PRECEPT STUDY

A longitudinal cohort study of maternal cardiovascular and biomarker changes in fetal growth restriction and pre-eclampsia

Dr Jasmine Wan Ying Tay

Department of Metabolism, Digestion and Reproduction

PhD Thesis

Supervisors: Prof Christoph Lees

Prof Philip Bennett

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Declaration of originality

I declare that this thesis is my own work. Where other people’s work has been used, this has been appropriately referenced.

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Chapter 1 Abstract

Pre eclampsia (PE) and fetal growth restriction (FGR) are perceived as placenta mediated disorders. However, large epidemiological studies has shown that women diagnosed with PE have significantly higher risk of cardiovascular disease in later life. Hence, the pathophysiology of PE and FGR might be more closely linked to the maternal heart than previously thought.

Maladaptation to haemodynamic changes in pregnancy could herald the manifestation of PE and FGR. Traditionally, PE is classified into “early”, onset prior to 34 weeks gestation; and “late” onset after 34 weeks. This arbitrary classification was decided because early PE usually co-exist with FGR and therefore clinically distinct from late PE.

Women across gestation (24-40 weeks) with PE, FGR,PEFGR and normal outcomes were recruited. At each visit, maternal cardiovascular and arterial function was studied, ultrasound examination performed and serum/urine collected. Those with PE and/or FGR participated in an exercise step test.

In PE, there was increased cardiac output (CO) and lower peripheral vascular resistance (PVR). Conversely, pregnancies with FGR, regardless of whether PE co-exists, showed increased PVR, independent of gestational age. Arterial function was abnormal in all pathological cases.

Low maternal CO and high maternal PVR are associated with raised impedance in the maternal uterine and fetal umbilical arteries.

Metabonomic characterisation and BAFF /PAF immunological marker support the distinction of PE from PEFGR.

In conclusion, this study has established distinct cardiovascular haemodynamic and serum profile in normal, PE and FGR pregnancies, irrespective of gestational age. Maternal cardiovascular profile is associated with utero-placental and fetal Doppler changes. These findings have implications on clinical choice of antihypertensives in pregnancy. It also raises the possibility of improving fetal outcomes via manipulation of maternal cardiac function pharmacologically.

There were no obvious differences in cardiovascular adaptation neither in relation to exercise nor postnatally between the groups.
Chapter 2  Introduction

Papers related to this chapter

2.1 Background of Study

Pre-eclampsia (PE) is a disorder characterised by high blood pressure and proteinuria that occurs in pregnancy, affecting the mother, the baby or both. Globally, PE affects 5-8% of pregnancies and remains one of the leading causes of maternal and perinatal mortality and morbidity worldwide. Traditionally, PE and FGR have been perceived as placenta mediated disorders. Delivery of the fetus and placenta is thought to “cure” these pregnancy specific conditions. PE is classically associated with the following risk factors – nulliparity, advanced maternal age, BMI >35, chronic hypertension, PE in previous pregnancy, multiple pregnancy and pre-pregnancy medical conditions such as diabetes, antiphospholipid syndrome, chronic kidney disease, systemic lupus erythematosus.

![Figure 2-1 Schematic diagram of placentation and feto-maternal circulation](image-url)
The underlying pathophysiology of PE has never been fully understood, but inadequate trophoblast invasion leading to uteroplacental mal-perfusion is thought to underlie both PE and FGR (3, 4). This is supported by histological work undertaken by Lyall et al which reported major defects in myometrial spiral artery remodelling in preeclampsia and FGR that is linked to clinical manifestation of pathological outcomes in pregnancy (5).

**Figure 2-2 Spiral artery remodelling**

Successful trophoblast invasion and remodelling of the spiral artery in normal pregnancy. Lack of trophoblast invasion and spiral artery remodelling is seen in preeclampsia. Figure reproduced from Maynard with permission (6).
A systematic review and meta-analysis by NICE, suggests that PE is associated with increased risk of future maternal cardiovascular disease (CVD); including hypertension (x4), ischaemic heart disease (x2), vascular dementia and stroke. A variety of adverse effects have been reported in children born to women with PE, including elevated blood pressure (BP), vascular dysfunction, and increased stroke risk.

Recent work by Foo et al showed that women who subsequently developed preeclampsia/fetal growth restriction had lower cardiac output and high peripheral vascular resistance when assessed pre-conception (7). This raises the possibility that some women are predisposed to PE and or FGR due to pre-existing cardiovascular profile, even before conception and development of a functioning utero-placental unit (8).

There is now increasing support from epidemiological observations, clinical trials and biological studies that describe two different aetiologies of PE, subsequently manifesting as two separate phenotypes (9, 10). This is divided into an early-onset phenotype which is associated with poor placentation and fetal growth restriction, and a late-onset phenotype, which is not thought to be related to placental causes.

In general, early-onset PE (defined as PE diagnosed before 34 weeks gestation) represents approximately 20% of all cases of PE, including the most severe cases (11). This form is linked to immune and placental mal-adaptation and is characterised by early sympathetic dominance in the cardiovascular system, elevated circulating markers of endothelial dysfunction, inadequate trophoblast invasion of the uterine spiral arteries, and early onset of fetal complications such as iatrogenic pre-term birth, and low birth weights (12-14). In large
screening models, uterine artery Doppler has good positive predictive value in screening for early onset PE (15).

In contrast, late-onset PE (defined as PE diagnosed after 34 weeks gestation) is the most common, encompassing more than 80% of all cases (11), and is commonly observed on the background of pre-existing maternal morbidities such as chronic hypertension, renal disease and obesity. Significantly, fetuses born of a late-onset PE pregnancy have a slightly higher birth weight and increased placental mass compared to those with early-onset PET (16).

Interestingly, the two phenotypes can also be distinguished based on maternal cardiovascular and haemodynamic status. It is increasingly evident that maternal cardiovascular status plays a crucial role in the aetiology of PE and/or FGR; investigative and therapeutic approaches of which will be beneficial in cases where defective placentation is not a sole causative factor (17). Of potential significance, some haemodynamic changes are detectable several weeks prior to the onset of symptoms, and thus could have wide-reaching applications in clinical practice with regards to risk stratification, targeted antenatal care, and preventative therapies.

2.1.1 Cardiac Output in normal and pathological pregnancy outcome

In order to understand how cardiac output evolves in pregnancies with pathological outcomes, we first need to ascertain normal ranges at different gestations of pregnancy. A recent meta-analysis of cardiac output in normal pregnancy illustrated normal ranges at different gestations bands (18). In general, cardiac output (CO) values are significantly increased at 6 weeks gestation from pre-pregnancy, with a simultaneous decrease in systemic
vascular resistance, the latter of which drives down MAP. The CO rise plateaus in the second and third trimester with values approaching 40% more compared to pre-pregnancy levels. The rise consists of 20% increase in stroke volume and heart rate, as well as increasing left ventricular mass.

Two distinct conditions can be characterised based on maternal cardiovascular status at the time of diagnosis of PE and/or FGR (19). Early onset PE is frequently associated with a high peripheral resistance and low cardiac output state. In contrast, late onset PE with smaller numbers of associated FGR is usually associated with low peripheral resistance and high cardiac output. This separation was an arbitrary one, and was first described in relation to the ability of uterine artery Doppler to identify early PE but its relatively poor sensitivity for late PE (20). Interestingly, the most comprehensive and recent systematic review concluded that studies of cardiovascular function in gestational hypertension and PE show conflicting results (21).

Of clinical importance, if high and low CO forms of PE do exist—the former with low and the latter with high systemic vascular resistance and corresponding high and low intravascular volume states—then treatment that is contra indicated in one form may be therapeutically appropriate in the other: for example beta blockers, calcium channel blockers and even diuretics. Currently management is based on a ‘one size fits all’ philosophy, with the NICE hypertension in pregnancy guideline not considering the underlying cardiovascular parameters when recommending pharmacological treatment (22).

In FGR pregnancies (with or without PE), women have a lower cardiac index (a ratio of CO and body surface area) and higher peripheral resistance when compared to unaffected
pregnancies (23). This suggests defective maternal adaptation: a diminished response to increasing physiological demands of pregnancy.

2.1.2 Arterial Function in normal and pathological pregnancy outcome

Pulse wave velocity (PWV) and augmentation index (AIX) is widely used outside pregnancy as a surrogate measure of arterial stiffness and subsequently as a predictor of future cardiovascular disease (24-26). Parameters to assess localise and global arterial function are summarised in a consensus report from the European Network for Non-invasive Investigation of Large Arteries (27).

There is strong evidence that maternal vascular dysfunction is seen in pregnancies complicated by PE and/or FGR (28). Parameters used to assess arterial function include AIX and PWV; with endothelial function commonly measured using flow mediated dilatation in the upper arm.

In normal pregnancies, augmentation index widely decreases in the second trimester then gradually increases closer to term (29, 30). This then return to pre-pregnancy baseline in the postpartum period (31). Pulse wave velocity largely follow a similar trend (32).

A large systematic review of 23 studies evaluated the effect of preeclampsia on arterial stiffness (33). Women with preeclampsia had elevated arterial stiffness both during and after pregnancy. This effect was much greater in PE when compared to gestational hypertension (GH). Interestingly, clinically severity of preeclampsia was also positively associated with arterial stiffness (33). In studies performed as early as 11 weeks gestation, significantly higher levels of PWV and Alx have been observed in those women who subsequently developed PE later in their pregnancies (34, 35). Studies done where arterial assessment was combined with
other maternal variables such as central systolic blood pressure have raised the possibility for these indices to be considered as a screening test to diagnose early sub-clinical changes and predict subsequent development of early and late-onset pre-eclampsia (34, 36).

A relationship between PWV in the third trimester and birth weight in normal pregnancies was found, with an increase of 1 m/sec in PWV correlating with a decrease in birth weight centiles by 17.6% (37). No specific studies reported on arterial function in FGR. However, a study incorporating small for gestational age babies found no differences in PWV recorded in the first trimester (38). Aix was normal in women with normotensive SGA pregnancies; but was elevated in women who later presented with pre-eclampsia and SGA fetuses (39).

Arterial function impairment could in some ways explain the increased cardiovascular risk factor in later life for women with PE and or FGR in their pregnancies, as post partum, AIX and PWV remains elevated in some of these women (29).

Figure 2-3 Augmentation Index formula

Chart adapted from Enkhmaa (40)
2.1.3 Uterine Artery Doppler in relation to pregnancy outcome

Aside from central haemodynamic studies, it is also possible that maternal cardiovascular status is reflected by measurements of uterine artery Doppler impedance (41, 42). Uterine artery performed in the second trimester has long been established as a useful screening tool in the prediction of early PE, SGA, placental abruption and stillbirths associated with the above conditions (43). Whilst uterine artery Doppler on its own is a good screening tool for early onset PE; the addition of maternal clinical factors improved the overall prediction of late onset PE (44). When combined with maternal clinical factors, uterine artery Doppler performed at 23 weeks gestation gives a 67.5% detection rate of PE (45).

A high uterine artery resistance in mid-trimester has also been observed to be significantly associated with decreased birth weight (p<0.0001) In distinguishing the different phenotypes of PE, uterine artery resistance index >90th centile in early-onset PE, late-onset PE and controls were 63.6%, 15.5% and 8.8% respectively (46).

![Figure 2-4](image)

*Figure 2-4  A – Normal uterine artery Doppler waveform; B – Notching on uterine artery Doppler*
2.1.4 Fetal Doppler changes in relation to pregnancy outcome

The changes in the fetal circulation assessed by Doppler are classically ascribed to placental dysfunction and damage (3). Classically, increased impedance in the fetal umbilical artery and a reduction in cerebral impedance in the fetus is characterised by abnormal Doppler waveform detected non-invasively in ultrasound scanning. This could represent adaptation of the fetal circulation to a chronically hypoxic state (47, 48). The increase in vascular resistance within the umbilical arteries in compromised pregnancy may result from either structural changes or functional adaption within the umbilical-placental bed. This observed sustained increase in fetal peripheral vascular resistance leads to increased impedance of blood flow returning to the placenta, clinically diagnosed by increased umbilical artery Doppler pulsatility index (PI) in compromised pregnancy (47, 49). The most severe changes in the fetal circulation are defined by absence or reversal of umbilical artery end diastolic flow (EDF).

![Figure 2-5 Normal fetal umbilical artery Doppler waveform and PI](image-url)
Figure 2-6 Fetal umbilical artery Doppler with positive end diastolic flow but high pulsatility index

Figure 2-7 Fetal umbilical artery Doppler with absent end diastolic flow
Figure 2-8 Fetal umbilical artery Doppler with reversed end diastolic flow
2.1.5 Biomarkers in normal and pathological pregnancy outcomes

Biomarkers can be used for diagnosis of a disease, as a marker of risk profile for disease prevention, as a potential drug target, or as a potential measure of a drug response or to predict outcome (50). Maternal metabolic and biochemical profile undergoes large changes during pregnancy. Pre-eclampsia (PE) and fetal growth restriction (FGR) are frequently attributed to placenta mal-adaptation. This in turn leads to abnormal expression of biomarkers in pathological pregnancies.

Many candidate biomarkers exist in the prediction of PE. Plasma placental growth factor (PLGF) is a placental derived angiogenic factor that has been found to be abnormally low in women with PE when compared to gestation age matched cohort (14). It has also been shown to have a high sensitivity and good negative predictive value as a screening test for development of PE in the third trimester (51, 52). Other anti-angiogenic factors of note include sFlt-1, thought to be commonly produced by the “hypoxic placenta” in PE, leading to high levels in early onset PE (53). When used in conjunction, the sFlt-1/PLGF ratio appears to improve the diagnostic and predictive value in relation to patients at risk of PE and or FGR (54). An increased level of circulating soluble endoglin levels have been shown two to three months before the onset of PE (13). Placental endoglin is thought to be up-regulated in pathological pregnancies with PE, therefore releasing soluble endoglin into the maternal circulation (55).

Metabonomics is a study of low molecular weight molecules (metabolites) present in the metabonome of a cell, tissue or organism. Metabolites are the final downstream product of
gene expression. Metabolic dysfunction is thought to reflect or relate to cardiovascular status, both in terms of phenotypic overlap (for example in adult metabolic syndromes), and pre-clinical risks (women with pre-existing metabolic disease such diabetes or hyperlipidaemia have higher risk of PE).

The potential role of metabolite analysis in association with pathological pregnancy has not been truly explored. Metabolic dysfunction (such as high levels of triglycerides, low density lipoproteins and cholesterol, and essential hypertension) is thought to have significant associations between the risk of PE, and women’s pre-pregnancy cardiovascular risk (56).

Currently, studies have demonstrated differences in maternal plasma from time of, or just before the onset of PE. A 2-phase discovery/validation study utilising 120 cohort samples (SCOPE cohort) obtained at 14-16 weeks gestation identified 40 significantly elevated and 5 reduced plasma molecules in women who subsequently developed PE, as compared to matched unaffected controls (57, 58). Of note, samples from the first and third trimester were excluded from that study.
2.2 Hypothesis

We hypothesised that:

1. Early-onset PE in association with FGR, or FGR at any gestation is associated with a lower CO in relation to unaffected pregnancies of the same gestation.

2. Clinical deterioration is characterised by worsening in maternal cardiovascular characteristics.
2.3 Aims of Study

The study objectives are as follows:

1) To investigate difference in cardiac output (delta CO) between women with unaffected pregnancies compared to those who develop PE and/or have FGR babies at 4-weekly gestation bands.

2) To investigate Augmentation index (Alx), pulse wave velocity, peripheral vascular resistance and central blood pressure (from gestation of diagnosis till post-delivery) in relation to gestation at onset of PE and/or FGR at 4 different gestation bands.

3) To investigate the longitudinal changes in cardiac output (delta CO) from diagnosis to decision to deliver in women with PE and/or FGR in relation to gestation of disease onset.

4) To investigate the difference in cardiac output (delta CO) in response to exercise testing in those who have PE and/or FGR.

5) To investigate longitudinal differences in serum and urine metabolite profile and biomarker at diagnosis and at time of delivery between women with unaffected pregnancies compared to those who develop PE and/or have FGR babies at different gestations.

6) To compare measurements and reproducibility of cardiovascular parameters using different non-invasive techniques.
Chapter 3 Methods

3.1 Study Design

3.1.1 Subjects

The study population comprised of women booking at Queen Charlotte’s and Chelsea Hospital (QCCH) or referred for specialist opinion. Women were recruited via antenatal clinic, antenatal ward, day assessment unit, centre for fetal care and labour ward.

Participants who were not initially booked at Queen Charlotte’s & Chelsea Hospital were expected to have continuing care at QCCH via intrauterine transfer.

3.1.1.1 Inclusion Criteria

Pregnant woman regardless of parity aged 18-44 years from 24-40 weeks gestation in 4 groups:

- Normotensive – blood pressure < 140/90.

- PET – defined as new onset blood pressure > 140/90 and urinary protein : creatinine ratio >30mg/mmol.

- FGR – defined as abdominal circumference less than 10th centile and umbilical PI more than 95th centile on ultrasound scan.

- Combination of PE and FGR.
3.1.1.2 Exclusion Criteria

- Fetal abnormality.
- Multiple pregnancy.
- Chronic hypertension at booking.
- Known underlying maternal cardiac condition.
- Women who in the opinion of the researcher by virtue of language or learning impairment would be unable to give fully informed consent to the study.

3.1.2 Recruitment

Potential participants underwent a screening interview with the clinical research fellow to ensure they meet the inclusion and exclusion criteria. If eligible, a patient information sheet was provided to them, and they were invited for a first study visit. Participants were divided into the following groups by the following gestation bands.

<table>
<thead>
<tr>
<th>Gestation at recruitment (weeks)</th>
<th>PE</th>
<th>FGR</th>
<th>PE with FGR</th>
<th>Controls (normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 – 27+6</td>
<td></td>
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<tr>
<td>28 – 31+6</td>
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<td>32 – 35+6</td>
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<tr>
<td>36 – 40</td>
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</tr>
</tbody>
</table>

*Table 3-1 Gestational ‘bins’ of recruitment by groups (PE, FGR, PEFGR and normal)*
We aimed to recruit 160 women, of whom, in each gestation bracket, 25 will have normal ongoing pregnancies, 5 will have FGR only, PE only or FGR and PE. There were no previous published studies on which to base our sample size calculation. We have used empirical observations to guide our recruitment plan. Post hoc sample size calculations were undertaken after completion of recruitment.
3.2 Study Timeline

Study timeline:

- **Months 0-6**: Completion of project proposal and ethics application. Setting up data collection, maternal vascular lab equipment and ultrasound scanning suite.

- **Months 7-30**: Active study recruitment of control patients (some with longitudinal components) and patients with pathological pregnancy outcomes (longitudinal assessments done where delivery is not imminent).
Schematic diagram of control participants. If recruited in the second trimester, participants were invited back for further study visits if they consented.

**Study protocol - controls**

*Figure 3-1 Study protocol schematic for healthy controls*
Schematic diagram of pathological participants. After the first visit, the subsequent visit was timed to be as close to “decision to deliver” as possible. They were also invited back for postnatal reassessment.

Figure 3-2 Study protocol schematic for pathological pregnancy outcomes
3.3 Ethical Approval

This study has received favourable ethical opinion from NRES-Committee, London Riverside (REC Reference 15/LO/0341) and NHS R&D approval from the Imperial College Joint Research Compliance Office (15HH2516).
### 3.4 Study Visits

**First study visit:**

The study was based at Queen Charlotte's & Chelsea Hospital (Imperial College NHS Trust). The cardiovascular assessments and blood sampling were performed in the cardiovascular lab, whilst the ultrasound scans were performed in either the Centre for Fetal Care or the Research Ultrasound Suite, all of which are located in Queen Charlotte's & Chelsea Hospital.

