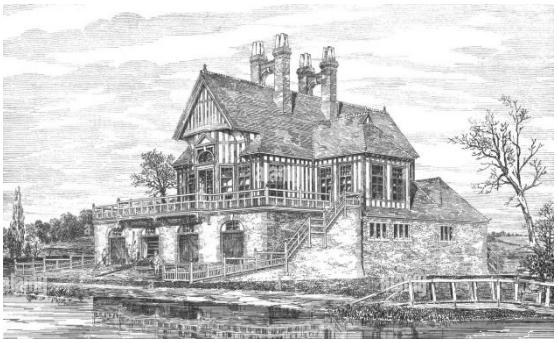
The retinal vascular changes observed in our cohort further underscore the lasting microvascular impact of hypertensive pregnancies. Both the women with preeclampsia

and gestational hypertension exhibited lower arteriovenous ratios and narrower arterioles and venules compared to the normotensive women at 6-12 months and 15-25 years postpartum. These findings are consistent with impaired endothelial function and altered vascular remodelling.

These changes in retinal microvasculature were observable as early as the first year after pregnancy, suggesting that the women have experienced an initial insult during pregnancy or had pre-existing differences in microvasculature which then increased their predisposition for hypertensive pregnancy. Although, any further decline in arterial narrowing appears minimal, these women still had worse values at 15-25 years postpartum than women with normotensive pregnancies, supporting my initial hypotheses that women with hypertensive pregnancies have a fixed microvascular phenotype.

Future research is needed to further explore the role of microvascular changes in the development of hypertensive pregnancies and to investigate the long-term cardiovascular implications for women with a history of hypertensive pregnancy.

CROSS-SECTIONAL AND LONGITUDINAL CARDIAC ANALYSIS AT 15-25 YEARS POSTPARTUM



5

I. Overview and Aims

This chapter examines the long-term cardiovascular impact of hypertensive pregnancy disorders through the analysis of longitudinal data collected in women with normotensive and hypertensive pregnancies at two postpartum time points: five to ten years and 15-25 years post their index pregnancy. The longitudinal design used enables the temporal evaluation of subtle cardiovascular changes within the same individuals. By characterising these trajectories, this chapter aims to clarify whether pregnancy-related cardiovascular changes represent transient adaptations or the early stages of progressive pathological remodelling.

The cohort included here were originally enrolled in the Preeclampsia Vascular Study (PVS). Prior analyses of this cohort revealed that at five to ten years postpartum, women with hypertensive pregnancies displayed a distinct cardiac geometry to women with normotensive pregnancies[122]. Namely, these women had higher left ventricular mass index (49.9 ± 7.1 versus 46.0 ± 6.5 g/m^2; p = 0.001) and higher left atrial volume index (40.4 ± 9.2 versus 37.3 ± 7.3 mL/m^2; p = 0.03), with differences persisting even

after adjustment for blood pressure. Computational modelling further revealed a distinct pattern of left ventricular mass distribution which was independently associated with a hypertensive pregnancy history[122].

In addition to structural changes, women with prior hypertensive pregnancy disorders showed early impairments in diastolic and systolic function. Measures such as left ventricular ejection fraction (NT: 65.6 ± 5.4%, HT: 63.7 ± 4.3%, $p = 0.03$), the ratio of early to late (E/A) ventricular filling velocities (NT: 1.52 ± 0.45, HT:1.34 ± 0.35; $p = 0.003$) and global longitudinal strain (NT: -19.94 ± 3.59%, HT: -18.31 ± 4.46%, $p = 0.02$) were all significantly lower in women with hypertensive pregnancies compared to normotensive women at five to ten years postpartum. Additionally, end- diastolic volumes (70.3 ± 9 versus 74 ± 9.5 mL/m^2; $p = 0.01$) and end- systolic volumes (24.3 ± 5.1 versus 26.9 ± 5.0; $p = 0.002$) indexed to body surface area were significantly lower in women with hypertensive pregnancy disorders[122].

These findings raise important questions on whether subclinical cardiac changes stabilise, regress or accelerate as women with hypertensive pregnancy disorders progress into midlife. Existing epidemiological studies consistently show that women with histories of hypertensive pregnancy are at increased risk of chronic hypertension[230], heart failure[90], coronary artery disease[153] and stroke[101]. Yet the mechanistic pathways linking a hypertensive pregnancy to these clinical endpoints remain poorly defined.

This work aims to bridge the gap between pregnancy and cardiovascular clinical endpoints by seeing how cardiac indices evolve over time and whether a hypertensive pregnancy history predicts accelerated cardiovascular decline. I hypothesise that the differences in cardiac structure and diastolic dysfunction previously observed in the PVS cohort will persist a decade later at the time of the HELPFUL study. Additionally, I

hypothesise that women with a hypertensive pregnancy history will show an accelerated rate of functional and structural cardiac decline.

II. Analysis, Design and Population

This chapter consists of two complementary analyses designed to capture the persistence and progression of cardiovascular changes after hypertensive pregnancy.

The first piece of work involves a cross-sectional analysis of women at 15-25 years post index pregnancy, who are enrolled in the HELPFUL study. This cohort is comprised of women who either had one or more hypertensive or normotensive pregnancies during their lifetime, with at least one of their pregnancies being 15-25 years ago. The index pregnancy used in HELPFUL was the pregnancy defined previously in the PVS Study. For most participants, this was their first pregnancy. For some women, the indexed pregnancy was not their first, meaning the follow-up extended beyond 25 years. All participants with cardiac imaging data collected in the HELPFUL study were included in the cross-sectional analysis. Detailed information on recruitment, inclusion/exclusion criteria and data availability are provided in *Chapter four,* and a full study protocol can be found in *Appendix B.*

The second part of this work involves a longitudinal analysis in a subset of the PVS cohort who also participated in the HELPFUL study. These women underwent cardiac imaging or echocardiography assessments at both timepoints. Since cardiac magnetic resonance imaging and echocardiography were not included in the original PVS protocol and they were introduced later through a study amendment, only participants who underwent cardiac imaging at both timepoints were included in the analysis.

Ethical approval for PVS was obtained in 2010 (08/H0604/127) with data collection occurring between the years of 2010 and 2015, when the participants were approximately five to ten years post their index pregnancy. Ethical approval for the HELPFUL study (22/LO/0781) was obtained in May 2023, with data collection occurring between June 2023 and June 2024, when the women were approximately 15-25 years post their index pregnancy.

III. Recruitment from the Preeclampsia Vascular Study to the HELPFUL Study

To facilitate the long-term follow-up, the PVS team previously maintained a secure database on a high compliance system containing addresses and contact information for participants who consented to future contact. Using these databases, I sent out an initial invitation letter *(Appendix C)* to eligible individuals, which included a participant information leaflet *(Appendix D)* with clear information about the study procedures and how to get in touch for further details. After the initial letter was sent, I followed up with the women by phone or email to confirm their interest in the study, to ensure that they had received the participant information leaflet and to address any questions.

IV. Primary and Secondary Outcomes for the Cardiac Analysis

The primary and secondary cardiac outcomes for both the cross-sectional and longitudinal analyses are outlined in *Table 10.* Left ventricular mass was selected as the primary structural outcome and the E/A ratio as the primary functional outcome. These outcomes were chosen for two reasons. First, they showed significant differences in the Preeclampsia Vascular Study[122]. Second, they are well-established, sensitive clinical

markers of cardiac remodelling and diastolic function, both of which are strongly associated with cardiovascular disease risk[231-232].

For the cross-sectional analysis, the primary outcome was left ventricular mass indexed to body surface area. Left ventricular mass was indexed to allow for standardised comparisons between individuals of different body sizes. However, in the longitudinal analysis, unindexed left ventricular mass was the primary outcome to track absolute within-person changes over time. This approach avoided masking true increases in cardiac mass which may occur if changes in body size are also incorporated into the index. Nevertheless, as weight gain may influence the findings, indexed values were also analysed.

Secondary outcomes were chosen to provide a broader characterisation of the cardiac phenotype. These included measures of systolic performance (ejection fraction, stroke volume, cardiac output, cardiac index), diastolic function (medial and lateral tissue doppler velocities and deceleration time), ventricular geometry (left and right ventricular volumes, global wall thickness, right ventricular mass), right heart function (ejection fraction, tricuspid annular plane systolic excursion, outflow) and hemodynamic adaptation (heart rate and left ventricular outflow tract velocity time integral).

Table 10. Primary and secondary outcomes for the cross-sectional and longitudinal cardiac analyses. Outcomes are reported for magnetic resonance imaging (left) and echocardiography (right) with cross-sectional outcomes reported in the first half of the table and longitudinal outcomes reported in the second half of the table.

Cross-Sectional Analysis		
Outcome	**Magnetic Resonance Imaging**	**Echocardiography**
Primary	Left Ventricular Mass Index	Mitral Valve E/A Ratio
Secondary	Left Ventricular Ejection Fraction	PA Acceleration Time, V_2 Max velocity and Max PG
Secondary	Left Ventricular End-Diastolic Volume Index	Mitral E- and A- wave Max velocity and deceleration time
Secondary	Left Ventricular End-Systolic Volume Index	Medial Peak S' Velocity
Secondary	Left Ventricular Stroke Volume	Medial Peak E' Velocity
Secondary	Left Ventricular Cardiac Output	Medial Peak A' Velocity
Secondary	Global Mean Wall Thickness	Lateral Peak S' Velocity
Secondary	Heart Rate	Lateral Peak E' Velocity
Secondary	Right Ventricular Mass Index	Lateral Peak A' Velocity
Secondary	Right Ventricular Ejection Fraction	Medial, Lateral and Mean E/E′ Ratio
Secondary	Right Ventricular End Diastolic Volume Index	Tricuspid Annular Plane Systolic Excursion

Secondary	Right Ventricular End-Systolic Volume Index	Right Ventricular S′ Velocity and Ejection Time
Secondary	Right Ventricular Stroke Volume	Aortic Valve Maximum Velocity, Mean Gradient, Maximum Pressure Gradient, Mean PG, VTI
Secondary	Right Ventricular Cardiac Output	Left Ventricular Outflow Tract V1 Maximal Velocity, Mean Velocity, Maximum PG, Mean PG and VTI

Longitudinal Analysis

Outcome	**Magnetic Resonance Imaging**	**Echocardiography**
Primary	Left Ventricular Mass	Mitral Valve E/A Ratio
Secondary	Left Ventricular Mass Index	Mitral Valve E- wave Maximum Velocity
Secondary	Left Ventricular Ejection Fraction	Mitral Valve A- wave Maximum Velocity
Secondary	Left Ventricular End-Diastolic and End-Systolic Volume (Indexed and unindexed)	Mitral Valve Deceleration Time
Secondary	Left Ventricular Stroke Volume and Cardiac Output	Medial A’ Velocity
Secondary	Global Mean Wall Thickness	Lateral Peak E' Velocity
Secondary	Heart Rate	Lateral Peak A' Velocity

Secondary	Right Ventricular Mass (Indexed and unindexed)	Lateral E/E' Ratio
Secondary	Right Ventricular Ejection Fraction	Tricuspid Annular Plane Systolic Excursion
Secondary	Right Ventricular End-Diastolic and End-Systolic Volume (Indexed and Unindexed)	Right Ventricular S' Velocity
Secondary	Right Ventricular Stroke Volume and Cardiac Output	Left Ventricular Outflow Tract V1 VTI

Max = Maximum, PA = Pulmonary Artery, PG = Pressure Gradient, Velocity Time-Integral, E/A = Early to Late Ventricular filling velocities.

V. Methods

Both the PVS and the HELPFUL study were conducted at the Cardiovascular Clinical Research Facility at the John Radcliffe Hospital, Oxford, UK. Similar protocols were used for baseline anthropometric and clinical assessments such as blood pressure measurements, height and weight. Although the research setting was consistent, there were some differences in the models of the echocardiography and magnetic resonance imaging machines used. These models are described in detail in the relevant sections below. There were also some software updates and hardware replacements which occurred over time as part of routine clinical practice.

The HELPFUL study data was collected by myself, Prenali Sattwika and a team of researchers, whilst the PVS data were collected by Harry Boardman, Merzaka Lazdam and their team. Detailed methods of the PVS including participant recruitment, diagnostic criteria, and imaging protocols have been published previously[86,122] and a full description of the HELPFUL study visit is included in *Chapter 4* and *Appendix B*.

In brief, both study visits included informed consent, anthropometric assessments, blood sampling, blood pressure measurements, echocardiography, cardiovascular magnetic resonance imaging, and pulse wave analysis. At the end of each visit, participants were provided with a health and lifestyle questionnaire to complete which was kept almost identical across both studies to maximise comparability, with some updates to account for age-related changes in the population and societal shifts such as the introduction of e-cigarettes.

VI. Data Sources and Linkage

Baseline pregnancy and early postpartum data were extracted from a secure PVS database. This dataset included detailed records of pregnancy outcomes, as well as the cardiovascular assessments that were conducted at five to ten years postpartum. To ensure accurate linkage between the participants' pregnancy histories and their long-term cardiovascular outcomes, I manually reviewed and validated the dataset by cross-referencing entries with the original PVS questionnaires and verifying the consistency of each participant's data across sources.

Following this process, I then systematically matched each PVS participant to their corresponding longitudinal records in the HELPFUL study by using identifiers such as study ID, participant name and date of birth. I subsequently used the data collected in the HELPFUL questionnaires to construct an integrated longitudinal dataset with updated demographic and clinical information.

VII. Echocardiography Acquisition and Analysis

Echocardiography was performed and analysed in accordance with the British Society of Echocardiography guidelines[233], by a team of trained echocardiographers. In PVS, the images were acquired using a Toshiba Artida and Philips iE33 machine. Meanwhile, in the HELPFUL study, the Philips EPIQ CVx and iE33 systems were used. All machines were high-end ultrasound systems designed for cardiac imaging and equipped with two-dimensional phased-array transducers. The scans were acquired by trained sonographers with the participants resting in the left lateral decubitus position and they were digitally stored for offline, anonymised analysis using IntelliSpace Cardiovascular software (Phillips, Netherlands).

Standard pulsed-wave and continuous-wave doppler techniques were used to quantify flow velocities and pressure gradients across the mitral, aortic, and pulmonary valves. The mitral valve E- wave and A- wave maximal velocities, E/A ratio, and deceleration time were measured using pulsed-wave doppler in the apical four-chamber view. Tissue doppler imaging was employed to evaluate medial and lateral annular velocities, including peak S', E', and A' velocities, from which the average E/E′ ratio was calculated.

Pulmonary artery acceleration time, V_2 max velocity, and pressure gradients were derived from pulsed and continuous wave doppler of the right ventricular outflow tract. Right ventricular function was assessed through right ventricular ejection time, tricuspid annular plane systolic excursion, and S′ velocity at the tricuspid annulus using m-mode and tissue doppler imaging.

Aortic valve velocities, pressure gradients, and velocity-time integrals were measured using continuous-wave doppler, whilst left ventricular outflow tract velocities and velocity-time integrals were assessed using pulsed-wave doppler in the apical five-chamber and long-axis views. All measurements were averaged over multiple cardiac cycles.

VIII. Cardiac Magnetic Resonance Imaging Acquisition

Both studies followed similar cardiovascular magnetic resonance imaging protocols using the same standardised settings, including the use of retrospective electrocardiography gating with a precordial three-lead electrocardiography setup, as well as the acquisition of steady-state free precession images during breath-hold at end expiration.

The short-axis stack was used to calculate left and right ventricular mass, end-diastolic and systolic volumes, stroke volume and cardiac output for both studies. This was obtained using the following parameters: a repetition time of 45.72 ms, an echo time of 1.07 ms, a flip angle of 60^{o}, in-plane resolution of 2.8 x 2.0 mm, a slice thickness of 8.0 mm and 25 calculated phases, yielding a temporal resolution of 40 ms at a heart rate of 60 beats per minute.

The short-axis stacks were acquired at 1cm intervals, covering the entire left and right ventricles and guided by the 2-, 3- and 4- chamber views. The 2-, 3- and 4- chamber views were acquired using the following parameters: a repetition time of 39 ms, echo time of 1.1 ms, flip angle of 60^{o}, in-plane resolution of 1.9 x 1.9 mm, a slice thickness of 7.0 mm and 25 calculated phases, yielding a temporal resolution of 40 ms at a heart rate of 60 beats per minute.

The key difference in imaging between the PVS and the HELPFUL study was the magnetic resonance imaging scanner used. The PVS used a 1.5 Tesla Avanto FIT (Siemens Medical Solutions, Erlangen, Germany) with a 12-channel surface array body coil, whereas the HELPFUL study employed a 3.0 Telsa scanner (PRISMA, Siemens Medical Solutions, Erlangen, Germany) with an 18-channel surface array body coil. Although differences in magnetic field strength and the coil used can affect image quality and resolution, prior validation has shown that the cardiac indices used in this work are comparable across the scanners [234].

IX. Cardiac Magnetic Resonance Imaging Analysis

Image analysis was performed using CVI42 version 6.3 (Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada). To ensure consistency in the image analysis over time and

to take advantage of the advances in imaging methods and software over the past decade, I recontoured all of the HELPFUL and PVS scans used in this work by using the same standardised approach, under the guidance of Prenali Sattwika and Adam Lewandowski.

Before starting the analysis, I reviewed the CVI42 database to confirm the availability of all of the PVS and HELPFUL scans. I then matched up the scans based on the participants' dates of birth and documented this in a spreadsheet. Once I had verified that every scan was accounted for, I anonymised the spreadsheet by removing any identifying information such as pregnancy status and I randomised the order of the cases to ensure blinding. I then rematched these once the contouring process was complete.

Ventricular borders were contoured using the CVI42 software through a semi-automated process, followed by manual adjustments to ensure accuracy. At end-diastole, right and left epicardial and endocardial borders were contoured. At end-systole only endocardial borders were contoured *(Figure 24)*. Any trabeculations and papillary muscles were excluded from the myocardial mass in line with the Oxford Centre of Cardiovascular Magnetic Resonance Imaging's protocol's, which are based on the Journal of Cardiovascular Magnetic Resonance's guidance[235]. Image contrast was adjusted for each slice to distinguish epicardial fat from myocardium.

End-systole was visually identified as the phase with the smallest ventricular cavity area, while end-diastole was identified as the phase with the largest ventricular cavity area. For the left ventricle, the basal slice at end-diastole was defined as the first short axis slice in which at least 50% of the circumference was enclosed by ventricular myocardium. This selection was validated by confirming that the cavity remained enclosed by myocardium for three adjacent end-diastolic frames. The same basal slice was then reviewed for end-systole and included only if the myocardium continued to surround the cavity. Additional

validation was performed by reviewing adjacent systolic frames, neighbouring slices above and below, and cine loops.

Identification of the right ventricular basal slice and structures was aided by cine viewing and through anatomical landmarks. Right ventricular walls were differentiated from atrial walls by their increased thickness, trabeculation and systolic inward motion. At end-systole, the atrioventricular groove was identified by its excursion. Due to tricuspid valve through-plane motion, the upper posterior right ventricle often appeared in the basal slice at end-diastole but corresponded to the right atrium at end-systole.

Basal contours were traced up to the pulmonary valve. The interventricular septum was considered part of the left ventricle and therefore excluded from right ventricular mass calculations. Apical slices at both end-diastole and end-systole were defined in the last short-axis view with a residual left ventricular cavity visible. Myocardial mass was calculated by summing the myocardial areas across the image stack and multiplying the total by the myocardial tissue density (1.05 g/cm^3). Left ventricular mass was then indexed to body surface area which was calculated using the Mosteller formula[197]:

$$Body\ Surface\ Area\ (m^2) = \sqrt{\frac{[height\ (m)\ x\ weight\ (kg)]}{3600}}$$

End-diastolic and end-systolic volumes were calculated by summing the ventricular areas across the image stack for each respective phase. Stroke volume was calculated as the difference between end-diastolic and end-systolic volumes, and ejection fraction was derived by dividing the stroke volume by the end-diastolic volume.

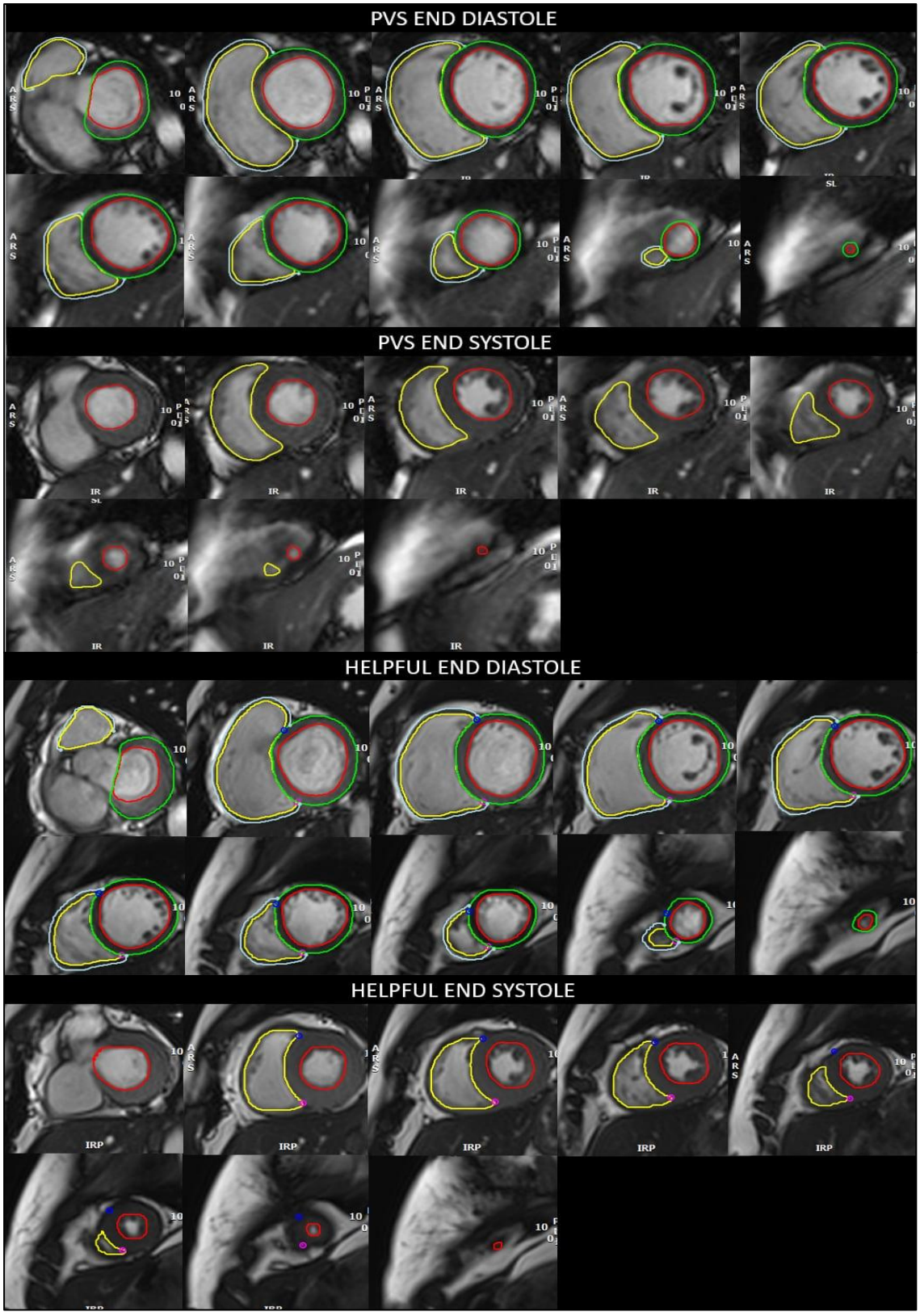


Figure 24. Cardiac magnetic resonance short-axis contours at end-diastole and end-systole in the same participant at two postpartum timepoints. Segmentation of the left ventricle can be seen in red and green contours and the right ventricle in yellow

contours. The images from PVS at five to ten years post index pregnancy are shown in the top panels and the images captured in the HELPFUL study at 15-25 years post index pregnancy can be seen in the bottom panels.

Left ventricular global mean wall thickness was measured at the mid-ventricular short-axis level during end-diastole. Measurements were obtained orthogonal to the endocardial-epicardial borders across six standard myocardial segments: anterior, lateral, inferior, inferolateral, inferoseptal and anteroseptal *(Figure 25)*. Segmental definitions and measurement protocols followed standard international guidelines[223]. A global mean wall thickness was then derived as the average of the six segmental values.

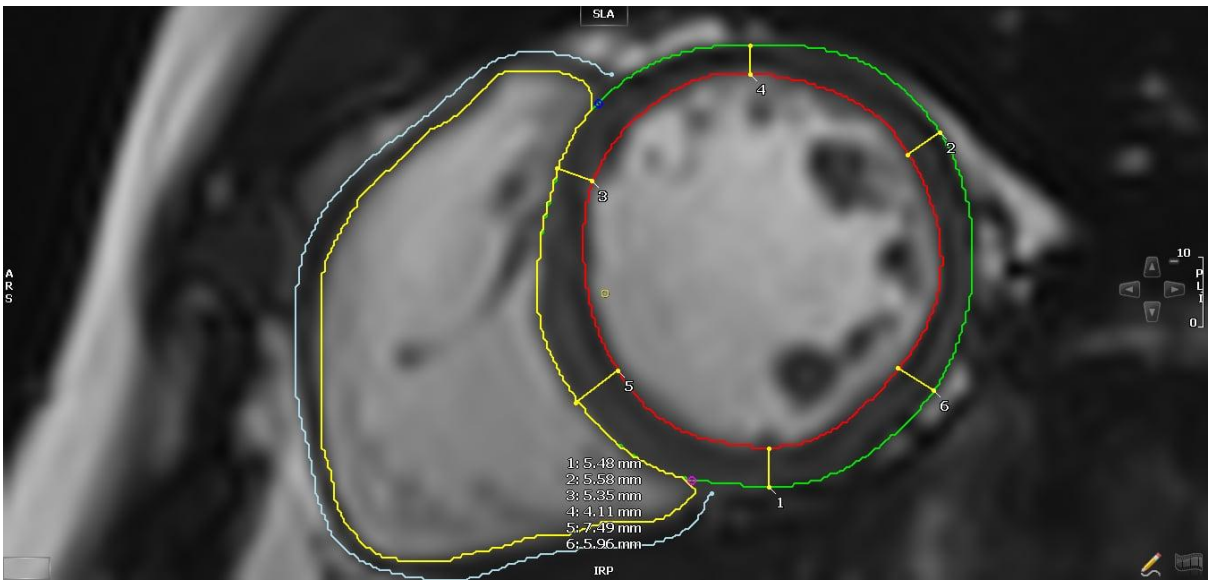


Figure 25. Segmentation of the left ventricle to measure global mean wall thickness. Myocardial wall thickness is measured at six standardised segments: anterior, lateral, inferior, inferolateral, inferoseptal and anteroseptal (yellow radial lines), with values displayed in millimetres. The red contours surround the left ventricle, and the blue contours surround the right ventricle.

X. Statistical Analysis

All analyses were performed using R (version 4.5.1) and R Studio (version 2025.05.1 + 513). Data distributions were tested for normality using the Shapiro-Wilks test for continuous variables where n is less than or equal to 50 and through visual observation using Q-Q plots and histograms for larger samples. Normally distributed continuous variables were reported as means and standard deviations. Continuous variables with skewed distributions were reported as medians and interquartile ranges. Categorical data were presented as counts and percentages.

For the recall of bias analysis and cross-sectional comparisons across hypertensive and normotensive groups, the Welch's independent samples t-test was used for normally distributed variables, and the Wilcoxon rank-sum test was used for variables with skewed distributions. Before carrying out the tests, F-tests were performed to evaluate the equality of variances. Differences across counts and percentages were compared using a two-sample test for equality of proportions, also known as the z test, with 95% confidence intervals for the difference in proportions calculated.

For longitudinal within-subject comparisons, paired t-tests were applied when change scores were normally distributed and the Wilcoxon Signed-Rank Test was used when they were skewed. Prior to analysis, the normality of the change distributions was assessed using the Shapiro-Wilks test.

To compare group differences in the primary outcomes, linear mixed effects models were used to account for repeated measures within the women. Group (hypertensive vs normotensive) and timepoint (five to ten years vs 15-25 years postpartum) were included as fixed effects, with a random intercept for participant ID. Left ventricular mass and E/A ratio were used as dependent variables. Interaction terms between group and

timepoint were specified to test for differential changes between hypertensive and normotensive women. Model assumptions were checked using residual diagnostics.

XI. Results - Study Population

Of the 268 women initially enrolled in the PVS, two were deceased prior to the recruitment of the HELPFUL study. One death was secondary to a subarachnoid haemorrhage and the other was due to a frontal lobe haemorrhagic stroke. This resulted in 266 participants that were eligible to be contacted. A total of 121 women agreed to participate in the HELPFUL study, reflecting an acceptance rate of approximately 45%. Recruitment progressed quickly, averaging just over ten participants per month *(Figure 26)*. Of the remaining 145 women, 98 were either unresponsive or accepted the invitation to participate after study recruitment had closed, 44 women declined participation, and three women had not provided consent during PVS to be re-contacted for future studies.

The reasons for declining the invitation to participate included logistical challenges such as the location or timing of the study, personal preferences and unrelated health issues. Some women were unresponsive because of changes in their address which prevented the delivery of their letters, and others could not be reached as their contact details had changed since the previous study. The reasons why the other women were unresponsive remains largely unknown.

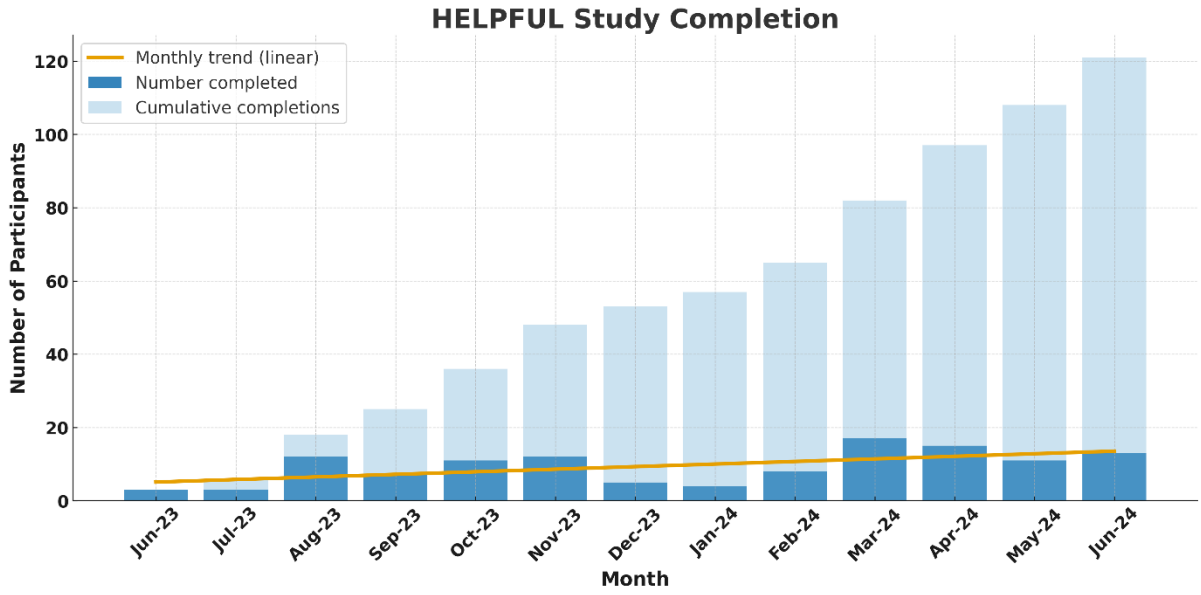


Figure 26. HELPFUL study completion by month (June 2023 - June 2024). Dark-blue bars show the number of participants completing a visit each month; light blue bars show the cumulative visit completions. The orange line indicates the linear trend in monthly visit completions.

All 121 HELPFUL participants underwent at least one form of cardiac assessment and were eligible for inclusion in the cross-sectional analysis. Transthoracic echocardiography was acquired in all participants. However, some parameters were unmeasurable due to suboptimal image quality. Per-parameter denominators (n) are reported in the results tables.

Cardiac magnetic resonance imaging was performed in 114 women but after quality control, 104 datasets were included. For the longitudinal analyses, paired imaging at both PVS and HELPFUL were available in 77 women for echocardiography and 70 women for cardiac magnetic resonance imaging. A detailed overview on recruitment numbers is summarised in *Figure 27*.

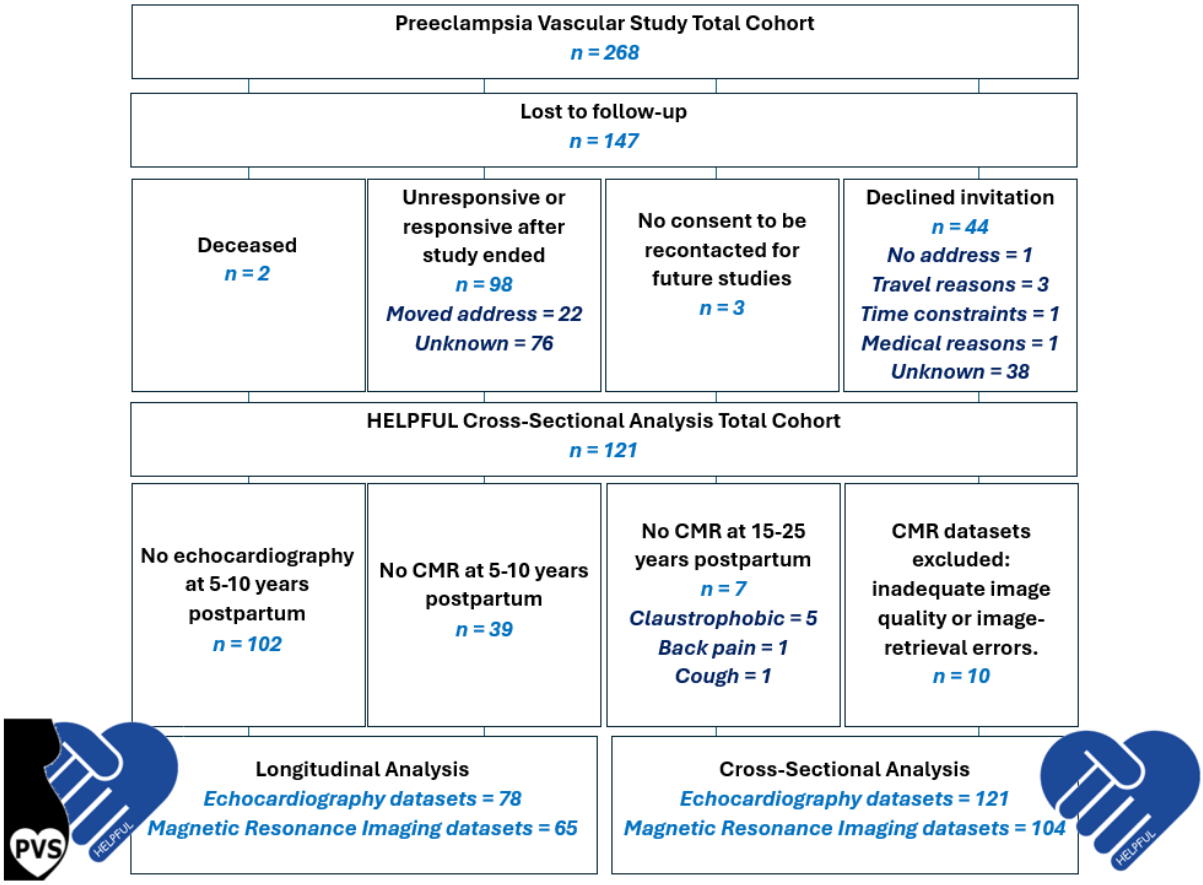


Figure 27. Flowchart of participant recruitment and inclusion in the cross-sectional and longitudinal cardiac analyses for the PVS and HELPFUL studies. CMR = Cardiac Magnetic Resonance.

XII. Pregnancy and Antenatal Characteristics

Of the 121 women enrolled in the HELPFUL study, 45 women (37.2%) had normotensive pregnancies, and 76 women (62.8%) experienced a hypertensive pregnancy. Within the pregnancy hypertension group, ten women (13.2%) had gestational hypertension, 24 women (31.6%) had early-onset preeclampsia, and 37 women (48.4%) had late-onset preeclampsia. A small number of women had more than one diagnosis across different pregnancies. This included three women (3.9%) who experienced both early- and late-onset preeclampsia and two women (2.6%) who had both gestational hypertension and preeclampsia. There were also ten women (13.2%) who had additional diagnoses of

HELLP syndrome and three women with additional diagnoses of eclampsia (3.9%). All of the normotensive cohort only had normotensive pregnancies.

The antenatal and pregnancy characteristics of the cohort are summarised in *Table 11*. The average time since first birth was 23 years for the normotensive cohort and 22.7 years for the hypertensive pregnancy cohort, with no significant differences across groups ($W = 1690$, $p = 0.89$). Most participants were multiparous, with only a small proportion of women being primiparous, (NT: 8.9%, HT: 13.2%). The mean number of pregnancies was higher in the normotensive pregnancy group (3.1 versus 2.6 pregnancies) as was the mean number of complete pregnancies (2.5 versus 2 pregnancies).

The average gestational age at delivery across all previous pregnancies was 38 weeks in the normotensive group and 37.5 weeks in the hypertensive pregnancy group. Meanwhile age at first pregnancy was 31.4 years in the hypertensive pregnancy group versus 29.1 years in the normotensive pregnancy group.

To assess follow-up bias, index-pregnancy characteristics recorded at the time of PVS for the entire cohort were compared with those of the participants who subsequently took part in the HELPFUL study *(Table 11)*. The distributions of key pregnancy characteristics were broadly similar between groups, with no significant differences across groups (all $p > 0.05$), suggesting that the women who returned for the follow-up were representative of the wider cohort. Accordingly, the loss of some women to the follow-up is unlikely to have introduced major bias into the longitudinal analysis.

Table 11. General and indexed pregnancy characteristics of the women in the Preeclampsia Vascular Study and the HELPFUL study cohorts.

General Pregnancy characteristics of the HELPFUL Cohort		
Variable	**Normotensive (N = 45)**	**Hypertensive (N = 76)**
Age at first delivery, years	29.3 ± 4.9[a]	31.4 ± 4.6[a]
Total number of pregnancies	3 ± 2[b]	2 ± 1[b]
Total complete pregnancies	2 ± 1[b]	2 ± 0[b]
Total number of complete hypertensive pregnancies	0 ± 0[b]	1± 1[b]
Total number of complete normotensive pregnancies	3 ± 2[b]	0.5 ± 0[a]
Primiparous, No., (%)	4 (8.9)	10 (13.2)
Average weeks of delivery across pregnancies, weeks	39.8 ± 3[b]	37.5 ± 3.1[b]
Time since first pregnancy, years	23 ± 6[b]	22 ± 5[b]

Pregnancy characteristics at Indexed pregnancy						
Variable	**PVS Normotensive (N = 104)**	**HELPFUL Normotensive (N = 45)**	**Test Statistic and P Value**	**PVS Hypertensive (N = 164)**	**HELPFUL Hypertensive (N = 76)**	**Test Statistic and P Value**

BMI at booking (kg/m^2)	23.2 ± 4.1[b]	22.5 ± 4.7[b]	W = 2019 P = 0.39	25 ± 6[b]	25 ± 5.5[b]	W = 3794.5 P = 0.97
Age at delivery (years)	32 ± 8[b]	32.7 ± 4.8[a]	W = 2080 P = 0.91	32 ± 6.8[b]	32 ± 4.6[a]	W = 5306.5 P = 0.72
Gestational age at delivery (weeks)	39 ± 6[b]	39.5 ± 3[b]	W = 2060 P = 0.67	37 ± 4[b]	37 ± 3.8[b]	W = 5060 P = 0.51
Birthweight of offspring (kg)	3.3 ± 1.4[b]	3.5 ± 0.9[b]	W = 2088 P = 0.39	2.6 ± 1.4[b]	2.9 ± 1.2[b]	W = 4589 P = 0.27
Preterm, No., (%)	29 (27.9%)	10 (22.2%)	Z = 0.74 P = 0.46	71 (43.3%)	30 (39.5%)	Z = 0.46 P = 0.65
Smoking during pregnancy, No., (%)	10 (9.6%)	7 (15.6%)	Z = 0.9 P = 0.37	9 (5.5%)	4 (5.3%)	Z = 0.08 P = 0.94
Time since delivery at PVS (years)	9 ± 1.9[a]	9.3 ± 1.8[a]	W = 8198.5 P = 0.71	9.2 ± 2.5[b]	8.9 ± 2.5[b]	W = 6324.5 P = 0.73

Values are presented as [a]Mean ± Standard deviation, [b]Median ± Interquartile range, or counts and percentages as appropriate. BMI: body mass index; HELPFUL: Hypertension Explored in Long-term Postpartum Follow-up of Later Life; No.,/N = Number, PVS: Preeclampsia Vascular Study.

XIII. Demographic and Clinical Characteristics

The demographic and clinical characteristics measured during the HELPFUL study are reported in *Table 12*. The women in both groups were predominantly non-Hispanic white (100% in the normotensive cohort and 94.7% in the hypertensive pregnancy cohort) and from low deprivation backgrounds (8.5 versus 9 multiple deprivation indices). The mean age at HELPFUL study enrolment was 51.9 years in the normotensive cohort (range: 41-60 years) and 53.6 years in the hypertensive pregnancy cohort (range: 38 to 62 years), with no significant differences across groups ($t = -1.217$, $p = 0.23$).

There were also no significant differences in height ($t = 1.44$, $p = 0.15$), nor weight ($W = 1545$, $p = 0.37$) across groups. On average, the women with prior normotensive pregnancies had a body mass index of 24.97 kg/m^2 which was in the healthy range according to the centre for disease control and prevention guidelines[237]. Meanwhile, the women with prior hypertensive pregnancies had an average body mass index of 26.6 kg/m^2, which placed them into the overweight category[237]. However, these differences in body mass index did not reach statistical significance ($W = 1449.5$, $p = 0.16$).

Women with previous normotensive pregnancies had average systolic and diastolic blood pressures of 115 and 69 mmHg, respectively. Meanwhile, the hypertensive pregnancy group had average systolic and diastolic blood pressures of 124 and 73.7 mmHg, respectively. Systolic blood pressure readings were significantly lower in the normotensive group compared to the hypertensive pregnancy group ($W = 1119$, $p = 0.002$), but not diastolic blood pressure ($W = 1430$, $p = 0.13$).

In terms of body composition, women in the normotensive and hypertensive pregnancy groups had comparable average body fat masses (22.8 kg vs 25.1 kg; $W = 1455.5$, $p = 0.38$), body fat percentage (34.9 % vs. 36.2 %; $t = 1.02$, $p = 0.31$) and visceral fat area (98.9

cm^2 vs 109.32 cm^2; W = 1492, p = 0.5). Likewise, there were no significant differences in skeletal muscle mass across groups (25.73 kg vs 25.2 kg; t = 0.85, p = 0.4).

Lifestyle behaviours were also similar. Around half of women in both cohorts reported current or past smoking or vaping (51.1% NT vs 44.7% HT), while alcohol consumption patterns showed most participants reporting 0 - 14.9 units per week (75.5% vs 87.7), with only a minority exceeding the recommended amount of alcohol per week (24.5% NT vs 12.3% HT).

Table 12. Anthropometric, demographic, body composition, blood biomarkers and the recreational activities of women included in the HELPFUL study. Anthropometry, blood samples and body composition measures were taken during the HELPFUL study and data on ethnicity and recreational activities were collected through questionnaires provided during the HELPFUL study.

Variable	Sample Size (N)	NT (N = 45)	HT (N = 76)
Anthropometry and participant characteristics			
Age, years, Mean ± SD[a]	121	51.9 ± 5.4[a]	53.6 ± 4.3[a]
Height, m, Mean ± SD[a]	121	1.7 ± 0.06[a]	1.6 ± 0.05[a]
Weight, kg, Median ± IQR[b]	121	69 ± 22.3[b]	71.7 ± 21.8[b]
BMI, kg/m^2, Median ± IQR[b]	121	25 ± 4.1[b]	26.6 ± 7.4[b]
Waist: hip ratio (cm: cm), Mean ± SD[a]	121	0.8 ± 0.06[a]	0.9 ± 0.07[a]
Mid left Arm circumference (cm), Median ± IQR[b]	121	29 ± 5[b]	29 ± 4.42[b]
Clinical SBP, mmHG, Median ± IQR[b]	121	115 ± 14.4[b]	124 ± 18.5[b]
Clinical DBP, mmHG, Mean ± SD[a], Median ± IQR[b]	121	69 ± 8[b]	73.7 ± 8.6[a]
Mean Arterial Pressure, Mean ± SD[a], Median ± IQR[b]	121	87.5 ± 9.4[b]	94.4 ± 10.8[a]
Multiple Deprivation Indices, Median ± IQR[b]	121	8.5 ± 2.3[b]	9 ± 2.5[b]
Ethnicity			

Asian or Middle Eastern, No., (%)	121	0 (0)	3 (2.9)
Hispanic or Latino, No., (%)	121	0 (0)	0 (0)
Caribbean, African or other, No., (%)	121	0 (0)	1 (1.3)
Non-Hispanic White/Eastern European, No., (%)	121	100 (100)	72 (94.7)
Body Composition			
Skeletal muscle mass (kg), Mean ± SD[a]	118	25.7 ± 3.4[a]	25.2 ± 3.2[a]
Body fat mass (kg), Median ± IQR[b]	118	22.8 ± 13.9[b]	25.1 ± 15.7[b]
Percentage body fat (%), Mean ± SD[a]	118	34.9 ± 8.6[a]	36.2 ± 8.4[a]
Visceral fat area (cm^2), Mean ± SD[a], Median ± IQR[b]	118	98.9 ± 60.6[b]	109.3 ± 36.9[a]
Recreational Activities			
Current or previous smoker or vaper, No., (%)	121	22 (51.1)	34 (44.7)
Never smoked or vaped, No., (%)	121	23 (49.9)	42 (56.7)
No/Low alcohol consumption (0 - 7 units), No., (%)	118	28 (62.2)	49 (67.1)
Medium alcohol consumption (7.1 - 14.9 units), No., (%)	118	6 (13.3)	15 (20.6)
High alcohol consumption (15+ Units), No., (%)	118	11 (24.4)	9 (12.3)

Bloods			
Albumin (g/L), Mean ± SD[a], Median ± IQR[b]	119	42.4 ± 2.6[a]	41.9 ± 2.6[b]
Bicarb (mmol/L), Mean ± SD[a], Median ± IQR[b]	119	19 ± 2.3[b]	19.2 ± 1.9[a]
Calcium (mmol/L), Mean ± SD[a]	119	2.3 ± 0.08[a]	2.3 ± 0.08[a]
Chloride (mmol/L), Mean ± SD[a]	119	107.6 ± 2.1[a]	107.1 ± 2[a]
Total cholesterol (mmol/L), Mean ± SD[a]	119	5.4 ± 0.9[a]	5.6 ± 1.1[a]
Creatinine (umol/L), Mean ± SD[a]	119	67.8 ± 8.1[a]	66.7 ± 10.6[a]
C Reactive Protein (mg/L), Median ± IQR[b]	119	1 ± 1[b]	1.3 ± 2.3[b]
Ferritin (microg/L), Median ± IQR[b]	119	59.8 ± 72.4[b]	54.2 ± 61.3[b]
Glucose (mmol/L), Median ± IQR[b]	119	5.1 ± 1.6[b]	5.1 ± 0.8[b]
HDL Chol (mmol/L), mean ± SD[a], Median ± IQR[b]	119	1.7 ± 0.3[a]	1.6 ± 0.5[b]
Insulin (pmol/L), Median ± IQR[b]	119	36 ± 23[b]	45 ± 23.7[b]
Iron (micromol/L), Median ± IQR[b]	119	17 ± 8.1[b]	18.3 ± 8.8[b]
Phosphate (mmol/L), Mean ± SD[a], Median ± IQR[b]	119	1.1 ± 0.1[a]	1.1 ± 0.1[b]
Potassium (mmol/L), Mean ± SD[a], Median ± IQR[b]	119	4.2 ± 0.3[a]	4.2 ± 0.3[a]
Sodium (mmol/L), Mean ± SD[a]	119	140.5 ± 1.8[a]	140.5 ± 2[b]

Transferrin (g/L), Mean ± SD[a]	119	2.7 ± 0.4[a]	2.7 ± 0.4[a]
Triglycerides (mmol/L), Median ± IQR[b]	119	0.8 ± 0.4[b]	1 ± 0.7
Urea (mmol/L), Mean ± SD[a], Median ± IQR[b]	119	4.6 ± 1[a]	4.6 ± 1.6[b]

Values are presented as [a]Mean ± SD, [b]Median ± IQR or counts and percentages. BMI = Body Mass Index, DBP = Diastolic Blood Pressure, NT = Normotensive, N = Number, SBP = Systolic Blood Pressure, SD = Standard Deviation, IQR = Interquartile Range,

XIV. Medication Usage

Medication usage reported at the time of the HELPFUL study is summarised in *Table 13*. Nearly half of the women (47.9%, n = 58) in the HELPFUL cohort reported taking prescription medications at 15-25 years postpartum. Meanwhile, 52.1% (n = 63) of the women (NT: 57.8%, n = 26; HT: 48.7%, n = 37) were not taking any regular prescribed medications. Hormonal replacement therapy and over-the counter supplements were excluded from the prescribed medication category as they do not generally target specific diseases.

The most prescribed medications were antihypertensive drugs with 21 women (17.4%) taking at least one. The proportion of antihypertensive drugs used were higher in the hypertensive pregnancy cohort compared to the normotensive cohort (p = 0.03), with 17 women (22.4%) with prior hypertensive pregnancies taking antihypertensives at the time of the HELPFUL visit. Meanwhile, only four normotensive women (8.8%) were prescribed antihypertensives and they were all only using a single prescribed agent. Among women with previous hypertensive pregnancies, eight women (10.5%) were prescribed multiple antihypertensives, whilst nine women (11.8%) were only prescribed one. The proportion of women taking multiple antihypertensives in the hypertensive cohort was significantly higher than the normotensive cohort (p = 0.003).

Angiotensin-converting enzyme inhibitors were the most frequently reported antihypertensive drug. These were used by ten women with prior hypertensive pregnancies (13.2%) and three women with previous normotensive pregnancies (6.7%). Calcium channel blockers were the most next used. These were used by six women with previous hypertensive pregnancies (7.9%) but no normotensive women, closely followed

by Angiotensin II receptor blockers which were prescribed in three women with prior hypertensive pregnancies (3.9%) and one normotensive woman (2.2%).

Antidepressants and anxiolytics were also quite common in this population being reported in 16 women (13.22%) (NT: 11.1%, n = 5; HT: 14.5%, n = 11). Similarly, asthma and respiratory medications were reported in 12 women (9.92%) (NT: 8.88%, n = 4, HT:10.52%, n = 8). Smaller proportions of women reported using aspirin or other antiplatelet agents for cardiovascular risk management (Total: 1.65%, n = 2, NT: 0%; n= 0, HT: 2.6%, n = 2), lipid-lowering medications such as statins (Total: 4.96%, n = 6, NT: 4.4%, n = 2; HT: 5.3%, n = 4) and antidiabetic medication (Total: 3.31%, n = 4, NT: 2.22%, n =1, HT: 3.95%, n =3).

Over-the-counter supplements were fairly uncommon, only being reportedly used by seven women (5.8%) (NT: 4.4%, n = 2; HT: 6.6%, n = 5). However, the prevalence of these supplements may be underestimated given the reliance on self-report, particularly for intermittent or non-prescribed products. Meanwhile, prescribed vitamins were reported in five women (4.13%) (NT: 4.44%, n = 2, HT: 3.95%, n = 3). In addition, 40 women (33.1%) (NT: 31.1%, n = 14; HT: 34.2%, n = 26) were also using hormonal contraceptives or hormone replacement therapy at the time of the HELPFUL study visit.

Table 13. Current medication usage reported by women included in the HELPFUL study. Information on medication usage was collected through questionnaires given to the participants at the end of the HELPFUL study and also through questions asked on the day of the study visit.

	Medication			
Variable	**NT (N = 45)**	**HT (N = 76)**	**Z score**	**P Value**
Supplements and vitamins, No., (%)	2 (4.4)	5 (6.6)	0.48	0.63
Prescribed vitamins	2 (4.4)	3 (4)	0.14	0.89
e.g. Cyanocobalamin, Iron				
Asthma and respiratory medications, No., (%)	4 (8.9)	8 (10.5)	0.88	0.74
Thyroid medications, No., (%)	1 (2.2)	3 (4)	0.95	0.56
Antihypertensives, No., (%)	4 (8.9)	17 (22.4)	2.21	0.03*
Single antihypertensives used, No., (%)	4 (8.9)	9 (11.8)	0.57	0.57
Multiple antihypertensives used, No., (%)	0 (0)	8 (10.5)	3	0.003*
ACE inhibitors, No., (%)	3 (6.7)	10 (13.2)	1.31	0.19
e.g., Ramipril, Perindopril, Lisinopril,				

Angiotensin II receptor blockers, No., (%)	1 (2.2)	3 (3.9)	0.59	0.56
e.g., Candesartan, Losartan				
Calcium channel blockers, No., (%)	0 (0)	6 (7.9)	2.56	0.01*
e.g., Amlodipine, Lacidipine, Nifedipine,				
Other antihypertensives, No., (%)	0 (0)	5 (6.6)	2.33	0.02*
e.g., Doxazosin, Indapamide, Bisoprolol or unknown				
Hormonal, hormone replacement	14 (31.1)	26 (34.2)	0.34	0.73
therapy/menopause, No., (%)				
Cardiovascular, No., (%)	0 (0)	2 (2.6)	1.45	0.15
e.g. Clopidogrel, Warfarin				
Statins or high cholesterol medications, No., (%)	2 (4.4)	4 (5.3)	0.25	0.8
e.g. Atorvastatin, Ezetimibe, Pravastatin Sodium				
Mood, No., (%)	5 (11.1)	11 (14.5)	0.62	0.53
e.g. Fluoxetine, Mirtazapine, Venlafaxine, Sertraline, Citalopram, Transodone, Amitriptyline, Mirtazapine, Gialopram, Nortriptyline				

Pain, No., (%)	1 (2.2)	4 (5.3)	1.13	0.26
e.g. Duloxetine, Gabapentin, Naproxen, Codomal				
Neurological, No., (%)	1 (2.2)	5 (6.6)	1.44	0.15
e.g.. Tegretol Retard, Naratriptan, Sumatriptan, Rizatriptan, Lamotrigine, Methylphenidate				
Antidiabetic, No., (%)	1 (2.2)	3 (4)	0.59	0.56
e.g. Empagliflozin, Humulin, Metformin, Dapagliflozin, Tirzepatide				
Gastrointestinal/colonic medications, No., (%)	3 (6.7)	7 (9.2)	0.56	0.58
e.g. Omeprazole, Mebeverine hydrochloride, Hyoscine butylbromide, Lansoprazole, Mesalazine, Budenoside				
Immunologcal/rheumatology, No., (%)	1 (2.2)	3 (4)	0.59	0.56
e.g. Hydroxychloroquine, Methotrexate, Amitryptline, codeine, Alendronate				

Allergy medication, No., (%) *e.g. Dymista, Loratadine, Cetirizine, Piritin, clobetasol propionate*	4 (8.9)	3 (4)	1.01	0.31
Retinal Hypertension, No., (%) *e.g. Pilocarpine*	0 (0)	1 (1.3)	1.01	0.31
No Prescribed Medications, No., (%) *(Except Hormonal Replacement Therapy and over-the counter supplements)*	26 (57.8)	37 (48.7)	0.98	0.33

Values are presented as counts and percentages. ACE = angiotensin converting enzyme, No., = Number, NT = Normotensive, HT = Hypertensive.

XV. Echocardiography Cross-Sectional Results

The echocardiographic findings for the HELPFUL cohort are summarised in *Table 14*. Data completeness among 121 women was as follows: 114 women had at least one pulmonary artery measurement (94%); 117 women had at least one mitral valve function parameter measured (97%); 119 women had at least 1 tissue doppler imaging index measured (98%); 116 women had at least one right ventricular function parameter measured (96%); 116 women had at least one aortic valve haemodynamic parameter measured (96%) and 115 women had all left ventricular outflow tract parameters measured (95%).

Table 14. Echocardiography cross-sectional results of the women included in the HELPFUL Study.

Variable	Sample Size (N)	NT	HT	Test Statistic	P Value
Pulmonary Artery Function					
Acceleration time (seconds)	114	0.15 ± 0.02[a]	0.15 ± 0.03[b]	W = 1667	0.41
V2 Maximum velocity (cm/ seconds)	111	86.9 ± 27.3[b]	86.4 ± 15.9[b]	W = 1366.5	0.68
Maximum pressure gradient (mmHg)	111	3 ± 1.7[b]	3 ± 1.1[b]	W = 1338	0.56
Mitral Valve Function					
E- wave peak velocity (cm/ seconds)	117	70.8 ± 11.4[a]	69.7 ± 16.8[b]	W = 1619	0.99
A- wave peak velocity (cm/ seconds)	114	61.1 ± 13.5[a]	67.8 ± 13.7[a]	T = -2.57	0.01*
Deceleration time (seconds)	115	0.2 ± 0.04[a]	0.2 ± 0.04[b]	W = 1774.5	0.19
E/A ratio	112	1.2 ± 0.4[b]	1.1 ± 0.3[b]	W = 1904	0.04*
Tissue Doppler Imaging					
Medial peak S velocity (cm/ seconds)	119	7.1 ± 1.1[a]	7.2 ± 1.4[b]	W= 1498	0.36
Medial peak E' velocity (cm/ seconds)	117	8.7 ± 2.3[b]	8.3 ± 2[a]	W = 1839.5	0.19
Medial A' velocity (cm/ seconds)	117	8.3 ± 1.7[a]	9 ± 1.9[a]	T = -1.9523	0.05

Medial E/E' Ratio	114	8.3 ± 1.9[a]	8.5 ± 2.6[b]	W = 1338.5	0.27
Lateral peak S velocity (cm/ seconds)	119	8.7 ± 2[a]	8.9 ± 2.2[a]	T = -0.42	0.67
Lateral peak E' velocity (cm/ seconds)	119	12.5 ± 3.4[a]	11.5 ± 3.1[a]	T= 1.63	0.11
Lateral peak A' velocity (cm/ seconds)	119	8.3 ± 2[a]	9.5 ± 2.8[b]	W = 1031	0.0005*
Lateral E/E' ratio	119	5.8 ± 2.2[b]	6.2 ± 2.1[b]	W = 1377	0.24
Mean E/E' ratio	114	7.2 ± 1.7[a]	7.4 ± 1.9[b]	W = 1312	0.21
Right Ventricular Function					
Ejection time (seconds)	108	0.4 ± 0.03[a]	0.3 ± 0.03[a]	T = 0.21	0.83
Tricuspid annular plane systolic excursion (mm)	104	2.3 ± 0.4[a]	2.4 ± 0.3[a]	T = -2.42	0.01*
S' Velocity (cm/ seconds)	116	11.4 ± 1.8[a]	11.4 ± 2[a]	T = -0.15	0.88
Aortic Valve Haemodynamics					
Maximum velocity (cm/ seconds)	116	122 ± 22.9[b]	128.3 ± 29[b]	W = 1306.5	0.12
Mean gradient (cm/ seconds)	116	83.6 ± 11.1[a]	85.9 ± 16.7[b]	W = 1372.5	0.23

Maximum pressure gradient (mmHg)	116	6 ± 2.2[b]	6.5 ± 2.6[b]	W = 1317	0.13
Mean pressure gradient (mmHg)	114	3.1 ± 1[b]	3.3 ± 1.3[b]	W = 1295	0.15
Velocity time interval (cm)	114	26.1 ± 4.2[a]	25.8 ± 6.3[b]	W = 1355.5	0.28
Left Ventricular Outflow Tract Function					
V1 maximum velocity (cm/ seconds)	115	103.7 ± 22.8[b]	103.9 ± 27.1[b]	W = 1513.5	0.73
Mean velocity (cm/ seconds)	115	68.4 ± 11[b]	70.1 ± 17[b]	W = 1520	0.75
V1 Maximum pressure gradient (mmHg)	115	4.3 ± 1.9[b]	4.3 ± 2.3[b]	W = 1524.5	0.77
Mean pressure gradient (mmHg)	115	2.1 ± 0.7[b]	2.2 ± 1[b]	W = 1521	0.76
V1 Velocity time interval (cm)	115	21.4 ± 5.2[b]	21 ± 6.1[b]	W = 1565	0.95

Values are presented as [a] Mean ± Standard Deviation, [b] Median ± Interquartile range, NT = Normotensive, HT = Hypertensive.

XVI. Pulmonary Artery Function

Pulmonary artery function was comparable across the normotensive and hypertensive pregnancy groups, with no significant differences observed in pulmonary artery acceleration time (NT: 0.15 ± 0.02 seconds, HT: 0.15 ± 0.03 seconds; p = 0.41), maximum velocity (NT: 86.9 ± 27.3, HT: 86.4 ± 15.9 cm/seconds, p = 0.68), or the maximum pressure gradient (NT: 3 ± 1.8 mmHg, HT: 3 ± 1.1 mmHg, p = 0.56).

XVII. Mitral Valve Function

Mitral inflow parameters were largely comparable between groups, with no significant differences in Mitral E-wave peak velocity (NT: 70.8 ± 11.4 cm/seconds, HT: 69.7 ± 16.9 cm/seconds, p = 0.99) or deceleration time (NT: 0.21± 0.04 second, HT: 0.2 ± 0.04 seconds, p = 0.19) across groups. However, women with hypertensive pregnancies demonstrated on average a significantly higher mitral valve A- wave peak velocity (NT: 61.1 ± 13.5 cm/seconds, HT: 67.8 ± 13.7 cm/seconds, p = 0.01, *Figure 28*).