At the first visit, the following was performed:

- Explained outline of study + what is expected from participant.
- Reconfirm with screening questions that the participant is eligible for the study. If so, written consent was obtained for participation in the study. Point of contact provided, and option given to withdraw from study at any stage.
- Complete with the participant, a questionnaire regarding demographic details, past medical, current pregnancy details, and recent medicinal intake (please see attached documents).
- Perform baseline non-invasive cardiovascular tests, exercise tolerance testing where appropriate.
- Obtain maternal anthropometry measurements (maternal weight, Body Mass Index).
- Fetal ultrasound scan.
- Obtain 20ml venous blood sample, and 10 ml urine sample.
- Provide each participant with unique study identity code. Personal data linking to each unique study code was kept in paper form in a confidential file, which was locked in a secure filing cabinet in Centre for Fetal Care.
Figure 3-3 Completing patient questionnaire with participant
Subsequent study visits

Control group:
Participants were offered subsequent visits if appropriate, spaced at least 4 weeks apart.
Repeat non-invasive cardiovascular assessments and fetal ultrasound scans were performed. At every visit, the participant completed a questionnaire relating to their recent medication intake. 20ml venous blood sample and 10ml urine sample were collected.

Pathological pregnancy outcome group:
Participants were initially seen once a week, with additional visits as appropriate. She had repeat non-invasive cardiovascular assessments, exercise testing and fetal ultrasound scans as appropriate. At every visit, the participant completed a questionnaire relating to their recent medication intake. 20ml venous blood sample and 10ml urine sample were collected at each visit.

Delivery:
Pregnancy outcome data obtained from medical records.

Post-partum:
Participant offered appointment at 6 weeks post-partum, and had repeat non-invasive cardiovascular assessments.

END OF PROTOCOL

Figure 3-4 Schematic diagram of follow up visits
3.5 Cardiovascular Assessment

There are multiple modalities used to measure cardiac output in pregnancy. The accepted gold standard is cardiac catheterisation then measurement via direct Fick method or thermodilution method. Obviously, this will not be acceptable to healthy women with ongoing pregnancies due to the additional risk of invasive testing. Recently, there have been many non-invasive devices widely used to measure cardiac output. This includes USCOM and NICOM (via continuous wave Doppler) (59) or Echocardiography measurement of cross sectional area and Doppler flow. Previous studies done in healthy non-pregnant population has validated the use of cardiac output and central BP using Vicorder vs Innocor (60).

To perform validation testing, data was combined with that from a parallel running study with a pre-conception longitudinal cohort (Conceive study). Bland Altman statistical tests was performed to ascertain limits of agreement between the two tests. As the tests cannot be used interchangeably, (27) only cardiac output measured on Innocor was reported as this has been previously validated in pregnancy (61) (Paper attached in Appendix 3a).

3.5.1 Innocor

The Innocor system utilises a inert gas rebreathing technique. Participants breathed in an oxygen (O\textsubscript{2}) enriched mixture containing soluble and insoluble gases (0.5% nitrous oxide, N\textsubscript{2}O; 0.1% sulfur hexafluoride), and a sensor sampled the proportion of soluble gas and O\textsubscript{2} absorbed across the lungs over several breathing cycles (total breathing time was roughly 30 seconds) via an infrared photoacoustic gas analyser embedded within the device. The amount
of N₂O absorbed was proportional to the pulmonary blood flow. This technique has already been used in pregnant women as N₂O is harmless at these concentrations for a very limited amount of time. With the use of the pulse oximeter, which determines oxygen saturation of haemoglobin in arterial blood, the amount of the shunt flow (blood flow which does not perfuse the ventilated part of the lungs) was calculated. Cardiac output was then derived using the Fick principle. Cardiac output was measured in the left lateral position in this study to avoid maternal aorto-caval compression.

*Figure 3-5 The Innocor machine*
Figure 3-6 The Innocor gas cylinder

Figure 3-7 Pictorial diagram showing principles of Innocor system
3.5.2 Vicorder

Vicorder® is an oscillometric devise used to analyse brachial pressure waveforms. Brachial artery waveforms are obtained using a fluid distension technique via cuff inflation on the upper arm. These waveforms are then transformed by a validated formula to give aortic AIX. Tonometry is the most widely used method in pregnancy. No comparative studies between Vicorder® and tonometry have been done in pregnancy. In a non-pregnant cohort of COPD patients, AIX measurements significantly correlated between Vicorder® and SphygmoCor®, however limits of agreement were only 10.42-9.02%, with co-efficient of reproducibility of 27.9%. Vicorder® values were lower but there was satisfactory agreement (62).

Figure 3-8 The Vicorder system
3.5.3 Testing protocol

All subjects were asked to refrain from caffeine for 4 hours prior to tests.

1) Brachial blood pressure and pulse rate (10 minutes)

Brachial blood pressure was measured using Omron-M7 blood pressure monitor on left arm in sitting position. Pulse rate was recorded. Two readings were obtained and average recorded.

2) Pulse wave velocity (PWV), central blood pressure (cBP), augmentation index (Aix), mean arterial pressure (MAP) (10 minutes)

With participant in left lateral lying position, PWV and cBP were measured using the Vicorder machine. Due to the left lateral position, the Vicorder leg cuff was applied to right leg, and the neck cuff applied over right carotid. Standard measuring tape was used to measure the carotid femoral distance.

Figure 3-9 Cuff based Vicorder testing in supine left lateral tilt
3) Cardiac output (CO) and stroke volume (SV) (5 minutes)

With participant lying in left lateral, CO & SV was measured using an inert gas re-breathing technique (Innocor, Innovision A/S, Denmark) at rest. The test was repeated with the participant standing upright. Nose clip was applied to ensure all breathes were through the Innocor mouthpiece.

![Image](image_url)

Figure 3-10 Non-rebreathing method with Innocor machine

4) Exercise testing – pathological pregnancy outcome group only (at first study visit, just before decision to deliver and postnatal) (10 minutes)

Participant underwent a simple 3 minute step test (Dundee Step Test (63)) that is well validated in cardiovascular testing. This involved stepping up with the dominant foot onto a platform 17.5cm high. The non-dominant foot is then stepped up, before both feet step
down one at a time. Rhythm was kept at a constant with a metronome (allowing 2 seconds for each completed step cycle).

Figure 3-11 Schematic diagram of Dundee step test

CO & SV were repeated during exercise. PR and brachial BP were then repeated immediately following completion of the step test.

3.6 Ultrasound

Trans-abdominal ultrasound scans were performed at first study visit. Thereafter, it was performed once weekly until delivery (more frequent if clinically appropriate) in the pathological pregnancy outcome group and at every study visit in the control group. The scans included routine fetal growth parameter, estimated fetal weights and Doppler studies (maternal uterine artery, fetal umbilical artery and middle cerebral artery). Reports of
participant’s routine dating and detailed anomaly scan was obtained for the study with their consent, from their hand-held maternity notes, or by contacting their healthcare provider.

All fetal scans was performed in Queen Charlotte’s & Chelsea Hospital by the clinical research fellow who was signed off for obstetric ultrasound competencies; or other appropriate clinical member of the team. Ultrasound equipment were already available on loan from Samsung UK and is a specific machine that was purely used for the purpose of research.

*Figure 3-12 Ultrasound scan for biometry and feto-maternal Doppler profile*
3.7 Metabolite & Biomarker Assessment – Blood and Urine samples

3.7.1 Serum and urine preparation

10ml blood and 5ml urine samples was obtained from participants at every study visit. Samples were stored on ice until they can be transferred to the satellite laboratory in the Women’s Health Research Centre (Queen Charlotte’s Hospital). Blood samples were centrifuged at 3000rpm for 10 minutes, and the plasma layer was then aliquoted and stored in -80°C freezers within 1 hour of collection. Urine samples were stored straight away at -80°C. A standardised set of nuclear magnetic resonance (NMR) and mass spectroscopy experiments were performed for sample comparing pathological pregnancy outcome group with control group. Appropriate candidate biomarkers were analysed to assess differences in these groups. This analysis was performed once recruitment was completed to minimise batch effect.
Chapter 4 Cardiovascular function in normal and pathological pregnancy outcomes

Abstract
Preeclampsia and fetal growth restriction are considered to be placentally mediated disorders. The clinical manifestations are widely held to relate to gestation age at onset with early- and late-onset preeclampsia considered to be phenotypically distinct. Recent studies have reported conflicting findings in relation to cardiovascular function, and in particular cardiac output, in preeclampsia and fetal growth restriction. We investigated maternal cardiovascular function in relation to clinical subtype in 45 pathological pregnancies (14 PE, 16 FGR, 15 PEFGR) and compared these with 107 healthy person observations. Cardiac output was the primary outcome measure and was assessed using an inert gas-rebreathing method (Innocor), from which peripheral vascular resistance was derived; arterial function was assessed by Vicorder, a cuff-based oscillometric device. Cardiovascular parameters were normalized for gestational age in relation to healthy pregnancies using Z scores, thus allowing for comparison across the gestational range of 24-40 weeks. Compared with healthy control pregnancies, women with preeclampsia had higher cardiac output Z scores (1.87 ± 1.35; P = .0001) and lower peripheral vascular resistance Z scores (-0.76 ± 0.89; P = .025); those with fetal growth restriction had higher peripheral vascular resistance Z scores (0.57 ± 1.18; P = .04) and those with both preeclampsia and fetal growth restriction had lower cardiac output Z scores (-0.80 ± 1.3 P = .007) and higher peripheral vascular resistance Z scores (2.16 ± 1.96; P = .0001). These changes were not related to gestational age of onset. All those affected by preeclampsia and/or fetal growth restriction had abnormally raised augmentation index and pulse wave velocity. Furthermore, in preeclampsia, low cardiac output was associated with low birthweight and high cardiac output with high birthweight (r = 0.42, P = .03). Preeclampsia is associated with high cardiac output, but if preeclampsia presents with fetal growth restriction, the opposite is true; both conditions are nevertheless defined by hypertension. Fetal growth restriction without preeclampsia is associated with high peripheral vascular resistance. Although early and late gestation preeclampsias are considered to be different diseases, we show that the hemodynamic characteristics of preeclampsia were unrelated to gestational age at onset but were strongly associated with the presence or absence of fetal growth restriction. Fetal growth restriction more commonly coexists with preeclampsia at early gestation, thus explaining the conflicting results of previous studies. Furthermore, antihypertensive agents act by reducing cardiac output or peripheral vascular resistance and are administered without reference to cardiovascular function in preeclampsia. The underlying pathology (preeclampsia, fetal growth restriction, preeclampsia and fetal growth restriction) defines cardiovascular phenotype, providing a rational basis for choice of therapy in which high or low cardiac output or peripheral vascular resistance is the predominant feature.

This chapter is based on the following papers

4.1 Introduction

Pre-eclampsia (PE) is not simply a pregnancy-specific syndrome: its implications on later life cardiovascular (64) and cognitive function (65) and healthcare cost (66) are only now being understood. Fetal growth restriction (FGR) has a close but poorly understood relationship with PE. Although PE and FGR frequently present in isolation, they may occur together, particularly at early gestation (67). The underlying pathophysiology of PE has never been fully understood, but the cause has often been attributed to the placenta as PE resolves completely after delivery. Inadequate trophoblast invasion leading to uteroplacental malperfusion is thought to underlie both PE and FGR (3, 4). However, this placental theory does not explain why women who had PE in their pregnancies have a higher cardiovascular risk in later life (68-70) or why women with pre-pregnancy cardiovascular risk factors have a higher risk of PE and FGR in pregnancy (71). Emerging data suggests that maternal cardiovascular function is impaired post-delivery in women with PE (72, 73) and high blood pressure prior to pregnancy increases the risk of PE developing (71). Nevertheless, PE and FGR are commonly referred to as ‘placenta mediated disorders’, suggested to arise through defective placentation.

Studies of cardiovascular function in pregnancy have shown inconsistent, and in some cases, contradictory results. The classic studies of Easterling suggested that PE was associated with a high CO state(74), though this relationship was thought to be explained, at least in part, by the increased body surface area (75). Other studies have suggested that PE should be subdivided into those where low or high CO is predominant (76). FGR with and without PE have a different cardiovascular profile (77, 78) though the relationship of these changes to healthy pregnancy or PE without FGR has not been studied. A recent systematic review
concluded that studies of cardiovascular function in gestational hypertension and PE show conflicting results. The authors concluded that “increased peripheral vascular resistance correlates with disease severity” but of note, the co-existence of FGR was not considered in majority of the studies (21).

Previous studies on cardiovascular function in PE have incompletely characterized FGR (79), recruited only within a particular gestation range (77) and/or not compared findings to a healthy reference pregnant population (19). Existing studies provide interesting insights to cardiovascular dysfunction in pathological pregnancies but leave important questions unanswered in relation to gestational effects and pregnancies where PE and FGR are combined.

Over the last two decades it has become customary to subdivide PE into “early” and “late” variants with an arbitrary 34-week gestational age ‘cut-off’. This distinction arose not because of a pathophysiological difference between the conditions, but because of the inability of screening by uterine artery Doppler to effectively identify cases of late onset disease (20, 80, 81). It is suggested that early and late variants have different underlying vascular characteristics, though in most studies, late PE is rarely or never associated with FGR (19). Adding further complexity, recent studies have suggested that PE at term is associated with babies with larger birth weight (75).

We sought to investigate the relationship between cardiovascular function and clinical phenotype of PE and FGR alone and in combination across a gestation range, with cardiac output being the primary outcome measure. Cardiovascular function changes with gestational age; therefore, in order to allow comparison we transformed all data in relation to that obtained from women with healthy pregnancies using the statistical technique of z-
scoring. This removed the need for a gestation matched cohort design and allowed all data points to be considered (79). In doing so, we performed comprehensive haemodynamic assessments from 24 weeks gestation onwards in women with healthy pregnancies, with PE in combination with FGR (PE and FGR), PE without FGR (PE only), and FGR without PE (FGR only), managed and monitored in a single maternity unit.
4.2 Study specific methods and population

4.2.1 Recruitment

The figure below illustrates recruitment flow chart for this chapter. This represents the complete cohort of all patients recruited to this study.

![Recruitment Flow Chart]

Figure 4-1 Recruitment Flow Chart
4.2.1 Methods

In this prospective cross-sectional study, women between weeks 24-40 of pregnancy were recruited from the antenatal clinic, labour ward, day assessment unit and antenatal ward of a tertiary level London teaching hospital between January 2015 – May 2017. The study protocol was approved by NHS National Research Ethics Committee, London Riverside and all participants gave written informed consent. Gestation of pregnancy was determined by measurement of crown-rump length between 11-13 weeks in early pregnancy.

Women were recruited at presentation or diagnosis where it was feasible to conduct a detailed cardiovascular examination and were not included if they were already in labour, undergoing induction of labour or imminent delivery was planned (Figure 1). We describe the diagnosis as those with PE alone ‘PE only’, FGR alone ‘FGR only’ and PE together with FGR ‘PE and FGR’. Women’s assessments are reported at the time of initial diagnosis of one of these conditions, and the diagnosis assigned at the time of study inclusion. One hundred and seven healthy person observations acted as control cases with no control subject assessed twice within the same epoch. PE was defined as blood pressure at diagnosis of >140/90 mmHg and urine protein creatinine ratio of >30. FGR was defined as fetal abdominal circumference or estimated fetal weight <10th centile (82) and umbilical Doppler PI >95th centile on ultrasound scan (83). Those women with known underlying cardiovascular conditions, multiple pregnancies and fetal anomalies were excluded from the study.

Participants underwent peripheral blood pressure measurement, comprehensive cardiovascular assessments and fetal ultrasound scans as detailed below.
4.2.2 Statistical Analysis

Statistical analyses were performed using SPSS (Version 24_0_0, 2016; SPSS Inc., Chicago, Illinois, USA). The Kruskal-Wallis test was used to compare the demographic characteristics between the four groups. The normality of distribution of the data was examined with the Shapiro-Wilk test and histograms and data are expressed as means ± SD or medians (interquartile range) for normally- and non-normally distributed data, respectively. We analysed the data using BMI, age and ethnicity as co-variates. Since cardiovascular function changes with gestational age, and in order to compare haemodynamic characteristics across groups with different gestational ages, we transformed all data in relation to that obtained from women with healthy pregnancies using the statistical technique of z-scoring. This removed the need for a gestation-matched cohort design and allowed all data points to be considered (77). Briefly, measurements were transformed to the corresponding Z scores with reference to means and SD values derived from Controls in four-weekly gestational epochs (24-27±6, 28-31±6, 32-35±6 and 36-39±6 weeks) for CO, PVR, Alx and PWV. The Z scores of each cardiovascular parameter were then compared using unpaired t-test. In our cohort, heart rate did not change with gestation and comparisons were made with untransformed data.
4.3 Results

Forty-five pathological pregnancies (14 ‘PE only’, 16 ‘FGR only’, 15 ‘PE and FGR’) were recruited, of whom three had a prior history of PE and or FGR. All women remained within the category to which they were first assigned at recruitment. A further 64 women with healthy pregnancies and normal pregnancy outcomes were studied across the gestation, yielding one hundred and seven healthy pregnancy observations. Kruskal-Wallis test showed no statistically significant differences between age and ethnicity within the 4 groups. The group with ‘PE’ had a higher booking BMI when compared to the control group (\( P =0.001 \)) [Table 4-1].

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>FGR</th>
<th>PE</th>
<th>PE and FGR</th>
<th>Kruskal-Wallis p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>107</td>
<td>16</td>
<td>14</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Gestational Age at recruitment Median (range)</td>
<td>32 (24-40)</td>
<td>32 (24-39)</td>
<td>36 (25-39)</td>
<td>30 (24-36)</td>
<td>0.50</td>
</tr>
<tr>
<td>Parity Median (range)</td>
<td>1 (0-3)</td>
<td>0 (0-2)</td>
<td>0 (0-2)</td>
<td>0.5 (0-3)</td>
<td>-</td>
</tr>
<tr>
<td>Age Median (IQR)</td>
<td>34 (31.5-36.5)</td>
<td>35 (31-39)</td>
<td>32 (27.5-36.5)</td>
<td>33 (31-35)</td>
<td>0.11</td>
</tr>
<tr>
<td>Booking BMI Mean (SD)</td>
<td>24 (3.2)</td>
<td>25.7 (5.6)</td>
<td>29.1 (4.5)*</td>
<td>25.8 (5.4)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* \( P =0.001 \) between controls and ‘PE’

*Table 4-1 Demographic characteristics at recruitment*
Cardiovascular parameters in pathological and control groups are presented in Table 4-2. The cardiovascular parameters in pathological pregnancies are presented in Table 4-3; Z scores were calculated in relation to control cases from healthy pregnancies which were subdivided into four weekly gestational epochs.