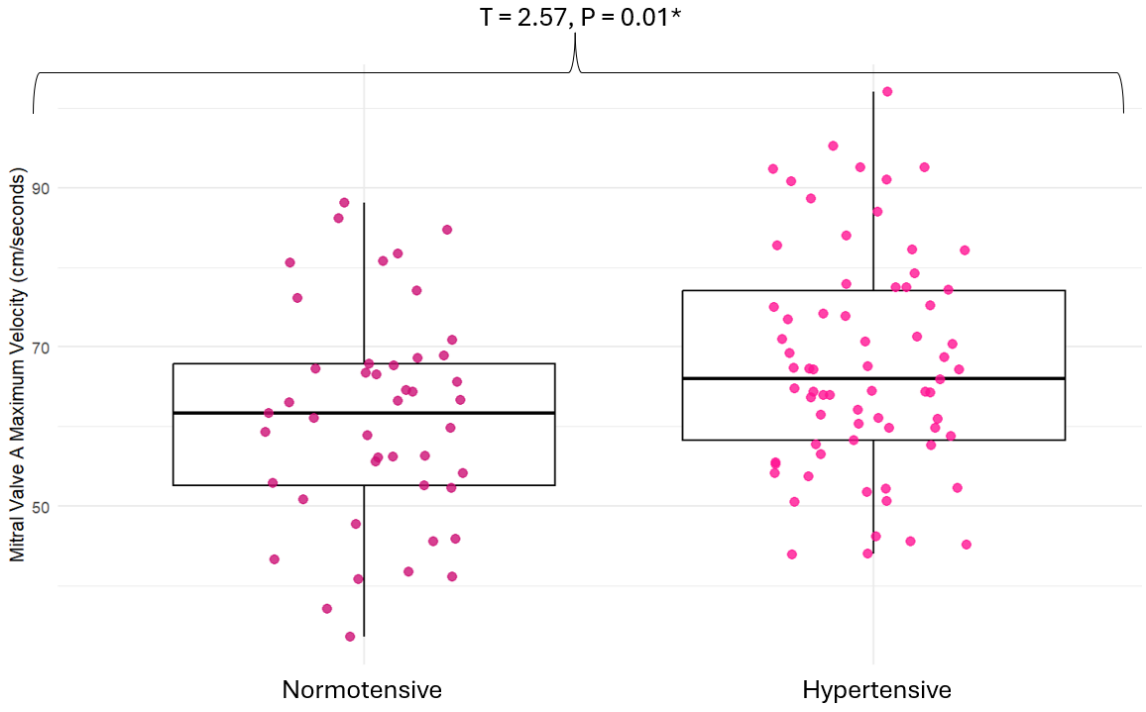


Figure 28. Boxplots showing the difference in mitral valve A- wave peak velocity (cm/seconds) across normotensive and hypertensive pregnancy groups. The boxplots are overlaid with dots representing each participant. The dots for the normotensive women are in dark pink and the dots for the women with prior hypertensive pregnancies are in light pink. The asterix represents significant differences.

Women with previous hypertensive pregnancies also demonstrated on average a significantly lower E/A ratio (NT: 1.2 ± 0.4, HT: 1.1 ± 0.3, p = 0.04, *Figure 29*) than the normotensive pregnancy group. Additionally, among the women with quantifiable E/A ratios (n = 112), eight (7.1%) had an E/A ratio less than 0.8, consistent with impaired relaxation, as per the American Society of Echocardiography and European Association of Cardiovascular Imaging guidelines[238]. By subgroup, this included six out of 69 women (8.7%) in the hypertensive pregnancy cohort and two out of 45 women (4.4%) in the normotensive pregnancy cohort.

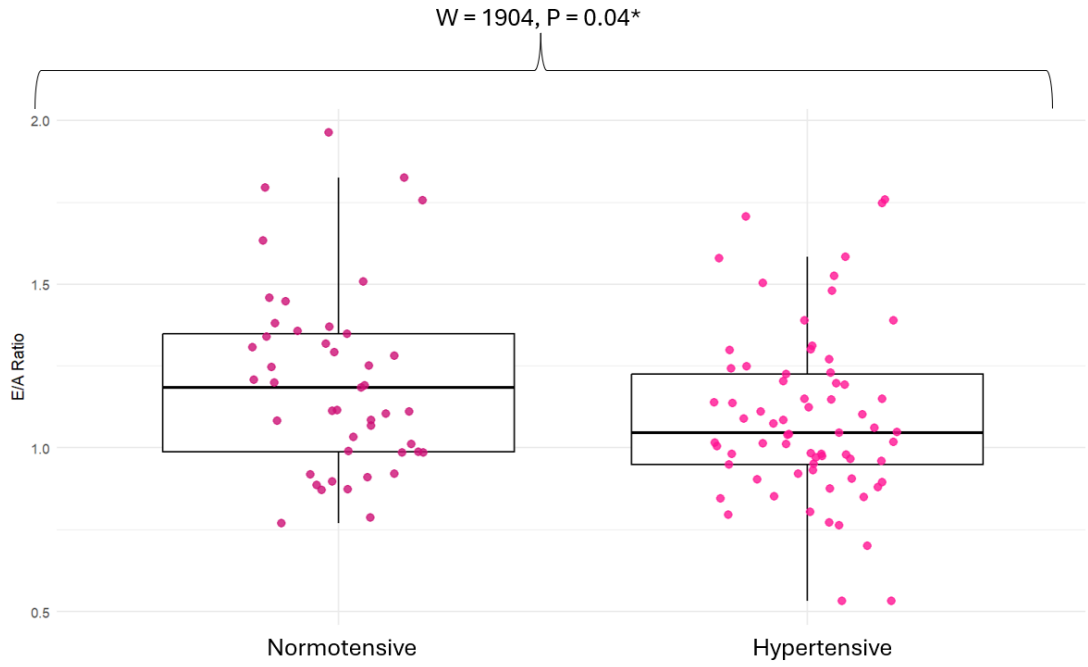


Figure 29. Boxplots showing the difference in E/A ratio across normotensive and hypertensive pregnancy groups. The boxplots are overlaid with dots representing

each participant. The dots for the normotensive women are in dark pink and the dots for the women with prior hypertensive pregnancies are in light pink. The asterix represents significant differences.

In a subgroup analysis of E/A ratios according to medication use in the women with previous hypertensive pregnancies. Average E/A ratio was 1.07 ± 0.3 in those on medication compared with 1.04 ± 0.3 in those not on medication. However, there were no significant differences between groups (W = 439, P = 0.63, *Figure 30*).

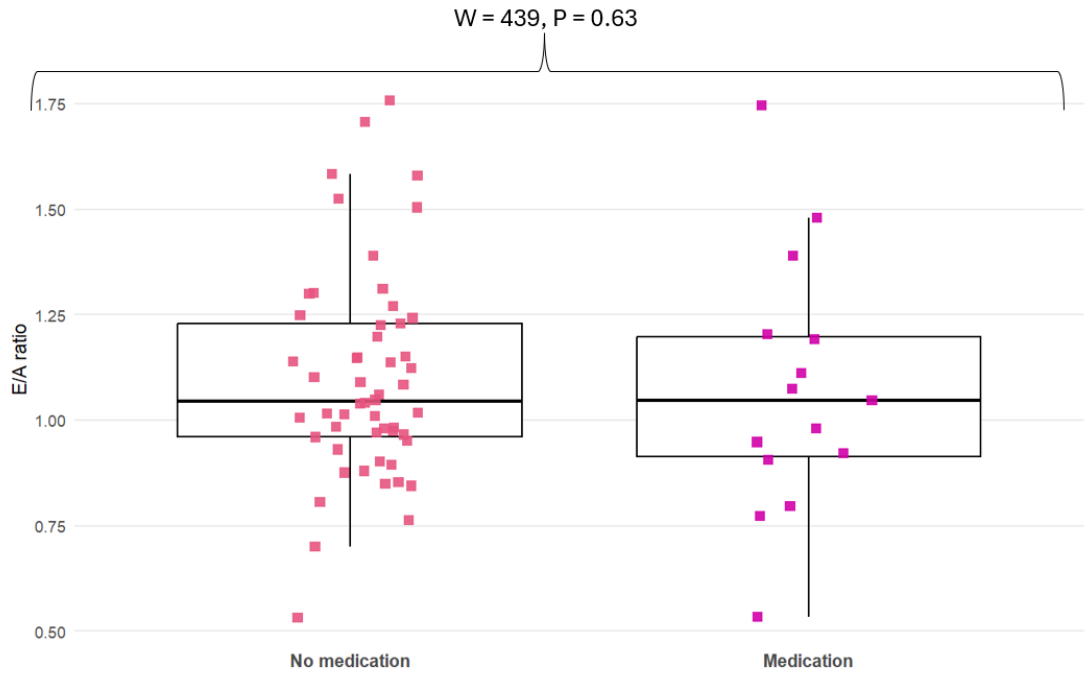


Figure 30. Boxplots showing the difference in E/A ratio across women in the hypertensive pregnancy group who currently report being on antihypertensive medication at the time of the HELPFUL Study. The boxplots are overlaid with squares representing each participant. The squares for the women not currently antihypertensive medication are in light pink and the squares for women who are currently on antihypertensive medication are in purple.

XVIII. Tissue Doppler Imaging

Tissue doppler imaging parameters were largely comparable across groups. There were no significant differences in medial S' velocity and medial E' Velocity or the medial E/E' ratio across groups (all $p > 0.05$). However, there was a trend towards increased medial A' velocity in the hypertensive pregnancy group compared to the normotensive pregnancy group (NT: 8.32 ± 1.92, HT: 9 ± 1.91 cm/seconds, $p = 0.05$, *Figure 31)*.

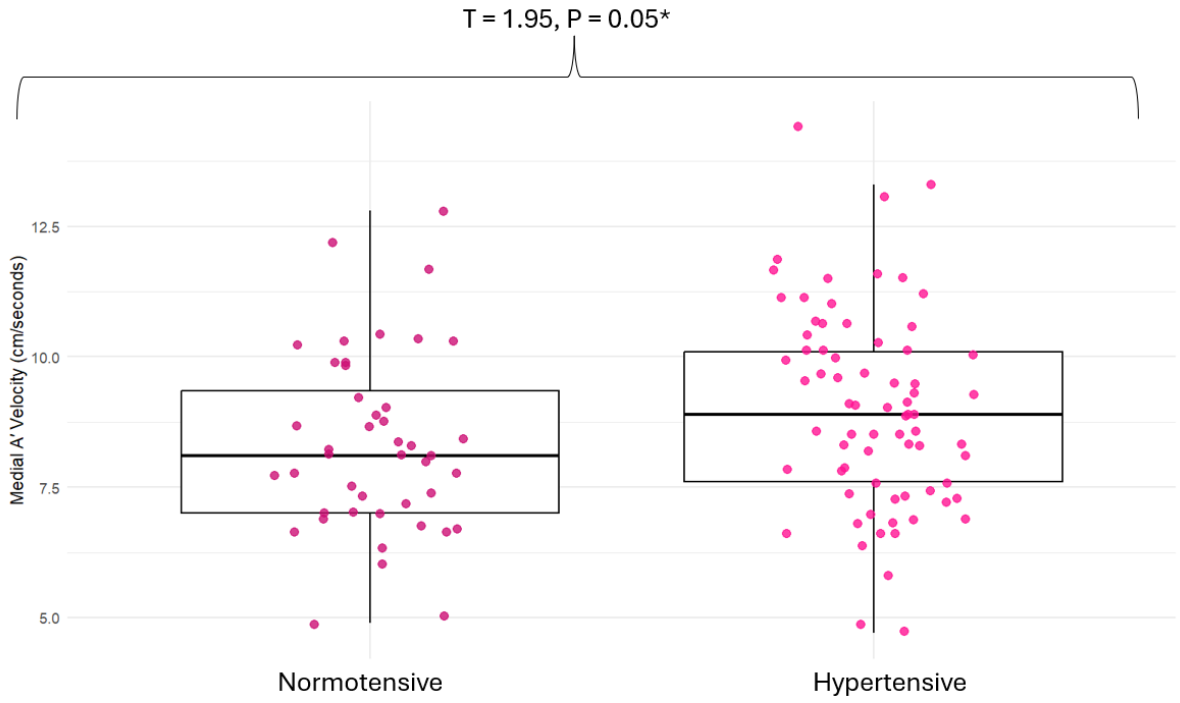


Figure 31. Boxplots showing the difference in Medial A' Velocity across normotensive and hypertensive pregnancy groups. The boxplots are overlaid with dots representing each participant. The dots for the normotensive participants are in dark pink and the dots for the women with prior hypertensive pregnancies are in light pink. The asterix represents significant differences.

Moreover, lateral tissue doppler indices were also similar between groups, with no significant differences in lateral S' velocity, lateral E' velocity, lateral E/E' ratio or the mean E/E' ratio (all $p > 0.05$). However, the women with prior hypertensive pregnancies

demonstrated significantly higher lateral peak A′ velocity compared to the women with previous normotensive pregnancies (NT: 8.3 ± 2.02, HT: 9.5 ± 2.75 cm/seconds, p < 0.001, *Figure 32)*.

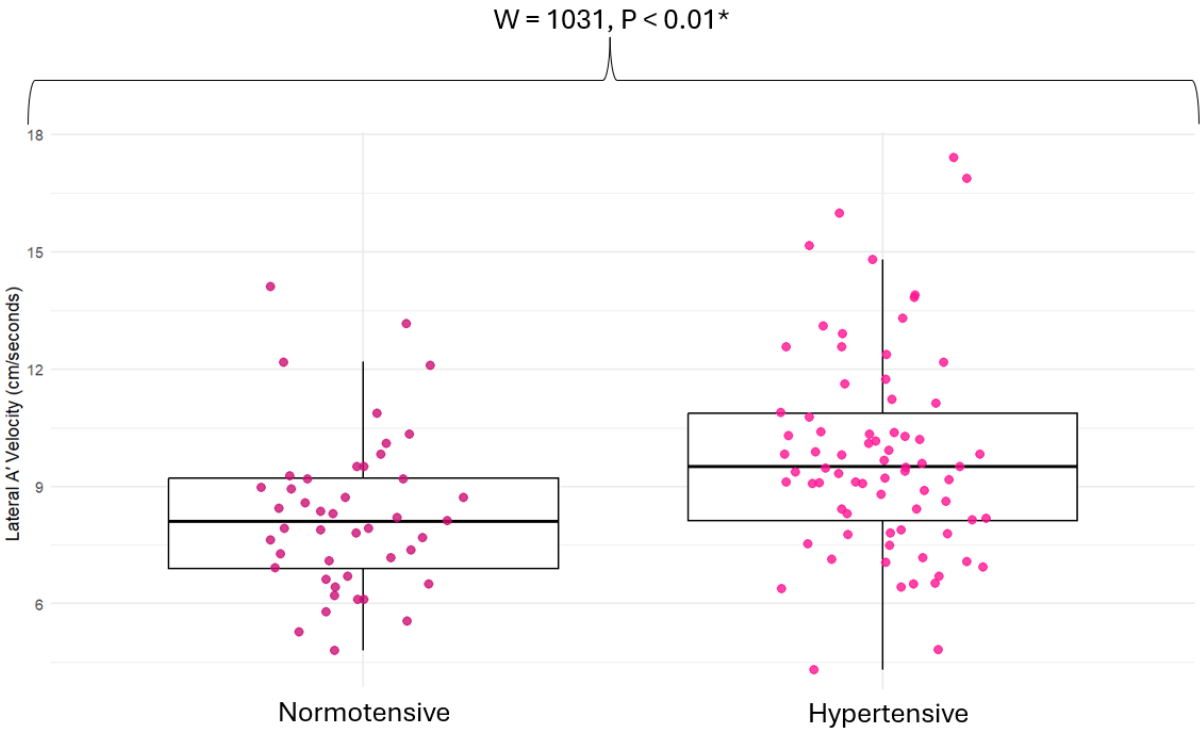


Figure 32. Boxplots showing the difference in Lateral Peak A' Velocity (cm/seconds) across normotensive and hypertensive pregnancy groups. The boxplots are overlaid with dots representing each participant. The dots for the normotensive women are in dark pink and the dots for the women with prior hypertensive pregnancies are in light pink. The asterix represents significant differences.

XIX. Right Ventricular Function

Right ventricular function was largely similar across groups. There were no significant differences in right ventricular ejection time (NT: 0.4 ± 0.03, HT: 0.3 ± 0.03 seconds, p = 0.83) or right ventricular S' velocity (NT: 11.4 ± 1.8, HT: 11.4 ± 2 cm/seconds, p = 0.88). However, tricuspid annular plane systolic excursion was modestly but significantly

higher in the hypertensive pregnancy group compared to the normotensive pregnancy group (NT: 2.3 ± 0.4, HT: 2.4 ± 0.3 mm, p = 0.01, *Figure 33).*

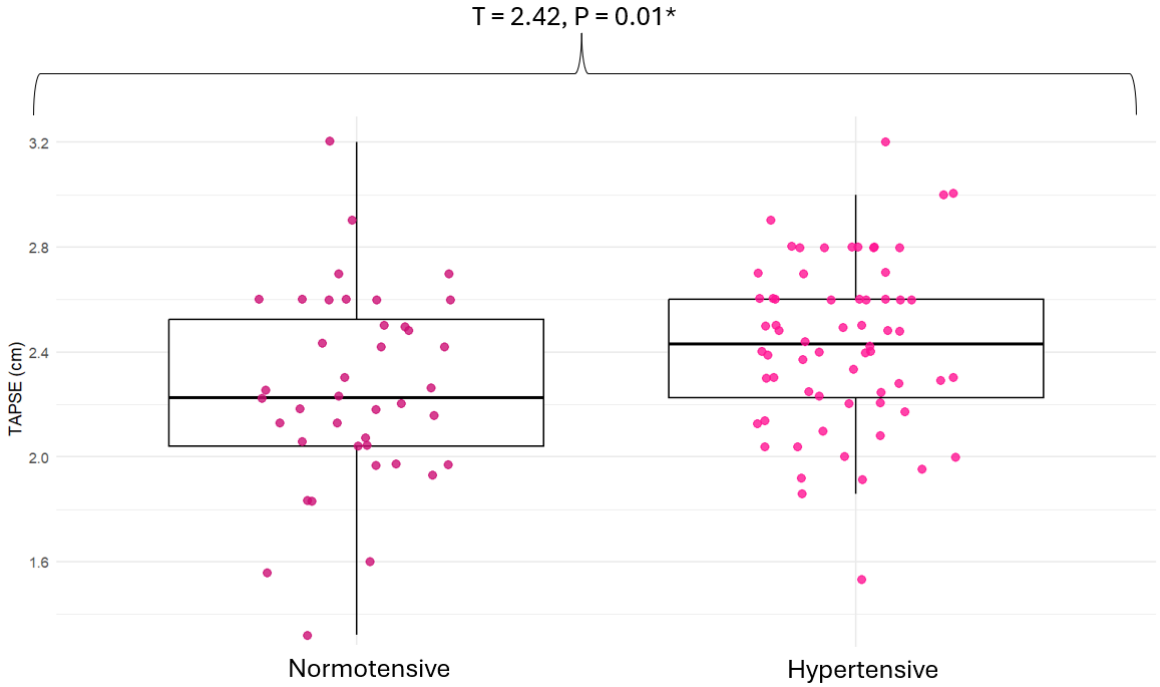


Figure 33. Boxplots showing the difference in tricuspid annular plane systolic excursion (mm) across normotensive and hypertensive pregnancy groups. The boxplots are overlaid with dots representing each participant. The dots for the normotensive women are in dark pink and the dots for the women with prior hypertensive pregnancies are in light pink. The asterix represents significant differences

XX. Aortic Valve Haemodynamics

Aortic valve haemodynamics were comparable between groups, with no significant differences in aortic peak velocity (NT: 122 ± 22.9 cm/secs, HT: 128.3 ± 29 cm/seconds, p = 0.12), aortic mean velocity (NT: 83.6 cm/secs, HT: 85.9 cm/seconds, p = 0.23), maximum transvalvular pressure gradient (NT: 6 ± 2.2 mmHg, HT: 6.5 ± 2.6 mmHg, p = 0.13), mean transvalvular pressure gradient (NT: 3.1 ± 1 mmHg, HT: 3.3 ± 1.3 mmHg, p =

0.15) or velocity time integral (NT: 26.1 ± 4.2 cm, HT: 25.8 ± 6.3 cm, p = 0.15).

XXI. Left Ventricular Outflow Tract Function

Similarly, left ventricular outflow tract parameters did not differ significantly between groups. Left ventricular outflow tract maximum velocity (NT: 103.7 ± 22.8 cm/seconds, HT: 103.9 ± 27.1 cm/seconds, p = 0.73), mean velocity (NT: 68.4 ± 11 cm/seconds, HT: 70.1 ± 16.98 cm/seconds, p = 0.75), maximum pressure gradient (NT: 4.3 ± 1.9 mmHg, HT: 4.3 ± 2.3 mmHg, p = 0.77), mean pressure gradient (NT: 2.09 ± 0.7 mmHg, HT: 2.23 ± 1mmHg) and velocity time integral (NT: 21.4 ± 5.2 cm, HT: 21 ± 6.1 cm, p = 0.95) were all comparable across groups.

XXII. Echocardiography Longitudinal Results

Building on the cross-sectional findings which demonstrate differences in diastolic and systolic function, this section now evaluates cardiac parameters over time. This cohort included 78 women with paired measurements at both timepoints. Of these, 27 women had a history of normotensive pregnancy, and 51 women had a history of hypertensive pregnancy. The results are summarised in *Table 15*. Parameter-specific denominators (n) varied due to measurement availability and image quality.

Table 15. Echocardiography results of women who had measurements at both five to ten years postpartum in the Preeclampsia Vascular Study and at 15-25 years postpartum in the HELPFUL Study.

	5-10 Years pp		15-25 Years pp			5-10 Years pp		15-25 Years pp		
Variable	**N**	**Normotensive**	**N**	**Normotensive**	**Statistics**	**N**	**Hypertensive**	**N**	**Hypertensive**	**Statistics**
Mitral valve E peak velocity (cm/sec)	26	91.7 ± 18.9[a]	26	73.5 ± 13.1[a]	*T = 4.94* *P <0.001**	49	82.6 ± 17.14[a]	49	69.5 ± 18.5[b]	*V = 1001* *P < 0.001**
Mitral valve A peak velocity (cm/sec)	26	59.7 ± 20.9[a]	26	58.6 ± 12.8[a]	*T = 0.31* *P = 0.76*	46	59.9 ± 12.2[a]	46	66.9 ± 13.5[a]	*T = -3.38* *P = 0.002**
Mitral deceleration time (sec)	25	0.2 ± 0.04[a]	25	0.2 ± 0.04[a]	*T = -0.66* *P = 0.52*	48	0.2 ± 0.04[a]	48	0.2 ± 0.04[a]	*T = -0.79* *P = 0.43*
Mitral valve E/A ratio	26	1.7 ± 0.5[a]	26	1.3 ± 0.3[a]	*T = 4.48* *P <0.001**	46	1.3 ± 0.4[b]	46	1.1 ± 0.3[a]	*V = 958* *P <0.001**
Medial A' velocity (cm/sec)	26	8.3 ± 2.1[a]	26	8.2 ± 1.8[a]	*T = 0.41* *P = 0.69*	51	8.8 ± 2.4[a]	51	8.7 ± 2[a]	*T = 0.11* *P = 0.91*
Lateral peak E' velocity (cm/sec)	27	15.2 ± 2.7[a]	27	12.9 ± 0.4[a]	*V = 331* *P < 0.001**	48	14.2 ± 3.3[a]	48	11.1 ± 2.7[a]	*T = 6.13* *P < 0.001**

Lateral peak A' velocity (cm/sec)	27	8.16 ± 1.9[a]	27	7.9 ± 1.6[a]	*V = 171* *P = 0.83*	49	8.8 ± 2.3[a]	49	9.6 ± 2.5[a]	*T = -1.7* *P = 0.1*
Lateral E/E' ratio	26	6 ± 2.7[b]	26	5.7 ± 2.1[b]	*V = 215* *P = 0.32*	44	5.7 ± 2.4[b]	44	6.1 ± 1.8[b]	*V = 462* *P = 0.71*
TAPSE (mm)	17	2.5 ± 0.4[a]	17	2.3 ± 0.3[a]	*T = 2.73* *P = 0.01**	25	2.6 ± 0.4[a]	25	2.4 ± 0.3[a]	*T = 1.97* *P = 0.06*
RV S' velocity (cm/sec)	19	14.6 ± 2[a]	19	11.3 ± 1.5[a]	*T = 6.7* P < 0.001*	25	14.6 ± 2.3[a]	25	11.7 ± 2.2[a]	*V = 292* P < 0.001*
LVOT V1 VTI (cm)	20	23.7 ± 4.3[a]	20	23.5 ± 4.7[a]	*T = 0.21* *P = 0.83*	32	22.4 ± 4.3[a]	32	22.3 ± 5.8[a]	*T = 0.14* *P = 0.89*

LVOT = Left Ventricular Outflow Tract, RV = Right ventricular, Max = Maximum, TAPSE = Tricuspid Annular Plane Systolic Excursion, VTI = Velocity Time Interval, [a] represents mean ± standard deviation, [b] represents median ± interquartile range

XXIII. Mitral Valve Function

In the normotensive pregnancy group, mitral inflow velocities showed an average reduction in E-wave peak velocity of 18.2 cm/s, declining from 91.7 ± 18.9 cm/s at five to ten years post index pregnancy to 73.5 ± 13.1 cm/s at 15-25 years post index pregnancy ($p < 0.001$). This was accompanied by a reduction in the average E/A ratio of 0.4 (1.7 ± 0.5 to 1.3 ± 0.3; $p < 0.001$, *Figure 34*). Meanwhile A-wave peak velocity and deceleration time remained stable (both $p > 0.05$).

In the hypertensive pregnancy group, E-wave peak velocity decreased significantly over time with an average reduction of 13.1cm/s, declining from 82.6 ± 17.1 cm/seconds to 69.5 ± 18.5 cm/s ($p < 0.001$). This was coupled with a marked increase in A-wave peak velocity from 59.9 ± 12.2 cm/s to 66.9 ± 13.5 cm/s ($p = 0.002$). Together, these changes resulted in a significant reduction in the average E/A ratio of 0.2 (1.3 ± 0.4 to 1.1 ± 0.3; $p < 0.001$, *Figure 31*).

Among women with paired E/A measurements ($n = 72$), five (6.9%) met the prespecified threshold for impaired relaxation ($E/A < 0.8$) at the time of HELPFUL. All five were in the prior hypertensive pregnancy group. At baseline in PVS, their E/A values were 0.89, 0.95, 1.05, 1.35, and 1.49. None of these values were below 0.8, indicating worsening values over time. Of these women, two reported being on antihypertensive medication at the time of HELPFUL.

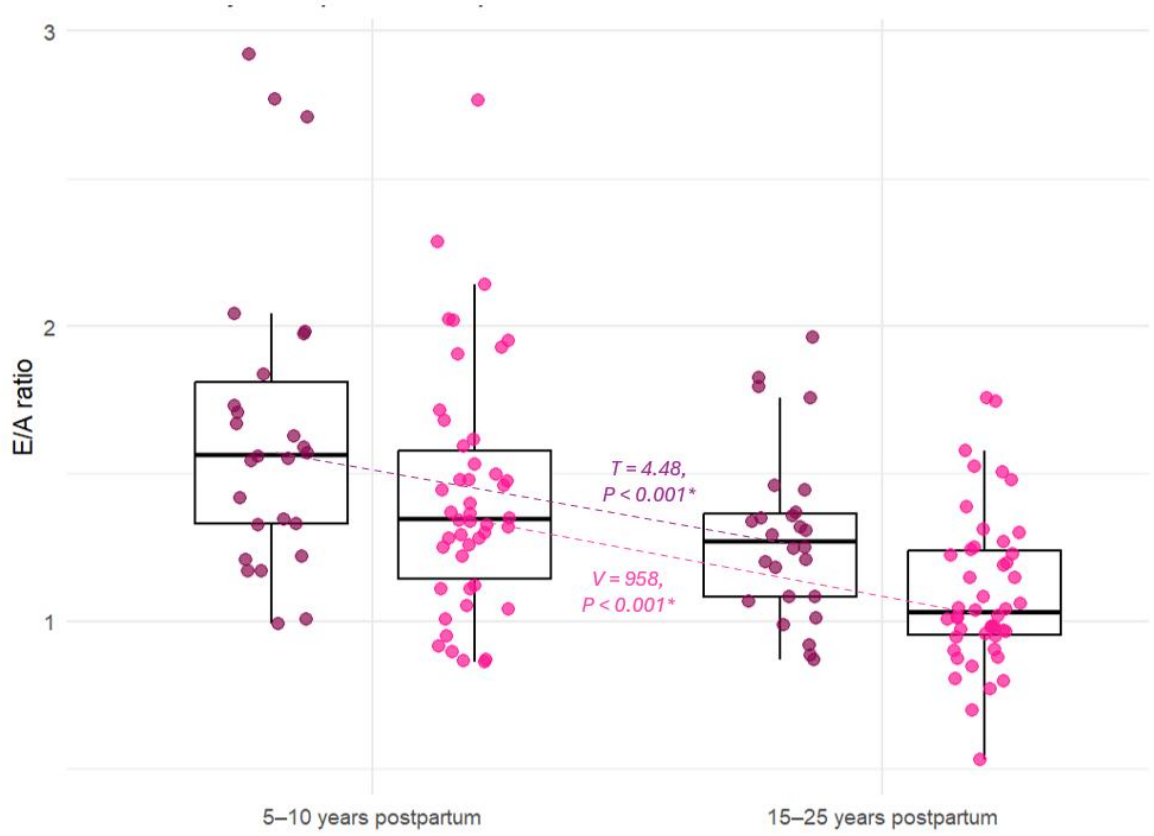


Figure 34. E/A ratio by hypertensive pregnancy status and follow-up time. Boxplots display the distribution of E/A ratios in women with a history of hypertensive pregnancy disorders (bright pink) and normotensive controls (dark pink) at five to ten years and 15-25 years postpartum. Boxes represent the interquartile range with the horizontal line indicating the median, whiskers extending to 1.5 × IQR, and individual points showing observed values. Significant differences across normotensive and hypertensive groups are shown in purple and pink, respectively. The asterix represents significant differences.

Using the mixed effects model of 72 women (144 observations), there was a significant main effect of group, $t(121.5) = 2.13$, 95 % CI [0.02, 0.37], $p = 0.036$ with the normotensive group showing higher E/A ratios compared to the hypertensive pregnancy group. A significant main effect of time postpartum was also observed, $t(70) = 5.41$, 95 % CI [0.21, 0.44] $p < 0.001$, indicating that E/A ratios were higher at five to ten years than at 15-25 years postpartum. However, the group × timepoint interaction was not significant, $t(70) = 0.37$, 95 % CI [-0.16, 0.23], $p = 0.72$ suggesting that the change in E/A ratio over time was similar for both groups.

In a subgroup analysis of the 46 women with hypertensive pregnancies, E/A ratios were compared according to medication usage. Of these, 36 women (78%) were not on antihypertensive medication, and 10 women (22%) were receiving treatment. At five to ten years postpartum, the median E/A ratio among those not receiving medication was 1.37 (IQR: 1.17-1.64) and for those on medication the mean E/A ratio was 1.36 ± 0.33. At 15-25 years postpartum, the median E/A ratio of women for those not receiving medication was 1.04 (IQR: 0.96-1.25), and the mean for those on medication was 1.13 ± 0.25.