<table>
<thead>
<tr>
<th></th>
<th>Gestation</th>
<th>Controls</th>
<th>FGR</th>
<th>PE</th>
<th>PE and FGR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CO standing</strong></td>
<td>24 – 27+6</td>
<td>6.2 (0.92)</td>
<td>5.56 (1.06)</td>
<td>7.23 (1.37)</td>
<td>5.22 (1.22)</td>
</tr>
<tr>
<td></td>
<td>28 – 31+6</td>
<td>6.0 (0.97)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 – 35+6</td>
<td>5.74 (1.13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 – 40</td>
<td>5.34 (0.84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PVR</strong> (dyn·s/cm^5)</td>
<td>24 – 27+6</td>
<td>1088.7 (217.9)</td>
<td>1311.6 (291.6)</td>
<td>1091.6 (232.2)</td>
<td>1648.5 (430.1)</td>
</tr>
<tr>
<td></td>
<td>28 – 31+6</td>
<td>1148.34 (270)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 – 35+6</td>
<td>1187.97 (200.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 – 40</td>
<td>1303.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aortic AIX</strong> (units)</td>
<td>24 – 27+6</td>
<td>14.22 (7.7)</td>
<td>22.2 (12.3)</td>
<td>29.7 (14.9)</td>
<td>25.8 (9.4)</td>
</tr>
<tr>
<td></td>
<td>28 – 31+6</td>
<td>11.3 (7.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 – 35+6</td>
<td>11.2 (8.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 – 40</td>
<td>14.1 (10.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PWV</strong> (m s⁻¹)</td>
<td>24 – 27+6</td>
<td>6.8 (0.9)</td>
<td>7.84 (1.32)</td>
<td>7.81 (1.38)</td>
<td>8.82 (1.37)</td>
</tr>
<tr>
<td></td>
<td>28 – 31+6</td>
<td>7.0 (1.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 – 35+6</td>
<td>7.0 (0.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 – 40</td>
<td>7.2 (0.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pulse</strong> (beats/ min)</td>
<td>24 – 27+6</td>
<td>80.4 (10.3)</td>
<td>78.7 (12.5)</td>
<td>83.8 (10.2)</td>
<td>76.1 (9.9)</td>
</tr>
<tr>
<td></td>
<td>28 – 31+6</td>
<td>83.5 (14.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 – 35+6</td>
<td>87 (13.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 – 40</td>
<td>86 (11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAP</strong></td>
<td>24 – 27+6</td>
<td>81.1 (7.1)</td>
<td>87.9 (9.6)</td>
<td>95.2 (7.2)</td>
<td>102.4 (10.8)</td>
</tr>
<tr>
<td></td>
<td>28 – 31+6</td>
<td>77.8 (8.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 – 35+6</td>
<td>79.1 (4.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 – 40</td>
<td>81.6 (7.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 4-2 Cardiovascular parameters (by gestation epochs for control cases) and overall values for pathological outcome cases [mean (SD)]*
Table 4-3 Z-scores of cardiovascular parameters by outcome group (controls are reference)

<table>
<thead>
<tr>
<th></th>
<th>FGR</th>
<th>PE</th>
<th>PE and FGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO standing</td>
<td>-0.35 (0.99)</td>
<td>1.87 (1.35) *</td>
<td>-0.80 (1.3) *</td>
</tr>
<tr>
<td>PVR standing</td>
<td>0.57 (1.18) *</td>
<td>-0.76 (0.89) *</td>
<td>2.16 (1.95) *</td>
</tr>
<tr>
<td>AIX</td>
<td>1.18 (1.44) *</td>
<td>1.52 (1.39) *</td>
<td>1.45 (1.09) *</td>
</tr>
<tr>
<td>PWV</td>
<td>0.87 (1.34) *</td>
<td>0.71 (1.47) *</td>
<td>2.01 (1.55) *</td>
</tr>
</tbody>
</table>

*denotes p<0.05 compared to corresponding control value

4.3.1 FGR only

In women with FGR only, PVR Z score was significantly higher (0.57 ± 1.18, p= 0.04) [Figure 4-2], and CO Z score was no different to healthy controls (-0.35 ± 0.99, p= 0.19) [Figure 4-3]. The Z scores of AIX (1.18 ± 1.44, p= 0.0001) [Figure 4-4] and PWV (0.87± 1.34, p= 0.002) [Figure 4-5] were higher than controls.

4.3.2 PE only

Women with PE only had a higher CO Z score than controls 1.87 ± 1.35, p= 0.0001) [Figure 4-3], a lower PVR Z score (-0.76 ± 0.89, p= 0.025) [Figure 4-2], higher AIX Z score (1.52 ± 1.39, p= 0.0001) [Figure 4-4] and higher PWV (0.71 ± 1.47, p=0.05) [Figure 4-5].

Of the 13 women with PE only, 7 women were examined before starting anti-hypertensive medication, 3 women were on labetalol and 1 on nifedipine. The Z scores for CO and PVR of
those examined on treatment versus those without were not different (p= 0.55 and p = 0.57, respectively).

4.3.3 PE and FGR

In PE and FGR cases, a lower CO Z score (-0.80 ± 1.3, p=0.007) [Figure 4-3] and higher PVR Z score (2.16 ± 1.95, p= 0.0001) [Figure 4-2] was observed compared to controls. There was higher AIx Z score (1.45 ± 1.09, p= 0.0001) [Figure 4-4] and higher PWV Z score (2.01 ± 1.55, p= 0.0001) [Figure 4-5].

Of the 14 women with ‘PE and FGR’; 3 were examined before starting anti-hypertensive medication, 4 were taking labetalol, 2 nifedipine and 3 both labetalol and nifedipine. Comparing the Z scores for CO and PVR of those examined on treatment versus those without, there were no differences (p=0.52 and p=0.99, respectively).
Figure 4-2 Z Score of Peripheral vascular resistance (standing) in outcome groups (Median/IQR)
Figure 4-3 Z Score of Cardiac output (standing) in outcome groups (Median/IQR)
Figure 4-4 Z Score of Augmentation index in outcome groups (Median/IQR)
Figure 4-5 Z Score of Pulse wave velocity in outcome groups (Median/IQR)
**Heart Rate**

There was no difference in observed heart rate between the four groups. ($p=0.26$) [Figure 4-6].

*Figure 4-6 Heart Rate in outcome groups (Median/IQR)*


**Gestation vs CO/PVR**

When compared across gestation (decimal weeks by estimated due date derived from dating scan), there was no relationship between gestation and either CO Z score (Pearson correlation = 0.16, p = 0.35) or PVR Z score (Pearson correlation = -0.21, p = 0.2) for PE only, FGR only and PE and FGR.

There was no difference between CO Z score (p=0.41) or PVR Z Score (p=0.18) in ‘PE only’ prior to 34 weeks and those after 34 weeks. In ‘PE and FGR’ cases, there was again no difference between CO Z score (p=0.34) or PVR Z score (p=0.76) in cases prior to 34 weeks and those after.

**CO vs Z score birth weight**

Within the group of women with PE (‘PE only’ and ‘PE and FGR’), CO was positively associated with Z score birth weight (r= 0.42, p=0.03). Within the group of women with ‘FGR only’, CO was not associated with Z score birth weight (r=0.27, p=0.31).

**Post Hoc power calculation**

Since this was novel work, a formal sample size calculation prior to undertaking the study was not possible and the enrolment period was guided by our previous pre-pregnancy studies in healthy women (84-86). However, a formal post hoc power calculation, assuming four unequal groups and a standard deviation for the CO z-score equivalent to the highest SD obtained in the three pathological pregnancy groups, gave >98% power to detect a significant overall difference in CO z-score, at an alpha level of 0.05.
4.4 Discussion

We report that women with ‘PE only’, ‘FGR only’ and ‘PE and FGR’ have distinctly different, and, in some cases, opposing cardiovascular characteristics. The predominant abnormalities in ‘PE only’ were a higher CO and lower PVR, while ‘FGR only’ was characterized by higher PVR than healthy pregnancies. When both PE and FGR occur together, the effect was a cardiovascular phenotype characterised by a markedly higher PVR and lower CO than FGR only. Previous studies of PE and FGR have reported inconsistent findings however none have studied haemodynamic findings across the whole gestation range in relation to healthy pregnancies recruited concurrently, nor have considered FGR, PE and PEFGR as separate entities.

Recently, a meta-analysis by Castleman (21) found that in gestational hypertensive diseases “severity of disease corresponded with increasing vascular resistance”. We suggest that this is an oversimplification in that according to our data it holds for PE with FGR; the converse being true for PE not affected by FGR (21). Irrespective of differences in CO and PVR, all three pregnancy complications were associated with abnormal arterial function assessed by increased augmentation index and increased pulse wave velocity.

The implications for understanding of the disease process is important, particularly in respect of the deeply ingrained, but empirical and arbitrary distinction between ‘early’ and ‘late’ PE with a “cut off” at 34 weeks (80, 87). It is true that the cardiovascular features of early and late PE were different, but this was purely by virtue of the association with FGR rather than because of the gestational age at onset of the disease. ‘PE only’ had the same cardiovascular
findings (high CO and low PVR) at early and late gestational ages, but if PE was associated with FGR, CO was low and PVR high, irrespective of gestational age. Importantly, in our study, pathological cases were similarly represented in numerical terms before and after 34 weeks; 24 and 21 cases respectively. The contradictory findings of previous studies, namely that gestational age is the determinant of haemodynamic profile in PE, may be explained because PE and FGR commonly co-exist at early gestational ages (88) and FGR is uncommonly encountered in late PE (75). PE is therefore more precisely defined by the presence or absence of FGR rather than by virtue of its gestational age at onset. We trace the origins of the categorization of ‘early’ and ‘late’ PE to a study on the sensitivity of second trimester uterine artery Doppler for these complications which was high for ‘early’ but low for ‘late’ disease. Interestingly, early PE was frequently found with FGR in that study.

In women with PE, with and without FGR, we found a significant positive association between CO Z score and birthweight normalized for gestation which was no different before or after 34 weeks. We did not specifically select women with PE, with and without FGR, but instead recruited 'all comers' and categorized FGR based on ultrasound and Doppler findings. Hence, there was no selection bias within women with PE as it might be argued that a larger fetus and placenta will 'drive' a larger CO. We previously reported that the incremental increase in maternal CO from prior to pregnancy to the mid-second trimester was positively associated with larger birthweight in normally grown babies (89) irrespective of the size of the fetus in the mid trimester. This suggests that fetal size on its own did not determine CO. A recent study in women with PE found that those with the lowest CO had the smallest babies (76). There is strong-albeit circumstantial-evidence that CO may be at least in part responsible for
birthweight, likely mediated through an effect on utero-placental perfusion. Though this is tempting to speculate, we cannot infer causality from our results.

Of direct clinical relevance is that the current management of hypertension in pregnancy is exclusively based on a ‘one size fits all’ philosophy where BP alone is the target of therapy irrespective of the underlying aetiology (90, 91). This approach does not distinguish between PE with FGR (low CO and high PVR) from PE without FGR (high CO). There is, however, a clear rationale in targeting therapy according to the underlying cardiovascular phenotype. Drugs with beta blocking activity are negatively chronotropic and reducing CO may be harmful in women with FGR where the utero-placental circulation is already impaired; previous studies have reported an association with small for gestational age babies (92-94). We report that PE only was typically associated with high CO: for these women, a beta blocker might be preferable whereas a drug leading to vasodilation primarily, such as a calcium channel antagonist, may be less effective. Conversely calcium antagonists are likely to be more effective in the high PVR state that characterizes PE with FGR. Though a model using haemodynamic parameters to determine response to labetalol in gestational hypertension has been reported (95), the effectiveness of anti-hypertensive agents with different modes of action has not. Indeed, if impaired utero-placental function is the by-product of a low CO-high PVR state such as that found in PE with FGR, then this should be amenable to targeted manipulation. Recent approaches have included expanding the intravascular compartment (96), with and without nitro-vasodilators (97) and calcium antagonists (98) with reported improvement in maternal cardiovascular and/or fetal parameters.

Arterial function was abnormal in ‘PE only’, ‘FGR only’ and the combination of both ‘PE and FGR’ as assessed by AIx and PWV, irrespective of whether CO and PVR were increased or
decreased. Aortic AIx is an index of wave reflection and may be considered as a measure of arterial function (33, 38, 99, 100). Raised AIx is an important predictor of future cardiovascular risk (101) and in hypertensive patients an independent predictor of future myocardial infarction (102). Although AIx is influenced by endothelial function, PWV describes aortic stiffness and is determined largely by the elastic component of large vessels. It is also known to be a reliable marker of cardiovascular risk in patients with hypertension (103).

The strength of this study is that detailed cardiovascular assessments were undertaken in women who were phenotyped according to accepted definitions for both PE and FGR. Importantly, our definition of FGR did not rely simply on fetal smallness (104), but required abnormal fetal umbilical artery Doppler. Our sample size allowed a high power to detect small differences in CO between the groups. A weakness is that as our protocol required time consuming examinations, not all women wished to or could take part, in particular where delivery or further therapy was being planned. This limited the number of women eligible for recruitment, but there was no attempt at selection other than through women consenting or otherwise for logistical and therapeutic reasons. Furthermore, though we intended to recruit women prior to starting anti-hypertensive treatment, these drugs were often started prior to hospital admission. Though we found no difference in CO and PVR within the ‘PE only’ and ‘PE and FGR’ groups for those on antihypertensive treatment, the modest numbers within these groups preclude discrimination between small effect sizes in anti-hypertensive agents and the duration of therapy.

Whilst not being validated specifically in pregnancy, Innocor has been validated extensively in healthy populations and those with diseases, including chronic heart failure and during haemodynamic perturbation caused by exercise as a method of assessing cardiac output (105-
In addition, our group has previously shown that this method has good reproducibility in pregnant women and is sensitive enough to detect longitudinal changes in CO both in very early pregnancy and throughout the gestation period (85, 89, 109). All our participants were examined in a similar position which limits any potential bias. Moreover, as the gas rebreathing method is not operator dependent, there is no inter-observer bias compared to echocardiography, which is the other widely used method. The Vicorder has been validated for use against SphygmoCor which is the most widely used technique to assess pulse wave velocity. There is no agreed gold standard non-invasive technique to assess pulse wave velocity in pregnancy (2).

In summary, we found no evidence to support the categorization of PE of “early” and “late” as different disease processes, though early onset PE was much more likely to be associated with FGR. The significance of this is that PE which is not associated with FGR has the same cardiovascular phenotype, namely high CO whether it is diagnosed at 24 or 36 weeks. The presence of FGR was associated with high PVR, whether or not in combination with PE. Whether the abnormal haemodynamic profiles that we have observed in the distinct subtypes of PE, FGR and PE combined with FGR are the cause of the clinical manifestation of hypertension and FGR, or the effect of an inherent underlying maternal cardiovascular dysfunction is unclear. This study raises an intriguing question as to whether the cardiovascular dysfunction seen in pathological pregnancies is a result of maladaptation to the increased cardiovascular demands of pregnancy in a woman with normal cardiovascular function, or is predisposed by an inherently abnormal pre-pregnancy cardiovascular status and its expression modulated through placental release of vasoactive substances.
Chapter 5 Relationship between maternal haemodynamic function and feto-placental Doppler

Abstract

The mechanism underlying fetal-placental Doppler changes in pre-eclampsia and/or fetal growth restriction are unknown though both are associated with maternal cardiovascular dysfunction. We sought to investigate whether there was a relationship between maternal cardiac output and vascular resistance and feto-placental Doppler in healthy and complicated pregnancy.

Women with healthy (n= 62), PE (Pre eclampsia) (n=13), PE+FGR (Pre eclampsia with fetal growth restriction) (n=15) or FGR (Fetal growth restriction) (n=17) pregnancies from 24-40 weeks were included. All underwent measurement of cardiac output (CO) using an inert gas rebreathing technique, and derivation of peripheral vascular resistance (PVR). Uterine and fetal Doppler indices were recorded: the latter were z-scored to account for gestation. Associations were determined by polynomial regression analyses.

Mean uterine artery pulsatility index (PI) was higher in FGR (Fetal growth restriction) (1.37, p=0.026) and PE+FGR (Pre eclampsia with fetal growth restriction) (1.63, p=0.001) but not PE (Pre eclampsia) (0.92, p=1) compared to controls (0.8). There was a negative relationship between uterine PI and CO ($r^2=0.101$; $p=0.025$) and umbilical PI z-score and CO ($r^2=0.078$; $p=0.015$), and positive associations between uterine PI and PVR ($r^2=0.150$; $p=0.003$) and umbilical PI z-score and PVR ($r^2=0.145$; $p=0.001$). There was no significant relationship between CO and PVR with cerebral Doppler.

Uterine artery Doppler is abnormally elevated in FGR with and without PE, but not in PE: this may explain the limited sensitivity of uterine artery Doppler for all these complications considered in aggregate. Furthermore, impedance within feto-placental arterial vessels is at least in part associated with maternal cardiovascular function. This relationship may have important implications for fetal surveillance and would inform therapeutic options in those pathological pregnancy conditions currently-and perhaps erroneously- attributed purely to placental mal-development.

Uterine and fetal placental Doppler indices are significantly associated with maternal cardiovascular function. The classical description of uterine and fetal Doppler changes being initiated by placental maldevelopment is a less plausible explanation for the pathogenesis of the conditions than that relating to maternal cardiovascular changes.

This chapter is based on the following papers

5.1 Introduction

Fetal growth restriction, with or without associated pre-eclampsia, is classically thought to be due to placental insufficiency (3), resulting in progressive fetal hypoxia and acidaemia and leading to compensatory changes in the fetal circulation (110). The interaction between pre-eclampsia, uteroplacental insufficiency and fetal growth restriction and circulatory changes is of direct clinical relevance as a major determinant of pre-eclampsia related healthcare costs arise from the neonatal costs of premature delivery (66). Delivery in early onset disease is often undertaken due to fetal growth restriction (111) and increasing severity of fetal growth restriction is associated with worse neonatal morbidity (104).

The fetal circulatory adaptation to chronic hypoxia detected non-invasively using Doppler ultrasound is characterised by increased impedance in the umbilical artery and a reduction in cerebral impedance in the fetus, the so called ‘brain sparing’ response (47, 48). Increased vascular resistance within the umbilical arteries in compromised pregnancy may result from either structural changes or functional adaption within the umbilical-placental bed. Both abnormal placental villous morphology (112, 113) and reduced villous count (114) are associated with fetal growth restriction and abnormal Doppler waveforms in the umbilical artery. Umbilical vasoreactivity also varies with oxygen tension and the pH of circulating blood (115), as well as the influence of vasoactive agents (116, 117). For instance, differences in the vasoconstrictor activity on umbilical arteries of noradrenaline (118), endothelin (116), thromboxane (119) and serotonin (120) have been described between control and pregnancies with abnormal umbilical artery flow velocity waveforms. However, the umbilical cord is not innervated by the autonomic nervous system (121).
Pregnancy affected by chronic fetal hypoxia triggers a maintained adaptive redistribution of the fetal cardiac output away from peripheral circulations towards essential vascular beds such as fetal brain (122) leading to the typical asymmetric fetal growth restriction (123). In addition, the sustained increase in fetal peripheral vascular resistance transfers to increased impedance of blood flow returning to the placenta, clinically diagnosed by increased umbilical artery Doppler pulsatility index (PI) in compromised pregnancy (47, 49).

Abnormalities in the early placental circulation (124) and structure have long been implicated in the pathogenesis of both pre-eclampsia and fetal growth restriction (125) and the concept of placental dysfunction has led to strategies to stratify high risk pregnancies (126). Though the theory of abnormal placentation is classically thought to explain abnormally high resistance uterine artery flow, little direct evidence supports a causative association. Our group has shown that healthy normotensive women who develop pre-eclampsia and/or fetal growth restriction have altered pre-pregnancy haemodynamics compared to those that have normal outcomes (7).

Early onset pre-eclampsia and fetal growth restriction commonly co-exist and are associated with abnormal maternal cardiovascular function, predominantly low cardiac output and high vascular resistance (19, 72). Our group has further refined this observation to establish that it is fetal growth restriction (with or without co-existing pre-eclampsia) which is associated with this low cardiac output-high peripheral vascular resistance maternal phenotype, irrespective of gestation (127). By contrast, pre-eclamptic pregnancies with appropriately-grown fetuses are associated with high maternal cardiac output-low peripheral vascular resistance; in other words the opposite maternal cardiovascular phenotype (128). This distinction between two type of pre-eclampsia are, we believe, critical to an understanding
of the corresponding changes in maternal cardiovascular function: if women with both forms of pre-eclampsia are considered together, the opposing changes in cardiac output and peripheral vascular resistance of these two 'clinical phenotypes' are negated by statistical averaging.

In this study we recruited a carefully-phenotyped cohort of pregnant women undergoing detailed cardiovascular and Doppler examinations and classified into four categories: pre-eclampsia, pre-eclampsia with fetal growth restriction and fetal growth restriction and healthy pregnancy. Cardiovascular function and Doppler indices changes with gestational age, therefore, in order to allow comparison we transformed all data in relation to that obtained from women with healthy pregnancies using the statistical technique of z-scoring (77). By adjusting parameters for the effect of gestational age changes in this way, we were able to investigate the relationship between maternal cardiovascular function and the classically described fetal cardiovascular changes during complicated pregnancy across the entire third trimester of pregnancy.
5.2 Study specific methods and population

We performed a prospective study, which included a cohort of pregnant women from 24 weeks of gestation affected by fetal growth restriction alone (“FGR” group), by preeclampsia alone (“PE” group) or by the combination of both (“PE+FGR” group) and a group of healthy unaffected pregnant women (“Control” group). Recruitment was at a single tertiary level referral hospital in London between January 2015 and June 2017. The study was approved by National Research Ethics Service Committee London Riverside (REC reference 15/LO/0341) and written consent was obtained. Participants were non-smokers, with maternal age between 18-44 years, body mass index (BMI) < 35 kg/m2 and had no comorbidities such as chronic hypertension, diabetes mellitus, cardiovascular or renal disease. Exclusion criteria were the presence of fetal malformations and multiple pregnancies. Women included were part of the PRECEPT study, whose cardiovascular function has been reported recently (127).

PE was defined as maternal blood pressure at diagnosis of > 140/90 mmHg and urine protein creatinine ratio of > 30. FGR was defined as fetal abdominal circumference or estimated fetal weight < 10th percentile (82) and umbilical Doppler PI > 95th centile on ultrasound scan (83). Participants with preeclampsia or fetal growth restriction were enrolled either at the time of first manifestation of the disease, or at the time of transfer of care to our hospital if they were booked elsewhere. Women included in the “Control group” had healthy pregnancies and were enrolled at different gestational ages, when they attended their routine antenatal clinic assessment. Gestational age was determined from measurement of crown-rump length at 11-13+0 weeks of gestation.
Maternal cardiovascular measurements were performed following a standardized protocol in all participants. Abstinence from caffeine for at least 4 hours before the assessments was required, and participants rested for 10 minutes in the research room before the tests. Cardiac output was obtained in the standing position with an inert gas rebreathing device (Innocor, Innovision A/S, Denmark) (85) previously validated against thermodilution for the measurement of cardiac output in non-pregnant populations (107).

Maternal blood pressure was measured using an automatic device (Omron™ M-7, OMRON Healthcare Europe B.V.) which has been validated in pregnancy (129). Blood pressure was measured on the right arm after five minutes of standing. Mean arterial pressure (MAP) was calculated by \([\text{diastolic pressure} + (\text{systolic pressure} - \text{diastolic pressure})/3]\). Maternal peripheral vascular resistance (PVR) was derived from MAP measured standing with the following formula, \(\text{PVR} = \text{MAP} \times 80 / \text{cardiac output}\) (130).

All women underwent serial ultrasound scans using Samsung WS80 (Samsung Medison, Korea) or GE Voluson E8 (GE, Zipf, Austria) within 72 hours from the maternal cardiovascular assessment. Fetal biometry and Doppler velocimetry were assessed in order to determine whether the fetal growth met the criteria for diagnosis of fetal growth restriction. Doppler vascular parameters examined were mean PI in the uterine artery (mean of right and left uterine arteries), umbilical artery, and fetal middle cerebral artery when indicated.
5.2.1 Statistical Analysis

In order to assess the relationship between maternal cardiovascular function and fetal vascular impedance, PI values in the fetal circulation (umbilical artery and middle cerebral artery) were transformed into the corresponding z-scores for gestational age, with mean and standard deviations obtained from widely used Doppler reference ranges (83). Uterine artery PI values were examined untransformed, since these values change little over the third trimester. Since maternal cardiovascular function also changes with gestational age, cardiac output and peripheral vascular resistance were also transformed into z-scores by comparing the values with a large cohort of measurements obtained in healthy pregnancies at different gestational epochs, as our group has recently described (127).

Statistical analyses were performed using SPSS (Version 24, SPSS Inc., Chicago, Illinois, USA). The Kruskal-Wallis test was used to compare the demographic characteristics and between the four groups. The associations between haemodynamic indices and PI were examined using polynomial regression analyses. Quadratic models were chosen after establishing that these provided the closest fit to the data, using curve-fitting analyses. Unless otherwise stated, data are expressed as means ± SD and P<0.05 was considered statistically significant.
## 5.3 Results

Subject characteristics are listed in Table 5-1. 45 pregnancies with pathological outcome (17 FGR only, 13 PE only and 15 PE + FGR) and a further 62 women with healthy pregnancies and normal pregnancy outcomes were recruited (controls). There were no statistically significant differences in gestational age or median age of the women between groups, although BMI at booking was significantly higher in women with PE than in controls (mean±SD 29.1±4.5 kg.m\(^{-2}\) vs. 24.0±3.52 kg.m\(^{-2}\), P=0.007). Of the 62 controls and 45 cases of pathological outcome, umbilical artery Doppler indices were available in all, middle cerebral artery Doppler indices in 15 controls and 35 pathological outcome cases, and uterine artery Doppler indices were available in 50 controls and 24 pathological outcome cases.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>FGR (Fetal growth restriction)</th>
<th>PE (Pre eclampsia)</th>
<th>PE+FGR (Pre eclampsia with fetal growth restriction)</th>
<th>Kruskal-Wallis p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of cases</strong></td>
<td>62</td>
<td>17</td>
<td>13</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td><strong>Gestational Age at recruitment, weeks Median (range)</strong></td>
<td>32 (24-40)</td>
<td>32 (24-39)</td>
<td>36 (25-39)</td>
<td>30 (24-36)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Parity, number Median (range)</strong></td>
<td>1 (0-3)</td>
<td>0 (0-2)</td>
<td>0 (0-2)</td>
<td>0 (0-3)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Age, years old Median (IQR)</strong></td>
<td>34 (31.5-36.5)</td>
<td>35 (31-39)</td>
<td>32 (27.5-36.5)</td>
<td>33 (31-35)</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Booking BMI, kg/m2 Mean (SD)</strong></td>
<td>24.0 (3.2)</td>
<td>25.7 (5.6)</td>
<td>29.1 (4.5)*</td>
<td>25.8 (5.4)</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Birthweight z score Mean (SD)</strong></td>
<td>0.61 (1.04)</td>
<td>-2.603 (0.86)</td>
<td>0.78 (1.96)</td>
<td>-2.5 (1.27)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Uterine artery PI Mean (SD)</strong></td>
<td>0.8 (0.24)</td>
<td>1.37 (0.51)**</td>
<td>0.92 (0.33)</td>
<td>1.63 (0.6)**</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 5-1 Maternal characteristics at recruitment

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77
Regression equations describing the relationship between maternal haemodynamic indices and Doppler impedance in the uterine and placental and fetal circulations are presented in Table 5-2.

<table>
<thead>
<tr>
<th>Association</th>
<th>$R^2$</th>
<th>P</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uterine Artery Pulsatility Index</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Output z score</td>
<td>0.101</td>
<td>0.025</td>
<td>$y = -0.115x + 0.044x^2 + 0.918$</td>
</tr>
<tr>
<td>Peripheral Vascular Resistance z score</td>
<td>0.150</td>
<td>0.003</td>
<td>$y = 0.142x - 0.009x^2 + 0.957$</td>
</tr>
<tr>
<td><strong>Umbilical Artery Pulsatility Index z score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Output z score</td>
<td>0.078</td>
<td>0.015</td>
<td>$y = -0.655x + 0.136x^2 + 1.161$</td>
</tr>
<tr>
<td>Peripheral Vascular Resistance z score</td>
<td>0.145</td>
<td>&lt;0.001</td>
<td>$y = 0.612x + 0.045x^2 + 1.058$</td>
</tr>
<tr>
<td><strong>Middle Cerebral Artery Pulsatility Index z score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Output z score</td>
<td>0.028</td>
<td>0.51</td>
<td>$y = 0.182x - 0.069x^2 - 0.392$</td>
</tr>
<tr>
<td>Peripheral Vascular Resistance z score</td>
<td>0.081</td>
<td>0.14</td>
<td>$y = -0.150x - 0.028x^2 - 0.355$</td>
</tr>
</tbody>
</table>

* $p=0.001$ between “Control” and “PE”  
** $p=0.026$ between “Control” and “FGR”  
*** $p=0.001$ between “Control” and “PE+FGR”

*Table 5-2 Associations between maternal haemodynamic indices and Doppler pulsatility indices*
5.3.1 Uterine artery Doppler

The relationships between uterine artery PI in the healthy control and pathological outcome groups are shown in Figure 5-1. Uterine artery PI was significantly elevated in women with FGR only or FGR+PE but not PE, when compared with women with healthy pregnancies.

![Figure 5-1 Uterine artery PI in women grouped according to pregnancy outcome](image)

Uterine artery PI independent of pregnancy health was inversely and non-linearly associated with maternal CO z-score ($R^2=0.101$, $P=0.025$, Figure 5-2), but directly, and non-linearly associated with PVR z-score ($R^2=0.150$, $P=0.003$, Figure 5-3).
Chapter 5

Figure 5-2 Scatter diagram of cardiac output z score vs uterine artery pulsatility index

Figure 5-3 Scatter diagram of peripheral vascular resistance z score vs uterine artery pulsatility index
Figure 5-4 Mean uterine artery PI (±SEM) vs cardiac output z score

Figure 5-5 Mean uterine artery PI (±SEM) vs peripheral vascular resistance z score
5.3.2 Umbilical artery Doppler

Similar trends were observed with umbilical artery z-scores, although the strengths of the associations were more modest (CO z-score: $R^2=0.078$, $P=0.015$, Figure 5-6; PVR z-score: $R^2=0.145$, $P<0.001$, Figure 5-7).

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*Figure 5-6 Scatter diagram of cardiac output z score vs umbilical artery pulsatility index z-score*
Figure 5-7 Scatter diagram of peripheral vascular resistance z score vs umbilical artery pulsatility index z score
Figure 5-8 Umbilical artery Pi z score (+SEM) vs cardiac output z score

Figure 5-9 Umbilical artery Pi z score (+SEM) vs peripheral vascular resistance z score
5.3.3 Middle cerebral artery Doppler

There were no associations identified between maternal haemodynamic indices and middle cerebral artery $P_i$ (Table 5-2). Cardiac output (Figure 5-10) and peripheral vascular resistance Z score (Figure 5-12) were compared against middle cerebral artery $P_i$ z score, and no correlation was seen.

*Figure 5-10 Scatter diagram of cardiac output z score vs middle cerebral artery pulsatility index z-score*
Figure 5-11 Scatter diagram of peripheral vascular resistance z score vs middle cerebral artery pulsatility index z-score
5.4 Discussion

We demonstrate a relationship between maternal cardiovascular function and fetal-placental Doppler indices in a mixed population of healthy pregnancies and those affected by pre-eclampsia and/or fetal growth restriction. Specifically, low maternal cardiac output and high maternal peripheral vascular resistance are associated with raised impedance in the maternal uterine and fetal umbilical arteries (131). Interestingly, there was no relationship with fetal cerebral Doppler impedance indicating that the mechanism is unlikely to be mediated by hypoxia.

Uterine artery Doppler is classically taken to represent placental development through spiral artery invasion (3), with high impedance reflecting inadequate trophoblast invasion (132) and narrow spiral arteries (133). Its sensitivity is particularly poor for pre-eclampsia and fetal growth restriction occurring at term (20, 81, 87, 134-136). By contrast in this cohort where pre-eclampsia and fetal growth restriction were carefully phenotyped and compared to healthy pregnancies, uterine Doppler is only abnormal where fetal growth restriction is present (with or without pre-eclampsia) and is normal in pre-eclampsia where fetal growth restriction is not present (137); our present work adds evidence of the connection between abnormal and normal uterine Doppler velocimetry and maternal cardiac output phenotype: FGR with or without PE and PE are associated with a low and high cardiac output respectively (127). This relationship was only possible to unravel as recruitment was from an entire gestation range and both maternal cardiovascular and feto-placental Doppler indices were adjusted to remove gestational age as a confounder. Though PE with FGR is certainly
commoner at early gestations (138), it is the condition that defines the cardiovascular phenotype rather than the gestation at onset.

Although a relationship between pregnancies affected with both fetal growth restriction and pre-eclampsia and reduced cardiac output/high vascular resistance has been known for a decade (23, 139), the mechanisms underlying this observation remain unclear. One scenario places the placenta as the primary cause of the haemodynamic changes. On the fetal side, there is a reduction in nutrient and oxygen delivery leading to growth restriction. The reduced umbilical vein oxygenation leads to fetal hypoxia, triggering redistribution of the fetal cardiac output away from peripheral circulations to maintain perfusion to the fetal brain, characteristically increasing impedance in the umbilical artery and a relative reduction in that of the middle cerebral artery (47, 48, 140). On the maternal side, increased placental vascular resistance and impedance of the tertiary villi increases maternal uterine artery impedance contributing to an increase in maternal peripheral vascular resistance (141). This in turn leads to an increase in maternal cardiac afterload, opposing maternal cardiac output and could explain the established relationship between adverse pregnancy outcome, impaired cardiac output and high vascular resistance.

An alternative scenario, supported by a recently published preconception study from our group is that a low maternal cardiac output/high vascular resistance state initiates reduced placental perfusion with oxygenated blood, triggering the consequences described above on the fetal and maternal side of the placenta (86). This sequence is compatible with the idea of maternal cardiovascular rather than primary placental dysfunction being the origin of complicated pregnancy. The quality of the maternal cardiovascular function may determine the quality of the placental and fetal circulation, linking maternal cardiac output with changes
in the maternal uterine and fetal umbilical circulations. Further evidence for maternal cardiac output changes driving, rather than responding to the maternal uterine and fetal circulation, is that the effects are dose dependent. In other words, a lower maternal cardiac output and higher peripheral vascular resistance are associated with both a higher umbilical and uterine artery impedance; both parameters are important descriptors of fetal growth restriction (142). This is consistent with the observation that incremental change in cardiac output from early pregnancy onwards is associated with birth weight (89, 143).

Interestingly, we report that a reciprocal relationship between maternal cardiac output and impedance in the uterine and umbilical arteries also exists in the whole cohort which includes healthy pregnancy. This suggests that the interplay between maternal cardiac output may be a normal physiological regulatory mechanism. In the lung, hypoxic pulmonary vasoconstriction, also known as the von Euler–Liljestrand mechanism, is a physiological response to alveolar hypoxia that ensures the distribution of pulmonary capillary blood flow to alveolar areas of highest oxygen partial pressure. Therefore, perfusion is matched to ventilation in poorly and richly oxygenated parts of the lungs. It could be argued that within the placenta, to ensure efficient materno-fetal transfer of flow-limited oxygenation, the equivalent would be to match the level of maternal uterine arterial oxygenation with the magnitude of placental perfusion. Therefore, a reduction in oxygen delivery to the placenta via the maternal uterine arteries is matched by an increase in placental vascular resistance. This will slow the passage of blood through the placenta, improving gaseous exchange, the reciprocal relationship between maternal cardiac output and placental vascular resistance representing a von Euler–Liljestrand mechanism within the placenta. Since maternal arterial blood pressure is determined by the product of cardiac output and peripheral vascular
resistance, a fall in maternal cardiac output is buffered by an increase in maternal peripheral vascular resistance to maintain maternal arterial blood pressure. Thus, it is plausible that a direct relationship between increased maternal peripheral vascular resistance and increased placental vascular resistance represents an analogous physiological mechanism. Low maternal CO equates to lower uterine blood flow and hence reduced oxygen availability to the fetal-placental unit-the oxygen content in blood is not reduced per se. This is important when one considers that human and animal data suggest that IUGR fetuses show lower oxygen extraction (144). This acute localized adaptation should be distinguished from chronic generalised hypoxia, which leads to pulmonary hypertension.

There are limitations to our interpretation of these findings. The correlations though highly significant are of moderate strength and suggest that contributions other than maternal cardiovascular function are important in modulating fetal-placental impedance. It may be that the relationships hold particularly in the case of pathological pregnancy associated with fetal growth restriction and pre-eclampsia; these cases are by their nature rare. Some Doppler values were missing-this was not a systematic bias but rather reflected the challenge of obtaining a full set of maternal and fetal cardiovascular observations in an acute setting.

These findings have relevance in surveillance of compromised pregnancies. If cardiac output does in part determine fetal circulatory changes, this raises a question about what might happen in the late third trimester when cardiac output reduces from its peak value (18). In normally grown fetuses that are stillborn, uterine and umbilical artery Doppler impedance have been shown to be higher than those liveborn (145). Might this reduction in maternal cardiac output imperil the utero-placental circulation and be a mechanism for unexplained still birth?
These findings also have potential therapeutic relevance. In later pregnancy, treatment with negatively inotropic drugs such as beta blockers have been associated with fetal growth restriction and stillbirth (94), perhaps through a direct effect on the utero-placental and fetoplacental circulations. Vasodilator drugs do not have a primary negatively inotropic mode of action and are effective in the treatment of acute hypertension in pregnancy (91) and plasma volume expansion combined with vasodilator therapy in women with high vascular resistance and fetal growth restriction is reported to increase fetal growth (96). This raises the possibility of intervening to optimize maternal cardiovascular function prior to or in established pre-eclampsia and/or fetal growth restriction.

Uterine artery Doppler’s relationship with pathological pregnancy has, from the first studies of three decades ago, been troubled by an apparent contradiction: its usefulness in screening ‘early onset’ PE and FGR but not for late onset complications (146). The explanation appears to be linked closely to the cardiovascular characteristics associated with the specific subtype of fetal growth restriction and/or pre-eclampsia. We suggest that the differential performance of uterine artery Doppler arises because it is most frequently abnormal in FGR and FGR associated with PE; these conditions co-exist particularly frequently prior to 34 weeks. Our data support that uterine artery Doppler impedance is no different from that of healthy pregnancy in ‘pure’ PE, unaffected by FGR, and this is more common at later gestation.