There were no significant cross-sectional differences between women prescribed or not prescribed medication at five to ten years postpartum ($t = 0.82$, $p = 0.42$) and 15-25 years postpartum ($t = 0.31$, $p = 0.76$). However, within-group analyses demonstrated that among women not taking medication, there was a significant decline in E/A ratio over time ($V = 601$, $p < 0.001$*, *Figure 35*), whilst for those women currently on medication, there was a borderline decline ($t = 2.25$, $p = 0.05$).

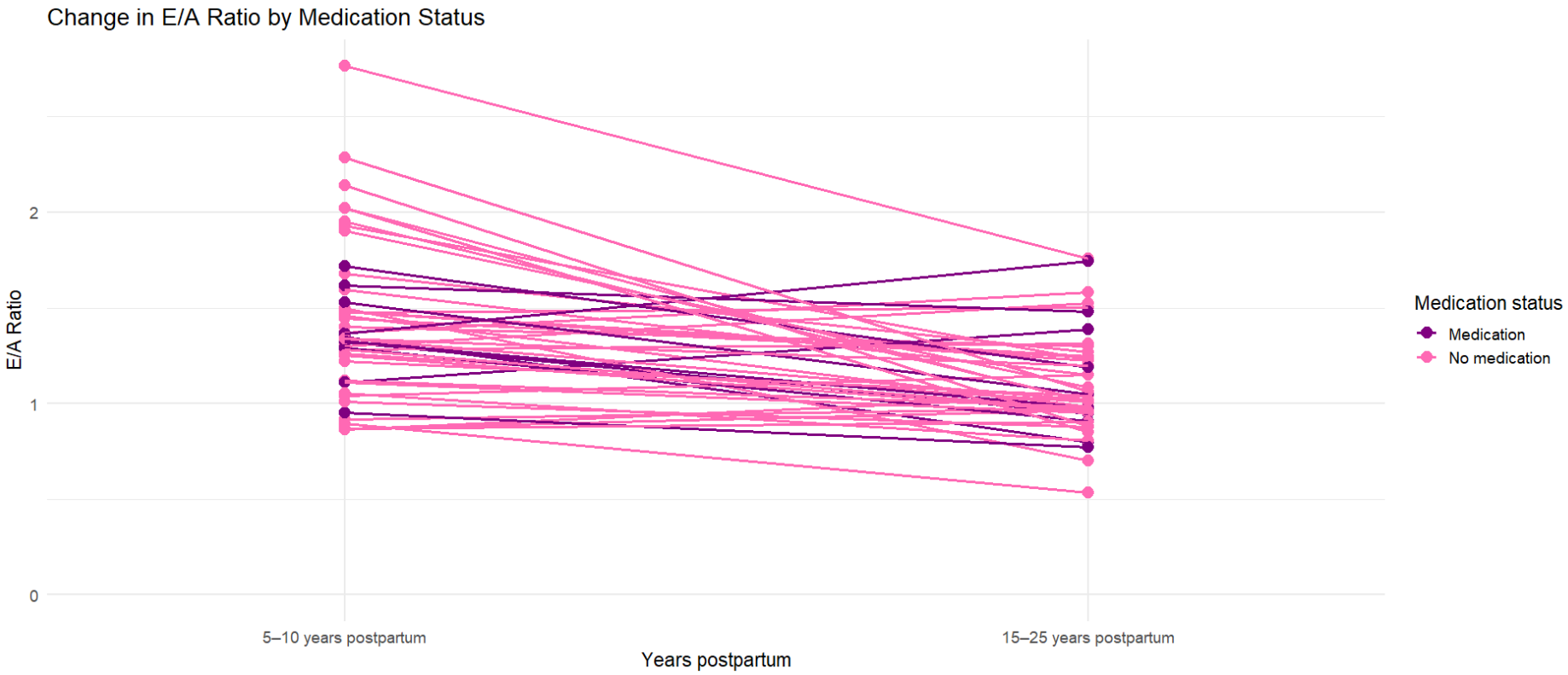


Figure 35. ***Change in E/A ratio by antihypertensive medication status from five to ten to 15-25 years postpartum.*** Paired line plots show individual participants' E/A ratios measured at two postpartum timepoints. Each line represents one woman, with pink indicating women who are not on antihypertensive medication (n = 36) and the purple lines indicating women on medication (n = 10). Lines generally trend downward, reflecting a reduction in E/A ratio over time. The decline was significant among women not on medication (Wilcoxon signed-rank V = 601, $p < 0.001$) and borderline significant among those on medication (paired t = 2.25, p = 0.05).

XXIV. Tissue Doppler Imaging

In the normotensive pregnancy group, medial A′ velocity remained stable ($p > 0.05$). As did the the lateral A′ velocity (8.16 ± 1.9 to 7.9 ± 1.6 cm/s, $p = 0.83$) and the lateral E/E′ ratio (6.0 ± 2.7 vs. 5.7 ± 2.1; $p = 0.32$). However, there was a significant decrease in lateral E′ velocity over time (15.2 ± 2.7 to 12.9 ± 0.4 cm/s; $p < 0.001$).

In the hypertensive pregnancy group, medial A' velocity and mitral valve deceleration time remained stable ($p > 0.05$). The lateral E/E′ ratio was also stable across timepoints (5.7 ± 2.4 vs. 6.1 ± 1.8; $p = 0.71$). However, lateral E′ velocity declined significantly (14.2 ± 3.3 to 11.1 ± 2.7 cm/s; $p < 0.001$). The lateral A′ velocity showed a non-significant upward trend.

XXV. Right Ventricular Function

In the normotensive pregnancy group, right ventricular function showed a small but significant reduction over time in tricuspid annular plane systolic excursion (2.5 ± 0.4 mm vs. 2.3 ± 0.3 mm; $p = 0.01$) and a decline in right ventricular S′ velocity (14.6 ± 2.0 cm/s vs. 11.3 ± 1.5 cm/s; $p < 0.001$).

In the hypertensive pregnancy group, tricuspid annular plane systolic excursion showed no significant change (2.6 ± 0.4 mm vs. 2.4 ± 0.3 mm; $p = 0.06$). As in the normotensive group, RV S′ velocity declined significantly over time from 14.6 ± 2.3 cm/s at five to ten years postpartum to 11.7 ± 2.2 cm/s at 15-25 years post index pregnancy, $p < 0.001$.

XXVI. Left Ventricular Outflow Tract Function

In both groups, left ventricular outflow tract velocity time integral remained stable over time, with no significant differences across groups (NT: 23.7 ± 4.3 cm vs. 23.5 ± 4.7 cm; p = 0.83, HT: 22.4 ± 4.3 cm vs. 22.3 ± 5.8 cm; p = 0.89).

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XXVII. Magnetic Resonance Imaging Cross-Sectional Results

Structural and functional cardiac magnetic resonance imaging parameters of women in the HELPFUL study are presented in *Table 16.*

Table 16. Structural and Functional Cardiac Magnetic Resonance Imaging Characteristics of women included in the HELPFUL Study.

Variable	*Sample Size (N)*	*Normotensive (Mean ± SD)*	*Hypertensive (Mean ± SD)*	*Test Statistic*	*P Value*
Left Ventricle					
Mass index (g/m^2)	104	43.8 ± 5.5	47 ± 8.5	W = 1012	0.06
Global mean wall thickness (mm)	104	6.2 ± 0.7	6.7 ± 1	W = 953.5	0.02*
Ejection fraction (%)	104	60.8 ± 4.7	61.2 ± 5.9	T = 0.32	0.75
End diastolic volume index (mL/m^2)	104	74.2 ± 8.6[a]	71.6 ± 11.5	T = 1.31	0.19
End systolic volume index (mL/m^2)	104	29.1 ± 5.2	27.9 ± 6.7	W = 1003	0.05*
Stroke volume (mL)	104	80.9 ± 12.8	79 ± 14.9	T = 0.62	0.53
Cardiac output (L/min)	104	5 ± 0.8	4.9 ± 1	W = 1231	0.64
Cardiac index ($L/min/m^2$)	104	2.8 ± 0.4	2.7 ± 0.6	W = 2698	0.15

Heart rate (bpm)	104	61.2 ± 8	62.5 ± 9.3	W = 1047.5	0.4
Right Ventricle					
Mass index (g/m^2)	104	17.4 ± 3.5	16.8 ± 2.8	W = 1400	0.51
Ejection fraction (%)	104	58 ± 4.7	58.8 ± 6	W = 1201.5	0.52
End diastolic volume index (mL/m^2)	104	76.3 ± 11.2	72.5 ± 11.9	T = 1.64	0.1
End systolic volume index (mL/m^2)	104	32.1 ± 6.5	27.9 ± 6.7	W = 1828	0.0001*
Stroke volume (mL)	104	79.1 ± 13	76.9 ± 15.1	T = 0.76	0.45
Cardiac output (L/min)	104	4.9 ± 0.8	4.8 ± 1	W = 1294	0.36
Cardiac index (L/min/m^2)	104	2.7 ± 0.4	2.7 ± 0.6	W = 1326	0.25

N = number, bpm = beats per minute, SD = standard deviation.

XXVIII. Left Ventricular Cross-Sectional Differences

Left ventricular mass index was higher in the hypertensive pregnancy group (47.0 ± 8.5 g/m^2) compared with the normotensive pregnancy group (43.8 ± 5.5 g/m^2), with a trend towards statistical significance (W=1012, p = 0.06, *Figure 36*).

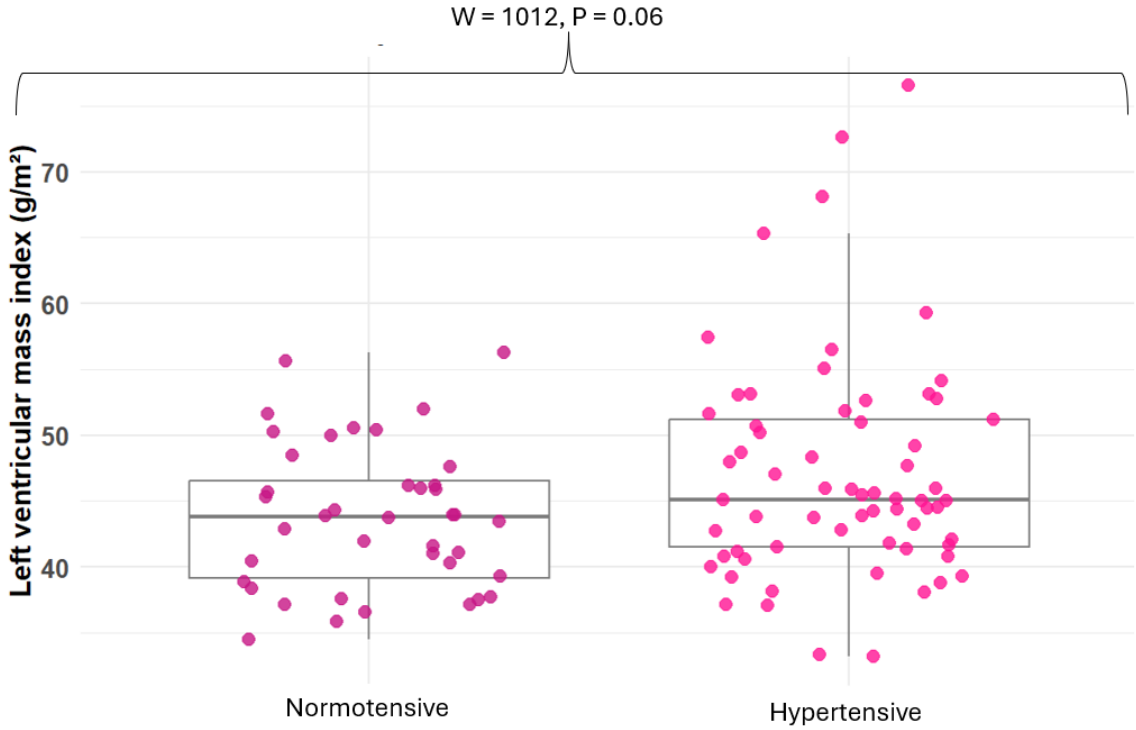


Figure 36. Boxplots showing the difference in left ventricular mass index (g/m^2) across normotensive and hypertensive pregnancy groups. The boxplots are overlaid with dots representing each participant. The dots for the normotensive participants are in dark pink and the dots for the hypertensive participants are in light pink.

In a subgroup analysis of left ventricular mass index according to medication usage, the women with previous hypertensive pregnancies had an average left ventricular mass index of 44.8 ± 8.9 g/m^2 compared with 48.3 ± 9.9 g/m^2 in those on medication. However, there were no significant differences between groups (W = 260, P = 0.21, *Figure 37*).

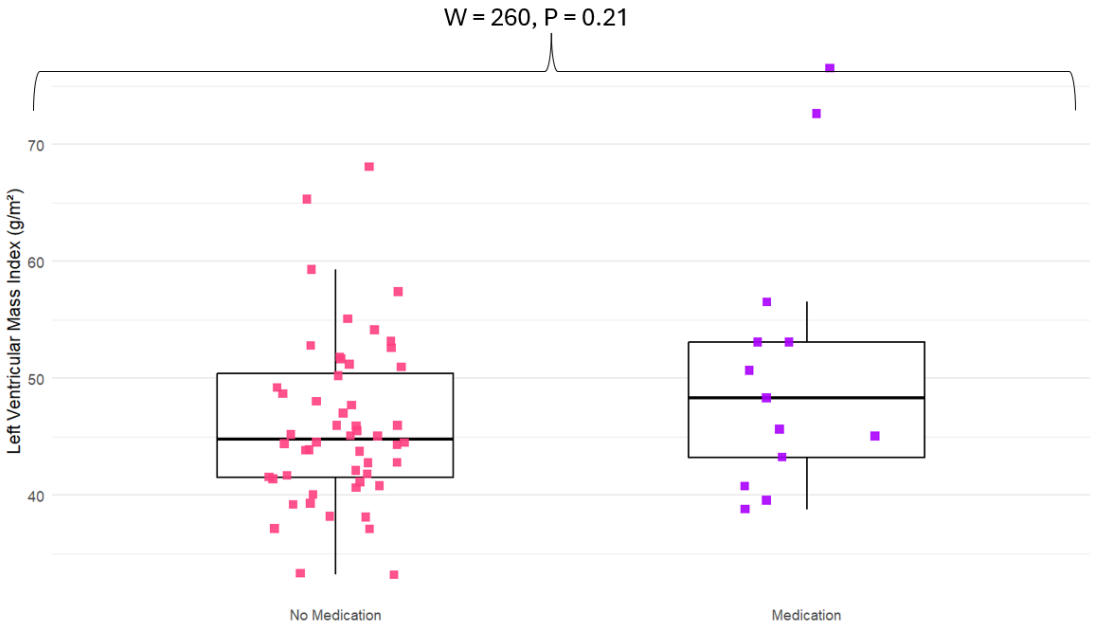


Figure 37. Boxplots showing the difference in left ventricular mass index (g/m²) across the hypertensive pregnancy group, with and without medication. The boxplots are overlaid with squares representing each participant. The squares for the hypertensive pregnancy group on no medication are in pink and the dots for the hypertensive pregnancy group on medication are in purple.

Global mean wall thickness was also significantly greater in the women with previous hypertensive pregnancies (6.7 ± 1.0 mm) than in the women with prior normotensive pregnancies (6.2 ± 0.7 mm), W = 953.5, p = 0.02, *Figure 38*. However, ejection fraction did not differ between groups (61.2 ± 5.9% vs. 60.8 ± 4.7%, T = 0.32, p = 0.75).

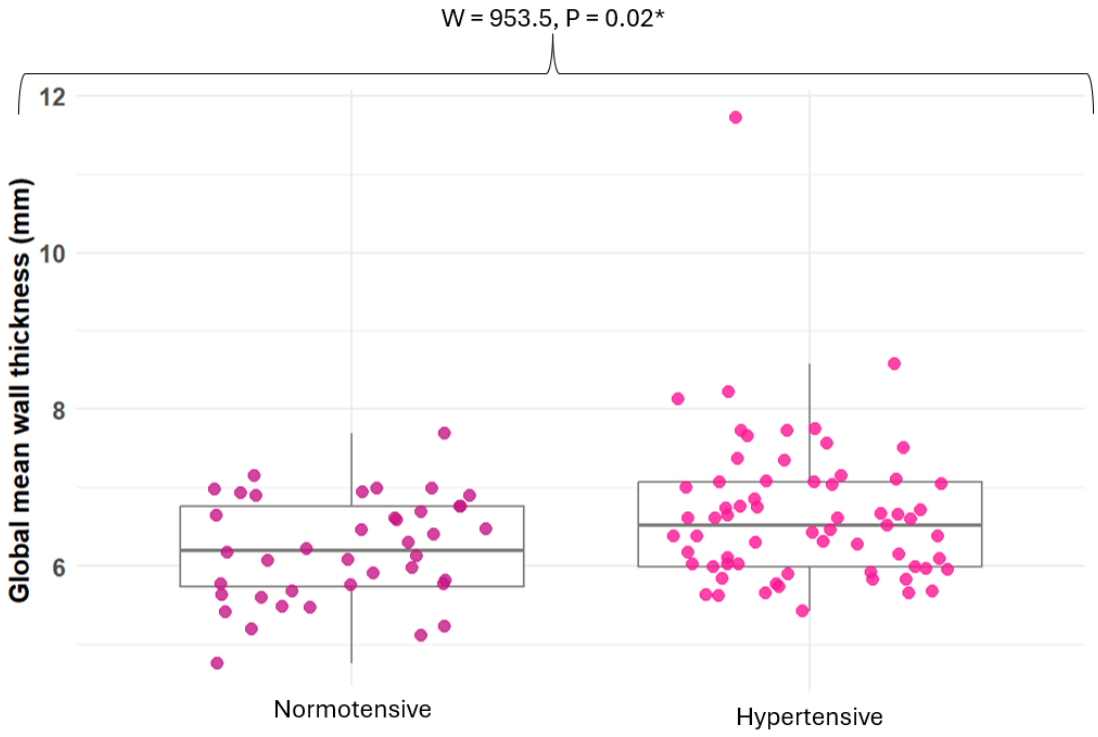


Figure 38. Boxplots showing the difference in global mean wall thickness across normotensive and hypertensive pregnancy groups. The boxplots are overlaid with dots representing each participant. The dots for the normotensive participants are in dark pink and the dots for the hypertensive participants are in light pink. The asterix represents significant results.

End diastolic volume index was on average lower in the hypertensive pregnancy group (71.6 ± 11.5 mL/m^2) compared with the normotensive pregnancy group (74.2 ± 8.6 mL/m^2), though this difference did not reach statistical significance (T = 1.31, p = 0.19). There was also a trend towards lower end systolic volume index in the hypertensive pregnancy group (27.9 ± 6.7 mL/m^2) compared with the normotensive pregnancy group (29.1 ± 5.2 mL/m^2), W = 1003, p = 0.05.

Stroke volume was similar between groups (79 ± 14.9 mL vs. 80.9 ± 12.8 mL, T = 0.62, p = 0.53), as was cardiac output (4.9 ± 1.0 L/min vs. 5.0 ± 0.8 L/min, W = 1231, p = 0.64) and cardiac index (2.7 ± 0.6 L/min/m^2 vs. 2.8 ± 0.4 L/min/m^2, W = 2698, p = 0.15). Heart

rate was slightly higher in the hypertensives pregnancy group (62.5 ± 9.3 bpm) compared with the normotensive group (61.2 ± 8.0 bpm), but the difference was not significant (W = 1047.5, p = 0.40).

XXIX. Right Ventricular Cross-Sectional Differences

For the right ventricle, there were no significant group differences observed in right ventricular mass index (NT: 17.4 ± 3.5 g/m^2 versus HT: 16.8 ± 2.8 g/m^2, W = 1400, p = 0.51). Similarly, right ventricular ejection fraction did not differ between groups (NT: 58.0 ± 4.7% versus HT: 58.8 ± 6.0%, W = 1201.5, p = 0.52). There were also no differences in right ventricular stroke volume across groups (79.1 ± 13.0 mL versus 76.9 ± 15.1 mL, T = 0.76, p = 0.45), right ventricular cardiac output (4.9 ± 0.8 L/min versus 4.8 ± 1.0 L/min, W = 1294, p = 0.36), nor cardiac index (2.7 ± 0.4 L/min/m^2 versus 2.7 ± 0.6 L/min/m^2, W = 1326, p = 0.25).

Right ventricular end- diastolic volume index was higher in the normotensive pregnancy group (76.3 ± 11.2 mL/m^2) compared with the hypertensive pregnancy group (72.5 ± 11.9 mL/m^2), though this did not reach significance (T = 1.64, p = 0.10). Additionally, end-systolic volume index was significantly higher in the normotensive pregnancy group (32.1 ± 6.5 mL/m^2) compared with the hypertensive pregnancy group (27.9 ± 6.7 mL/m^2) (W = 1828, p = 0.0001, *Figure 39*).

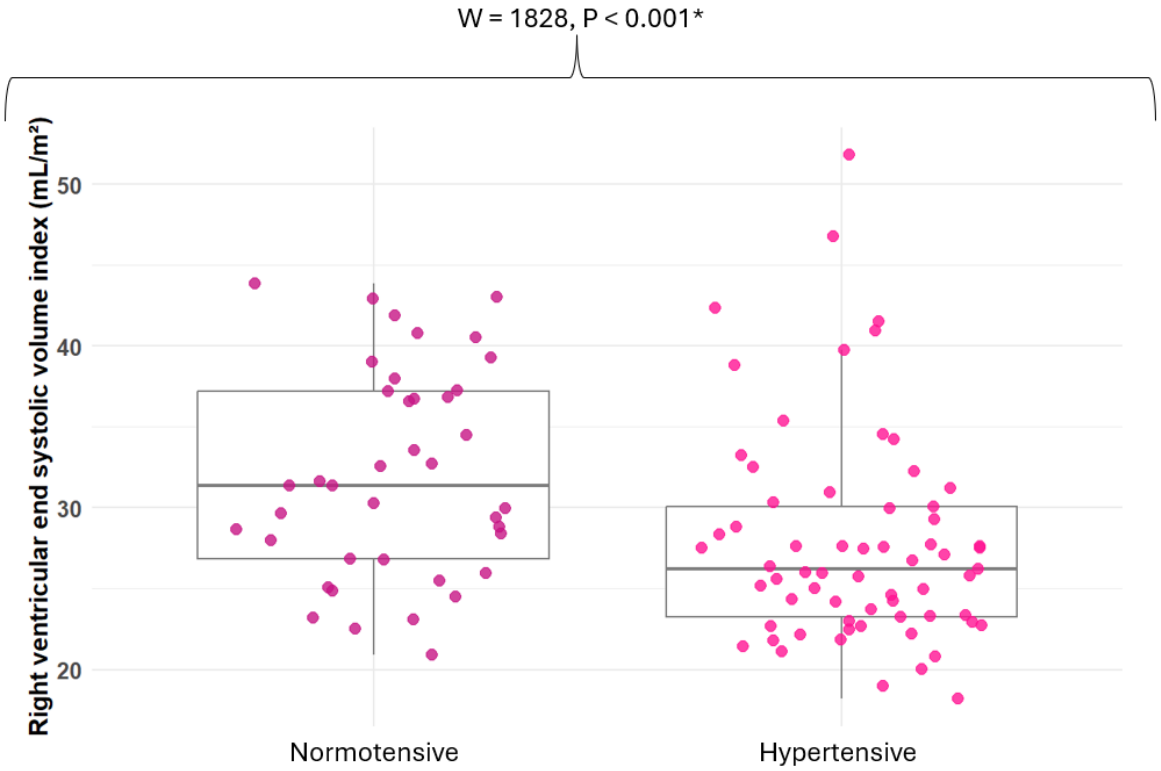


Figure 39. Boxplots showing the difference in right ventricular end systolic volume indexed to body surface area (ml/m²) across normotensive and hypertensive pregnancy groups. The boxplots are overlaid with dots representing each participant. The dots for the normotensive participants are in dark pink and the dots for the women with prior hypertensive pregnancies are in light pink. The asterix represents significant differences.

XXX. Magnetic Resonance Imaging Longitudinal Results

The results of the longitudinal analysis of the PVS and HELPFUL cohorts are presented in *Table 17.*

Table 17. Structural and functional cardiac magnetic resonance imaging characteristics of women who had measurements at both five to ten years postpartum in the Preeclampsia Vascular Study and 15-25 years postpartum in the HELPFUL Study.

Variable	Normotensive (5-10 years pp) ***N = 20***	Normotensive (15-25 years pp) ***N = 20***	Test Statistic and P Value	Hypertensive (5-10 years pp) ***N = 45***	Hypertensive (15-25 years pp) ***N = 45***	Test Statistic and P Value
			Left Ventricle			
Mass (g)	70.3 ± 10.5	77.1 ± 10.9	*T= 6.54* *P < 0.001**	72.6 ± 11.9	84.9 ± 18.8	*V = 34* *P < 0.001**
Mass index (g/m^2)	39.6 ± 4.3	43.1 ± 5	*T = 4.67* *P < 0.001**	40.9 ± 5	46.7 ± 8.6	*V = 83* *P < 0.001**
Global mean wall thickness (mm)	5.6 ± 0.6	6.2 ± 0.5	*T = 5.38* *P < 0.001**	5.9 ± 0.7	6.7 ± 1.1	V = 49 *P < 0.001**

Ejection fraction (%)	60 ± 5.3	60.2 ± 4.7	*T = 0.23*	59.8 ± 4.9	60.5 ± 5.9	*V = 397*
			P = 0.82			*P = 0.18*
End diastolic volume	140.7 ± 19.9	133.6 ± 20.2	*T = 3.02*	138.7 ± 18.5	127.5 ± 22.1	*T = 5.78*
(ml)			*P = 0.007**			*P < 0.001**
End diastolic volume	79.5 ± 8.6	75.4 ± 9	*V = 175*	78.5 ± 9.1	70.3 ± 11	*T = 6.12*
index (mL/m^2)			*P = 0.007**			*P < 0.001**
End systolic volume	56.7 ± 12.8	53.5 ± 12.9	*T = 2.11*	55.8 ± 10.9	50.3 ± 11.9	*V = 839*
(ml)			*P = 0.0047**			*P < 0.001**
End systolic volume	32 ± 7	29.8 ± 5.8	*T = 2.41*	31.6 ± 6	27.8 ± 6.4	*V = 868*
index (mL/m^2)			*P = 0.03**			*P < 0.001**
Stroke volume (ml)	84 ± 11.8	79.8 ± 10.6	*T* = 1.99	82.8 ± 12.2	77.2 ± 15.4	*T = 3.31*
			P = 0.06			*P = 0.002**
Cardiac output (L/min)	5.1 ± 0.7	4.8 ± 0.8	*T* = 1.27	5.3 ± 1	4.7 ± 0.9	*T = 3.98*
			P = 0.22			*P < 0.001**
Cardiac index	2.8 ± 0.4	2.8 ± 0.5	*T = 1.53*	3 ± 0.5	2.6 ± 0.5	*T = 4.27*
($L/min/m^2$)			*P = 0.14*			*P < 0.001**

Heart Rate (bpm)	60.6 ± 7.9	61.1 ± 7.9	*V = 79* *P = 0.54*	64.7 ± 10.4	62 ± 8.5	*T = 1.81* *P = 0.08*
Right Ventricle						
Mass (g)	26.2 ± 4.5	28.7 ± 3.7	*T = 2.93* *P = 0.009**	26.5 ± 5.1	29.4 ± 5.2	*T = 4.38* *P < 0.001**
Mass index (g/m^2)	14.8 ± 2	16.1 ± 2	*T = 2.37* *P = 0.03**	14.9 ± 2.4	16.2 ± 2.6	*T = 3.29* *P = 0.002**
Ejection fraction (%)	56.5 ± 4.2	58.4 ± 4.8	*T = 1.34* *P = 0.19*	57.3 ± 4.7	58.7 ± 5.7	*T = 1.41* *P = 0.16*
End diastolic volume (ml)	147.5 ± 20.3	136.1 ± 21.5	*T = 5.12* *P = < 0.001**	142.9 ± 22.1	127.6 ± 23.7	*T = 6.71* *P < 0.001**
End diastolic volume index (mL/m^2)	83.5 ± 9.9	76.3 ± 11.3	*T = 4.32* *P = < 0.001**	80.7 ± 10.2	70.2 ± 11.1	*T = 7.37* *P < 0.001**
End systolic volume (ml)	64.5 ± 12.5	57 ± 13.5	*T = 3.38* *P = 0.003**	61.7 ± 13	52.7 ± 12.12	*T = 6.04* *P < 0.001**

End systolic volume	36.5 ± 7	36.1 ± 7	*T= 0.86*	34.8 ± 6.5	29 ± 6.1	*T = 6.69*
index (mL/m^2)			*P = 0.39*			*P < 0.001**
Stroke volume (ml)	83.1 ± 10.5	79.1 ± 11.4	*T = 2.1*	81.6 ± 12.1	74.8 ± 15.1	*T = 4.1*
			*P = 0.045**			*P < 0.001**
Cardiac output (L/min)	5 ± 0.6	4.8 ± 0.9	*T = 1.06*	5.2 ± 0.9	4.6 ± 0.9	*T = 4.46*
			P = 0.3			*P < 0.001**
Cardiac index	2.8 ± 0.4	2.7 ± 0.5	*T = 0.52*	3 ± 0.4	2.5 ± 0.5	*T = 4.87*
($L/min/m^2$)			*P = 0.61*			*P < 0.001**

pp = postpartum, N = Number,

XXXI. Longitudinal Comparison of Left Ventricular Indices

At 15-25 years postpartum, left ventricular mass was significantly higher than at five to ten years post index pregnancy across both cohorts (NT mean difference: 6.8g, HT mean difference: 12.3g, *p < 0.001, Figure 27*). The same finding was observed for left ventricular mass index (NT mean difference: 3.5 g/m^2, *P* = 0.02, HT mean difference: 5.8 g/m^2, p < 0.001) and global mean wall thickness (NT mean difference: 0.6 mm, *P* < 0.001, HT mean difference: 0.8 mm, *P* < 0.001).

The linear mixed-effects model using left ventricular mass as a dependent variable, demonstrated that there was a significant main effect of group, $t(79.8) = -2.03$, $p = 0.045$, 95% CI [-15.41, -0.31], indicating that, on average, the normotensive group had lower left ventricular mass than the hypertensive pregnancy group. A significant main effect of time postpartum was also observed, $t(63) = -8.34$, $p < 0.001$, 95% CI [-15.26, -9.46], such that left ventricular mass increased from five to ten years to 15-25 years postpartum. Importantly, the group × timepoint interaction was significant, $t(63) = 2.09$, $p = 0.041$, 95% CI [0.34, 10.80], suggesting that the increase in left ventricular mass over time was steeper in the hypertensive pregnancy group compared with the normotensive pregnancy group *(Figure 40).*

Left ventricular end diastolic volume was significantly lower at 15-25 years postpartum compared to five to ten years postpartum in both the hypertensive pregnancy cohort and the normotensive cohort (NT mean difference: 7.7 ml, p = 0.007, HT mean difference: 11.2 ml, p < 0.001). Similarly end- diastolic volume index was significantly lower at 15-25 years postpartum across both cohorts (NT mean difference: 4.1 ml, p = 0.007, HT mean difference: 8.2 ml/m^2, p < 0.001) The same was found for left ventricular end systolic volume (NT mean difference: 3.2 ml, p = 0.005, HT mean difference: 5.5 ml, p < 0.001) and

end systolic volume index (NT mean difference: 2.2 ml/m^2, p = 0.03, HT mean difference: 3.8 ml/m^2, p < 0.001).