In conclusion, uterine and fetal placental Doppler indices are significantly associated with maternal cardiovascular function. Though we cannot ascribe causality, emerging evidence supports cardiovascular dysfunction preceding fetal growth restriction and pre-eclampsia—possibly even from prior to pregnancy rather than their resulting from the conditions. The
classical description of uterine and fetal Doppler changes being initiated by placental mal-development is a less plausible explanation for the pathogenesis of the conditions than that relating to maternal cardiovascular changes.
Chapter 6 Longitudinal and Postnatal Changes in pathological pregnancies

6.1 Introduction

Little is known about acute changes in maternal cardiac parameters once PE and/or FGR is diagnosed. Once the diagnosis of PE+FGR is made, on average, delivery will likely be indicated based on maternal or fetal indications within the following two weeks. In PE diagnosed at or after term, clinical management is largely to deliver the baby having stabilised the mother. It is tempting to speculate whether subtle changes in cardiovascular parameters can be observed prior to deterioration in maternal or fetal clinical condition. If this can be demonstrated, optimisation of clinical condition prior to delivery is therefore possible, e.g. in-utero transfer to a tertiary neonatal unit if applicable, timing of administration of intramuscular steroids for fetal lung maturation and magnesium sulphate infusion for neuroprotection. However, there are currently no studies in the literature examining cardiovascular parameters longitudinally once PE and or FGR has been diagnosed to immediately prior to delivery. Most longitudinal studies in the literature performed an initial assessment in the first trimester followed by a second assessment when pathology is diagnosed (147). They were largely designed to assess if maternal cardiovascular parameters may serve as an effective screening tool or to help with risk stratification of antenatal care. A study by Stott assessed longitudinal cardiovascular parameters changes 1 hour and 24 hours following treatment with labetalol found that cardiac output largely remained stable even post treatment, but there was a fall in heart rate and MAP (95).
Persistence of hypertension, residual cardiac dysfunction and abnormal endothelial function is well defined in women who had PE in their previous pregnancies (73, 148). Melchiore et al assessed women with PE in pregnancy at one year postpartum and demonstrated significant asymptomatic left ventricular moderate-severe dysfunction/hypertrophy (149, 150). Similarly, mean arterial blood pressure, though in normal range, remains elevated when compared to women with uncomplicated pregnancies. A separate study assessing women at 6 months post-partum after a diagnosis of severe PE reported that despite being clinically normotensive, biventricular dysfunction was present on echocardiogram examination (151).

This suggests that the cardiovascular changes seen acutely at diagnosis might not fully return to baseline even with cessation of pregnancy (152). No study has accurately characterised the immediate transformation of cardiac parameters following delivery of the baby. This knowledge is important as there is currently no evidence on which to base postnatal management of women with hypertension (153). The ISSHP (International society for the study of hypertension in pregnancy) recommends that in the immediate 3 days post-delivery, women should have four-hourly blood pressure and kept under observation.
6.2 Cardiovascular parameters at diagnosis to delivery to postnatal

For the analysis of this specific cohort, women were divided into two subgroups – PE with appropriately grown fetus and those with fetal growth restriction (with or without PE). This is because these two groups have opposing cardiovascular phenotypes (see Chapter 4).

Longitudinal measurements comparing diagnosis to “decision to deliver” was available in 5 patients. (PE, n=1; FGR +/- PE, n= 4) Decision to deliver was determined purely on clinical grounds, and usually represent a deterioration in maternal/ fetal conditions. In the pregnancy with PE, diagnosis to delivery interval was 6 weeks. In the pregnancies with FGR +/- PE, diagnosis intervals (in gestation weeks) were 4,4,2 and 1 respectively.

Postnatal measurements comparing diagnosis to postnatal period was available in 9 patients. (PE, n=2; FGR +/- PE, n=7) All assessments were completed between 6 to 8 weeks post-partum.

Due to the small numbers, descriptive statistics and paired t-test were used to describe the findings in each group. Longitudinal statistical analysis was not possible in this sample size. Comparison between pathological outcome groups and their deltas were made using 2-way repeated measures ANNOVA test with Graphpad software. The red line in each chart denotes the mean value derived from healthy pregnancy controls across the whole cohort studied.
6.2.1 Cardiac Output (Standing and lying)

In FGR+/-PE, the mean cardiac output lying (litres/min) fell from 6.6 to 5.9; p=0.18 (Figure 6-2) whilst the corresponding cardiac output standing also fell from 4.9 to 4.3; p=0.20 from diagnosis to delivery. (Figure 6-1) In the case with PE diagnosed at 25 weeks gestation, delivery was indicated for abnormal maternal biochemistry at 31 weeks. There was a drop in CO standing (litres/min) from 8.5 to 8.0. Similarly CO lying fell from 8.7 to 7.0. (Table 6-1)

When assessing delta CO, both the effect of time (lying, p=0.07; standing, p=0.28) and effect of disease (lying, p=0.20) on the differences observed from diagnosis to delivery were non-significant. However, when assessing changes by PE vs FGR +/- PE grouping, then effect of this separation contributed significantly to the differences in CO standing observed from diagnosis to delivery. (p=0.04)

Postnatally, cardiac output standing appeared to fall in both groups with delta (diagnosis to postnatal) in PE (mean -1.56litres/min) and FGR +/-PE (mean -0.41litres/min); p=0.25 likely representing normalisation to baseline.
<table>
<thead>
<tr>
<th></th>
<th>PE n=1</th>
<th>FGR +/- PE (mean) n=4</th>
<th>P value</th>
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<tr>
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<td>8.7</td>
<td>6.6</td>
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<td>P=0.20</td>
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<tr>
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<td>4.3</td>
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<td>334</td>
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</tr>
</tbody>
</table>

Units: $CO$ (l/m)  $TPR$ (dyn·s/cm$^5$)

Table 6-1 Cardiac output and total peripheral resistance from diagnosis to delivery

Figure 6-1 Cardiac output (standing) from diagnosis to delivery to postnatal
6.2.2 Total peripheral resistance (standing and lying)

In FGR+/-PE, the mean total peripheral resistance (TPR) standing (dynes/second/cm$^5$) increased from 1510 to 1834; $p=0.33$ (Figure 6-3) whilst the corresponding mean TPR lying also increased from 1162 to 1363; $p=0.27$ from diagnosis to delivery. (Figure 6-4)

In the case with PE, TPR standing (dynes/second/cm$^5$) increased by 41 and TPR lying increased by 133 from decision to delivery.
When assessing delta TPR, both the effect of time (lying, p=0.24; standing, p=0.49) and effect of disease (lying, p=0.41; standing, p=0.12) on the differences observed from diagnosis to delivery were non-significant.

Postnatally, women with PE positively incremented their TPR standing by a mean of 371; whilst TPR in women with FGR +/- PE also showed a modest increase of 107. (p=0.29)

Figure 6-3 Total peripheral resistance (standing) from diagnosis to delivery to postnatal
6.2.3 Mean arterial pressure

Mean arterial pressure (MAP, mmHg) was obviously elevated in both groups when compared to average MAP across the healthy cohort, as they comprised of women with diagnosis of PE. In the group with FGR +/- PE, the mean MAP increased from 95.4 to 97.8 from diagnosis to delivery, suggesting maternal decompensation. In PE, the woman studied was well established on antihypertensive treatment (labetalol) by the time decision to deliver was made, therefore her MAP fell from 105.6 at diagnosis to 96.6 at the time of delivery.
Postnatally, women with PE saw a mean fall in MAP of 10.7. In the other group with FGR +/- PE, MAP fell by a modest -1.6. This is largely skewed by 2 cases whereby MAP remained elevated well into the postnatal period.

Figure 6-5 Mean arterial pressure from diagnosis to delivery to postnatal
6.2.4 Heart rate

In general, there was a wide variation of heart rate amongst the cohort of women studied.

Mean heart rate appeared to remain constant from diagnosis to delivery in both groups (92 beats/min). This was similar to the average heart rate in the healthy cohort (88 beats/min).

In the postnatal period, heart rate in women with PE appears to fall on average by 6 beats/min; whilst those with FGR+/ PE kept their heart rate constant from diagnosis to the post-partum period.

Figure 6-6 Heart rate from diagnosis to delivery to postnatal
6.3 Arterial function at diagnosis to delivery to postnatal

Arterial function is known to be abnormal in all pathological cases. However, the degree of deviation from normality varies on an individual basis.

6.3.1 Augmentation index

In women with FGR +/- PE, augmentation index (AiX) increased from a mean of 13.5 at diagnosis to 20.3 just prior to delivery. In the woman with PE, similar trend was observed where her AiX increased from 2 to 21.

![Augmentation index from diagnosis to delivery to postnatal](image-url)
Postnatally, AIX fell by -15.5 in the women with PE but remained constant with a delta of 1.3 in women with FGE +/-PE.

6.3.2 Pulse wave velocity

In women with FGR +/- PE, mean pulse wave velocity (m/s) increased from 7.4 at diagnosis to 7.9 at delivery. Similarly, the woman with PE incremented her pulse wave velocity from 5.8 to 7.3.

Postnatally, pulse wave velocity remained constant in the women with PE. In the group with FGR +/- PE, pulse wave velocity fell by a modest -0.39 in the postpartum period.

Figure 6-8 Pulse wave velocity from diagnosis to delivery to postnatal
6.4 Discussion

There were no statistically significant changes in cardiac output, TPR, MAP, heart rate, augmentation index nor pulse wave velocity from diagnosis to delivery to 6 weeks postpartum when compared directly. However, repeated measures ANNOVA showed a positive effect on cardiac output (standing) when grouped by disease type; where despite taking into account the baseline opposing cardiac phenotypes of PE from FGR +/- PE, there remains significant differences in mean values from time of diagnosis to delivery to postnatal period.

It was difficult to draw firm conclusions from the data as numbers analysed were small. In particular, in PE, most of these cases were diagnosed at term and therefore longitudinal studies were not possible as the clinical management would usually involve immediate induction of labour once maternal condition is stabilised.

The longitudinal changes in CO illustrates the fact that pregnancies with PE follow a different trajectory in cardiovascular phenotype when compared to those with FGR +/- PE. It is plausible that the co-existence of fetal growth restriction changes the adaptive mechanism. The maintenance of utero-placental circulation requires the persistence of high TPR in an intravascularly deplete mother, and correspondingly, this will lead to a fall in CO when the severity of the pathology worsens, leading to a decision to deliver. In contrast in PE, TPR is already low as a result of high CO with features of volume overload; and therefore at extremis, unable to increase TPR further, the maternal CO falls as decompensation occurs.
The postpartum findings of this study can be considered in tandem with those of “Conceive study” using the same cardiovascular assessment protocol with a 6-week postnatal assessment. Findings are comparable where MAP showed a drastic drop from the third trimester however remained elevated postnatally in patients who had PE and or SGA babies (7). This is important as traditionally postnatal care provision arbitrarily ends six weeks postnatally and women are then discharged back to primary care. A snapshot at this time points clearly shows that cardiovascular parameters remain abnormal and could persist well into one year postnatal and beyond. Ambulatory blood pressure performed postnatally at 6-12 weeks has been suggested as a strategy to risk stratify those women with subclinical hypertension who are more likely to require pharmacological treatment (154). Where available, home blood pressure monitoring is an effective and easy way to provide adequate follow up and screen for those women that need more intensive care and tertiary referral.

Nonetheless, any diagnosis of PE or FGR should prompt lifestyle intervention and health education to reduce long term cerebral and cardiovascular risk factor in later life. Indeed, longer term follow up might be indicated as up to 40% of women develop essential hypertension between 1-2 years postnatally (149). Profiling of cardiac function in women with PE and FGR during the immediate postnatal period and establishing its associations with short- and long-term complications would be an important initial step to guide the development of postnatal care pathways.
Chapter 7 Exercise changes in pathological pregnancy

7.1 Introduction

Pregnancy can be regarded as a “stress test” that unmasks previously subclinical pathology. Underlying cardiovascular and metabolic maladaptation increases the risk of developing pregnancy complications such as gestational diabetes. Exercise serves to further exaggerate any subtle changes, and therefore could be useful in delineating severity of pathology. Data from animal and human studies suggest that pregnancy-associated and exercise-induced cardiac hypertrophy shares similar characteristics such as structural remodelling, reversibility and common signalling pathways. Therefore, whilst not directly comparable, acute exercise which alters cardiac loading conditions and haemodynamics can mimic the physiological changes observed during the latter half of pregnancy (e.g. increased CO, decreased peripheral resistance).

In order to understand basic cardiac physiological changes in exercise, the following formulas are key:

\[ \text{MAP} = \text{Cardiac output} \times \text{total peripheral resistance}. \]

\[ \text{Cardiac output} = \text{Heart rate} \times \text{stroke volume} \]

The maternal cardiovascular system undergoes adaptation during period of aerobic exercise. In healthy pregnancies, an increase in stroke volume and cardiac output is offset by peripheral vasodilation during acute resistance exercise therefore keeping blood pressure constant (155). Little is known about maternal haemodynamic function in response to exercise in pregnancies with PE and or fetal growth restriction. Exercising after a diagnosis of
hypertensive disorder in pregnancy might be beneficial by improving placental angiogenesis and endothelial function, (156) and aerobic exercise is not thought to be harmful (157). However, previous guidelines from the ACOG cautions against exercising once hypertension is diagnosed in pregnancy, due to concerns that exercise will further increase the mean arterial blood pressure. They also cite epidemiological studies linking aggressive exercise to pregnancies with fetal growth restriction (158). This is supported by a small study demonstrating a restriction of maternal activity over a period of 4 weeks in normotensive pregnant women reduced overall PVR and therefore potentially enhancing fetal growth (159).

7.2 Study specific methods and population

Ethical permission was granted to perform simple exercise step test on pathological cohort.

All patients with PE, FGR and PEFGR were questioned if they felt well enough to undergo light exercise for 3 minutes. Those who provided consent were briefed on the methods. They were informed to terminate the test if they felt unwell or unable to continue.

Dundee step test has been extensively validated in cardiovascular research outside pregnancy (63). It was originally designed for participants across all age groups regardless of cardiovascular fitness. The step measured 17.5cm and the step rate was set at 23 steps/minute.
The participants were instructed to step up and down the prescribed step in 4 distinct moves guided by a metronome set at 92 beats/minute. With the first ‘click’ of the metronome, the subject lifted the right foot onto the step, followed on with the left foot with the second ‘click’. With the third ‘click’, the subject lowered the right foot onto the floor and finally lowering the left foot with the fourth ‘click’. After 2.5 minutes, the investigator attaches the pulse oximeter, nose clip and hands the participant the mouthpiece for the Innocor machine. The participants are then asked to undergo Innocor testing at 3 minutes.
Figure 7-2 Performing Innocor testing at the end of exercise step test protocol
7.3 Results

In total, 12 participants underwent exercise testing (PE = 2, FGR = 5, PEFGR = 5). Comparison was made between cardiovascular parameter performed at rest and those immediately after exercise in each individual participant.

As the numbers are small, descriptive statistic and Kruskal-Wallis were used to compare the means and SD between the 3 groups.

7.3.1 Cardiac Output

In PE, the mean increase of CO from rest to post exercise was 7.25 litres/min (SD = 2.47). In FGR delta CO was 6.16 (SD = 1.51), whilst in PEFGR mean increase was 5.08 (SD = 1.53). Kruskal-Wallis showed no difference between the 3 groups. (p=0.53) There was no difference between the groups of PE vs FGR (p=0.49) nor PE vs PEFGR (p=0.20).
Figure 7-3 Cardiac output at rest and post exercise in PE, FGR, PEFGR
7.3.2 Total peripheral resistance

In PE, the mean reduction of total peripheral resistance (TPR) from rest to post exercise was 447.88 dynes/seconds/cm$^5$ (SD = 277.67). In FGR delta TPR was -920.01 (SD = 433.16), whilst in PEFGR mean change was -914.36 (SD = 294.14). Kruskal-Wallis showed no difference between the 3 groups. ($p=0.27$) There was no difference between the groups of PE vs FGR ($p=0.22$) nor PE vs PEFGR ($p=0.11$).

*Figure 7-4 Delta cardiac output from rest to post exercise*
Figure 7-5 Total peripheral resistance at rest and post exercise in PE, FGR, PEFGR
Figure 7-6 Delta total peripheral resistance from rest to post exercise

7.3.3 Mean arterial pressure

In PE, the mean increase of mean arterial pressure from rest to post exercise was 14.67mmHg (SD =1.41). In FGR delta mean arterial pressure increase was 6.87mmHg (SD =13.64), whilst in PEFGR mean change was 1.4mmHg (SD=19.7). Kruskal-Wallis showed no difference between the 3 groups. (p=0.66) There was no difference between the groups of PE vs FGR (p=0.41) nor PE vs PEFGR (p=0.48).
Figure 7-7 Mean arterial pressure at rest and post exercise in PE, FGR, PEFGR
7.3.4 Heart Rate

In PE, the mean increase of heart rate from rest to post exercise was 9 beats/min (SD = 2.8). In FGR delta heart rate was 12.4 (SD = 13.5), whilst in PEFGR mean change was -4.0 (SD=14.2). Kruskal-Wallis showed no difference between the 3 groups. (p=0.42) There was no difference between the groups of PE vs FGR (p=0.75) nor PE vs PEFGR (p=0.28).
Figure 7-9 Heart Rate at rest and post exercise in PE, FGR, PEFGR
Figure 7-10 Delta heart rate from rest to post exercise
7.4 Discussion

In PE, FGR and PEFGR, there were no significant statistical differences in increment of CO, TPR, MAP and heart rate from rest to post exercise. There were however trends emerging when the effect of exercise was examined in relation to cardiac output in 3 pathological pregnancy outcome groups. The post exercise increment of cardiac output from baseline was highest in PE > FGR >PEFGR. If it is indeed true that pure PE is linked to a hyperdynamic circulation, this could explain the bigger increment of CO from resting state. A successful increment of CO post exercise is vital to ensure that utero-placental circulation is maintained despite the increasing anaerobic demands of exercise.

Conversely, the fall of TPR appears to be biggest in PE when compared to FGR and PEFGR. This could be because TPR is significantly higher in FGR and PEFGR at rest; and therefore post exercise, absolute TPR values reached a similar nadir across all 3 groups.

Cardiac output typically increases when a healthy woman exercises in pregnancy, due to a corresponding increase in stroke volume and heart rate. This however, leads to a fall in TPR to keep the MAP constant. Reassuringly, MAP did not increase significantly post exercise in all 3 groups in our study. This is an important finding, as the precipitous increase in blood pressure is the basis of not recommending exercise in women with hypertensive disorders of pregnancy.
Heart rate appeared to increment appropriately post exercise in all 3 groups. This increment was less in PEFGR women, who might not be able to mount an adequate response to the increasing cardiovascular demands of exercise. In this group, the increase in stroke volume is likely to be the main contributory factor towards maintaining adequate cardiac output to perfuse the utero-placental circulation. At very high exercise intensities, there might also be blunted sympato-adrenal response, leading to failure of incrementing to a maximal heart rate.

Exercise has been proven to improve cardiovascular function and reduce rate of hypertension in men and women outside pregnancy (160). This could be by reducing insulin sensitivity and triglyceride levels. Certainly pre-pregnancy, exercise as a lifestyle intervention is logical as women who embark on a pregnancy normotensive, normo-glycaemic and with a normal BMI tend to have better pregnancy outcomes. Even in the first trimester, women who are physically active are found to be at a lower risk of developing PE compared to those who are sedentary (161). Potential mechanisms include improved endothelial function, better placentation, as well as by regulating maternal immune and inflammatory responses.

Exercise in pregnancy has been studied mostly in relation to prevention and treatment of gestational diabetes, with inconclusive results (162, 163). This is likely due to difficulty in assessing the impact of behavioural intervention on a multi-factorial disease. Indeed, assessment of cardiovascular parameters is limited by contributory factors such as gestational age, position during exercise, pre-pregnancy fitness and exercise intensity.
In pathological pregnancy, it is attractive to recommend exercise as an easy adjunctive treatment, seeing as currently the only definitive “cure” for PE and FGR is delivery of the baby. However, conflicting results have been shown in studies, with one study suggesting that vigorous physical activity in pregnancy is linked to development of PE (164). Conversely, a study done showed women who exercised regularly in pregnancy had a statistically significant higher level of placental growth factor (PLGF) thus the ability of restoring angiogenic balance in PE and improving pregnancy outcomes (165).