There were no significant differences in left ventricular ejection fraction or heart rate, across either group (p > 0.05), and there were no significant differences in stroke volume, cardiac output or cardiac index in the normotensive pregnancy group (p > 0.05). However, in the hypertensive pregnancy group cardiac output, cardiac index and stroke volume were significantly lower at 15-25 years postpartum (p < 0.001).

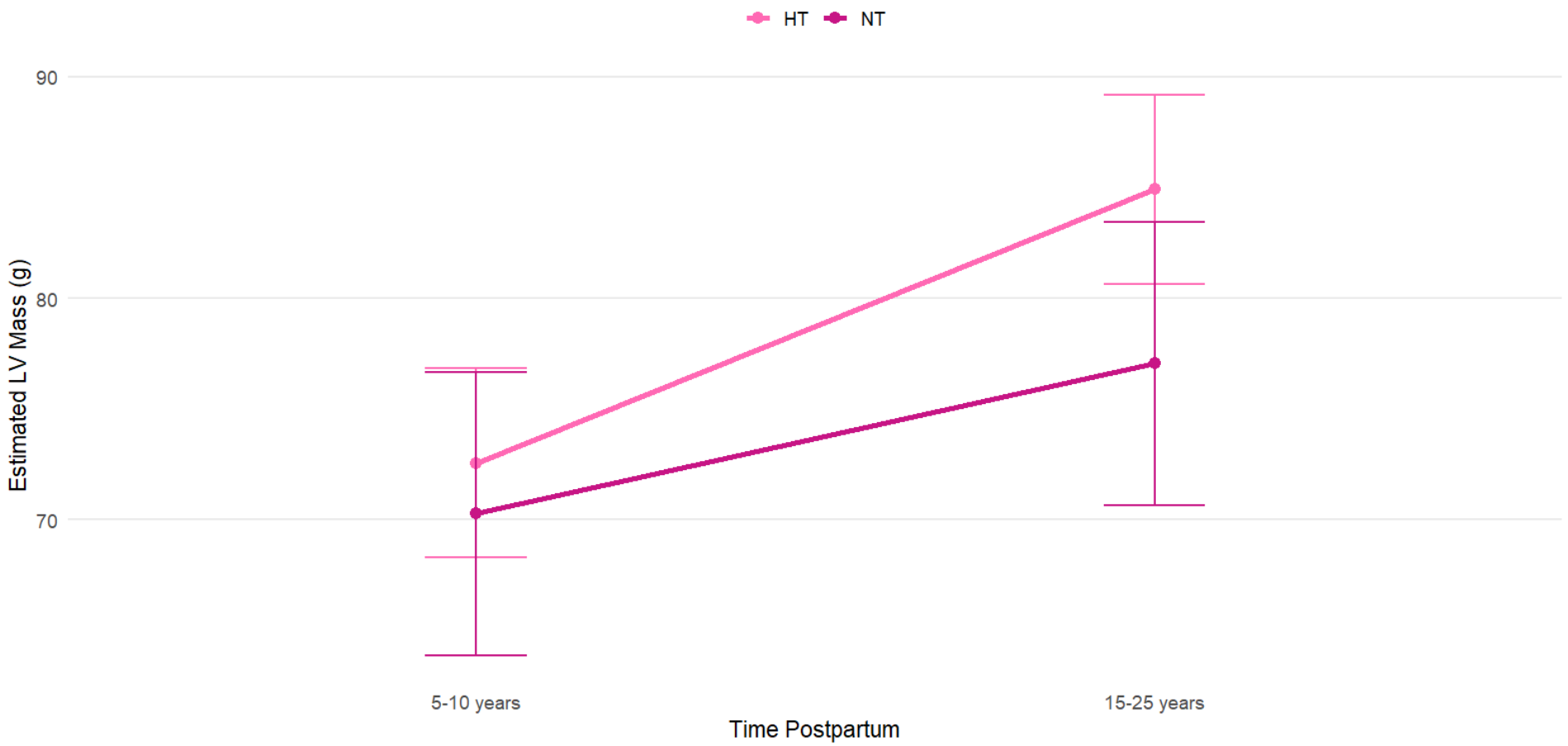


Figure 40. Estimated left ventricular mass by group and time postpartum. Estimated marginal means (± 95% confidence intervals) from the linear mixed-effects model are shown. Bright pink lines represent the hypertensive (HT) group and dark pink lines represent the normotensive (NT) group

XXXII. Longitudinal Comparison of Right Ventricular Indices

Right ventricular mass increased significantly in the normotensive and hypertensive pregnancy groups from five to ten and 15-25 years post index pregnancy, (NT mean difference: 2.5g, $p < 0.001$, HT mean difference: 2.9g, $p < 0.001$). The same was found for right ventricular mass index (NT mean difference: 1.3g/m^2, $p = 0.03$, HT mean difference: 1.3g/m^2, $p = 0.002$).

Right ventricular end diastolic volumes were significantly lower at 15-25 years postpartum compared to five to ten years post index pregnancy across both cohorts (NT mean difference: 11.4 ml, $p < 0.001$, HT mean difference: 15.3 ml, *$p < 0.001$*). Similarly, right ventricular end diastolic volume index was significantly lower at 15-25 years postpartum compared to 5-10 years postpartum across both cohorts (NT mean difference: 7.2 ml/m^2, $p < 0.001$, HT mean difference: 10.5 ml/m^2, $p < 0.001$). The same was found for end systolic volume in both cohorts (NT mean difference: 7.5 ml, HT mean difference: 15.3 ml, *$p < 0.001$*). End systolic volume index was only significantly lower in the hypertensive pregnancy cohort (NT mean difference: 0.4 ml/m^2, $p = 0.39$, HT mean difference: 5.8 ml/m^2, $p < 0.001$).

There were no significant differences in right ventricular ejection fraction across timepoints in either group (NT mean difference: 1.9, $p = 0.19$, HT mean difference: 1.4, $p = 0.16$), or cardiac output and cardiac index in the normotensive pregnancy group ($p > 0.05$). However, stroke volume, cardiac output and cardiac index (were all significantly reduced in women with previous hypertensive pregnancies ($p < 0.001$).

XXXIII. Discussion

This chapter presents the first longitudinal, multimodal cardiovascular phenotyping of women several decades after hypertensive and normotensive pregnancies. By integrating comprehensive transthoracic echocardiography and high-resolution cardiac magnetic resonance imaging, these findings provide a holistic characterisation of cardiac structure and function across two decades after pregnancy.

Cross-sectionally, women with a history of hypertensive pregnancy demonstrated significantly lower E/A ratios than women with normotensive pregnancies, indicative of impaired early diastolic filling. Approximately, 7% of the cohort met the formal echocardiographic criteria for grade I diastolic dysfunction. The lower E/A ratio was primarily attributable to elevated peak mitral A- wave velocities, accompanied by higher lateral annular A' velocities on tissue doppler imaging.

These findings are consistent with augmented atrial contribution to late diastolic filling and impaired early myocardial relaxation and collectively indicate subclinical diastolic impairment and early abnormalities in left ventricular compliance. Interestingly, the women with prior hypertensive pregnancy disorders also showed a significantly higher tricuspid annular plane systolic excursion, which possibly reflects compensatory right ventricular contractile adaptation in the context of increased afterload[241].

Structurally, the women with prior hypertensive pregnancy disorders also showed higher mean left ventricular global wall thickness and a trend towards elevated left ventricular mass index, compared to the normotensive pregnancy group. They also demonstrated lower left ventricular end systolic volumes and a trend towards lower left ventricular end-diastolic volumes, with no differences in ejection fraction or stroke volume. These patterns are consistent with the physiology of patients diagnosed with

early heart failure with preserved ejection fraction[242]. Similarly, in the right ventricle, women with prior hypertensive pregnancies showed significantly lower right ventricular end systolic volumes, and a trend towards lower end-diastolic volumes, with no significant differences in ejection fraction, stroke volume or right ventricular mass.

Longitudinally, there was an age-related decrease across both groups in diastolic dysfunction, as evidenced by reductions in the E/A ratio over time, with no differences in the rate of this decline across groups. Importantly, whilst the women with prior hypertensive pregnancy histories did not show a statistically significant acceleration of diastolic decline over time, all women who newly crossed below the diagnostic threshold for diastolic dysfunction were from the hypertensive pregnancy group. Additionally, the E/A ratio was lower in the hypertensive pregnancy group at both timepoints. These findings indicate that subclinical susceptibility occurs early and persists within this population. Peak E' and right ventricular S' velocity also declined across both groups, indicating age-associated myocardial relaxation impairment. Meanwhile, peak mitral A velocity only significantly increased in the hypertensive pregnancy group and tricuspid annular plane systolic excursion only decreased in the normotensive pregnancy group.

In terms of structure, longitudinally, both cohorts showed increases in left ventricular mass and global wall thickness, alongside reductions in left ventricular end-diastolic, end-systolic and stroke volume. These findings are consistent with age-related concentric remodelling. However, only the hypertensive pregnancy cohort showed significant declines in left ventricular cardiac output and cardiac index, suggesting cumulative haemodynamic compromise. Meanwhile left ventricular ejection fraction and heart rate showed no differences. In the right ventricle, similar trends were observed with right ventricular mass increasing across both cohorts, whilst end-diastolic, end-systolic and

stroke volume decreased. Notably, right ventricular cardiac output and end systolic volume index only decreased in the hypertensive pregnancy group.

Furthermore, women with histories of hypertensive pregnancies showed consistently higher left ventricular mass and global mean wall thickness at both timepoints compared to the normotensive pregnancy group. Further analyses of the cohort then confirmed that these women showed accelerated increases in left ventricular mass over time, compared to women with normotensive pregnancies. Although postpartum cardiovascular changes have been described previously in women with hypertensive pregnancies[90,105-123,125-134], most available studies are cross-sectional or include only limited follow-up, constraining inference about the timing, trajectory, and reversibility of cardiac remodelling. These findings show that women with prior hypertensive pregnancy disorders not only maintain persistently adverse cardiac phenotypes decades postpartum but also exhibit accelerated left ventricular hypertrophic remodelling over time.

Taken together, the pattern of persistently lower E/A ratios, higher A' velocities, increased left ventricular wall thickness and smaller ventricular volumes observed in this chapter is consistent with the 1.5 - 3 fold increased risk of experiencing a cardiovascular event in women with hypertensive pregnancy disorders[4]. Coupled with the higher systolic blood pressure observed in this population, these findings point to increased concentric remodelling and impaired relaxation; phenotypes which independently predict the incidence of cardiovascular events[239]. These findings are also consistent with the disproportionately high incidence of heart failure with preserved ejection fraction in women hypertensive pregnancy disorders[240].

The absence of a statistically significant acceleration in the decline of the E/A ratio implies that diastolic impairment stabilises early postpartum and thereafter progresses

in parallel with normal aging, rather than showing a distinct acceleration. This supports the hypothesis that myocardial relaxation abnormalities and concentric remodelling are established soon after hypertensive pregnancy and that they represent a fixed subclinical phenotype which predisposes to overt dysfunction in later life.

Collectively, these findings underscore the need for long-term cardiovascular surveillance and early preventive management in women with a history of hypertensive pregnancy disorders. Regular echocardiographic monitoring, optimisation of blood pressure, and targeted lifestyle interventions may mitigate progression toward clinically diagnosed heart failure with preserved ejection fraction and other related cardiovascular morbidity.

XXXIV. Future Planned Analyses

Building on the current longitudinal, multimodal cardiovascular phenotyping, I plan to do future analyses extending the findings to atrial structure and function, myocardial strain, and relative wall thickness. Additionally, another future aspect of this work will be to examine whether there are any predictive biomarkers in these women, and whether there are any other relevant markers which alter the trajectory of accelerated cardiovascular disease progression.

Importantly, as not all women with a history of hypertensive pregnancy go on to develop clinical cardiovascular disease, this work could be useful for differentiating those at higher risk and for characterising early markers of adverse outcomes. Ultimately, these insights can support the development of more accurate, individualised risk prediction models and facilitate the implementation of targeted preventive strategies aimed at

reducing cardiovascular morbidity in this population.

XXXV. Strengths and Limitations

The main strength of this work lies in its longitudinal design, which allows for repeated assessments of the same individuals over time. This approach minimises interindividual variability and strengthens the capacity to draw causal inferences regarding the relationship between hypertensive pregnancies and subsequent cardiovascular disease. By facilitating within-subject comparisons, the longitudinal design enables the detection of subtle, subclinical cardiac changes that may precede overt cardiovascular pathology. Such a framework enhances the understanding of temporal trajectories of disease progression and the underlying pathophysiological mechanisms linking hypertensive disorders of pregnancy to long-term cardiovascular risk.

Another notable strength of this study is the long follow-up period of up to 25 years postpartum. This timeframe allowed for comprehensive tracking of cardiovascular adaptations and outcomes across decades following pregnancy. This is the first longitudinal study to examine cardiovascular change at such an extended timeframe. This strengthens the current field of literature by providing a more detailed analysis at such a late postpartum timepoint.

Another strength of this work is the detailed cardiac phenotyping, achieved using advanced magnetic resonance imaging and echocardiographic techniques. These methods enabled a thorough characterisation of both left and right ventricular structure and function, allowing for a sensitive assessment of myocardial remodelling, tissue characteristics, and hemodynamic responses.

Nevertheless, several limitations should be acknowledged. A key methodological constraint arises from differences in imaging protocols between cohorts: the PVS study utilised a 1.5 Telsa magnetic resonance imaging scanner, whereas the HELPFUL study used a 3 Tesla scanner. Variations in field strength may have introduced minor discrepancies in image resolution and signal-to-noise ratios. However, prior validation has shown cardiac indices to be comparable across the scanners[234]. Furthermore, all cardiac analyses were completed by myself, so there are were no differences in the analysis techniques used or inter-observer bias.

Another limitation relates to the sample size available for the longitudinal analysis. While sufficient for detecting within-subject changes over time, the cohort size may have limited statistical power to detect more subtle subgroup effects or to fully explore the influence of potential confounders. Additionally, the study population were predominantly Caucasian and from low deprivation backgrounds, which may restrict the generalisability of the findings to more ethnically diverse populations. Future studies should aim to include participants from a broader range of ethnic and socioeconomic backgrounds to improve external validity and to better understand potential differences in cardiovascular adaptation and risk across populations.

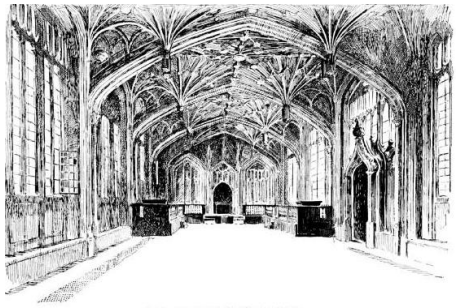
XXXVI. Conclusions

This is the first work to analyse women with hypertensive pregnancies longitudinally for over two decades postpartum. The findings demonstrate that women with a history of hypertensive pregnancy show a consistently worse cardiac phenotype over time, compared to women with normotensive pregnancies. This is characterised by concentric remodelling and diastolic dysfunction compared with those with normotensive

pregnancies. These changes represent early, subclinical alterations that may underpin the higher risk of heart failure with preserved ejection fraction in this population. Recognition of pregnancy history as an important cardiovascular risk factor and implementation of targeted long-term surveillance may be key to reducing future cardiovascular morbidity in this cohort.

OVERALL CONCLUSIONS AND BROADER WORK

6



I. Summary of Thesis Aims and Findings

This thesis set out to address two central questions. The first question was whether the elevated long-term disease risk in women with hypertensive pregnancies can be seen through early structural changes across multiple organ systems in the puerperium and the first year postpartum. This question was addressed in chapters two, three and four. The second question aimed to address whether these early target organ alterations place affected women on a steeper trajectory towards later disease. This question was addressed in chapters four and five. Collectively, the results presented here provide empirical evidence addressing these hypotheses.

Regarding the first hypothesis, I found consistent evidence that there are early structural changes across the heart, kidneys, brain and retinal microvasculature in women with hypertensive pregnancies. The first piece of evidence that I presented supporting this finding was the results of my systematic literature review in chapter two. This review demonstrated that within the first year postpartum, there is evidence of left ventricular and atrial remodelling as well as diastolic dysfunction[103-108]. These structural changes are independently related to a hypertensive pregnancy history, regardless of blood pressure at the time of measurement[122]. In addition, there was evidence of increased white matter

disease and lower cortical gray matter volume early postpartum[173], as well as higher levels of retinal arteriolar narrowing[146-149].

The review also highlighted that there are functional kidney changes such as increased microalbuminuria, higher proteinuria and reduced estimated glomerular filtration rates during pregnancy[4]. These functional markers represent alterations in renal haemodynamics, glomerular permeability and tubular load, and reflect changes in filtration pressure and protein handling during pregnancy[65]. While these parameters improve in the early postpartum period[159], epidemiological studies consistently show that women with hypertensive pregnancy disorders remain at increased risk of developing chronic kidney disease and end-stage renal failure later in life[15-20]. This paradox led me to the question of whether there are any subclinical structural changes present in the kidneys shortly after pregnancy which represent a pathological substrate between transient pregnancy associated dysfunction and long-term renal disease.

To address this, in chapter three, I used magnetic resonance imaging techniques to compare total kidney volumes and corticomedullary differentiation in women with hypertensive and normotensive pregnancies at 6-12 months postpartum. The use of magnetic resonance imaging provided non-invasive, high resolution, and highly quality images for assessing renal structure. This allowed me to evaluate tissue characteristics and subtle morphological alterations in detail. These changes would not normally be easily detectable using standard clinical measures such as ultrasound.

The findings of this work demonstrate that the women with preeclampsia in our cohort had lower total kidney volumes indexed to body surface area, compared to their normotensive counterparts. This difference was clinically significant and not surprising considering that proteinuria and renal dysfunction are hallmark features of

preeclampsia[4]. In addition, total kidney volume correlated with estimated glomerular filtration rate at delivery, but not postpartum. These findings suggest that despite overt functional improvements in kidney function, there are subclinical structural changes present which could explain the increased risk of kidney disease later in life.

Surprisingly, the differences in total kidney volume were not related to underlying structural tissue integrity, with corticomedullary differentiation only being lower in women with gestational hypertension. Given that the differences in total kidney volume were not related to corticomedullary differentiation, it is highly possible that women with preeclampsia have smaller kidney volumes before pregnancy. However, it is unknown whether smaller kidney volumes represent pre-existing susceptibility or a consequence of pregnancy-related injury. Unfortunately, this question could not be directly tested in my work due to a lack of pre-pregnancy data.

Nonetheless, the findings of this work offer promising directions for future research and potential intervention strategies. Additionally, they carry important clinical implications. Imaging could serve as a valuable tool for identifying women at increased risk of preeclampsia or later disease, enabling closer monitoring and targeted preventative interventions, with the potential to reduce hospitalisation time and associated NHS costs. Another intriguing possibility is whether augmenting kidney volumes or improving estimated glomerular filtration rates prior to or during pregnancy could reduce the risk of preeclampsia, although this remains speculative and warrants further mechanistic investigation.

Given the breadth of organ involvement following hypertensive pregnancy disorders, it is unlikely that these changes occur in isolation. Instead, it is plausible that there is a shared pathophysiological mechanism among these organs. Microvascular dysfunction,

in particular, is emerging as a critical driver of remodelling across different organs. The heart, brain and kidneys rely heavily on an intact microvascular network for normal function, and endothelial disruption, characterised by reduced nitric oxide availability, oxidative stress and anti-angiogenic imbalance is increasingly recognised as a major driver of chronic organ injury[205].

Although, my literature review found evidence of microvascular changes during pregnancy and early postpartum[79], it remained unknown whether microvascular changes represent transient pregnancy-related phenomena or fixed lasting phenotypes. Therefore, in chapter four, I examined cross-sectional differences in the retinal microvasculature, across two cohorts of women with hypertensive and normotensive pregnancies, at postpartum timepoints of 6-12 months and 15-25 years. The use of identical retinal imaging protocols, equipment, research teams and analysis techniques enhanced the validity of the comparisons by minimising measurement variability. This enabled the interpretation of differences attributable to physiological or pathological changes across two postpartum timepoints for the first time.

One of the most striking findings from this piece of work was the evidence of premature microvascular ageing in women with hypertensive pregnancies. At just 6-12 months postpartum, their average retinal arteriolar calibre was comparable to values usually seen in women over the age of 55, despite a mean cohort age of only 33 years. These women also showed greater venular narrowing than their normotensive counterparts, a change typically linked to ageing[228-229]. Together, these results suggest that hypertensive pregnancy disorders may accelerate vascular ageing.

Furthermore, even though the differences in women with hypertensive pregnancies compared to women with normotensive pregnancies were more pronounced at 6-12

months postpartum than at 15-25 years postpartum, retinal arteriolar and venular calibre were consistently narrower in women with hypertensive pregnancies at both timepoints. This was evident in women with both gestational hypertension and preeclampsia, with more marked arteriolar narrowing in women with gestational hypertension, even after adjustment for body mass index and time since delivery. These findings suggest that there is a fixed microvascular phenotype in women with hypertensive pregnancy disorders, which may be an underlying factor in multi-organ dysfunction. If microvascular narrowing truly reflects an accelerated ageing process, this could explain the convergence of brain, cardiac, and renal dysfunction in this population.

Nevertheless, one limitation of this work was its cross-sectional design. Although, I used consistent methodology and analysis protocols across the two groups; two different cohorts were studied at each timepoint. As a result, I was unable to directly track subtle organ changes over time. Therefore, in the next phase of my research, I employed a longitudinal design to evaluate subtle cardiovascular changes within the same individuals across timepoints. This piece of work involved recruiting women from an already established cohort of women.

These women were involved in the Preeclampsia Vascular Study which was originally designed to evaluate cardiac structure and function at five to ten years post index pregnancy. The previous analyses of this cohort revealed that women with a prior hypertensive pregnancy had a distinct cardiac geometry characterised by increased concentric remodelling, diastolic dysfunction and a unique pattern of left ventricular mass distribution[119].

I followed these women up at approximately 15-25 years post their index pregnancy in a new study called HELPFUL. During the HELPFUL study visit, participants underwent a

full multi-organ assessment which included magnetic resonance imaging of the brain, heart, liver and kidneys, as well as various other clinical assessments. This study has generated a rich dataset that enables both longitudinal and cross-sectional analyses, and although my thesis only focuses on the retinal and cardiac findings from this cohort, analyses of the other organ systems are planned for the near future.

In terms of the cardiac analysis, I first conducted a cross-sectional analysis of the entire HELPFUL cohort to compare whether the structural differences previously observed in the Preeclampsia Vascular Study are still present at 15-25 years postpartum. Overall, I found that women with prior hypertensive pregnancy disorders showed increased global wall thickness compared to their normotensive cohorts, as well as a trend towards increased left ventricular mass index. There were also significant differences in left and right ventricular end-systolic volumes indexed, as well as differences in several diastolic function parameters such as the E/A ratio. However, there were no differences in ejection fraction.

These differences in cardiac structure and function are consistent with the findings found at 5-10 years postpartum in the Preeclampsia Vascular Study, showing that there is a fixed cardiac phenotype in women with hypertensive pregnancies, which is consistent with the increased risk of cardiovascular mortality in this population[64]. The findings are also consistent with a phenotype of heart failure with preserved ejection fraction, which is disproportionately high in women hypertensive pregnancy disorders[240] and currently lacking effective treatments.

Furthermore, women with hypertensive pregnancies showed lower E/A ratios and higher left ventricular mass index at both timepoints, with accelerated progression of left ventricular concentric remodelling. The accelerated increase in left ventricular mass

observed reinforces the critical need for ongoing cardiovascular surveillance and early interventional strategies in this population. Additionally, although I found no evidence of a steeper decline in the E/A ratio over time, the women who crossed the threshold for diastolic dysfunction were not previously below the threshold at five to ten years post index pregnancy. These findings suggest that diastolic impairment is established earlier after hypertensive pregnancy and tracks over time, rather than accelerating progressively. This stable but adverse phenotype highlights pregnancy as a critical inflection point that sets long-term cardiac trajectories and puts these women at higher risk of cardiovascular disease later in life.

The observation that diastolic dysfunction does not appear to deteriorate more quickly over time but instead diverge early after a hypertensive pregnancy and then remain stably adverse, has important clinical implications. It suggests that the critical window for intervention is not decades later when symptomatic disease emerges, but rather in the early postpartum period when these structural changes first become established. Preventative strategies, whether lifestyle, pharmacological, or imaging-based risk stratification are therefore likely to be most effective if implemented soon after pregnancy.

This trajectory is further reinforced by the retinal findings, where vascular calibre was already markedly narrower at 6-12 months postpartum, resembling values seen in much older women. Similarly, women with preeclampsia already had lower total kidney volumes at 6-12 months postpartum and women with gestational hypertension had lower corticomedullary differentiation values.

It is currently unknown whether these structural phenotypes can be easily modified. Within my work, I completed subgroup analyses comparing women with physician

optimised blood pressure control in the puerperium to those who were given standard NHS care. I found no differences in retinal calibre, kidney volume or corticomedullary differentiation between the two subgroups, suggesting that these structural changes are not easily reversible, or that the type of intervention was not as effective for kidney and microvascular health. However, the fact that short-term blood pressure interventions have been shown to modify cardiovascular maladaptations previously[95], offers promising avenues for further research.

It may be that the vascular and renal structural adaptations associated with hypertensive pregnancy represent more permanent remodelling rather than transient hemodynamic responses. In contrast, the myocardium appears to retain a degree of plasticity and functional recovery following blood pressure optimisation[95]. These findings suggest that postnatal interventions alone may be insufficient to reverse established microvascular or renal remodelling, again underscoring the importance of early identification and prevention of hypertensive pregnancy disorders.

II. Future Directions and Research Priorities

Future research could be directed towards developing and targeting interventions for women with renal insufficiency and microvascular maladaptation's during and before pregnancy. Additionally, research should focus on early postpartum interventions for promoting cardiac recovery and mitigating long-term cardiovascular risk in women with hypertensive pregnancy disorders. These interventions should be targeted at the most high risk groups such as those with a family history of preeclampsia, pre-existing renal disease, or other indicators of vascular vulnerability.

Future research could also aim to develop comprehensive risk prediction models that integrate pre-pregnancy characteristics with later life organ-specific phenotypes to improve identification of women at greatest risk of adverse cardiovascular and renal outcomes following hypertensive pregnancy. Incorporating factors such as maternal age, body mass index, family history, baseline renal function, and vascular biomarkers alongside imaging-derived measures of cardiac, renal, and retinal structure could enable more precise risk stratification and more tailored interventions. The utilisation of large, longitudinal datasets such as the HELPFUL cohort will be important in this endeavour.

Building on this, recent advances in machine learning have opened new avenues for quantifying hypertension related multi-organ damage. A large-scale UK Biobank study (n = 27,099) introduced HyperScore, a metric that models hypertension progression across multiple organ systems using over 500 imaging and clinical variables[243]. By capturing subtle, system-wide physiological alterations, the HyperScore framework provides a promising foundation for the development of unified, data-driven risk stratification tools. Incorporating such multi-organ, machine learning based approaches alongside pregnancy specific cohorts like HELPFUL could substantially enhance our ability to predict, monitor, and ultimately mitigate long-term cardiovascular and renal sequelae in women with hypertensive pregnancy disorders.

Finally, another promising avenue for future research lies in the application of artificial intelligence to enhance imaging acquisition and analysis techniques. While many of the methods used in this work, such as manual kidney contouring, required significant researcher input; future efforts should focus on developing automated and reproducible pipelines. Automation would not only increase efficiency and reduce observer variability but also enable the inclusion of larger and more diverse cohorts, thereby improving the

scalability, generalisability, and overall understanding of maternal cardiovascular and renal adaptation.

III. From Hypothesis to Evidence-Based Trajectories

At the beginning of my thesis, I proposed a set of theoretical trajectories to illustrate how hypertensive pregnancy disorders might influence long-term multi-organ health (*Figure 7*). These initial models were based on available evidence in the literature, but there were gaps that this thesis aimed to address. Now, with the evidence generated across multiple organs in this thesis, I have revisited and refined the trajectory models to integrate the findings found in my thesis. While some trajectories aligned closely with my original hypotheses, other trajectories differed, offering new insights into when target organ alterations emerge and whether they remain stable or accelerate over time.

IV. Renal Trajectory

The initial renal trajectory that I proposed was based solely on proteinuria, reflecting a functional marker of renal health. This trajectory accounted for the known increased levels of proteinuria that occur in pregnancy in women with hypertensive pregnancy disorders, particularly in women with preeclampsia[4]. The findings from my literature review additionally demonstrated that proteinuria levels return to baseline postpartum[159]. This was despite women with hypertensive disorders of pregnancy having a higher risk of developing chronic kidney disease and end-stage renal failure later in life[15-20].

However, my work has since shown that structural and functional renal parameters follow distinct trajectories. While functional markers such as proteinuria and estimated glomerular filtration rates often improve after delivery[159], structural differences such as total kidney volumes in women with preeclampsia and corticomedullary differentiation in women with gestation hypertension remain evident at 6-12 months postpartum[186].

In light of these findings, I have developed two new renal trajectories (*Figure 41)* to replace the initial simplified model that was based only on proteinuria. The first trajectory represents functional parameters such as proteinuria and estimated glomerular filtration rate. These appear to worsen during pregnancy, reflecting acute stress on the kidneys, but then they show partial or full recovery in the early postpartum period[79]. However, this apparent recovery may mask underlying risk, as women with hypertensive pregnancies remain at elevated long-term risk of chronic kidney disease despite normalisation of these functional markers[15-20].

The second trajectory represents structural parameters such as total kidney volume in women with preeclampsia. Unlike functional markers, these changes are already evident

at 6-12 months postpartum and show no sign of reversal[186]. This suggests that a fixed renal phenotype may be established early, potentially reflecting pre-existing susceptibility or incomplete recovery from pregnancy related injury. This fixed structural phenotype likely persists beyond pregnancy and may provide the pathological substrate linking pregnancy complications to later disease.

Future studies incorporating both pre-pregnancy imaging and long-term postpartum follow-up will help refine the renal trajectory, providing further understanding that will help improve health outcomes for women with hypertensive pregnancy disorders.