Our study provides a snapshot of cardiovascular adaptation to exercise in pathological pregnancy. To more accurately assess fitness levels in pregnancy, women should have a regime tailored taking into account their VO2 max (maternal maximal oxygen uptake) (166). Previously, this was not thought to be safe due to concerns with utero-placental mal-perfusion; however examination of fetal umbilical and maternal uterine Doppler post exercise has been shown not to be adversely affected by exercise (167, 168).
Chapter 8 Biomarkers in normal and pathological pregnancy outcomes

8.1 Immunological markers – BAFF (B cell activating factor)

Abstract

The objective of this study was the analysis of B-Cell Activating Factor (BAFF) levels in pregnancies affected by PE, and in pregnancies affected by fetal growth restriction without hypertensive disorders and its possible correlation with pulse wave velocity and cardiac output.

This was a prospective study of 69 women at 24-40 weeks gestation. Haemodynamic function was assessed in those with Pre-eclampsia (PE, n = 19), fetal growth restriction (FGR, n = 10) and healthy pregnancies (n = 40). Maternal venous BAFF levels at recruitment were measured using ELISA. We analysed the relationship between BAFF and cardiac output (CO), and BAFF and PWV (pulse wave velocity); the gold standard for assessing arterial stiffness. PWV was measured with an oscillometric device and CO using inert gas rebreathing technique. PWV and CO were converted to gestation adjusted indices (z scores).

BAFF was higher in PE (p = 0.03) but not in FGR (p = 0.83) when compared to healthy pregnancies. There was a positive correlation between BAFF levels and z score PWV (r = 0.25, p = 0.04), but not CO (r = -0.01, p = 0.91). BAFF levels did not change with gestational age. (r = 0.012, p = 0.925).

These findings provide evidence of a possible contribution of BAFF to both maternal inflammation and arterial dysfunction associated with PE. Though no relationship was found with another disorder of placentation: normotensive FGR, this condition is not thought to be associated with maternal inflammation.

The following section is based on the following publication:

Tay J, Costanzi A, Basello K, Piuri G, Ferrazzi E, Speciani AF, Lees CC

Maternal Serum B Cell activating factor in hypertensive and normotensive pregnancies

8.1.1 Introduction

Pre Eclampsia (PE) is a multi-system disorder that affects the pregnant mother and sometimes the fetus. The underlying pathophysiology is not fully understood, though the most widely accepted hypothesis relates to inadequate physiologic transformation of the uterine spiral arteries secondary to poor trophoblastic invasion of maternal tissues. This leads to release of placental factors into the maternal circulation with endothelial damage caused by pro-inflammatory cytokines derived from visceral fat, dysbiosis and placental oxidative stress. Though the link between inflammation and abnormal vascular function is hypothesized, there is limited mechanistic evidence to support this (169, 170).

B-cell activating factor (BAFF) is a cytokine that belongs in the Tumour Necrosis Factor (TNF) family, with a role in proliferation and differentiation of B cells. BAFF is an immune-stimulant and in many autoimmune conditions, BAFF levels are elevated. Previous studies in rat models have demonstrated the role of B cell involvement in PE via activation of pro-inflammatory cytokines (171). More recently, elevated first trimester BAFF levels have been shown to be associated with hypertensive disease in later pregnancy (172). An increase in BAFF leads to elevated B cells and subsequently autoantibody and immunoglobulin secretion which is associated with vascular and cardiac remodelling in hypertension (173). Moreover, BAFF could also be involved in the development of human placenta and functions as an autocrine and paracrine hormone towards local placental cells (174). However, it is unclear as to whether BAFF is implicated in the disease process of hypertensive pregnancies or whether it may have a role as a biomarker. Hypertension in pregnancy is associated with arterial dysfunction. Large artery stiffness is evidenced by higher pulse wave velocity when compared to normotensive pregnancies (33). Pulse wave velocity (PWV) is the non-invasive gold
standard measure of arterial stiffness and is predictive of future cardiovascular risk (175). We sought to analyse BAFF values in pregnancies affected by PE, and those affected by fetal growth restriction; and to investigate the relationship between BAFF and pulse wave velocity and cardiac output in normotensive and hypertensive pregnancies.

### 8.1.2 Study specific methods and population

In this prospective study from April 2015 through to April 2017, 69 women between 24-40 weeks gestation were recruited. They were subdivided to three groups - pre-eclampsia (PE), fetal growth restriction (FGR) and those with healthy pregnancies. Those with multiple pregnancies, chronic medical problems, known underlying cardiovascular conditions and fetal anomalies were excluded. PE was defined as new onset hypertension after 20 weeks with blood pressure of >140/90 mmHg and urine protein creatinine ratio of >30. Fetal growth restriction was defined as fetal abdominal circumference <10th centile and umbilical Doppler pulsatility index (PI)>95th centile on ultrasound scan (67, 83, 176). None of the women in the PE group satisfied the diagnosis for FGR and none of those with FGR satisfied the criteria for PE, hence the groups were mutually exclusive.

Arterial function was assessed by the Vicorder system (Skidmore Medical, Bristol, UK). Whilst lying in the left lateral supine position, three cuffs were attached to the left upper arm, the left upper thigh and over the left carotid pulse respectively. The linear carotid femoral distance was measured with a standard measuring tape. Pulse wave velocity was recorded. With the patient standing upright, cardiac output was measured with Innocor (Innovision A/S,
Denmark) (85, 177), a non-invasive inert gas rebreathing technique previously validated against thermodilution (107).

Maternal venous blood was obtained from peripheral venepuncture and serum stored at -80 Celsius. Upon completion of all sample collection, samples were thawed and pipetted in 50 microlitres on dried blood spot cards (50 microlitres per spot), then let air-dry 30 minutes. Following that, 6 mm punch circles were collected in 1.5 ml tubes and PBS was then added for elution (25 microlitres per 6 mm circle). Tubes were vortexed and the supernatants were recovered into new tubes for processing. BAFF was measured in triplicate according to the corresponding ELISA kit protocol (R&D Systems, Minneapolis, USA). The manufacturer reports lower limits of detection of 2.68 pg/ml with intra-assay precision of 5.7% and inter-assay precision of 10.5%.

The main outcome measures were the association between BAFF levels in PE and FGR. Descriptive non-parametric statistics was applied for basic demographic and Kruskal- Wallis p-value was used to test differences between groups. BAFF concentration in PE, FGR and healthy pregnancies was analysed by a univariate test and reported as box and whiskers plot.
8.1.3 Results

Nineteen patients with pre-eclampsia (PE) and ten patients with fetal growth restriction (FGR) were recruited with forty cases of healthy pregnancies. Kruskal-Wallis test showed no statistically significant differences between maternal age and gestational age at recruitment.

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<td>12 (30%)</td>
<td>7 (70%)</td>
<td>11 (57.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 8-1 Demographic table of participants

We found that BAFF levels were higher in women with PE (mean 0.22 ng/ml, SD 0.06) compared to healthy controls (0.18 ng/ml, SD 0.06 - p=0.03). BAFF levels were no different in women with normotensive FGR (0.18ng/ml, SD 0.06) (p=0.83). [Figure 8-1] There was no correlation between BAFF levels and gestation of sampling within this third trimester cohort. (r=0.012, p=0.925)
PWV (Z score corrected for gestation) was higher in PE (1.049, SD 1.49) when compared to healthy controls (0.039, SD 0.879) (p=0.0018). There was no difference in PWV between FGR cases and healthy controls. (p= 0.15) There was a correlation between PWV (Z score corrected for gestation) and BAFF levels in the cohort (r=0.25, p=0.04). [Figure 8-2]
There was no significant difference in CO (Z score corrected for gestation) between PE (0.41, SD 1.65) and healthy controls (-0.01, SD 1.09) (p=0.25). Furthermore, CO did not correlate with BAFF levels (r=0.01, p=0.91).
8.1.4 Discussion

BAFF is a key modulator of B cell function and was higher in PE when compared to healthy pregnancies or FGR. PWV was higher in PE than FGR and healthy controls; in the whole group there is a significant positive association between PWV and BAFF. This suggests a plausible link might exist between inflammation and arterial dysfunction particularly as inflammation is thought to be related specifically to pre-eclampsia but not normotensive FGR. The relationship between PWV and BAFF is of potential importance as PWV is a known marker of cardiovascular risk in hypertension and has been used as a predictor of future cardiovascular mortality (103). Our findings support those previously reported where arterial stiffness is found to be elevated in pre-eclampsia (33). There is conflicting data regarding cardiac output changes in pre-eclampsia, some studies suggesting higher CO and others low CO (21, 128, 177). This may explain the lack of association between CO and BAFF in our cohort which was not sufficiently large to subdivide for different clinical phenotypes of preeclampsia (178).

BAFF plays a key role in regulating immune system. This cytokine, belonging to the TNF family, has pro-inflammatory action (179) and modulates many functions of the immune system and in particular of B cells. Autoimmune diseases including haemolytic anaemia, systemic lupus erythematosus and rheumatoid arthritis have been associated with an increase in BAFF serum levels (180-182). In addition, BAFF plays a role in the development of the human placenta (174) and this activity is probably mediated by its action on the endothelium (183). Other studies hypothesize the role of this inflammatory molecule in the pathogenesis of atherosclerosis (184) and the mechanisms leading to hypertension through a greater production of angiotensin II (185).
Though inflammation has been much discussed as underlying the pathogenesis of PE (186), there is little understanding of the mechanism by which this might be and how inflammation affects endothelial function. PE and FGR are thought to be placental disorders (3, 187), however it is becoming clear that they are associated with different cardiovascular phenotypes (19, 78). Through BAFF’s key role in B cell activation (188) and a recent report of its possible use as an early biomarker of PE (172), it is a plausible candidate linking inflammation and the arterial dysfunction that characterizes PE but not the placental insufficiency that characterizes FGR.
8.2 Immunological markers – PAF (Platelet activating factor)

8.2.1 Introduction

In PE, many angiogenic factors have been proposed to play an important role in altering maternal vascular status (186). Platelet activating factor (PAF) was first proposed in the early 90s as a potential biomarker expressed in PE (189). It is a phospholipid that plays an important role in thromboxane production, platelet aggregation and destruction, all of which are implicated in potential pathogenesis of PE.

Other related studies have previously examined PAF RNA expression in placentas post-delivery and found no difference in those from women with PE when compared to those with healthy pregnancies (190). However, plasma studies from neonates of mothers with PE showed significantly higher levels of PAF (191). Those studies were limited as earlier laboratory technique had to rely on indirect measurements of PAF concentrations through inhibitory functions.

More recently, in a study of 11 women in the third trimester, PAF levels was found to be significantly higher in women with PE compared to healthy pregnancies (192). PAF in relation to FGR has never been studied previously.
8.2.2 Study specific methods

In this prospective study from April 2015 through to April 2017, 69 women between 24-40 weeks gestation were recruited. This was the same cohort as that recruited to the BAFF study above. [Table 8-1] Women were subdivided to three groups - pre- eclampsia (PE), fetal growth restriction (FGR) and those with healthy pregnancies.

Maternal venous blood was obtained from peripheral venepuncture and serum stored at -80 Celsius. Upon completion of all sample collection, samples were thawed and pipetted in 50 microlitres on dried blood spot cards (50 microlitres per spot), then let air-dry 30 minutes. Following that, 6 mm punch circles were collected in 1.5 ml tubes and PBS was then added for elution (25 microlitres per 6 mm circle). Tubes were vortexed and the supernatants were recovered into new tubes for processing. PAF was measured in triplicate according to the corresponding ELISA kit protocol (R&D Systems, Minneapolis, USA).

Analysis was undertaken within a control cohort and our cohort to ensure that there was no gestation effect in PAF levels. (ANOVA F=2.053 P=0.12 R square=0.1461)
8.2.3 Results

In women with PE, PAF values were higher (14.74 ± 5.74) than in those with healthy pregnancy. (8.61 ± 1.48, p= 0.002)

*Figure 8-3 PAF levels in Pre eclampsia vs healthy cohort*
In FGR, PAF values were higher (14.89 ± 3.745) than in healthy controls (6.399 ± 1.817); (p=0.04).

![PAF level in normal vs FGR](image)

*Figure 8-4 PAF levels in fetal growth restriction vs healthy cohort*

ROC curve analysis was performed using PAF levels in participants with PE. PAF proved to be a plausible screening tool for diagnosing PE. For values higher than 10 ng/ml the sensitivity was 90% with a specificity of 70% (area 0.8125 p = 0.0024).
Figure 8-5 ROC curve analysis of PAF in diagnosis of PE
8.2.4 Discussion

PAF levels were significantly higher in both PE and FGR when compared to healthy pregnancies. It performed well as a screening tool for PE, with a high level of sensitivity and specificity.

PAF may serve as a marker for the increased thromboxane production, with its resultant vasoconstriction and platelet aggregation. This is frequently seen in clinical manifestation of PE. An increase in PAF expression also suggests presence of a chronic inflammation status and higher oxidative stress, which may potentially link to related metabolic complications in later life. The elevated PAF in pathological cases could be linked to pathogenic mechanism of platelet activation, and could provide plausible explanation of the recently demonstrated beneficial use of aspirin as a prophylaxis to reduce the incidence of preterm PE (193).

PAF could potentially be adopted as a screening tool to detect PE and or FGR prior to onset of clinical symptoms. Given the small numbers in our cohort, larger studies will be needed to examine longitudinal changes, as well as confirm its reproducibility as a screening test in a larger group of women with PE and/or FGR.
8.3 Metabonomics – exploratory work

8.3.1 Introduction

Metabolomics has been widely regarded as the next prominent emerging field of research whereby metabolome profiling is used to identify potential biomarkers which could be translated into clinical use. Biomarkers can be used as screening tests to predict susceptibility to PE and or FGR, or indeed, be used to monitor disease progression (194).

Using metabolomics as a phenotyping approach is seen as being complementary to transcriptomics and proteomics. However, small changes in upstream processes amplify changes in metabolites, potentially making it more sensitive than other omics techniques.

Human metabolome is also considered to be more dynamic in reflecting changes in the human phenotype, and therefore an improved indicator of evolving pregnancy complications.

Metabolites are routinely characterised as small molecules of less than 1 kDa in size. These can be detected in bodily fluids including serum, plasma and urine using techniques such as mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy.

A recent study identified a sub-panel of metabolites associated with PE. However, this association was much harder to prove in FGR (195). No studies have characterised maternal cardiovascular function and metabonomic profile in PE and FGR, and their inter-relationship.
8.3.2 Study specific methods and population

The same cohort were recruited for this part of the study, where the participants consented and samples successfully obtained.

A total of 33 pathological pregnancies (PE, FGR and PEFGR) and 66 healthy pregnancies were sampled. Where participants are seen more than once, only the sample at the first visit was incorporated into the analysis.

Following phlebotomy, whole blood was collected into lithium heparin tubes, which were left horizontally on ice for 30 min before being centrifuged at 1200 x g at 4°C for 10 min. Supernatants were aliquoted and immediately stored at -80°C until NMR analysis. Midstream urine samples were collected in a universal sample and placed immediately on ice before being aliquoted and frozen at -80°C within 30 min of collection.

Sample preparation

Plasma and urine samples were prepared and analysed using standardised and optimised protocols. Briefly, plasma samples were thawed at room temperature before 300 μL aliquots were mixed with 300 μL of buffer (Na2HPO4*7H2O, 0.75 M, pH 7.4), containing 4.6 mM 3-(trimethylsilyl) propionate-2,2,3,3-d4 (TSP) as an internal chemical shift reference, 6.2 mM sodium azide (NaN3)/H2O (w/w) as a bacteriostatic agent and 10% (v/v) D2O to provide a lock signal for the magnetic field. Following centrifugation (12000 x g, 4°C, 5 min) to remove solids, 550 μL of sample was transferred into 5 mm SampleJet NMR tubes and immediately loaded onto a refrigerated SampleJet robot (Bruker Biospin, Germany) and temperature kept
constant at 4°C until analysis. Pooled quality control (QC) samples were generated from a composite of all samples and used to assess potential batch effects.

For urine analysis, samples were thawed at room temperature before 540 μL of urine was mixed with 60 μL of urine phosphate buffer (KH2PO4, 1.5 M, pH 7.4 made up in D2O containing 5.8 mM TSP and 2 mM NaN3) before being centrifuged (12000 x g, 4°C, 5 min). Aliquots of 550 μL were then transferred into SampleJet 5 mm NMR tubes. To monitor batch effects, urine QC samples were also ran.

**NMR Spectroscopy**

Proton (1H) NMR spectra for all samples were acquired following standardised protocols using a Bruker Avance III 600 spectrometer equipped with a BBO probe. For plasma samples, mono-dimensional (1D) CPMG and standard 1D spectra were acquired in automation using the standard pulse sequences. For urine samples, a standard one dimensional water pre-saturation pulse sequence [relaxation delay-90°-t1-90°-tm-90°-acquire free induction decay (FID)] was applied in automation. All spectra were calibrated, phased and baseline corrected automatically using TopSpin (v2.1, Bruker BioSpin, Rheinstetten, Germany). Spectra were then visually inspected and manually adjusted where necessary using Amix (v3.9.11, Bruker BioSpin, Rheinstetten, Germany).

**Data processing and analysis**

Data modelling were carried out using SIMCA P+ (v14.0, Umetrics, Umea, Sweden). Multivariate analysis of NMR data was initially performed using PCA to assess co-variance in the datasets. The knowledge discovery by accuracy maximisation (KODAMA) algorithm was then performed to facilitate identification of clustering patterns descriptive of underlying
metabolic phenotypes in the cohorts (PE, PEFGR, FGR and controls). PLS-discriminant-analysis (PLS-DA) was used to assess differences in metabolic profiles associated with the cohort group of PE, FGR, PEFGR and healthy controls. Correlations between the most significantly altered metabolites in plasma and urine were calculated with Pearson’s product moment correlation analysis (rho, R software).
8.3.3 Results - Plasma

There was evidence of clustering of samples into disease groups when principle components were used. The OPLS-DA analysis clustered the samples into their respective disease groups, suggesting that there were differences in measured serum metabolites between the healthy and PE, FGR and PEFGR samples.

![Figure 8-6 Serum metabolite PLS-DA Analysis by clinical outcome subset](image-url)
The relative concentration of the 44 identified metabolites in all serum samples were compared in both the discovery and validation datasets using univariate analyses.

**Figure 8-7 Relative concentration of serum metabolites as used in PLSDA modelling**
There was a clear separation on score plots representing metabolomic profiles of those patients with PE and those with PEFGR.

Figure 8.8 Score plot of PLSDA analysis comparing PE and PEFGR
There was a distinct metabolomic profile when comparing pregnancies with PE and those with FGR.

**Figure 8-9** Score plot of PLSDA analysis comparing PE and FGR
8.3.4 Results - Urine

The OPLS-DA analysis clustered the samples into their respective disease groups, suggesting that there were differences in measured urine metabolites between the healthy and PE, FGR and PEFGR samples.

Figure 8-10 Urine metabolite PLS-DA Analysis by clinical outcome subset
The relative concentration of the 66 identified metabolites in all urine samples were compared in both the discovery and validation datasets using univariate analyses.

**Figure 8-11 Relative concentration of urine metabolites as used in PLSDA modelling**
8.3.5 Discussion

PE, PEFGR and FGR have distinct cardiovascular phenotypes. It appears that these phenotypes can also be defined by the plasma and urine metabonome. Comparison of a grouping by clinical diagnosis revealed significant differences in metabolite concentrations.

This exploratory work also highlighted potential candidate metabolites that could prove useful as future biomarkers. This will have important implications in differentiating between disease processes and raises the prospect of better understanding of the underlying pathogenesis of these condition. In the long run, this profiling raises the possibility of metabolites being used to monitor disease progression as well as being used as a screening tool to predict development of pathological conditions in pregnancy. As urine is already routinely analysed for protein creatinine content as a diagnostic criterion of PE, knowledge of novel metabolome could be used as adjunct to further delineate gestational proteinuria from evolving PE in pregnancy.
Chapter 9 Conclusion

The aim of this thesis was to investigate maternal cardiovascular function and biomarkers in pre eclampsia and fetal growth restriction.

- PE is associated with high cardiac output and low peripheral vascular resistance, regardless of gestation of diagnosis.
- FGR is associated with high peripheral vascular resistance.
- Where PE co-exist with FGR, cardiac output is significantly lower and vascular resistance higher, regardless of gestation of diagnosis.
- PE, FGR and PEFGR all show abnormal arterial function as evidenced by higher augmentation index and pulse wave velocity.
- Maternal cardiac output and vascular resistance is significantly correlated with uterine artery Doppler and fetal umbilical artery Doppler but not MCA Doppler.
- BAFF levels are higher in pregnancies with PE when compared to normal.
- BAFF levels are correlated with pulse wave velocity measurements.
- PAF is higher in pregnancies with PE and FGR when compared to normal.
- Metabolomic profiles are distinct in PE, FGR, PEFGR and healthy controls.
- There were insufficient numbers to draw firm conclusion on exercise and longitudinal changes in maternal cardiovascular and arterial function in pathological pregnancies.
Clinically, it is my hope that bedside maternal cardiovascular parameter assessment will become mainstream routine practise in the near future. This is useful during initial diagnosis, in order to characterise the phenotype associated with the clinical manifestation of hypertension. Following on from this, appropriate antihypertensives can be chosen to target the underlying cardiovascular maladaptation driving the pathological process. At present, hypertension treatment remains untargeted and arbitrarily guided by NICE guidelines in the UK, which does not make the distinction between differing phenotypes driving the pathophysiology of PE. This is in contrast to established metabolic hypertension clinics or in intensive care units, whereby non-invasive assessment of cardiovascular parameters is routinely used to guide pharmacological choice and indeed monitor response to treatment.

Postulating further, cardiovascular parameters could also be manipulated to prolong pregnancy gestation and delay progression of pathology. Using results obtained from the current study, effect of pharmacological or other novel therapy could be studied to validate these hypotheses. For example, by using nitric oxide donors and other potent vasodilators to reverse the high PVR seen in the most severe cases of FGR and PEFGR.

Expanding the studied cohort to include those at highest risk of developing hypertensive disorder in pregnancy (such as women with pre-existing chronic hypertension, high BMI, co-existing diabetes or higher order multiple pregnancy) could enrich the findings of the current study. The role of ethnicity in the pathophysiology of hypertension and response to pharmacological treatment could also be studied in a larger cohort.
The impact of exercising in unmasking pathological adaptation of cardiovascular function will be fascinating if the study was performed at a larger scale. This study has shown that simple step test is acceptable and well tolerated by women with pathological pregnancy. Expanding this to incorporate gestation matched healthy controls could yield interesting findings. At present, conflicting advice are given to women about exercising in pregnancy. Understanding normal cardiac adaptation to exercise provides sound evidence base to any proposed guidance. In addition, if exercise is proven to modulate cardiovascular parameters, pre-pregnancy exercise can be recommended as an intervention in healthy women and those at higher risk of developing PE and FGR.

Emerging biomarkers including those reported in this study (BAFF, PAF and metabolomic candidate biomarkers) could prove to be the future of screening for disease prior to clinical manifestation and indeed, could be utilised to monitor disease progression after a diagnosis is made. Combined with current established biomarkers, this could also increase the positive predictive value of any proposed screening test.

The easy availability of non-invasive systems to assess maternal cardiovascular function at the bedside makes collaboration across research centres a real possibility. With the establishment of the “International working group on maternal hemodynamics”, research in this field is thriving with plans to share research ideas and enrich patient cohort.
References


86. Lees CC, Marlow N, van Wassenaer-Leemhuis A, Arabin B, Bilardo CM, Brezinka C, et al. 2 year neurodevelopmental and intermediate perinatal outcomes in infants with very


activation, and acute atherosis in the basal plate of the placenta. American journal of obstetrics and gynecology. 2017;216(3):287.e1-.e16.


Appendix 1. Publications & Presentations

Appendix 1a Publications


- Foo L, Tay J, Lees CC, et al., 2015, Hypertension in Pregnancy: Natural History and Treatment Options, Current Hypertension Reports, Vol: 17, ISSN:1522-6417


Title: Early and late preeclampsia are characterized by high cardiac output, but in the presence of fetal growth restriction, cardiac output is low: insights from a prospective study

Author: Jasmine Tay, Lin Foo, Giulia Masini, Phillip R. Bennett, Carmel M. McEniery, Ian B. Wilkinson, Christoph C. Lees

Publication: American Journal of Obstetrics and Gynecology

Publisher: Elsevier
Date: May 2018
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Title: Uterine and fetal placental Doppler indices are associated with maternal cardiovascular function

Author: Jasmine Tay, Giulia Masini, Carmel M. McEniery, Dino A. Giussani, Caroline J. Shaw, Ian B. Wilkinson, Phillip R. Bennett, Christoph C. Lees

Publication: American Journal of Obstetrics and Gynecology

Publisher: Elsevier
Date: January 2019
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Title: Maternal Serum B Cell activating factor in hypertensive and normotensive pregnancies


Publication: Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health

Publisher: Elsevier

Date: July 2018

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Appendix 1b Presentations

- **Fetal umbilical artery Doppler end-diastolic flow is associated with changes in maternal cardiovascular function: a three-centre study using different techniques**
  Oral communication at ISUOG conference, Berlin, October 2019
  Shortlisted for Young investigator Award

- **Maternal uterine and fetal umbilical artery Doppler impedance is associated with maternal hemodynamic function**
  Oral communication at ISUOG conference, Singapore, October 2018

- **Fetal middle cerebral artery Doppler impedance is not related to maternal haemodynamic function**
  Short oral presentation at ISUOG conference, Singapore, October 2018

- **Maternal uterine artery Doppler changes in pre-eclampsia and fetal growth restriction**
  Oral poster at ISUOG conference, Singapore, October 2018

- **Why early and late PE are the same condition**
  Invited speaker at 3rd international congress in maternal haemodynamics, Cambridge, April 2018

- **Maternal haemodynamics in PE**
  Invited speaker at Expert Fetal Medicine, Royal college of Physicians, February 2018

- **Maternal cardiovascular function in PE, FGR and PE +FGR**
  Oral presentation at Maternal haemodynamics conference, Milan, January 2017
• Are there changes in cardiac output and total peripheral resistance measurements amongst pregnancies with fetal growth restriction when compared to healthy pregnancies?
  Oral communication at ISUOG conference, Rome, September 2016

• Are there changes in augmentation index and pulse wave velocity measurements amongst pregnancies with fetal growth restriction when compared to healthy pregnancies?
  Oral poster at ISUOG conference, Rome, September 2016

• Cardiac output and total peripheral resistance in pregnancies with fetal growth restriction
  Oral presentation at 2nd international Maternal haemodynamics conference, Rome, May 2016

• Is maternal arterial function impaired in pregnancies with fetal growth restriction
  Oral presentation at 2nd international Maternal haemodynamics conference, Rome, May 2016

• Non-invasive measurement of cardiac output in pregnancy: A comparison between Innocor and Vicorder
  Oral presentation at Maternal haemodynamics working group, Maastricht, December 2015

• Cardiac output in relation to early miscarriage
  Oral presentation at Maternal haemodynamics working group, Maastricht, December 2015

• An overview of the Precept and Conceive Studies
  Invited oral presentation at Action for Pre-Eclampsia London expert meeting, London, November 2015

• The Precept Study
  Invited oral presentation at O&G/Genetics clinical research network meeting, London, September 2015

• Precept Study – Early cases
  Oral presentation at Maternal haemodynamics working group, Cardiff, May 2015
Appendix 2 Additional research papers arising from study

Appendix 2a Innocor and Vicorder comparison study

The following paper (196) amalgamated data from this study with another pre-conception cohort (Conceive) (7) to consider differing methods used in assessing maternal cardiovascular function, and the limits of agreement between the tests.

ABSTRACT

Objectives

We aimed to describe cardiac output (CO) trend from pre-pregnancy to postpartum using an inert gas rebreathing (IGR) device, and compare these measurements to those obtained by a pulse waveform analysis (PWA) technique, both cross-sectionally and longitudinally.

Methods

Non-smoking healthy women, aged 18-44 years, with BMI <35 were included in this prospective observational study. CO measurements were collected at different time points (pre-pregnancy, at four different gestational epochs and post-partum) using IGR and PWA. A linear mixed model analysis tested whether the longitudinal change in CO differed between techniques. Bland Altman analysis and intra-class correlation coefficient (ICC) were used for cross-sectional and a 4-quadrant plot for longitudinal comparison.

Results

Of 413 participants, 69 had a complete longitudinal assessment throughout pregnancy. In this latter cohort, the maximum CO rise was seen at 15.2 weeks with IGR (+17.5% from pre-
pregnancy) and at 10.4 weeks with PWA(+7.7% from pre-pregnancy). Trends differed significantly (p=0.0093). Cross-sectional analysis was performed in the whole population of 413 women: mean CO were 6.14 and 6.38 L/min for PWA and IGR, respectively, percentage of error was 46% and ICC 0.348 with similar results at all separate time points. Longitudinal concordance was 64%.

Conclusions

Despite differences between devices, maximum CO rise in healthy pregnancies is more modest and earlier than previously reported. The two methods of CO measurement do not agree closely and cannot be used interchangeably. Technique-specific reference ranges are needed before they can be applied in research and clinical settings.
INTRODUCTION

Maternal haemodynamic indices, including cardiac output (CO), change markedly during pregnancy. Indeed, a recent meta-analysis reports that in normal pregnancies, CO increases from pre-pregnancy, peaking at 31% in the early third trimester[1]. Of clinical relevance is that women who develop pregnancy complications, especially pre-eclampsia and fetal growth restriction (FGR), have abnormal CO values which can identify different patterns of diseases if combined with a comprehensive assessment of the central haemodynamics and arterial function[2].

The reference methods for quantifying CO, such as pulmonary artery catheterisation using thermodilution and direct Fick measurement, are invasive and not applicable in healthy pregnancy. For this reason, several non-invasive techniques for CO measurement have been tested in obstetric research. Cardiac magnetic resonance imaging is becoming increasingly recognized as a reference technique, although its use in pregnancy is mostly limited to women with complex cardiac or aortic disease or small pathophysiological studies[3-4]. As such, transthoracic echocardiography (TTE) using pulsed wave Doppler in the left ventricular outflow tract (LVOT) is considered a surrogate reference technique for CO measurement in pregnant women. This is because it is the only non-invasive method that has been validated against the invasive procedures[5], although this was in a limited sample of 34 women, suffering from severe pregnancy complications which required invasive monitoring. However, TTE is strongly operator dependent and, as such, prone to considerable inter-observer variability. It requires specifically trained operators and probes rarely available in the obstetric setting and is influenced by the patient’s habitus which could have an effect on the quality
and reliability of the assessment. Moreover, differences in the accuracy of CO estimation depending on the calculation method used have been reported [6-8].

Several other easy to use and operator-independent devices for CO measurement have been developed, including the Innocor® (Innovision A/S, Denmark), based on inert gas rebreathing (IGR), and Vicorder® (Skidmore Medical, Bristol UK), based on pulse waveform analysis (PWA). The former has previously been validated against thermodilution, direct Fick and cardiac magnetic resonance imaging methods for measurement of CO[9-12] in non-pregnant subjects, and the Innocor technique is robust in non-pregnant patients with restrictive ventilation disorders that may mimic restrictive patterns due to pregnancy[13]. The latter has been validated for the assessment of central blood pressure against invasively measured aortic blood pressure (BP)[14] and compared with tonometry[15], and magnetic resonance imaging[16] for pulse wave velocity. However, as yet, measurements of CO derived from this PWA-based device have not been compared with other techniques.

In the clinical environment, haemodynamic assessments are rarely based on single-point measurements, so cross-sectional comparisons are of limited use. As such, examining changes with time or in response to interventions or specific therapies are more informative; for example the measurement of CO and other parameters in disease states such as pre-eclampsia and FGR and also acute circulatory insults at delivery such as regional anaesthesia. To interpret these changes especially in the context of therapy monitoring requires an understanding of the characteristics of different measurement techniques, and of the relationship of CO with gestation.

Furthermore, there is a wide normal range and variety between individuals depending on characteristics such as age, height and weight. Hence there is a need for longitudinal
assessment of haemodynamic parameters across a gestational range within the same subject, particularly as it has only once before been performed with the purpose of comparing different devices-and then in 10 patients [8].

The aim of this study was to evaluate longitudinal trends in CO, measured with two non-invasive and user-independent methods, in a large population of non-pregnant healthy women trying to conceive who subsequently became pregnant and were followed with repeated measurements at different time points throughout gestation to the post-partum period. This allowed paired measurements to be used to show how the incremental changes in CO between the devices were related.

The two devices were subsequently assessed for their agreement in measuring both cross-sectional (at the same time) and longitudinal (at different time points) CO changes to determine how generalizable the techniques were, using IGR as the reference technique.

METHODS

Non-smoking healthy women aged between 18-44 years with a BMI <35 were recruited either when they were planning a pregnancy or at different gestational ages in pregnancy from two prospective observational studies investigating maternal cardiovascular changes in pregnancies conceived naturally. Both studies were approved by National Research Ethics Committees (“CONCEIVE”: East of Scotland Research Ethics Service, REC reference 14/ES/1046; “PRECEPT”: National Research Ethics Service Committee London Riverside, REC reference 15/LO/0341) and written consent was obtained from all participants. Women were recruited between 2014-2017 using social media and from in-patient and out-patient clinics in a tertiary inner city maternity unit serving a multi-ethnic population. Those with underlying
medical conditions, pregnancy complications such as pre-eclampsia, pregnancy induced hypertension, FGR, fetal malformations or twin pregnancies were excluded.

Two analyses were undertaken: longitudinal and cross sectional. Women who conceived were offered longitudinal assessments throughout pregnancy on CO at fixed time points (preconception, 6, 10, 22, 34 weeks and 6 weeks post-partum). The CO measurements of those women who had a complete data set of assessments were used for the comparison of CO trend with both devices. Those who were assessed in pregnancy only or were not assessed at all time points were included in the cross-sectional analysis.

All CO measurements were obtained following the same protocol. Patients were asked to refrain from caffeinated drinks for at least 4 hours prior to assessments and all CO measurements were performed in a temperature-controlled room after the participant had rested and acclimatised in the room for 10 minutes. Assessments were carried out in recumbent left lateral position to avoid aorto-caval compression during the exam at late gestational epochs.

PULSE WAVEFORM ANALYSIS

CO was firstly measured via PWA using Vicorder® (Skidmore Medical, Bristol UK). Brachial BP was obtained by digital oscillometry using a brachial cuff. This has previously been shown to compare favourably with a validated oscillometric sphygmomanometer[14]. Immediately afterwards, the device recorded brachial pressure waveforms by applying a volume displacement technique using the same cuff statically inflated to 70 mmHg. A brachial-to-aortic transfer function was then applied to derive the central (aortic) pulse waveform from
which central BP and heart rate were obtained, and the stroke volume, and hence CO, estimated using a proprietary algorithm.

INERT GAS REBREATTHING

The assessment of CO with an IGR technique was performed with the Innocor® system (Innovision A/S, Denmark). Participants breathed in an oxygen (O₂) enriched mixture containing soluble and insoluble gases (0.5% nitrous oxide, N₂O; 0.1% sulphur hexafluoride, SF₆), and a sensor sampled the proportion of soluble gas and O₂ absorbed across the lungs over several breathing cycles (roughly 30 seconds) via an infrared photoacoustic gas analyser embedded within the device. The amount of N₂O absorbed was proportional to the pulmonary blood flow. This technique has already been used in pregnant women as N₂O is harmless at these concentrations for a very limited amount of time[17]. With the use of the pulse oximeter, which determines oxygen saturation of haemoglobin in arterial blood, the amount of the shunt flow (blood flow which does not perfuse the ventilated part of the lungs) was calculated. CO was then derived using Fick’s principle.

Statistical analysis

Statistical analyses were carried out using IBM SPSS version 24, and R Statistical Software version 3.3.2. The trend of CO data through all time points was described using a linear mixed model analysis with CO as the response variable, time as a fixed variable and a random effect for patient, modelled with natural cubic splines with 4 internal knots. For the analysis we used the actual time of visit at any approximate time point. The 95% confidence interval (CI) was
calculated based on the Bootstrap method. The trajectories of CO obtained with IGR and PWA were compared considering a p-value <0.05 as statistically significant.

Bland Altman analysis was used to compare cross-sectional CO measurements between both devices considering IGR as the reference technique. Accuracy (bias, mean difference between mean CO obtained with each method), precision (standard deviation, SD, of differences), 95% limits of agreement (LOA), bias +/- 1.96 SD of bias), percentage of error and intra-class correlation coefficient (ICC) were calculated for the whole population and for each of the time epochs, separately.

The differences between CO values obtained with each device at different time points (ΔCO) were compared using a four-quadrant plot. An exclusion zone of 0.5 l/min was applied as changes below this threshold are traditionally considered to fall within the accuracy and precision noise ratio of the measurement systems, based on an average adult human CO of 5 l/min and are of less clinical significance. Concordance rate (proportion of data points in the plot which are in agreement regarding the direction of change in CO), angular bias (mean of the angles of the (0,0;x,y) line for each ΔCO to the 45° line) and radial LOA (the symmetric angle around the 45° line in which 95% of the data points fall) were determined, as recommended by Saugel et al, 2015[18]. A concordance rate of 90% between the devices, angular bias < ± 5° and radial LOA < ± 30° were considered to show good agreement.

RESULTS

Between November 2014 and January 2017, we recruited 435 participants (Figure 1).

Of those, 22 were subsequently excluded due to pregnancy complications. Sixty-nine patients had complete measurements from prior to pregnancy through to postpartum and their measurements were compared for the longitudinal analysis of CO trend. The total number of
measurements obtained from all participants and time points with both devices was 1141 (358 from non-pregnant participants; 198 in the early first, 166 in the late first, 166 in the second and 173 in the third trimester, 80 in the post-partum period) and they were all included in the cross-sectional comparison. Table 1 shows the demographic characteristics of all the participants included and those selected for the longitudinal analyses only.

Table 1. Demographic characteristics of the participants.

<table>
<thead>
<tr>
<th></th>
<th>Participants included in longitudinal comparison and analysis of CO trend</th>
<th>Participants included in cross-sectional comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. of participants</td>
<td>69</td>
<td>413</td>
</tr>
<tr>
<td>Maternal age, mean (SD)</td>
<td>33 (3.86)</td>
<td>34 (4.61)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>24.18 (3.65)</td>
<td>24.47 (3.95)</td>
</tr>
<tr>
<td>Caucasian, n. (%)</td>
<td>47 (68.1)</td>
<td>275 (66.6)</td>
</tr>
<tr>
<td>Nulliparous, n. (%)</td>
<td>35 (50.7)</td>
<td>237 (57.4)</td>
</tr>
</tbody>
</table>

CO, cardiac output; BMI, body mass index.