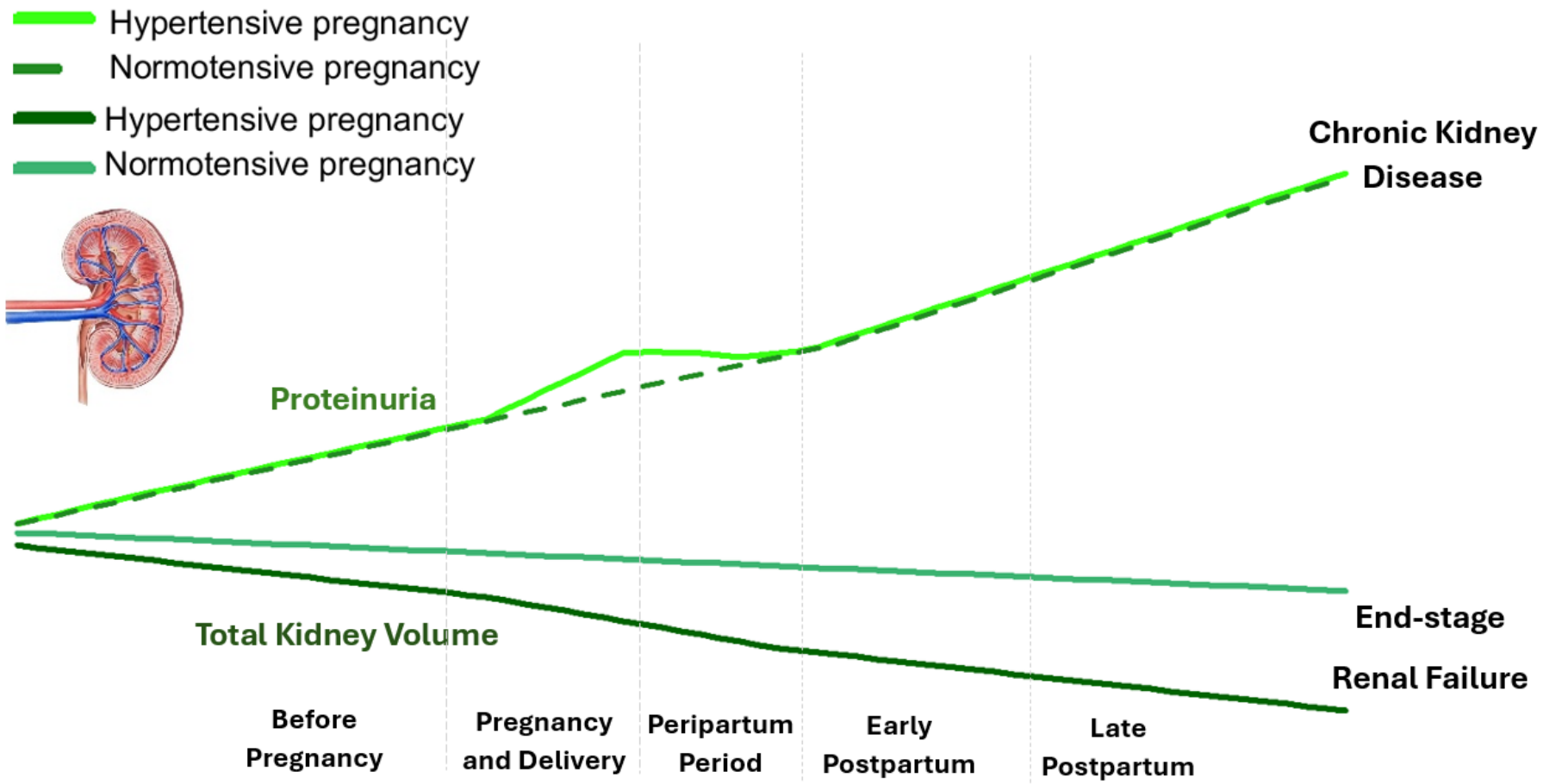


Figure 41. Evidence-based trajectory of renal organ maladaptation's across hypertensive and healthy pregnancy. These are trajectories based on the evidence presented in my work - see: *Cutler. H.R., et al., 2025, Subclinical Postpartum Renal Structure After Hypertensive Pregnancy Disorders. Hypertension, doi/10.1161/HYPERTENSIO NAHA.125.25130*[180]*.* Each line represents a different trajectory, with a key in the upper left corner.

V. Microvascular Trajectory

In my initial theoretical trajectory, I predicted that microvascular changes would emerge gradually, reflecting cumulative endothelial injury over time. Instead, my findings demonstrate that women with hypertensive pregnancies have narrower arterioles and venules at 6-12 months postpartum, calibres more typical of women two decades older, and these differences persist decades later. This reframes the microvascular trajectory, suggesting that there is accelerated ageing compressed into the postpartum period, followed by a plateauing into a fixed adverse microvascular phenotype.

The new microvascular trajectory therefore depicts a sharp early decline in calibre during pregnancy, rather than a slow, progressive narrowing across the life course (*Figure 42)*. After this initial drop, the trajectory appears to stabilise into a fixed adverse phenotype that persists for decades. It is possible that hypertensive pregnancy triggers rapid acceleration of vascular ageing which then resets the baseline level of microvascular health, leaving women on a parallel but lower trajectory compared to those with normotensive pregnancies. By representing the microvasculature in this way, the revised model captures both the abrupt, early impact of pregnancy and the long-term persistence of a compromised vascular state.

This new trajectory highlights the need for early intervention. Microvasculature changes often precede overt clinical manifestations and end organ changes. By identifying and addressing these alterations during pregnancy and early postpartum, there is the opportunity to preserve vascular function, slow disease progression, and improve long-term multi-organ health outcomes.

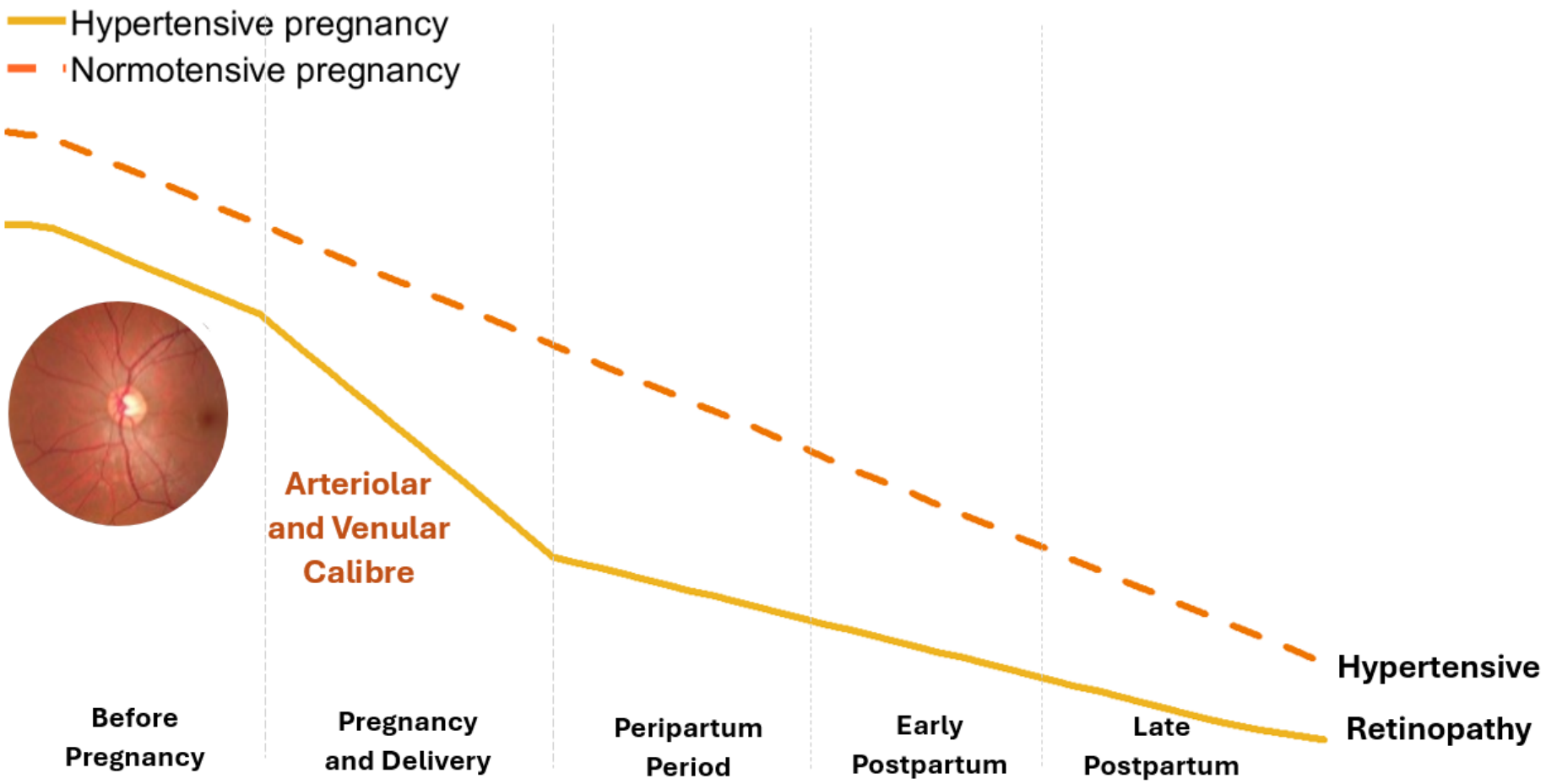


Figure 42. Evidence-based trajectory of microvascular maladaptation's across hypertensive and healthy pregnancy. This trajectory is based on the evidence presented in my thesis. Each line represents a different trajectory, with a key in the upper left corner.

VI. Cardiac Trajectory

My initial trajectory for the cardiac profile was based on the very large body of research that is already available regarding cardiac changes post hypertensive disorders of pregnancy. As highlighted in my literature review, research into cardiovascular remodelling and dysfunction spans the pre-pregnancy period, gestation and the early and late postpartum phases[79]. Based on this literature, my initial trajectory depicted an increase in left ventricular mass index during pregnancy which although partially remodels postpartum, does not completely return to normal. These women are then on accelerated trajectories towards cardiovascular disease later in life.

My research in this thesis added to the body of existing literature by being the first to provide a longitudinal, detailed cardiovascular assessment extending decades postpartum, using both magnetic resonance imaging and echocardiography. This research demonstrated two important findings. First, there was an accelerated increase in left ventricular mass within the hypertensive pregnancy cohort. Second, there was no evidence of a steeper decline in the E/A ratio over time in women with hypertensive pregnancies compared to women with normotensive pregnancies.

Nonetheless, in the longitudinal analysis, all of the women with an E/A ratio equivalent to grade I diastolic dysfunction were in the hypertensive pregnancy cohort, and their E/A ratios were not previously within this threshold. This suggests that despite the women not showing a steeper decline in diastolic dysfunction, the initial insult during pregnancy puts them on a trajectory with a lower baseline that runs parallel to the trajectory of women with normotensive pregnancies.

These findings reframe our understanding of the cardiac trajectory. The first findings support our initial hypothesis that left ventricular mass increases at an accelerated rate in women with hypertensive disorders of pregnancy, following the initial insult during pregnancy. Therefore, I have made no changes to the initial cardiac trajectory regarding left ventricular mass index (*Figure 43)*. However, the E/A ratio findings do not appear to show continuous acceleration of cardiac function. Instead, these findings suggest that the initial insult during pregnancy sets these women on a lower baseline trajectory, which slowly declines over time. Ongoing analyses within this cohort aim to further refine and validate these proposed trajectories, providing a clearer understanding of long-term cardiac adaptation following hypertensive pregnancy disorders.

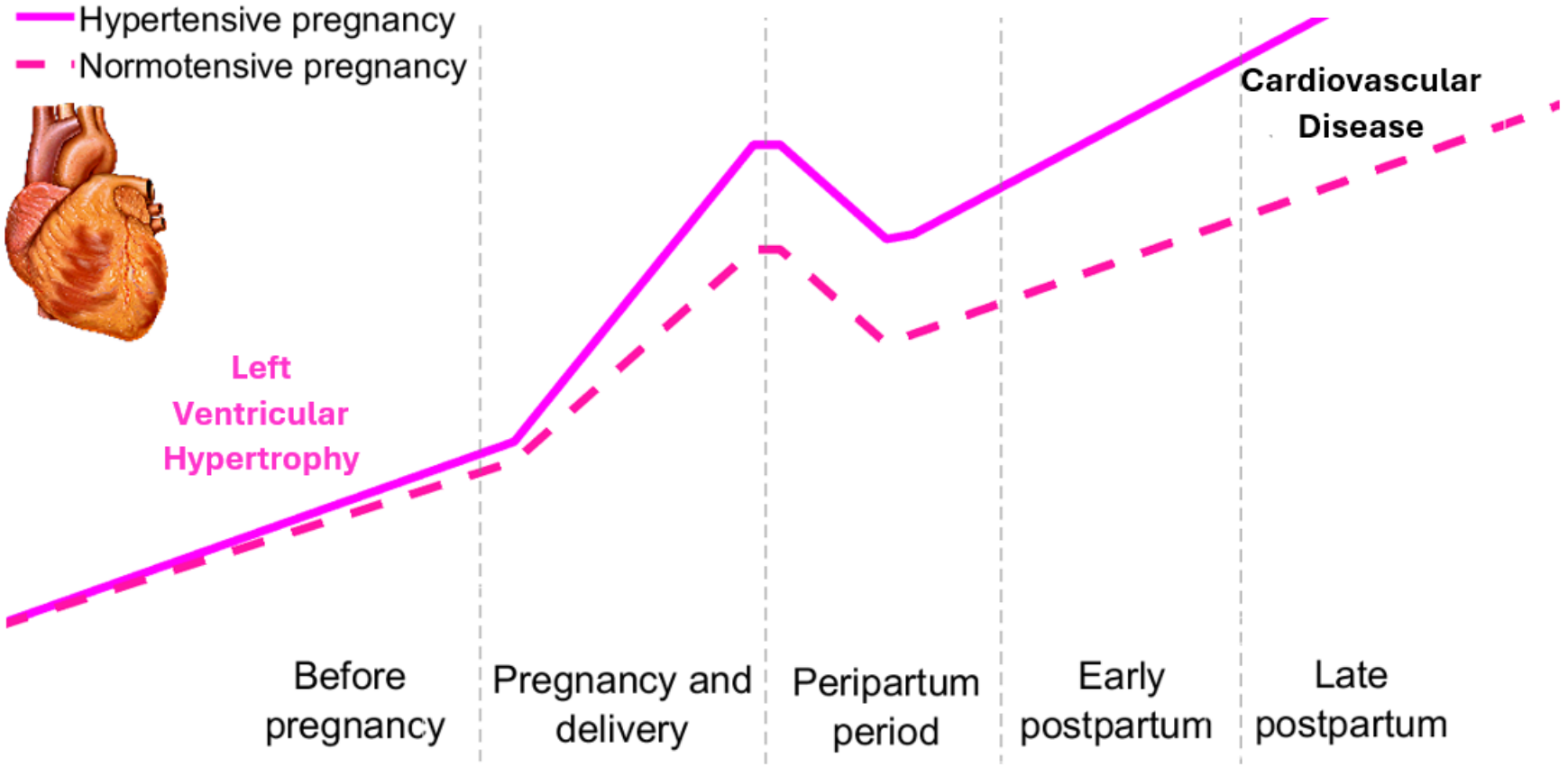


Figure 43. Evidence-based trajectory of cardiac remodelling across hypertensive and healthy pregnancy. This trajectory is based on the evidence presented in my thesis. Each line represents a different trajectory, with a key in the upper left corner.

VII. Conclusions

This thesis contributes to redefining the long-term health trajectory of women following hypertensive pregnancy disorders, across multiple organ systems. By integrating detailed cross-sectional and longitudinal assessments of the heart, microvasculature, and kidneys, this research provides a comprehensive picture of how pregnancy complications leave a durable imprint on maternal physiology. While prior studies have often been limited to the early postpartum period, this work extends the timeline decades beyond pregnancy, using advanced imaging and functional measures to characterise subtle but persistent changes in organ structure and function.

These insights reframe hypertensive pregnancy not merely as a transient complication but as an early life event that sets women on a distinct health trajectory. The thesis therefore not only advances mechanistic understanding of the interplay between pregnancy and chronic disease risk but also identifies critical opportunities for early intervention, shaping the future direction of both clinical practice and preventive research.

This work highlights pre-pregnancy, during pregnancy and immediately postpartum as critical windows for surveillance and intervention, with the potential to mitigate the elevated burden of multi-organ disease in later life. Whilst interventions like those used in POP-HT have been shown to reverse cardiovascular remodelling in women with hypertensive pregnancies, further research is needed to understand the interventions best suited for other organs.

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APPENDICES

A

LIFESTYLE AND MEDICAL HISTORY QUESTIONNAIRE

The HELPFUL Study
Questionnaire

Study Code: *Participant identification number:*

H	L	P	—			

Thank you for filling out this questionnaire.

You can decline to answer a question(s) if you wish.

Questions about your medical history

Are you taking any medicines, tablets or pills regularly? YES NO

If yes, what are they?:

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

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

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

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

Have you been diagnosed with any chronic illness(es)? YES NO

If yes, please provide details:

……

……

Have you ever been told that you have high blood pressure? YES NO

Have you ever been treated for high blood pressure? YES NO UNSURE

If yes, how were you treated (including the name of any medication)? ……

If female, were you pregnant when you had high blood pressure? YES NO

If yes, please provide additional details if known: ……

Have you ever been told that you have diabetes? YES NO

Have you ever been treated for diabetes? YES NO UNSURE

If yes, how were you treated (including the name of any medication)? ……

If female, were you pregnant when you had diabetes? YES NO

If yes, please provide additional details if known: ……

Have you ever been told that you have high cholesterol? YES NO

Have you ever been treated for high cholesterol? YES NO UNSURE

If yes, how were you treated (including the name of any medication)? ……

Have you gone through menopause? YES NO UNSURE

If yes, at what age

……

Questions about your family medical history

Are there an illnesses that run in your family? YES NO UNSURE

If yes, what is (are) the illness(es)

……

Have any members of your family ever suffered from. Please tick all that apply.

	Mother		Father		Sibling 1		Sibling 2	
	Mother	age of onset	Father	age of onset	Sibling 1	age of onset	Sibling 2	age of onset
Angina	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
Heart attack	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
High blood pressure	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
Stroke	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
Type I diabetes	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
Type II diabetes	☐	☐☐	☐	☐☐	☐	☐☐	☐	☐☐
High blood cholesterol								

*If you have more than two siblings, please refer to supplementary page at the end of the questionnaire.

Questions about your smoking

Do you currently smoke cigarettes, e-cigarettes, cigars or a pipe regularly? (e.g. at least 1 cigarette a day for the last 6 months) YES NO

If yes, how many cigarettes do you smoke each day at present? ..... cigarettes

how many puffs do you take from an e-cigarette each day at present? ..... e-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

Did your mother smoke while she was pregnant with you? YES NO UNSURE

If yes, how long into the pregnancy did she smoke for? ..... months

If yes, how many cigarettes did she smoke each day? ..... cigarettes

how many cigars did she smoke each day? ..... cigars

how many ounces of tobacco in a pipe did she smoke each day? ..... ounces

Did your other parent/legal guardian smoke while you were in utero (i.e. during pregnancy)? YES NO UNSURE

If yes, how long into the pregnancy did they smoke for? ..... ……months

If yes, how many cigarettes did they smoke each day? …… cigarettes

how many cigars did they partner smoke each day? ..... cigars

how many ounces of tobacco in a pipe did they smoke each day? ..... ounces

Questions about your exercise

Do you currently participate in any regular activity or program designed to improve or maintain your physical fitness? YES NO

If yes, which activity(ies)? ……

For how many hours a week do you take part in these activities, on average? …….

Questions about your diet

How often do you eat the following foods?

	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	.....	.....	.....	.....	.....	.....

Weekly alcoholic unit intake (units per week) ……

Single small shot of spirits * (25ml, ABV 40%) = 1 unit

Alcopop (275ml, ABV 5.5%) = 1.5 units

Small glass of red/white/rosé wine (125ml, ABV 12%) = 1.5 units

Bottle of lager/beer/cider (330ml, ABV 5%) = 1.7 units

Can of lager/beer/cider (440ml, ABV 5.5%) = 2 units

Pint of lower-strength lager/beer/cider (ABV 3.6%) =2 units

Standard glass of red/white/rosé wine (175ml, ABV 12% = 2.1 units

Pint of higher-strength lager/beer/cider (ABV 5.2%) = 3 units

Large glass of red/white/rosé wine (250ml, ABV 12%)= 3 units

*Gin, rum, vodka, whisky, tequila, sambuca. Large (35ml) single measures of spirits are 1.4 units.

Early Life Feeding

How were you fed for the first six months of life (please circle)? **Breast-fed only** **Bottle-fed only (formula)** **Mixture of both**

If **breast-fed only** for the first six months:

a. Until what age were you fed exclusively on breast milk? ……

b. What age did you stop having any breast milk? ……..

c. Did you ever receive donor breast milk (i.e. from a milk bank)? YES NO

i. If yes, for how long? …….. ……

If **bottle-fed (formula) only** for the first six months:

a. Until what age were you fed exclusively on formula milk? ……

b. What age did you stop having any formula milk? ……

c. What type of formula milk were you fed? ……

If **mixture of both breast milk and formula milk** in the first six months:

a. Did you ever receive exclusive breast milk feeding? YES NO

b. What age did you start the mixed diet? ……

c. What type of formula milk were you fed? ……

What age did you start having solid food in your diet (i.e. food other than breast milk or formula)? …… months

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 davs. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

____ days per week

No vigorous physical activities → Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

____ hours per day ____minutes per day

Don't know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

____ days per week

No moderate physical activities → Skip to question 5

4. How much time did you usually spend doing moderate physical activities on one of those days?

____ hours per day ____minutes per day

Don't know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time? ____ days per week

No walking → Skip to question 7

6. How much time did you usually spend walking on one of those days?

____ hours per day ____ minutes per day

Don't know/Not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time.
This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

____ hours per day ____minutes per day

Don't know/Not sure

Thank you for completing this questionnaire.

SUPPLEMENTARY PAGE

Have any members of <u>your family</u> ever suffered from. Please tick all that apply.

Sibling 3 Sibling 4 Sibling 5 Sibling 6

	Sibling 3	age of onset	Sibling 4	age of onset	Sibling 5	age of onset	Sibling 6 age of onset
Angina	☐	☐☐	☐	☐☐	☐	☐☐	☐☐☐
Heart attack	☐	☐☐	☐	☐☐	☐	☐☐	☐☐☐
High blood pressure	☐	☐☐	☐	☐☐	☐	☐☐	☐☐☐
Stroke	☐	☐☐	☐	☐☐	☐	☐☐	☐☐☐
Type I diabetes Type II	☐	☐☐	☐	☐☐	☐	☐☐	☐☐☐
diabetes	☐	☐☐	☐	☐☐	☐	☐☐	☐☐☐
High blood cholesterol							

B

HELPFUL STUDY PROTOCOL

Study Title: HELPFUL (Hypertension Explored in Long-term Post-partum Follow-up in Later Life)

Ethics Ref: 22/LO/0781

IRAS Project ID: 312727

Date and Version No: 26/06/2024, V4.0

Principal Investigator: Professor Paul Leeson,

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Funder: Medical Research Council

Chief Investigator Signature: 

Conflicts of Interest:

Professor Leeson, Professor Lewandowski and Dr Lapidaire are named inventors on a patent filing related to the use of artificial intelligence for disease progression modelling related to this project. Dr Lapidaire is named as an inventor on a patent filing underlying the VITA ear imaging device that will be used in this project. The device is not yet CE marked. The data obtained from this study will not be used as part of a CE marking application.

Confidentiality Statement:

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

TABLE OF CONTENTS

KEY CONTACTS

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Funder(s)	Medical Research Council
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LAY SUMMARY

Women who experience high blood pressure during pregnancy are at increased risk of developing cardiac and vascular diseases later in life. They show changes in their heart, brain, and blood vessels long before they develop high blood pressure. We therefore think that these changes develop slowly over the course of the life of the woman and establish their risk of later disease.

Through better understanding of the pattern of changes across multiple parts of the body over extended periods of time, we aim to identify how advanced the underlying disease is for an individual and how the disease is likely to develop over the next few years. By comparing the rate of change across different parts of the body, we can investigate how one area affects another.

Data including images of the heart, brain and blood vessels will be acquired in women 10 to 25 years after their pregnancy. The initial analysis will focus on assessing differences between women who have had a normotensive pregnancy and those who have had a hypertensive pregnancy, both at a single timepoint and in changes within individuals over the following 15 years.

This dataset will then be used in conjunction with previously acquired data in women who have experienced a hypertensive pregnancy to find out how patterns emerge across the whole body. Participants who previously participated in the PVS Study (REC 08/H0604/127) will be asked to provide consent to link their individual data from the PVS study to this study. We will combine information from different measures at the same time and use the machine learning models to learn the patterns of change that occur as someone progresses from a healthy to a diseased state. This will allow us to identify patterns of hypertensive disease development and may open doors to better interventions and therapies tailored to individuals.

SYNOPSIS

Study Title	Hypertension Explored in Long-term Post-partum Follow-up in Later Life		
Internal ref. no. / short title	HELPFUL		
Study registration	Study to be registered on ClinicalTrials.gov		
Sponsor	University of Oxford Research Governance Ethics and Assurance, University of Oxford Boundary Brook House, Churchill Drive, Headington, OX3 7GB Email: c rgea.sponsor@admin.ox.ac.uk		
Funder	Medical Research Council 58 Victoria Embankment London EC4Y 0DS 01793 416200		
Study Design	Observational		
Study Participants	Women who have experienced either a hypertensive or normotensive pregnancy 10 to 25 years prior to the study visit.		
Sample Size	100 women who have experienced a hypertensive pregnancy and 100 women who have experienced a normotensive pregnancy.		
Planned Study Period	01/11/2022 to 01/11/2042		
Planned Recruitment period	01/11/2022 to 01/11/2027		
	Objectives	Outcome Measures	Timepoint(s)
Primary	To compare cardiac alterations across women who have had either a hypertensive or normotensive pregnancy 10-25 years prior to the	Cardiac magnetic resonance imaging (MRI) to assess changes in left and right ventricular mass.	Pregnancy/PVS Study and Visit 1 (10-25 years post pregnancy)

	study.		
Secondary	To complete a cross-sectional analysis of the whole body in women who have had either a hypertensive or normotensive pregnancy 10-25 years prior to the study.	**Brain** Neural magnetic resonance imaging (MRI) to assess changes in brain volumes, white matter hyperintensities, cerebrovascular morphology and blood volume and perfusion. **Heart** Echocardiography assessment of cardiac left and right ventricular mass, right and left ventricular end- diastolic volumes, right and left ventricular systolic and diastolic function, electrocardiogram (ECG) assessment of heart rate and rhythm. **Vasculature** Aortic distensibility by MRI and aortic/central blood pressure measurements (blood pressure cuff) and retinal and ear vascular imaging. **Liver and Kidneys** MRI of the liver to quantify intra-hepatic lipid content, steatohepatitis, and hepatic fibrosis, and MRI renal imaging to quantify renal alterations. **Blood Sampling** Whole, plasma, and serum blood samples to quantify circulating angiogenic, metabolic and vascular markers **Lung function** Spirometry measurements and cardiopulmonary exercise test (CPET) to measure oxygen uptake and carbon dioxide exchange kinetics across submaximal and peak exercise, as well as heart rate recovery. **Physical activity** Objective measure of physical activity (7 days of wrist worn activity monitor).	Visit 1 7-days post Visit 1

Secondary	To determine whether changes in women 10-25 years post hypertensive or normotensive pregnancy are predicted by measurements performed at time of pregnancy or at other points between pregnancy and follow-up.	**Pregnancy** Clinical measures of disease severity and pregnancy characteristics collected from medical records. **Clinical measures 5 to 10 years post-partum** Prior measures of the heart, brain, vascular system and other clinical measures collected at previous study visits within the Pre-eclampsia Vascular Study (PVS).	Pregnancy, PVS study and visit 1 of follow-up
Secondary	To combine information from different measures at the same time and use machine learning models to learn the patterns of change that occur as women progress from a healthy to a diseased state.	Computational models that combine multiple imaging data sources to describe phenotypes related to hypertensive pregnancy.	Post visit 1
Secondary	To investigate whether metrics measured 10-25 years post hypertensive or normotensive pregnancy can predict future cardiovascular risk.	NHS Digital Follow-Up (including myocardial infarction, stroke, TIA, angina, hospital procedures, mortality)	For up to 15 years

ABBREVIATIONS

Define all unusual or 'technical' terms related to the project.

CCRF	Cardiovascular Clinical Research Facility
CPET	Cardiopulmonary exercise test
CRF	Case Report Form
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
PIL	Participant/ Patient Information Leaflet

REC	Research Ethics Committee
MRI	Magnetic Resonance Imaging
NHS	National Health Service
OCMR	Oxford Centre for Clinical Magnetic Resonance Research
PVS	Pre-eclampsia Vascular Study
RGEA	Research Governance, Ethics & Assurance
SOP	Standard Operating Procedure
VITA	Vascular Imaging Tool for the Auricle

BACKGROUND AND RATIONALE

Hypertension during pregnancy

Hypertension during pregnancy [1] occurs in over 10% of women [2] and is associated with disease progression at a multi organ level and up to a four-fold increased risk of developing a range of cardiovascular disorders in later life including myocardial infarction, stroke, and heart failure [3]. In view of this increase in risk, post-partum review of women who have had a hypertensive pregnancy is advised, followed by annual cardiovascular health assessment [4-9]. However, during this early post-partum period, women are often asymptomatic, their blood pressure can temporarily normalise and currently there is little personalised advice that can be provided.

As a result, loss of follow-up is frequent, particularly in countries without nationalised health care [8]. This is until the women present with established hypertension, stroke, or myocardial infarction. Indeed, in a UK priority setting partnership of families, researchers and healthcare workers, long-term consequences of hypertensive pregnancy, and the best way to prevent them, were identified as two of the most important priorities for future research [10].

Identifiable hypertensive disease progression

Strong evidence is emerging that during the post-partum period there is identifiable progression of hypertensive-related disease within the heart, brain, and vasculature. By undertaking imaging studies in women during the five to fifteen years after a hypertensive pregnancy we, and others, have demonstrated that left ventricular structure is altered from early post-pregnancy and is associated with significant diastolic impairment [11]. These cardiac differences are coupled with later vascular changes extending from central aortic stiffening to peripheral microvascular rarefaction and extend to cerebrovascular small vessel white matter disease [12, 13].

Most of these findings derive from small cohorts of women comparing single phenotype measures. To address this potential limitation we have previously performed multi-organ, multi-modality imaging to determine whether a panel of imaging markers were consistently associated with a hypertensive pregnancy history. This replicated prior single phenotype observation relates to cardiac structure, cerebrovascular disease, and microvascular rarefaction but, additionally highlighted that these frequently co-occur and vary independent of blood pressure at time of assessment [13]. When identified in mid-life, the changes we found have,

previously, been individually related to future risk of vascular events independent of blood pressure [14-16].

Rationale for study population

In-depth imaging derived phenotype data in women more than fifteen years following their hypertensive pregnancy is scarce and longitudinal changes in these phenotypic patterns have not been fully explored. In addition, it is unknown whether these phenotypes are also evident in women with environmental and health-related behaviours in whom advanced disease stages and severe complications are more prevalent and who may gain particular benefit from more accurate disease diagnosis. Thus, this study aims to examine a sample of women 10-25 years post-index hypertensive or normotensive pregnancy from a range of backgrounds. This will help to further understand hypertensive disease progression across the heart, brain, and vasculature over the life course.

6. OBJECTIVES AND OUTCOME MEASURES

	Objectives	Outcome Measures	Timepoint(s)
Primary	To compare cardiac alterations across women who have had either a hypertensive or normotensive pregnancy 10-25 years prior to the study.	Cardiac magnetic resonance imaging (MRI) to assess changes in left and right ventricular mass.	Pregnancy/PVS Study and Visit 1 (10-25 years post pregnancy)
Secondary	To complete a cross-sectional analysis of the whole body in women who have had either a hypertensive or normotensive pregnancy 10-25 years prior to the study.	**Brain** Neural magnetic resonance imaging (MRI) to assess changes in brain volumes, white matter hyperintensities, cerebrovascular morphology and blood volume and perfusion. **Heart** Echocardiography assessment of cardiac left and right ventricular mass, right and left ventricular end- diastolic volumes, right and left ventricular systolic and diastolic function, electrocardiogram (ECG) assessment of heart rate and rhythm **Vasculature** Aortic distensibility by MRI and aortic/central blood pressure measurements (blood pressure cuff) and retinal and ear vascular imaging. **Liver and Kidneys**	Visit 1

		MRI of the liver to quantify intra-hepatic lipid content, steatohepatitis, and hepatic fibrosis, and MRI renal imaging to quantify renal alterations. **Blood Sampling** Whole, plasma, and serum blood samples to quantify circulating angiogenic, metabolic and vascular markers **Lung function** Spirometry measurements and cardiopulmonary exercise test (CPET) to measure oxygen uptake and carbon dioxide exchange kinetics across submaximal and peak exercise, as well as heart rate recovery. **Physical activity** Objective measure of physical activity (7 days of wrist worn activity monitor).	7-days post Visit 1
Secondary	To determine whether changes in women 10-25 years post hypertensive or normotensive pregnancy are predicted by measurements performed at time of pregnancy or at other points between pregnancy and follow-up.	**Pregnancy** Clinical measures of disease severity and pregnancy characteristics collected from medical records. **Clinical measures 5 to 10 years post-partum** Prior measures of the heart, brain, vascular system and other clinical measures collected at previous study visits within the Pre-eclampsia Vascular Study (PVS).	Pregnancy, PVS study and Visit 1 of follow-up
Secondary	To combine information from different measures at the same time and use machine learning models to learn the patterns of change that occur as someone progresses from a healthy to a diseased state.	Computational models that combine multiple imaging data sources to describe phenotypes related to hypertensive pregnancy.	Post visit 1
Secondary	To investigate whether metrics measured 10-25 post hypertensive or normotensive pregnancy predict future cardiovascular risk.	NHS Digital Follow-Up (including myocardial infarction, stroke, TIA, angina, hospital procedures, mortality)	For up to 15 years

STUDY DESIGN

Study design and Aims

This is an observational study of women with and without a previous hypertensive pregnancy. Participants will attend one 4-hr visit at the John Radcliffe Hospital, with the option to split the study visit over two days for convenience. Participants will also be invited to take part in a longer-term follow-up as part of the study. This part of the study does not involve any study visits or further contact with the research team. Instead, we will follow up their health outcome data for up to 15 years using NHS digital. There is no requirement for participants to consent to take part in this longer-term follow up and if they decide to take part, they are free to withdraw consent at any time without giving a reason.

Participant profile

We will study a group of 200 women, 10 to 25 years post pregnancy to extend data coverage over time and age and to ensure broad representation of hypertensive disease state during mid-life in women. This will be achieved by a follow-up of participants from our previous Preeclampsia Vascular Study (PVS) (08/H0604/127) originally seen at 6 to 13 years post-index hypertensive or normotensive pregnancy. If required, we will restart our identification and recruitment methods, previously used in PVS, based on obstetric records to identify, and recruit additional women who had pregnancies 10 to 25 years prior, to ensure that the sample size is reached. All participants will undergo a standardised imaging protocol as described in the general overview.

Study visit and measures

Study participants will attend approximately one 4-hour visit on a single day or split over multiple visits, at the participants' convenience to complete all measures. Participants will first be given the opportunity to answer any questions and they will provide written consent for the study. Participants' anthropometric measures (waist-to-hip ratio, height and weight, body composition, and resting blood pressures) will then be taken, as well as a blood sample collection. After this, the participants will have an echocardiography scan and will undergo microvascular imaging (retina and ear) followed by spirometry and cardiopulmonary exercise testing. Participants will then be asked to undergo a magnetic resonance imaging (MRI) scan. The study visit will conclude with participants being provided with a wrist-worn physical activity monitor to wear for 7 days following the study visit. A flowchart for the planned visit procedures can be found in Appendix A at the end of this protocol.

PARTICIPANT IDENTIFICATION

Study Participants

200 women will be recruited from either the PVS study or obstetric records, of which 100 will have experienced a hypertensive pregnancy and 100 will have experienced a normotensive pregnancy.

Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study
- Female who had a pregnancy 10 to 25 years prior
- 30 to 70 years of age
- Able (in the investigator's opinion) and willing to comply with all study requirements.
- Adequate understanding of verbal and written English
- Inclusion as a case (hypertensive pregnancy) or control (normal pregnancy) in the PVS study, or with the following features in obstetric records, as detailed below:

  Controls (normal pregnancy) only

  Index pregnancy:
    - Normal obstetric history (previous and index pregnancy)
    - Diastolic BP consistently below 90mmHg
    - Systolic BP consistently less than 140mmHg
    - No more than a trace of proteinuria

  Cases only (Hypertensive Pregnancy)

  Index pregnancy:

  Gestational Hypertension and Pre-eclampsia
    - New onset hypertension after 20 weeks gestation in index pregnancy
    - Diastolic BP 90mmHg on two separate occasions within a 24 hour period

  Pre-eclampsia or Hypertension with Superimposed Pre-eclampsia
    - Raised blood pressure (as above)
    - And new onset proteinuria of:
    - 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

Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- Pregnant or lactating when they are due to attend for study visit 1
- Less than six months postpartum
- Planning to donate blood within two weeks prior to study visit 1
- Evidence of congenital heart disease or significant chronic disease relevant to cardiovascular or metabolic status
- Any significant disease or disorder which, in the opinion of the investigator, might influence the participant's ability to participate in the study

For exclusion of MRI component only:

- Unsuitable for MRI based on participant screening. The participant may still be included in other parts of the study.

PROTOCOL PROCEDURES

See appendix A for a schedule of study procedures.

Recruitment

9.1.1. Pre-eclampsia Vascular Study (PVS)

Individuals will be recruited from the PVS at the University of Oxford. As part of the follow-up of this study cohort, the PVS study team have maintained a database of addresses and contact information for participants in the PVS cohort who have expressed interest to participate in future studies and have consented to being contacted. They will contact eligible participants who took part in the PVS study and agreed to be contacted about future research with an initial letter or email signed by the Principal Investigator of the PVS study and the HELPFUL Participant Information Leaflet (PIL). This will provide them with information about the study and contact details for the HELPFUL study team in the Division of Cardiovascular Medicine, University of Oxford should they wish to receive more information about the study. If interest is expressed via a reply slip, potential participants will be re-contacted by email or telephone after a minimum of 24 hours to discuss any queries. Eligible participants will be invited to attend the study visit and complete the study informed consent process. Participants who are contacted will also be given the option to opt in or out of being contacted for future studies. If there is no response to the initial invite after 4 weeks, the PVS team will contact the participant by a phone call to confirm receipt of documents.

9.1.2. Direct recruitment from obstetric records

Potential participants will also be identified from the John Radcliffe Hospital Maternity records by the clinical team from obstetric records. All mothers who interact with the maternity services are included on the clinical database and data on complications of pregnancy such as hypertension or pre-eclampsia are included. All mothers recorded with a pregnancy complicated by pre-eclampsia or gestational hypertension 10 to 25 years ago for the duration of the study will be identified based on ICD-10 case codings (011 - pre-existing hypertension with superimposed pre-eclampsia, 013 - gestational hypertension, 014 - gestational hypertension with significant proteinuria and 015 - eclampsia). Maternity records for these mothers will be reviewed by the clinical team and only those who fulfil the ISSHP (International Society for the study of Hypertension in Pregnancy) criteria for definition of pre-eclampsia and gestational hypertension will be included. All mothers with a pregnancy 10 to 25 years ago, not complicated by pre-eclampsia (ICD-10 codes 014/015) or elevated blood pressure during pregnancy will also be identified. Potential participants who could act as either cases or controls will be contacted at the same time, starting with those identified from the 1998 records.

Individuals identified by the clinical team from obstetric records will be contacted via their contact address validated against the John Radcliffe case note records. Where this address is found no longer to be current, an application will be made to trace the individual via the NHS Strategic Tracing Service. This will be an initial letter from the clinical team and HELPFUL

Participant Information Leaflet (PIL), providing them with information about the study and contact details for the HELPFUL study team in the Division of Cardiovascular Medicine, University of Oxford should they wish to receive more information about the study. If interest is expressed via a reply slip, potential participants will be re-contacted by email or telephone after a minimum of 24 hours to discuss any queries. Eligible participants will be invited to attend the study visit and complete the study informed consent process. If there is no response to the initial invite after 4 weeks, the clinical team will contact the participant by a phone call to confirm receipt of documents.

Identification of potential participants and recruitment will continue until the sample size is reached. During recruitment, the age and parity of mothers who take part will be monitored, and if necessary, invitations selectively sent to control mothers who more closely match the participants with hypertensive pregnancy (based on control group age being within +/- 4 years and equal recruitment into parity-based groupings 1,2-3, 4+).

Screening and Eligibility Assessment

Potential participants from the previous ethically approved PVS and those recruited directly from maternity records will be approached by the research teams as described in section 9.1. Following provision of the participant information leaflets, study eligibility will be assessed during a telephone call to potential participants. The study investigator will seek verbal consent to ask potential participants about their medical history. This is to clarify if there are significant contraindications, diseases, or disorders which, in the opinion of the investigator, might influence the individual's ability to participate in the study. Verbal consent and inclusion/exclusion criteria will be documented on a case report form (CRF).

Informed Consent

Eligible individuals willing to participate in the study will be provided with the Participant Information Leaflet, detailing the study information in advance of the informed consent process. A paper copy of the study questionnaire will be given to the participants via email/post prior to the study visit to allow them to check some of the questions about family history, and in particular, birth history. The participants can use this time to gather the information needed to answer the questions, which will then be completed as part of the study visit. The questionnaires will be identified by participant number and no identifiable information will be sent through the post or email.

Only after participants have had a reasonable period of time (not less than 24h) to read the study information and discuss with friends and family, will they be invited to schedule a time for their study visit. The informed consent process will be completed on the day of the study visit.

The participant must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed. Should it be necessary to reconsent participants this will be done electronically. Participants will receive the study information leaflet, a reconsent form and an email explaining why their reconsent is being sought. A copy of their reconsent will be provided to participants.

During the informed consent process written and verbal versions of the Participant Information Leaflet and Informed Consent form will be presented to the participants detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and

constraints of the protocol and potential risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

Participants will also be invited to take part in a longer-term follow-up as part of the study. This part of the study does not involve any study visits or further contact with the research team. Instead, we will follow up their health outcome data for up to 15 years using NHS digital. There is no requirement for participants to consent to take part in this longer-term follow up and if they decide to take part, they are free to withdraw consent at any time without giving a reason.

Opportunity to ask questions about the study or consent process will be provided. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the delegated study investigator who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the study site and another retained in their medical notes (if applicable). During the consent process participants will be invited to declare their interest in participation in future or related studies.

Enrolment

The study is not randomised. Participants will be enrolled as they are consented. At enrolment, all participants will be assigned an ID in sequential order. Each participant will be invited for one study visit. Enrolment for each individual will be only to complete the single visit.

9.5. Blinding and code-breaking

There is no blinding in the study, and no code breaking procedure.

Description of study intervention(s), comparators, and study procedures (clinical)

There is no study intervention or comparator.

Description of study procedure(s)

Study Visit

Study procedures will take place in the Oxford Cardiovascular Clinical Research Facility (CCRF) and the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR), which are part of the Division of Cardiovascular Medicine, University of Oxford at the John Radcliffe Hospital in Oxford. A study visit will take up to 4 hours. We will ask participants not to have any food or snacks at all for at least six hours before they come in for their study visit.

Anthropometry and Blood Pressures (15 minutes)

Assessments include height, weight, BMI and waist-to-hip ratio. Participants will have their clinic pressure checked after five minutes' rest using the automated mode of a sphygmomanometer. Five blood pressure readings will be taken over 10 minutes.

Echocardiogram (cardiac ultrasound) scan (15 minutes)

Cardiac ultrasound imaging will be performed by a trained sonographer to evaluate cardiac structure and function.

Resting electrocardiogram (ECG): (5 minutes)

ECG sensors will be attached to the participant, so heart rate and rhythm can be measured at rest.

Retinal imaging (10 minutes)

Retinal photography will be completed using a digital camera and imaging software following an established protocol. Imaging is non-invasive with no requirement for topical drops and does not require removal of contact lenses.

Ear imaging (10 minutes)

Ear imaging will be completed using the VITA (Vascular Imaging Tool for the Auricle). This is a prototype device developed by study co-investigators. The study using the device has been provided ethical approval (by the Central University Research Ethics Committee R76459/RE001). Imaging is non-invasive. The device will be clipped onto the ear and digital (transmission) images will be taken when the device is in place. A polarisation lens may then be slotted in the device to enable a type of imaging where the light is split by polarization into two rays taking slightly different paths. This enables measurement of thickness/inner diameter of blood vessels.

The device is not CE marked and was developed in house. This study will not be used to obtain CE marking. There are plans to commercialise this device eventually (some aspects of it have been patented), but at the moment no company has been set up. The inclusion of this device is not vital for the study, but it is part of one of the aims of the project to create a way to monitor

cardiovascular disease using affordable measures. We used capillaroscopy in previous studies to measure small vessel health, but this was not reliable enough due to inter-user variation. A pilot study in VITA has shown that this device is easier to use for researchers and can also take images of small vessels of sufficient quality.

The vessel imaging device (VITA) clips onto the auricle (the visible part of the ear outside the head). This has a number of advantages over other imaging; (i) the auricle is accessible on both sides and relatively thin, enabling detection of light through the ear, (ii) the auricle can withstand much brighter light exposure resulting in a brighter and clearer image, (iii) the clipping mechanism on VITA is attached to the ear, which is particularly advantageous in children and certain clinical populations with difficulty remaining still (e.g. people with dementia).

All components of the device are produced commercially and have thus undergone all standard rigorous safety checks. The ear is exposed to a simple backlight, emitting visible light frequencies to which our bodies are exposed to on a daily basis (red and green: wavelengths 630 and 523 nm). The optical powers are safe: for comparison, the intensity of the backlight is less than standard LED bicycle lights. The device is powered from a 12V micro-USB charger, and all circuit boards are enclosed and run at safe low voltages (<12V). The device may use strobed lights, but only when attached to the ear (so the participant and the researcher will not be exposed to strobed light when the device is used as per instructions. A previous version of this device has been tested in 60 participants in a previous trial (REC approval R76459/RE001) with no adverse effects. The device is made from commercially EU safety compliant components and runs at safe low voltages (<12V). For the VITA ear imaging device, the tip (which touches the ear of the participants) and the handle (which the researcher will hold) will be cleaned between participants.

All equipment and devices will be cleaned at points of contact with participant or researcher. PPE will be provided for staff and relevant protection for participants at the study site.

Cardiopulmonary Exercise Testing (CPET) (40 minutes)

Cardiac function and oxygen requirements in response to incremental increase in workload will be measured via cardiopulmonary exercise testing on an exercise bike. A blood pressure cuff and ECG sensors will be attached to participants so heart rate and electrocardiography (ECG) can be monitored before, during and after the exercise testing. Echocardiographic measures will also be assessed during the CPET. The exercise protocol is a validated incremental protocol with established use in clinical and research practice. The exercise protocol is currently utilised in ongoing ethically approved studies conducted by the Division of Cardiovascular Medicine and is performed on a stationary bike. Exercise testing will consist of an incrementing workload (increasing resistance to pedal against) to assess exercise capacity at up to 80% of maximal heart rate, the test will last 10 to 15 minutes. After reaching up to 80% of their maximal heart rate, a recovery period will be conducted for five minutes to allow the participants to recover. CPET measures will be continued during this time.

Break and Lifestyle Questionnaire (15 minutes)

Afterwards, participants will have a 15-minute break where snacks and water will be provided. Participants will be asked to fill in a lifestyle and medical history questionnaire during this time.

Magnetic Resonance Imaging (MRI) (60 minutes)

Once contraindications to magnetic resonance imaging (MRI) are excluded by use of the facility's screening forms, the risks of undergoing a scan are minimal. A trained scanner operator or radiographer will go through a list of possible risks with the participant before scanning. The MRI scanner consists of a large powerful magnet. Magnetic resonance imaging uses no ionising radiation. There are, however, potential hazards associated with MRI and the scanning of participants including the presence of surgical implants, participants' clothing, jewellery (such as body piercings, some tattoos) bodily habitus, or medical conditions. A comprehensive list of potential risks has been compiled, and the participant should be checked against this by the operator, prior to entering the controlled areas of the MRI scanners.

MRI will be used to quantify kidney, aorta and brain structure and volume, as well as cardiac structure and function using a 3Tesla (3T) scanner once the participant has been deemed to be scanner safe. Established protocols will be used and performed by trained operators. Participants will lie in a supine position and a dedicated cardiac coil placed around their chest. Images are obtained using a combination of breath hold and non-breath hold sequences and ECG gating. During the actual scanning procedure, the scanner produces loud banging noises, and the participant will be fitted with suitable hearing protection (earplugs and protective headphones). There is a small mirror that will allow them to see out of the scanner. During the experiment, the participant will be able to communicate with the operator in the control room. In addition, they will be given a call button, which allows them to alert the operator at any time. People with a history of claustrophobia may be excluded from participation in the study. All participants will still be introduced carefully to the scanner and allowed to leave at any stage, should they wish to do so. Once in the scanner, participants will be able to indicate immediately if they wish the scanning to cease by pressing a call button in their hands.

Vicorder® Vascular Measures and Central Blood Pressures (10 minutes)

Resting measures of vascular stiffness including pulse wave velocity and central blood pressure will be collected using a non-invasive device (Vicorder®). The Vicorder® is a cuff-based device that is CE marked and is being used for it's intended purpose.

Blood sampling (10 minutes)

A venous blood sample (approximately 25mls, or 5 teaspoons) will be taken at rest and include samples for a) whole blood, plasma and serum lipid and inflammatory marker analysis and b) analysis of biochemistry and metabolism and c) analysis of biomarkers associated with inflammation, angiogenesis, and endothelial activation.

Fitting of Accelerometer Activity Monitoring Devices (5 minutes)

At the end of the study visit, an activity monitor will be fitted to the participant's wrist. This wrist-worn accelerometer is similar in design to a wrist-worn watch. It consists of an activity monitor placed inside a rubber wristband. It is shock and waterproof so participants can shower/swim with it on. Wrist-worn accelerometers have high compliance and reliability and are validated measures of physical activity. Participants will be asked to wear accelerometers for 7 days, 24 hours per day. However, they can shower with the accelerometer. Stamped addressed envelopes will be provided to return devices.

Long term follow-up

Participants will be asked if they would like to consent to a 15-year follow-up via NHS Digital in order to collect evidence of cardiovascular events and relevant investigations, including myocardial infarction, stroke, TIA, angina, hospital procedures and mortality. This data will be reviewed regularly to identify any participants that have been admitted to hospital and to obtain health related data. The data will be pseudonymised and added to a secure research database so that longitudinal changes in hypertensive disease progression can be compared across the course of the participant's life. If the participant wishes to opt out of the digital follow-up they can express this at the time of recruitment or through contacting the researchers after their active involvement in the study is completed.

Sample Handling

Blood samples will be centrifuged and the whole blood plasma and serum will be separated and stored. in a de-identified format in secure freezers at -80 degrees Celsius at the University of Oxford, under the custodianship of the Division of Cardiovascular Medicine. The samples may be used in future research, here or abroad, which has ethics approval, potentially including commercial organisations. A copy of the consent will be retained from participants who consented for their samples to be used in future research. All other remaining human materials that have not been depleted will be disposed of at the end of the study in accordance with the Human Tissue Authority Code of Practice.

Early Discontinuation/Withdrawal of Participants

During the course of the study a participant may choose to withdraw early from the study treatment at any time. This may happen for several reasons, including but not limited to:

- The occurrence of what the participant perceives as an intolerable AE.
- Inability to comply with study procedures
- Participant decision

Participants may choose to stop study assessments but may remain on study follow-up.

Participants may also withdraw their consent, meaning that they wish to withdraw from the study completely.

In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Loss of capacity
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant deviation from the study protocol
- Withdrawal of consent

Should the participant withdraw, we may be unable to destroy the data if it has already been de-identified, as outlined in the confidentiality section. Data already collected would not be used in the final study analysis except where analysis of their data has already been integrated into interim results.

The type of withdrawal and reason for withdrawal will be recorded in the CRF.

If the participant is withdrawn due to an adverse event, the Investigator will arrange for telephone calls until the adverse event has resolved or stabilised.

Definition of End of Study

The end of active participant involvement in the study will be the completion of the follow up period when the last participant dataset has been analysed and queries resolved. However, we will also ask participants for consent to a 15-year long term follow up via NHS Digital in case there are any clinical events relevant to our study data. End of study will be final receipt of NHS Digital follow up data.

SAFETY REPORTING

Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other ‘important medical events’ may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC (Research Ethics Committee) that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was ‘related’ (resulted from administration of any of the research procedures) and ‘unexpected’ in relation to those procedures. Reports of related and

unexpected SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA (Health Research Authority) report of serious adverse event form (see HRA website).

STATISTICS AND ANALYSIS

11.1. Statistical Analysis Plan (SAP)

The plan for the statistical analysis of the study is outlined below. There is not a separate SAP document in use for the trial.

Description of the Statistical Methods

Within the PVS datasets we will undertake comparisons of different imaging phenotypes across women with normotensive and hypertensive pregnancies to understand the extent of disease progression post hypertensive pregnancy. We will use standard regression analysis to study the association of hypertensive pregnancy and current lifestyle factors on disease progression within the heart, brain and vasculature. Furthermore, we will attempt to determine how phenotype varies across different cohorts of women. As multiple datasets will be available, we will be able to test whether differences identified in one study can be replicated in independent datasets.

Sample Size Determination

Analysis of covariance (ANCOVA) will be used, and power estimated for differences in right ventricular mass index (RVMI), adjusted for age and sex, as well as left ventricular mass indexed to body surface area, LV wall thickness, LV and RV systolic and diastolic function, brain volumes and white matter hyperintensities, cerebrovascular morphology, aortic compliance, microvascular structure, hepatic morphology, lung function, exercise capacity, and circulating angiogenic, metabolic and vascular markers.

Sample size calculations are at 90% power for a two-sided test at α = 0.05 using the following formula: $ni = 2\frac{(Z_1-\frac{\alpha}{2}+Z_1-\beta)}{ES}^2$. To detect a 0.32g/ml RVM/EDV difference, in line with a conservative estimate of differences in adults with different pregnancy backgrounds, we require 19 participants per group[16]. The planned sample sizes of 100 hypertensive pregnancy vs 100 normotensive pregnancy for the 10-25 years post-partum follow-up are thus sufficient. Additionally, we require a larger sample size than the initial power analysis to allow us to assess whether mass varies across different subgroups of women according to their severity of hypertensive disease during pregnancy.

DATA MANAGEMENT

The plan for the data management of the study are outlined below. There is not a separate Data Management document in use for the study.

Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

Access to Data

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

Data Recording and Record Keeping

Personal data will be recorded electronically on data forms that are entered into an access-restricted computer, encrypted and located on the secure University of Oxford High Compliance system, a service for Clinical Trials Units (CTUs) and Medical Division Departments that securely access applications and store sensitive data.

Study data will be recorded in a pseudo-anonymised manner, and processed electronically where applicable; participants would not be identifiable from this. All participants will be identified by a code number (Study ID number) on case report forms (CRFs), paper questionnaires, scan images (i.e. ultrasound and MRI) and any electronic databases i.e. their identity will remain unknown. All source documents will have all identifiers removed and replaced by only the study ID number.

All documents containing personal data (the code list/code break document and ICFs) will be stored securely in lockable cabinets and are only accessible by study staff and authorised personnel. The study investigator is responsible for keeping these documents encrypted where possible and kept securely to ensure that in case of an emergency, participants can be identified and contacted.

Should it be necessary to reconsent, then a third party would be involved in the processing of the data. Microsoft forms is trusted by the University for confidential information, so will be used for the purpose of obtaining reconsent. Once completed by the participant, a printed copy of the online consent form will be stored securely in lockable cabinets, only accessible by study staff and authorised personnel. A copy will also be stored digitally in the secure University of Oxford High Compliance system, and a further copy will be stored in the participant's medical records.

Electronic data will be held on secure University of Oxford network drives/hard disks/ servers on password protected computers within locked offices with restricted access. Backup copies of files will be made weekly and stored on a different secure server/external hard drive. These back up locations will be subject to the same security principles as the primary locations. When datasets are complete, the primary copy will remain at the study site where it will be transferred onto optical media, e.g. DVD/external drive and undergo archiving for hard copy data. A copy will also be stored on AIMES, a digital storage service specialised in healthcare data. Any copies leaving the study site will be completely anonymised.

All study data will in turn be entered onto a password protected, electronic cloud based software kept in England, called 'Castor EDC'. Castor EDC is an electronic data capture and management system that permits secure multi-site access. The server is based in the UK and it complies with relevant laws to ensure that the data is held securely. A security assessment has been carried out for Castor EDC by Oxford University's Info Security Team. The participants will be identified by a unique study specific participant ID in this database also and will not be directly identifiable from this. The pseudonymised data will be kept on the Castor system for 15 years, to allow for follow-up data, before it is removed by the principal investigator.

Personal details will be stored for 12 months after the study has finished. Contact details and copy of the consent form, of those participants, who have consented to be approached for future research, will be stored for up to 10 years following the end of the study separately from the study information. Blood samples taken in the course of the research will be disposed of at the end of the research in accordance with the HTA's code of practice unless it is possible to transfer them to another project following ethical approval.

Data Sharing

With the consent of the participant, personal data (namely participant's NHS number, Date of Birth and post code) will be shared securely between the main study site at the University of Oxford and NHS digital in order to request health outcome data from participants. NHS digital will return the requested information to the data controller (University of Oxford) in pseudo-anonymised form which is only identifiable to the data controller.

A secure RDSF (Research Data Storage Facility) will be used to securely transfer clinical data gathered from the case report forms (CRFs), questionnaires, laboratory results, and output data generated from the scan images and cardiovascular tests between the University of Oxford and collaborators. The RDSF is a set of disks and servers housed in two separate data centres. Connection is via the University network or VPN. No personal identifiable information will be transferred between sites. Pseudo-anonymised study data may be shared with collaborators, other educational establishments, and commercial organisations. Data sharing agreements will be used.

QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP (Good Clinical Practice), relevant regulations and standard operating procedures. Images will be acquired as part of primary and secondary outcomes. Echocardiography will be analysed offline by a member of the research team, trained in the analyses of echocardiography. MR images will be analysed by an appropriately trained individual, trained in interpreting MR scans. An initial quality review of data will be conducted as soon as possible after it has been collected to make sure it is of an acceptable quality for analysis. This review will be conducted by a performing investigator for each of the measures.

Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. Study monitoring will be based on a risk assessment. The risk assessment will be focussed on minimising risk to study participants, reducing hazards, and ensuring patient safety, data integrity and compliance with GCP and the Data Protection act.

Study monitoring

Regular monitoring will be performed according to a risk based monitoring plan. Data will be evaluated for compliance with the protocol and completeness in relation to source documents and CRFs. Direct access will be granted to authorised representatives from the Sponsor within the appropriate department and host institution for monitoring and/or audit of the study to ensure compliance with regulations. Following written standard operating procedures, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

Study Committees

There are no oversight committees for this study as it is an observational study without an intervention.

PROTOCOL DEVIATIONS

Any deviations from the protocol will be documented in a protocol deviation form and filed in the study master file. A standard operating procedure will be in place describing the procedure for identifying non-compliances, escalation to the central team and assessment of whether a non-compliance /deviation may be a potential Serious Breach.

SERIOUS BREACHES

A “serious breach” is a breach of the protocol or of the conditions or principles of Good Clinical Practice which is likely to affect to a significant degree -

(a) the safety or physical or mental integrity of the trial subjects; or

(b) the scientific value of the research.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the approving REC committee and the relevant NHS host organisation within seven calendar days.

ETHICAL AND REGULATORY CONSIDERATIONS

Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

Approvals

Following Sponsor approval of the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA (where required) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Other Ethical Considerations

There will be no involvement of vulnerable participants or participants who are unable to consent for themselves.

Unexpected health related findings

It is possible that the study investigations could uncover unexpected disease findings on the MRI or ultrasound scans, or other research procedures, such as blood tests. CCRF and the MRI scanning facility OCMR, have an established Standard Operating Procedure (SOP) to be followed in the instance of an unexpected abnormality found on a research scan. If any evidence of an unexpected abnormality is detected on the MRI or echocardiography scans the investigators will not attempt to interpret them. They will contact the site MRI or echocardiography technologist, consultant or radiologist for conformation of an abnormality prior to disclosing any problem to the participant. For any abnormalities confirmed, a designated clinical specialist will discuss the implications with the participant and may arrange for further investigations as necessary. Participants will be aware from the PIL that research scans and procedures are not for diagnostic purposes, and therefore are not a substitute for a clinical appointment. Investigators will gain permission from the participant to contact their general practitioner (GP) directly so that the GP can then arrange appropriate management.

Phlebotomy

Risk to Participants: Common risks associated with phlebotomy are pain during the procedure and bruising (with associated pain afterwards). These risks will be minimised by ensuring that all staff are fully trained in phlebotomy. All participants will be fully informed about these risks in the Participant Information Leaflet. The worry associated with taking blood may cause some participants to feel unwell or faint before, during or after the procedure. The risk associated with this will be reduced by having an adequately equipped facility for performing the procedure (see above) and having a staff member trained in basic life support. Although phlebotomy is a very safe procedure, it does create a puncture wound on the skin which may very rarely lead to infection around the puncture site. The risk of this will be minimised by ensuring strict hygiene during the procedure and by not recruiting participants who are at increased risk of infection. In the event that a participant reports symptoms of an infection (local redness, swelling, pain or discharge of pus) they should be referred to their GP or to A+E urgently.

Risk to Researchers/Other Staff: Taking blood cares a risk of needle stick injury to the phlebotomist, which in turn carries a risk of exposure to blood borne infections. This risk will be minimised by a) ensuring staff are adequately trained, b) ensuring staff have been vaccinated against, and show immunity to Hepatitis B and c) having a local policy for needle stick injury which describes the process of being assessed for and receiving post exposure prophylaxis.

Echocardiogram cardiac ultrasound scans

Although echocardiography carries no risk of physical harm to the participant, there is a risk to personal discomfort or embarrassment to the participant is minimised by offering a choice of

male or female echocardiographer to carry out the scan, and providing gowns to maintain modesty and dignity at all times.

MRI

MRI is a safe and non-invasive technique with no known risk when appropriately supervised. It does not involve ionising radiation (X-rays). Potential participants with ferromagnetic objects in their bodies or with implanted devices which can be damaged by the magnet will be excluded by carefully screening all subjects for ferromagnetic objects, metal implants and other metal (e.g. shrapnel injury) every time prior to entering the scanner environment.

The OCMR is fully equipped for resuscitation (including defibrillation) in the unlikely event of a medical emergency during scanning and doctors performing and/or supervising the scans are trained in Advanced Life Support.

While most people do not experience discomfort in a MRI environment, the enclosed space of the scanner can potentially feel uncomfortable, especially for more elderly participants. Discomfort from lying still for a long period of time will be minimised with comfortable padding and positioning. People with a history of claustrophobia would be excluded from participation in the study. Participants will be given a chance to see the scanner before the study starts. Whilst in the scanner, participants are able to use the alarm button or can squeeze a bulb placed in their hands if they wish to communicate with the operator or to interrupt the scanning at any stage of the scanning process. As the MRI scanner is noisy, participants would be given ear-plugs and/or acoustically shielded headphones (3T and 7T) or earplugs optionally (1.5T) to minimize the noise and aid communication between participants and investigators.

Participants will be asked to wear ”pyjama-style” top and trousers for the MRI scans that preserves their modesty while remaining loose in the scanner to avoid potential burns from synthetic clothing. To help maintain participant dignity they will be asked to leave their underwear on, so long as it has no metal parts (e.g. zips, bras clasps or studs). Participants will be asked to change in a changing room near to the scanner and will be given a locker to securely store their belongings. If they are unable to change into the outfit by themselves a member of staff will be on hand to offer assistance.

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing is very safe and is even used in people with heart failure. However, as with all forms of hard exercise, very occasionally some people have significant changes in their heart rate and rhythm that requires medical attention. Although the risk of this happening is small, the test is carried out in a room equipped with emergency monitoring, emergency medications and resuscitation equipment. Medical personnel will be available throughout the testing. Participants' heart rate and electrocardiography (ECG) are monitored before and during the exercise testing and the exercise test will be stopped if there are any concerns.

Ear Imaging

The auricle imaging device has not been widely used or tested before, but no adverse reactions and side effects have occurred so far or would be expected.

NHS Digital Follow-Up

Participants will be asked to consent to NHS Digital follow for up to 15 years after the study visit. There is no requirement for participants to consent to take part in this longer-term follow up and if they decide to take part, they are free to withdraw consent at any time without giving a reason

If the participant decides that they do not want their study data to be linked with the NHS Digital follow up then they can withdraw without affecting their current medical care. They can do this by contacting the study team, who will require the participant's identifiers, data (namely participant's NHS number, date of birth and post code), then inform NHS Digital that they no longer wish to be part of the cohort. NHS Digital will then remove their identifiers from the study.

Through consenting to NHS Digital follow up, participants will provide permission for the study investigators at the University of Oxford to access their medical records and to store them on a secure database linking their personal details, clinical notes and research findings. The database will be stored on an access-restricted computer, encrypted and located on the secure University of Oxford High Compliance system, a service for University of Oxford Clinical Trials Units (CTUs) and Medical Sciences Division Departments that securely access applications and store sensitive data. In order to keep research records up to date throughout the duration of the study we will send identifying information including unique Study ID numbers, NHS numbers, date of birth and postcodes to NHS Digital. This will allow them to link up with patient files and send back information such as hospital episode Statistics, admitted patient care, accident, and emergency information, as well as civil registry mortality data.

As University of Oxford researchers, we are responsible for looking after the participants' information and using it properly. Thus, the minimum possible personally identifiable information will be used. The University of Oxford will keep identifiable information for up to one year after the study (including the follow-up) has finished. This excludes research documents with personal information, such as consent forms, which will be held for 5 years after the end of the study.

The data that we receive and analyse from NHS Digital will be identified by a trial number only, and will not be identified by name, date of birth, NHS number or address. With the trial number, we will link to the original study database, and information collected during the earlier trial visits. The information received from NHS Digital will be imported into a database held securely by the University of Oxford and used solely for academic research purposes. Before analysing this complete dataset (including information already provided by trial participants with information from NHS Digital) personal identifiers will be removed.

Importantly, whilst the information received is specific to each trial participant, no individual person will be identifiable in any publication arising from this work. None of the study data, including any participant personal data, will be transferred, or shared with any third parties,

third countries or international organisations. No data will be used for automated decision making.

Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

Transparency in Research

Prior to the recruitment of the first participant, the study will have been registered on a publicly accessible database, clinicaltrials.gov. The HRA also publish research summaries at https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/

Participant Confidentiality

The study will comply with the UK General Data Protection Regulation (UK GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s), with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

Expenses and Benefits

Reasonable travel expenses for any visits will be reimbursed on production of receipts, or a mileage allowance provided as appropriate. For each visit, participants will be provided with a small snack and £30 to thank them for their time.

FINANCE AND INSURANCE

Funding

The study funding is provided by the Medical Research Council.

Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the Medical Research Council. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

20. ARCHIVING

During the study electronic data will be held on secure network drives/hard disks/servers on password protected computers within locked offices. Backup copies of files will be made weekly and stored on a different secure server/external hard drive. These back up locations will be subject to the same security principles as the primary locations. When datasets are complete, the primary copy will remain at the study site where it will be transferred onto optical media, e.g. DVD/external drive and undergo archiving for hard copy data. Any copies leaving the study site will be completely de-identified and require authorisation to do so by the PI.

At the end of the study, electronic files containing the anonymised data will be transferred to a hard drive and will be stored securely in commercial archiving facilities sub-contracted by the Division of Cardiovascular Medicine, University of Oxford to provide an archiving service. The data will be stored for 15 years and then securely destroyed.

21. REFERENCES

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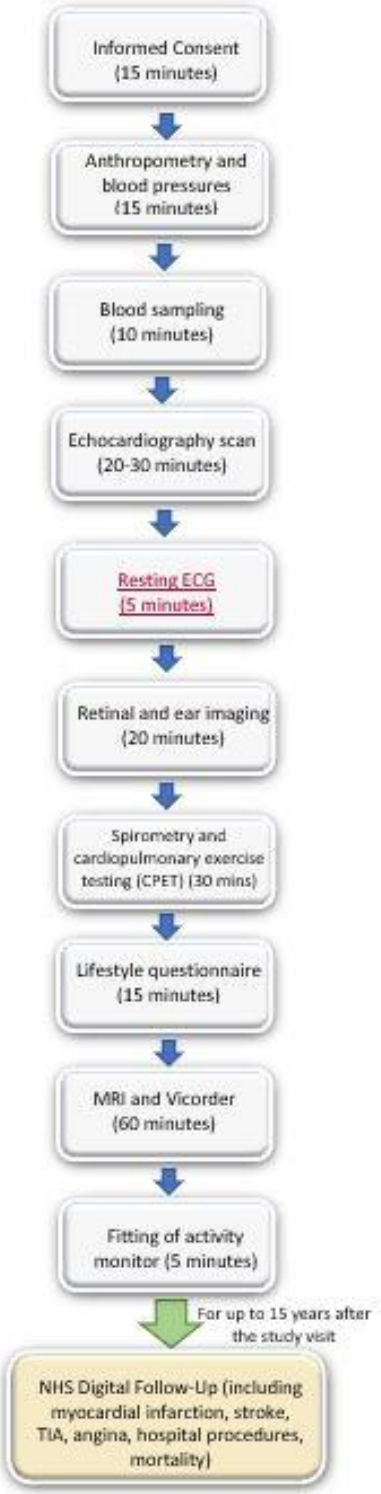
22.APPENDIX A: STUDY FLOW CHART



23. APPENDIX B: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
Non-substantial amendment 01	1.0	5 April 2023	Ronja Bahadori	To inform the HRA that we would now like to use an OID for the contractual arrangements with OUH.
Substantial amendment 01	2.0	13 June 2023	K. Suriano	To change the wording in the protocol describing the cardiopulmonary exercise testing methodology.
Substantial amendment 02	3.0	20 November 2023	K. Suriano	1. Update to the section 9.1.1 of the study protocol to allow research team to contact participants by phone call to ensure research team reaches the recruitment targets. 2. Update to the invitation letter to make it clearer to participants that both normotensive and hypertensive groups are being contacted. The following statement is added: 'As part of the PVS study you may have been part of the normotensive or hypertensive pregnancy cohort'. Furthermore, if there is no response to the initial invite after 4 weeks, the PVS team will contact the participant by a phone call to confirm receipt of documents (this is in line with the update to the protocol to make sure recruitment target is reached). 3. Update to the study questionnaire to allow to ask participants whether they have gone through menopause and what age they went through menopause. We have also added some questions about e-cigarettes to the series of questions about smoking. This information will be used as part of our final statistical analysis for the study. 4. Update the protocol and PIL to add in detail regarding resting ECG as well as echocardiography measures during the CPET.

Substantial Amendment 03	4.0	26.06.2024	K.Suriano	Addition of the option to reconsent participants electronically

C

HELPFUL STUDY INVITATION LETTER

DIVISION OF CARDIOVASCULAR MEDICINE,
RADCLIFFE DEPARTMENT OF MEDICINE

Invitation Letter - The HELPFUL Study

Hypertension **E**xplored in **L**ong-term **P**ost-partum **F**ollow-**U**p in **L**ater Life (HELPFUL)

<Name>

<Address>

Previously you kindly helped the Division of Cardiovascular Medicine at the University of Oxford through participating in our Pregnancy Complications Vascular Study (PVS) and agreed to be approached about other research. This study explored the effects of raised blood pressure (hypertension) during pregnancy and early delivery on the heart and vasculature. **As part of the PVS study you may have been part of the normotensive or hypertensive pregnancy cohort.**

We are now undertaking a follow-up study called HELPFUL to understand the pattern of changes that occur across the body in women who have had hypertensive pregnancies over a longer time period. Therefore we would like to ask for your help again. Through completing this study, we can learn more about how hypertension and cardiovascular disease develop over a long period of time. This will allow us to understand why these diseases develop in certain groups of women and therefore open doors to better interventions and therapies tailored towards individuals.

The project will involve methods similar to those used previously in our PVS study, including ultrasound imaging, magnetic resonance imaging, retinal and ear imaging, cardiopulmonary exercise testing and blood pressure monitoring. These methods do not involve x-rays, or any harmful radiation. A Participant Information Leaflet is included with this letter, which will give you more information about the HELPFUL study.

If you would like to know more about this study, please fill in the form and return it in the stamped addressed envelope provided. Alternatively, you can contact the research team in Oxford directly by emailing HELPFUL@cardiov.ox.ac.uk or by telephoning on 01865 572845. If you would like to know more about the study, then we will contact you within the next few weeks. If there is no response to the

initial invite after 4 weeks, we will contact you by a phone call to confirm receipt of documents.

We look forward to talking to you and to answering any questions that you may have. All information will be taken in the strictest confidence. If you take part, we will refund all travel expenses and we will provide a small gift token to thank you for your time and inconvenience.

Thank you for taking the time to read this letter.

This letter may be sent to you twice. If this is the second time you've received this letter and do not wish to respond, please disregard it.

Yours sincerely,

Professor Paul Leeson

Professor of Cardiovascular Medicine and C

onsultant Cardiologist

Oxford Cardiovascular Clinical Research Facility

Division of Cardiovascular Medicine, Radcliffe Department of Medicine

University of Oxford

D

HELPFUL PARTICIPANT INFORMATION LEAFLET

DIVISION OF CARDIOVASCULAR MEDICINE,
RADCLIFFE DEPARTMENT OF MEDICINE

Tel: +44(0)1865 572845

Email:paul.leeson@cardiov.ox.ac.uk HELPFUL@cardiov.ox.ac.uk

Hypertension **E**xplored in **L**ong-term **P**ost-partum **F**ollow-**U**p in **L**ater Life (**HELPFUL**)

Participant Information Leaflet

We are asking whether you would like to take part in a research study at the John Radcliffe (JR) Hospital in Oxford. Before you decide if you would like to join the study, it is important that you understand what the study is about, why the study is being done, and what it will mean if you decide to take part. Please take the time to read the following information carefully and to discuss it with friends, relatives, and your GP if you wish. If there is anything that is not clear, or if you would like more information, please ask us.

What is the purpose of the study?

Women who have hypertensive (high blood pressure) pregnancies are at increased risk of cardiovascular disease in later life compared to women who have no complications during pregnancy. The exact cause and way in which cardiovascular disease develops after a hypertensive pregnancy is not completely understood. Therefore, this study will use new imaging techniques of your heart and brain to learn more about the pattern of changes that occur across the body during later life. The data that we collect will help us to learn more about why hypertension and cardiovascular disease develop in certain groups of women. This will then allow better interventions and therapies to be created that are tailored uniquely towards individuals.

Why have I been invited?

We are hoping to recruit 200 participants to help us with this study, of which 100 will have experienced a hypertensive pregnancy and 100 will have experienced a normotensive (normal

blood pressure) pregnancy. You may have previously participated in a study at the University of Oxford and agreed to be approached about further research, or you might have been identified as appropriate to participate in this study from your John Radcliffe Hospital maternity records that have been reviewed by the clinical team.

Do I have to take part?

No, you do not. 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 will still retain any data already obtained from you unless you request otherwise.

What does taking part in the study involve?

Once we have checked that you are happy to take part, we will invite you to the John Radcliffe Hospital in Oxford. There is ONE study visit as part of the HELPFUL study which will last up to 4 hours. Please note that it is possible to split the visit over different days if needed or preferred by you. There are no follow-up visits planned at this time. No specific treatments are given and participation in the study does not change your medical care. At the end of the study visit, we will give you an activity monitor (similar in design to a wrist-worn watch like a “Fitbit”) which measures your activity. You will be asked to wear this for 7 days, 24 hours per day. You can shower with it on. Stamped addressed envelopes will be provided to return devices.

As part of this study, outcome measures will include prior measures of the heart, brain, vascular system and other clinical measures collected at previous study visits within the Pre-eclampsia Vascular Study (PVS), and clinical measures of disease severity and pregnancy characteristics collected from medical records.

As an optional part of your participation in the study, in addition to the information you provide about your health or is obtained from existing records, the research team in Oxford will ask for

information about your health from NHS Digital (including, but not limited to, NHS Digital and the Office of National Statistics) for the next 15 years. NHS Digital is the national information and technology partner to the health and social care system in the UK. We will send identifying information about you such as your name, date of birth, NHS number and postcode to NHS Digital (or other central NHS bodies) who can link this information to your centrally held records. NHS Digital provides (on behalf of the Office of National Statistics) information about study participants such as hospital admissions or deaths including the date and cause of death.

A summary of the study measures is outlined below, with more detail for each of the measures on the following page:

STUDY TIMELINE

Total time is approximately 3 - 4 hours

	Study Measure (see below for details of each measure)	**Approx. time (minutes)**
STUDY VISIT	Informed Consent	15
	Blood pressure and anthropometry measurements	15
	Blood sampling	10
	Echocardiography scan of the heart	30
	Resting electrocardiogram (ECG)	5
	Retinal imaging	10
	Ear imaging	10

Spirometry and cardiopulmonary exercise testing	30
Lifestyle questionnaire	15
Magnetic Resonance Imaging (MRI) and Vicorder	60
Fitting activity monitor	5
Total time for study measures	205

To achieve the most accurate assessment results, **we must ask you not to have any food or snacks at all for six hours before you come in for your study visit. You can drink water and we'll have snacks for you during your visit.** You can have breaks whenever you want throughout the visit.

We also ask that you wear loose clothing (e.g. gym shorts and a t-shirt) and gym shoes to make the study measures easier. Wired bras will not be suitable for some of the assessments. There are changing areas in our facilities if you would like to change into gym clothes during the study visit.

Study Measures:

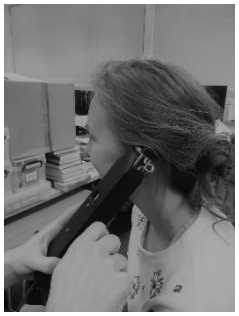
1. *Informed consent*: After confirming that you have understood what is involved, we will ask you to sign the consent form (15 minutes).
2. *Anthropometry:* We will measure how tall you are and how much you weigh, and we will take some measurements of your waist, hips and arms (5 minutes).
3. *Blood Pressure:* While you sit quietly, we will take five resting blood pressure measurements (10 minutes).
4. *Blood sampling:* The equivalent of 5 teaspoons (25 mls) of blood will be collected by a trained member of staff (10 minutes).

5. *Echocardiogram scan (Ultrasound of the heart):* We will do an ultrasound scan of your heart to assess structure and function. 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 transducer (a small microphone-like device) 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. to acquire these images. If you prefer someone of the same sex to perform this procedure, please let us know (30 minutes).

6. *Resting electrocardiogram (ECG):* ECG sensors will be attached to you so we can measure heart rate and rhythm (5 minutes).

7. *Retinal imaging:* Retinal photography will be completed using a digital camera and imaging software following an established protocol. This means we will take a digital photograph of the back of your eye and produce coloured images the blood vessels in the back of your eye. We won't need to put any drops in your eye and contact lenses wearers won't need to remove them (10 minutes).

8. *Ear imaging:* Ear imaging will be completed using the VITA (Vascular Imaging Tool for the Auricle). Imaging is non-invasive. The device will be clipped onto the ear and digital images of the light transmitted through the ear will be taken when the device is in place. A polarisation lens may be inserted in the VITA prototype between images (10 minutes).



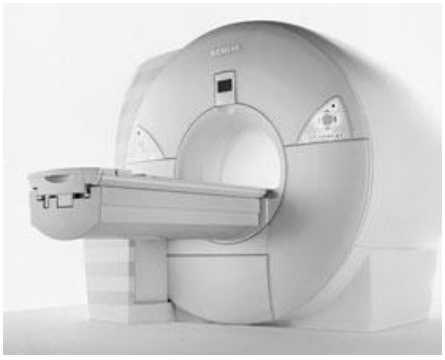
9. *Spirometry:* Spirometry is a way we test lung function. We will measure the amount of air you can breathe out from your lungs and how fast you can blow it out. You'll be asked to take a very deep breath and blow out as fast as you can into a mouthpiece, until no more air comes out. During the test, we will ask you to wear a soft nose clip to prevent air escaping through your nose (10 minutes).

10. *Cardiopulmonary Exercise Testing (CPET):* Exercise testing will be performed on a stationary bike where we will ask you to cycle against increasing resistance to assess

your peak exercise capacity. The test will last 10 to 15 minutes. During the exercise test, a blood pressure cuff and ECG sensors will be attached to you so we can measure blood pressure and heart rate measurements before, during and after the test. An ultrasound scan of your heart will also be taken during the exercise test. After reaching your maximum effort, a recovery period consisting of pedalling on the bike at a low resistance will be conducted for five minutes to allow you to recover. CPET measures will be continued during this time. The exercise protocol we use is well recognised and used in clinical and research practice (30 minutes).

11. *Lifestyle Questionnaire:* You will also be asked to fill in a validated lifestyle and medical history questionnaire during your visit (15 minutes). The questionnaire asks for details on things such as your early life feeding, and family medical history. We will email or post the study questionnaire to you prior to the study visit to allow you to check some of the questions about your family history, and in particular, your birth history. You can use this time to gather the information needed to answer the questions, which will then be completed as part of the study visit.

12. *Magnetic Resonance Imaging (MRI):* As part of this study, you will have an MRI scan of your heart and brain. The MRI scanner is shaped like a polo mint, with 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 may be asked to breathe in and out and hold your breath for several seconds for some of the scans. MRI is safe and non-invasive and does not involve any ionising radiation (x-rays). However, because MRI scanners use a large magnet to work, MRI scans are not suitable for everybody. Because of this, you will be asked a pre-screening safety questions to help determine if you are able to take part. For example, if you suffer from claustrophobia, you could not be scanned. If you are unsuitable for MRI based on the pre-screening you may still be included in other parts of the study.



Normally, MRI scanning for research purposes would not be performed without further investigation if you have a heart pacemaker, mechanical heart valve, mechanical implant such as an aneurysm clip, hip replacement, or if you carry other pieces of metal that have accidentally entered your body. While there is no evidence to suggest that MRI is harmful to unborn babies, as a precaution, the Department of Health advises against scanning pregnant women unless there is a clinical benefit. We do not test for pregnancy as routine so if you think you may be pregnant you should not take part in this study.

As some of the scans are noisy, we will give you earplugs, or headphones to make this quieter for you. It is important that these are fitted correctly as they are designed to protect your ears. In preparation for your scan and for your comfort and safety we may ask you to change into scrubs. You may keep your underwear and socks on, but we would ask ladies to remove their bras. Metal jewellery, including body piercings, must also be removed and if you have any tattoos, you will be asked about them in the pre-screening safety questions because some types contain materials that can interact with the magnetic field. Lockers are provided to secure your personal belongings and clothing (60 minutes).

During the MRI, measures of peripheral and central blood pressures will be measured using a non-invasive cuff-based device called a Vicorder®. (Central BP is the pressure in the aorta, which is the large artery into which the heart pumps.)

13. *Activity monitoring:* At the end of the study visit, we will spend 5 minutes fitting an activity monitor to your wrist. This wrist-worn monitor is similar in design to a wrist-worn watch like a “Fitbit” and measures your activity. You will be asked to wear this for 7

days, 24 hours per day. You can shower with it on. Stamped addressed envelopes will be provided to return devices.

Outside of study visit measures:

As discussed above, following the visit, you will be asked to wear the wrist-worn activity monitor for 7 days, 24 hours per day. You can shower with it on. Stamped addressed envelopes will be provided to return devices.

As part of this study, outcome measures will include prior measures of the heart, brain, vascular system and other clinical measures collected at previous study visits within the Pre-eclampsia Vascular Study (PVS), and clinical measures of disease severity and pregnancy characteristics collected from medical records.

You will also be invited to take part in a longer-term follow-up as part of the study. This part of the study does not involve any study visits or further contact with the research team. Instead, we will follow up your health outcome data for up to 15 years using NHS Digital. This longer-term follow-up is optional, and if you consent to take part in this part of the study, you are free to withdraw consent at any time without giving a reason.

What should I consider?

You will not be able to take part in this study if the chief investigator thinks your taking part would be unsafe for you or if ANY of the following study exclusion criteria apply:

- You are pregnant or lactating, or it is less than six months since you have given birth
- Any significant disease or disorder which, in the opinion of the investigator, might influence your ability to participate in the study
- Evidence of congenital heart disease or significant chronic disease relevant to cardiovascular or metabolic status
- You are planning on donating blood within two weeks of attending the study visit

Should you have any concerns, however, please discuss this with the study team.

Are there any possible disadvantages or risks from taking part?

As with any medical procedure or medication, there are some minor risks that are described below. Throughout the study visit, an experienced study investigator will be with you and will address appropriately any issues that may arise.

Blood samples: You may experience some bruising and discomfort at the site where you have your blood taken. Our staff are highly trained in blood taking and we will make sure you are as comfortable as possible.

MRI scan: Magnetic resonance imaging is very safe and there are no known significant side effects from the types of scanner that we use. The scan involves lying flat in a slightly confined space and a small number of people may find this too claustrophobic.

Echocardiogram: There is no risk of physical harm, however there may be a risk to personal discomfort or embarrassment to you. Our echocardiographers are highly experienced and will make sure to respect your modesty and dignity by providing gowns or scrubs.

Cardiopulmonary Exercise Testing: If there are any concerns regarding your safety during the exercise testing, you will be asked to stop. Study investigators are trained, at minimum, in Basic Life Support and doctors who are Advanced Life Support trained will be available during CPET testing. The site is fully equipped for resuscitation (including defibrillation) in the unlikely event of a medical emergency during the test.

Ear Imaging: This new imaging device is a research tool developed in Oxford and therefore has not been tested to UK safety standards. All components of the VITA device are produced commercially and are compliant with UK and EU safety standards checks. The ear is exposed to a simple backlight with an intensity less than standard LED bicycle lights. The device runs at safe low voltages (<12V).

What are the possible benefits of taking part?

There may be no direct benefit to you but your involvement in the study will aid in the understanding of cardiovascular changes of women with hypertensive pregnancies and may help provide tailored interventions and therapies to individuals in the future.

Will my General Practitioner/ family doctor (GP) be informed of my participation?

Your GP will not be informed of your participation, but may be contacted to follow up incidental findings that may be of clinical significance, such as high blood pressure or Echo or MR imaging findings.

Will my taking part in the study be kept confidential?

Yes, any information that is collected about you during the course of the research will be kept strictly confidential. We will use a participant code instead of your name. All documents will be stored securely and will only be accessible by study staff and authorised personnel.

Responsible members of the University of Oxford and the Oxford University Hospitals NHS Foundation Trust may be given access to data for monitoring and/or audit of the study to ensure that the research is complying with applicable regulations.

Will I be reimbursed if I take part?

We will be happy to cover reasonable travel expenses for the study visit. We will also provide you with a £30 voucher to thank you for your time.

What will happen to my data?

Data protection regulation requires that we state the legal basis for processing information about you. In the case of research, this is 'a task in the public interest.' The University of Oxford is the sponsor for this study, based in the United Kingdom, is the data controller and is responsible for looking after your information and using it properly.

We will be using information from you and your medical records, NHS Digital and other central NHS registries in order to undertake this study and will use the minimum personally-identifiable

information possible. We will keep identifiable information about you for 6-12 months after the study has finished. This excludes any research documents with personal information, such as consent forms, which will be held securely at the University of Oxford for 15 years after the end of the study.

As part of this study, outcome measures will include prior measures of the heart, brain, vascular system and other clinical measures collected at previous study visits within the Pre-eclampsia Vascular Study (PVS), and clinical measures of disease severity and pregnancy characteristics collected from medical records.

As an optional part of your participation in the study, in addition to the information you provide about your health, the research team in Oxford will ask for information about your health from NHS Digital (including, but not limited to, NHS Digital and the Office of National Statistics). NHS Digital is the national information and technology partner to the health and social care system in the UK. We will send identifying information about you such as your name, date of birth, NHS number and postcode to NHS Digital (or other central NHS bodies) who can link this information to your centrally held records. NHS Digital provides (on behalf of the Office of National Statistics) information about study participants such as hospital admissions or deaths including the date and cause of death. Only anonymised study data may be shared between the University of Oxford and collaborators. No personal identifiable information will be shared with any other organisations other than those listed here.

If you have been approached following clinical review of notes, the local NHS Trust will use your name and contact details, to contact you about the research study, and to oversee the quality of the study. They will keep identifiable information about you from this study according to local policy for retention of medical records.

Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Further information about your rights with respect to your personal data is available at https://compliance.web.ox.ac.uk/individual-rights

Should it be necessary to reconsent, then a third party would be involved in the processing of your data. Microsoft forms is trusted by the University for confidential information, so will be used for the purpose of obtaining reconsent.

You can find out more about how we use your information by contacting study investigators at HELPFUL@cardiov.ox.ac.uk.

What will happen to the samples I give?

Samples will be used to measure such things as blood sugar levels and lipid profiles. All samples will be retained securely for analysis and will be stored with a barcode number at the University of Oxford, under the custodianship of the Principal Investigator, in the Division of Cardiovascular Medicine. If any sample remains it will be used either by local researchers or in ethically approved research projects worldwide. If however you don't agree for your samples to be used in future research, your remaining biological samples will be disposed of at the end of the study in accordance with the Human Tissue Authority Code of Practice.

What will happen to the images and data I give?

Images and data collected will be analysed for this study and then with your consent will be retained securely for future analysis and stored pseudo-anonymously at the John Radcliffe Hospital and University of Oxford under the custodianship of the Department of Cardiovascular Medicine in secure facilities. If you agree, images and data may also be used in future research, here or abroad, which has ethics approval, and this may include research by commercial organisations All data will be identified by a code number and your identity will remain unknown. If you withdraw from the study for any reason, we will retain any data collected up to that point for use in research as detailed in this participant information sheet.

What will happen if I don't want to carry on with the study?

Study participation is voluntary and you may change your minds at a later stage. Withdrawal will not affect in any way any future clinical care you may receive.

Should you wish to withdraw, please let us know if we can keep the information we have collected about you so far. We may be unable to destroy the data if it has already been de-identified, as outlined in the confidentiality section. Data already collected would not be used in the final study analysis except where analysis of your data has already been integrated into interim results.

What will happen to the results of this study?

We hope that the results will be published in scientific journals and/or presented in scientific or other meetings for the benefit of the wider medical community. However, you will not be identified in any publication or presentation and your personal and clinical details will remain strictly confidential. Summarised results will be published in scientific journals and also summarised on our website, after completion of the study, for you to read: https://www.rdm.ox.ac.uk/about/our-clinical-facilities-and-mrc-units/cardiovascular-clinical-research-facility/ongoing-clinical-studies. You may also wish to follow us on Instagram and Twitter (@OxfordCCRF), where we post regular updates on our research activity.

What if we find something unexpected?

It is important to note that we do not carry out any of these assessments for diagnostic purposes, and therefore they are not a substitute for a clinical appointment. Rather, our study assessments are intended for research purposes only. However, in the unlikely event a researcher notices a potential abnormality in any of your study assessments, it will be discussed with a designated clinical specialist, who will determine its clinical significance. You will only be

informed if a doctor thinks it is medically important such that the finding has clear implications for your current or future health.

What if there is a problem?

The University of Oxford, as Sponsor, has appropriate insurance in place in the unlikely event that you suffer any harm as a direct consequence of their participation in the study. 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 (Tel: 01865 226845, email: paul.leeson@cardiov.ox.ac.uk) or you may contact the Research Governance, Ethics & Assurance Team at the University of Oxford on 01865 616480, or the director of RGEA: ctrg@admin.ox.ac.uk.

How have patients and the public been involved in this study?

The Oxford Cardiovascular Clinical Research Facility holds regular focus groups to gather feedback from previous study participants. The participant documents and study procedures have been prepared based on comments and input from previous study participants. We hope their feedback helps make HELPFUL a good project for the advancement of scientific knowledge while being a positive experience for our participants.

Who is organising and funding the study?

The study is being organised by the Division of Cardiovascular Medicine at the University of Oxford. The study is being paid for by the Medical Research Council.

Who has reviewed the study?

All research is looked at by an independent group of people, called a Research Ethics Committee to protect participants' interests. This study has been reviewed and given favourable opinion by London-Surrey Research Ethics Committee.

Participation in Future Research

We may ask if we can contact you about future studies. This is optional, 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 on a password protected computer on the Medical Sciences Division IT High Compliance system in the Division of Cardiovascular Medicine. You can withdraw your consent for future contact at any time.

Who should I contact if I have further questions?

Please contact the study team at HELPFUL@cardiov.ox.ac.uk or 01865 226845 if you have any questions. You may also wish to speak with the study Principal Investigator, Prof Paul Leeson, who can be contacted by telephone: 01865 226845 or email: paul.leeson@cardiov.ox.ac.uk.

THANK YOU FOR READING THIS INFORMATION.