1. **LONGITUDINAL ANALYSIS OF CO TRENDS WITH INERT GAS REBREATHING AND PULSE WAVEFORM ANALYSIS**

The comparison of trends of CO assessed with the two techniques at all time points is graphically represented in Figure 2. The longitudinal trajectory of CO recorded with the two devices differed significantly (p=0.0093). The maximum rise in CO was estimated to be reached at 15.2 weeks of gestation (95% CI: 10.36-17.14) using IGR, when the mean CO was 6.99 L/min (+ 1.05 L/min and + 17.5% compared with pre-pregnancy values), whereas using
PWA, the peak CO was estimated to be achieved at 10.4 weeks of gestation (95% CI 5.2-23.9; 6.54 L/min; + 0.47 L/min and + 7.7% compared to pre pregnancy values).

2. COMPARISON BETWEEN TECHNIQUES

2a. Cross-sectional comparison

The Bland-Altman analysis and ICC are presented in Table 2. Percentage of error ranged between 42% and 50%. The Bland-Altman plot for assessments obtained for the whole population is shown in Figure 3.

Table 2. Bland-Altman analysis and ICC for the total population and for each time epoch separately.

<table>
<thead>
<tr>
<th>Time Epoch</th>
<th>N.</th>
<th>Mean GA</th>
<th>Mean CO (SD) PWA</th>
<th>Mean CO (SD) IGR</th>
<th>Bias (L/min)</th>
<th>SD bias (L/min)</th>
<th>1.96 X SD Upper</th>
<th>1.96 X SD Lower</th>
<th>Percentage Error (%)</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-pregnancy</td>
<td>358</td>
<td>-</td>
<td>5.92 (1.21)</td>
<td>5.85 (1.13)</td>
<td>-0.06</td>
<td>1.40</td>
<td>2.74</td>
<td>2.68</td>
<td>-2.80</td>
<td>47</td>
</tr>
<tr>
<td>Early first Trimester</td>
<td>198</td>
<td>6.77</td>
<td>6.35 (1.30)</td>
<td>6.81 (1.45)</td>
<td>0.46</td>
<td>1.68</td>
<td>3.29</td>
<td>3.75</td>
<td>-2.83</td>
<td>50</td>
</tr>
<tr>
<td>Late first Trimester</td>
<td>166</td>
<td>10.65</td>
<td>6.42 (1.24)</td>
<td>6.74 (1.33)</td>
<td>6.58 (1.10)</td>
<td>0.32</td>
<td>1.41</td>
<td>2.76</td>
<td>3.08</td>
<td>-2.44</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>166</td>
<td>22.92</td>
<td>6.20 (1.78)</td>
<td>6.92 (1.45)</td>
<td>6.56 (1.12)</td>
<td>0.72</td>
<td>1.41</td>
<td>2.76</td>
<td>3.48</td>
<td>-2.04</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>173</td>
<td>34.46</td>
<td>6.12 (1.26)</td>
<td>6.26 (1.18)</td>
<td>6.19 (0.99)</td>
<td>0.14</td>
<td>1.41</td>
<td>2.76</td>
<td>2.90</td>
<td>-2.62</td>
</tr>
<tr>
<td>Postnatal</td>
<td>80</td>
<td>-</td>
<td>6.00 (1.23)</td>
<td>6.03 (1.24)</td>
<td>6.01 (1.00)</td>
<td>0.03</td>
<td>1.43</td>
<td>2.80</td>
<td>2.83</td>
<td>-2.77</td>
</tr>
<tr>
<td>Total population</td>
<td>1141</td>
<td>-</td>
<td>6.14 (1.25)</td>
<td>6.38 (1.36)</td>
<td>6.26 (1.07)</td>
<td>0.23</td>
<td>1.48</td>
<td>2.90</td>
<td>2.90</td>
<td>-2.90</td>
</tr>
</tbody>
</table>

CO, Cardiac output; PWA, pulse waveform analysis; IGR, inert gas rebreathing; SD, standard deviation; LOA, limits of agreement; ICC, intraclass correlation coefficient.
2b. **Comparison between two time points**

From the 69 patients whose measurements were analysed longitudinally, we obtained 1035 ΔCO measurements in total. Having opted for an exclusion zone of 0.5 L/min, we subsequently included 932 ΔCO measurements in the analysis. The concordance rate between the devices was 64%. The angular bias (0.6836°) and radial LOA (12.87°-77.13°) are shown graphically on the 4-quadrant plot in Figure 4.
DISCUSSION

We have evaluated changes in CO measured by IGR in a longitudinal cohort from before pregnancy, in pregnancy and the postpartum period and compared the measurements with those obtained by PWA. Though caution must be exercised in interpreting the findings, we show that the magnitude of change of CO, and gestation at which maximum changes occur, is lower than reported in previous studies, with both non invasive measurement techniques showing less than a 20% incremental increase from pre-pregnancy and the peak CO occurring before the third trimester. Indeed, the maximum CO change in pregnancy was estimated to be 17.5% with IGR and less than half that figure (7.7%) with PWA from pre-pregnancy. The gestational age at which the peak CO was detected also differed significantly (15.18 vs 10.36 weeks, respectively) based on curves fitted around the fixed measurement time points.

It is noteworthy that both these maximum changes occurred at earlier gestation and were considerably lower in magnitude than those reported by other authors, mainly from studies that were one to two decades old. Few studies have assessed the longitudinal trend in CO from pre-pregnancy (Table, Supplementary Material). All included relatively few participants (8-54) and followed different assessment protocols, with numbers of measurements varying from 2 to 10 during pregnancy and 0 to 3 postnatally.

The gestational age at maximum CO has been reported to range from late first to the third trimester. Two studies[19-20] detected a maximum increase in CO in the late third trimester, one showed the peak at 32 weeks of gestation[21] and others[17,22] in the late second trimester, at 26 and 23-24 weeks, respectively. However, the maximum rise in CO has been reported by one group at 16 weeks [23] and in two others at 12 weeks[24-25]. A recent systematic review[1], which included studies from 1996 to 2014, showed that peak CO occurs
in the early third trimester (when it increases by 31% compared to pre-pregnancy values). The pre-pregnancy CO value was, however, derived not only from longitudinal but mainly cross-sectional studies. We did not detect the same trend; on the contrary, we observed a decrease in CO in the third trimester. However, we measured this parameter once only in the second and third trimesters.

There is a demonstrable need for non-invasive operator independent techniques for CO determination in obstetrics. Up until now, ours is the largest study in which trends in CO measured by non-invasive devices have been compared over a large number of time points with complete statistical analysis. A recent study performed on 10 patients assessed prior to conception, at 12 and 34 weeks of gestation, showed a significant difference in CO values and trends depending on the method used[8]. In particular, CO estimated with LVOT velocity time integral (VTI) with TTE was larger than with other echocardiographic methods (2D-Teichholz and Simpson’s methods), whereas with impedance cardiography pre-pregnancy CO was higher and the change in CO during pregnancy lower than with VTI.

Cross-sectional comparative studies in pregnancy of non-invasive devices which have adopted TTE as the reference technique, have shown variable agreement with Doppler and bioreactance[26-27] and MRI has been investigated in the third trimester and postpartum showing good correlation with TTE parameters[28].

We were also able to compare a larger number of paired measurements with a cross-sectional analysis, in order to assess whether the two devices showed different levels of agreement in and outside pregnancy. In this analysis, a low bias indicating accuracy and narrow limits of agreement with percentage of error <30% reflecting precision and ICC > 0.75 are considered to reflect good agreement between the two methods[29,30]. Despite a good level of
accuracy, especially for non-pregnant patients and in the third trimester, wide LOA, percentage of error >42% and low ICC suggested that in our cohort, there was a moderate to poor cross-sectional correlation in CO measurements between PWA and IGR at all time points. In addition, we assessed the performance of the two devices in tracking changes within two different time points. For this analysis, two statistical methods have been widely described: the 4-quadrant plot and the polar plot. We used the advantageous components of both techniques by combining the visually intuitive 4-quadrant plot and concordance rate with the angular bias and radial LOA indicating not only the direction but also the magnitude of the changes and degree of agreement[18]. Despite very low angular bias (0.6836°), concordance rate was 64% and radial LOA were wide (12.87°-77.13°), thus exceeding to a great degree the thresholds of 90% and ± 30° considered to show acceptable concordance rate and radial LOA between techniques. These results strongly imply that PWA and IGR are not interchangeable when used longitudinally.

The strengths of our study are the large number of participants included and the availability of pre-conceptional data for the longitudinal assessment. Limitations include the fact that we did not attempt any comparison with an invasive technique, as our cohort contained only healthy women. In addition, the Vicorder device employs a generalized transfer function to derive the central pressure waveform from which CO is calculated, and other approaches, such as individualized transfer functions may have been preferable. We did not have access to the proprietary algorithm for calculation of CO by the Vicorder device and cannot, therefore, confirm its appropriateness from a theoretical perspective. Finally, we relied on using standard, rather than measured values haemoglobin concentration for the Innocor-derived hemodynamic measurements. Not a strength, nor a weakness, but of note is that
the CO measurements were always undertaken in the same order at all appointments, with the PWA device first, followed by the IGR device immediately after. We did not compare our measurements to TTE as we intended to examine only operator-independent techniques.

In summary, both techniques of CO assessment show a CO peak in the late first/early second trimester but of a magnitude lower than previously described. These techniques show moderate-poor agreement in cross sectional and longitudinal investigations in pregnancy. Although it is not possible to determine which technique is the more accurate from this study as both work on different principles and neither can be compared to invasive measurement in pregnancy, measurements obtained with inert gas rebreathing are more similar to those reported in the literature. With non-invasive and bedside cardiovascular assessment devices becoming ubiquitous, it is important to appreciate that there are differences between techniques, that they cannot be used interchangeably and gestation specific ranges might be appropriate for each technique. For these reasons, IGR is the preferable technique for CO measurement, although the PWA technique has the advantage of being operator-independent and does not rely heavily on consumables, so may be more useful in high throughput, or, possibly, low-income settings.
Appendix 2b Vicorder and novel ultrasound comparison study

The following paper is in press. The work was done as part of a BSc project (Swina Santhirakumaran) running parallel to my PhD.

Comparison study: Measuring Augmentation index using the Vicorder™ compared to a novel semi-automated technique

Abstract

Background

Augmentation index (Aix) is increased in pre-eclamptic pregnant women compared to normotensive pregnant women. Numerous devices including the Vicorder™ are currently available to measure Aix. However, the majority of these techniques require operator training and may not be appropriate to obtain measurements during pregnancy. A novel operating package uses an ultrasound semi-automated technique to calculate Aix.

This study aims to compare the Vicorder™ oscillometry technique and the novel Samsung™ HS70A ultrasound technique of determining Aix.

Methods

Informed and written consent was obtained from 66 normotensive pregnant women within 24-40 weeks gestational age for this study. The Vicorder™ device was used with participants in the left-lateral lying position to measure Aix. The novel semi-automated technique was used to obtain high-resolution B-mode ultrasound cine-loops of the right common carotid artery from which Aix was calculated.
Results

A Bland-Altman plot displayed low agreement between the two techniques; bias was 6.17%, limits of agreement were -13.72 and 26.06 and the percentage error was 32.01%. There was a significant correlation ($r=0.306$, $p=0.016$) ($\rho=0.238$, $p=0.065$) between the Aix measurements obtained by the two techniques. However, the Aix measurements acquired by the two techniques were significantly different ($z=-4.1484$, $p=0$).

Conclusions

The Vicorder™ and the novel semi-automated ultrasound technique to obtain Aix cannot be used interchangeably, which may reflect their different technological approaches to Aix measurement. A normal reference range specific to the novel semi-automated ultrasound technique would need to be established before clinical use.

Introduction

Pre-eclampsia, a maternal condition categorized by hypertension and proteinuria, is associated with greater arterial wall stiffness(1). Arterial wall stiffness has found to be increased in women who develop pre-eclampsia prior to, during and after the affected pregnancy compared to normotensive pregnant women (1). Furthermore, arterial stiffness has been shown to positively correlate with onset and severity of pre-eclampsia (2),(3). As a result of these findings there is gained interest in the of use of arterial stiffness measurements as a predictive test for pre-eclampsia.
Stiffening of the arteries is a natural consequence of ageing and is the result of a gradual deterioration of elastin, rise in collagen and thickening of the arterial wall (4). Pulse Wave Velocity (PWV) is considered the 'gold standard' for the assessment of arterial stiffness (5),(6). Another measure of arterial stiffness, Augmentation index (Aix), is an indirect assessor of arterial stiffness (7),(8), calculated by dividing augmentation pressure by pulse pressure. Studies have shown Aix is reduced in pregnancy compared to pre-pregnancy (10),(11) adopting a 'U-shaped relationship' with increasing gestational age, with the lowest Aix recorded at 25 weeks gestational age (12). Unlike PWV, Aix is measured from a single arterial site (13), therefore Aix can be a more rapid assessment of arterial stiffness compared to PWV. Although Aix only assesses local arterial stiffness and therefore may not accurately reflect the global stiffness of the arterial system (14).

Applanation tonometry, oscillometry and Doppler ultrasound are non-invasive techniques which can be used to determine PWV and Aix (1),(15). A novel semi-automated package utilizes ultrasound echo-tracking on longitudinal sections of arterial ultrasound recordings. This technique involves monitoring the movement of opposing segments of an arterial wall during an ultrasound recording to determine changes in vessel diameter (16), from which pressure changes can be calculated and used in PWA (17). The most commonly assessed superficial arteries for Aix measurement are the carotid and radial artery; although the Aix values obtained from different arteries correlate, their absolute values differ and therefore cannot be used interchangeably (18).
This study aimed to compare the novel semi-automated ultrasound echo-tracking technique with an oscillometry technique of Aix measurement.

Methods

Participants

66 pregnant women undergoing a normotensive pregnancy between 24 and 40 weeks gestational age (GA) participated in this study. Exclusion criteria for this study included: (1) multiple pregnancy, (2) a known maternal cardiovascular condition, (3) maternal hypertension defined as \( \geq 140\text{mmHg} \) systolic blood pressure (BP) and/or \( \geq 90\text{mmHg} \) diastolic BP, (4) foetal abnormality, (5) BMI \( \geq 40 \) or (6) self-reported smoking during pregnancy.

Data collection

The participant’s booking body mass index (BMI) and BP as recorded during their antenatal booking appointment was noted from their medical records. The participant’s current weight was measured at time of recruitment. Mean arterial pressure (MAP) was calculated as 

\[
\text{MAP} = \frac{2 \times \text{diastolic} + \text{systolic}}{3}.
\]

Oscillometry technique

Pulse wave analysis was carried out using the Vicorder™ (Skidmore Medical, Bristol, UK). The participant was required to lie in a left-lateral lying position, achieved by placing a pillow under the right side of the participant’s back to avoid aorto-caval compression from a gravid
uterus. A cuff was placed on the right upper arm and another cuff on the right leg as high as possible. A neck cuff was placed around the neck with the sensor positioned above the right carotid artery. The distance between the top of the right shoulder and the midpoint of the width of the leg cuff, as positioned on the participant’s right leg, was considered the carotid-femoral distance and recorded in centimetres. Carotid-femoral distance was recorded lateral to the participant’s abdomen as to avoid obtaining incorrectly augmented measurements due to a gravid uterus. The Vicorder™ device measured carotid-femoral PWV, BP, heart rate (HR) and Aix measurements. The participant was asked to refrain from talking while the neck cuff was active. Before obtaining measurements, participants were rested for approximately 10 minutes.

**Semi-automated ultrasound technique**

The novel semi-automated ultrasound technique to measure Aix was carried out using the Samsung™ HS70A ultrasound system (Samsung Medison, Seoul, South Korea). The recommended use of the novel technique was taught be a representative of the manufacturer.

The ultrasound system was used with a L3-12A probe to visualise the longitudinal section of the right common carotid artery (RCCA) whilst the participant turned their head leftwards and remained in the semi-recumbent position. Multiple high-resolution ultrasound cine-loops of the longitudinal section of the RCCA during a cardiac cycle was saved and the 2 cine-loops
that depicted the RCCA in the clearest, most horizontal position, as deemed by the operator, were selected for analysis.

A semi-automated technique was used to obtain Aix from the ultrasound device (Aix-S). Contour lines were plotted by the operator along opposing luminal sides of the adventitia layer of the carotid artery wall (Fig.2). Carotid waveforms were produced following automated arterial analysis. When necessary, the maximum and minimum diameter and the inflection point on the graph for a selected wave cycle were altered to the most appropriate position as determined by the operator and the Aix-S value was recorded.

Statistical analysis

Microsoft Excel and SPSS Version 23 were used for statistical analysis. Normal distribution of data was determined using Shapiro-Wilks test and histogram plots. Normally distributed data were displayed as means and standard deviations (SDs), whilst non-normally distributed data were displayed as medians and interquartile ranges (IQRs). For statistical analysis, all Aix values measured by the Vicorder™ (Aix-V) and PWV values were normalised to HR by including HR as an independent covariate as suggested by Stoner et al. (9). Pearson correlation coefficient was calculated to determine the relationship between variables. An Mann-Whitney U test was performed to assess the difference between Aix measurements obtained by the two different techniques. A p value <0.05 was regarded as statistically significant.
A Bland-Altman plot was produced to evaluate the agreement between the novel semi-automated ultrasound and Vicorder™ techniques to measure Aix. The Vicorder™ was used as the reference technique. Bias (mean difference between Aix-S and Aix-V), limits of agreement (LOA) (bias±1.96*standard deviation of bias), and percentage error were calculated. A percentage error smaller than 30% was considered to suggest good agreement between the two techniques as stated by Critchley’s criteria (19).

Results

66 pregnant women were recruited, of which 5 women were excluded due to multiple pregnancy, pathology, current smoking status or error in data recording. Demographics and overall results of the 61 participants included in the study are presented in Table 1.

Arterial assessment parameters by gestational age epochs.

GA did not have a significant correlation with Aix-V (rho=0.115, p=0.377) or Aix-S (rho=0.195, p=0.132). However, GA did have a significant positive correlation with PWV (rho=0.439, p=0.000). The mean Aix values at each gestational age epoch is displayed in table 2.

Augmentation index

Aix-V

No significant correlation was found between Aix-V and booking BMI (rho=0.198, p=0.127), BMI at time of recruitment to the study (rho=0.247, p=0.055), MAP (rho=-0.039, p=0.768) or maternal age (rho=0.034, p=0.797).

Aix-S
No significant correlation was identified between Aix-S and MAP (\(\rho=0.044, p=0.735\) or maternal age (\(\rho=0.228, p=0.077\)). However, a significant positive correlation was noted between Aix-S and booking BMI (\(\rho=0.354, p=0.005\)), and BMI at time of recruitment to the study (\(\rho=0.334, p=0.009\)). Repeated Aix-S measurements were obtained from 60 participants and the mean difference between the repeated measures was 11.93 (range=0.529, 44.321).

**Comparison of Aix-V & Aix-S**

There was no significant correlation between Aix-V and Aix-S (\(\rho=0.238, p=0.065\)) (Fig.3). An Mann-Whitney U test found that there was a statistically significant difference between Aix-V and Aix-S (\(z=-4.1484, p=0.00\)).

A Bland-Altman plot was constructed using the Vicorder™ as the reference technique (Fig. 4). The bias between Aix values obtained by the two techniques was 6.17%. The upper and lower LOAs were 26.06 and -13.72 respectively. Percentage error was calculated to be 32.01%, this does not fulfill Critchley’s criteria (percentage error <30%) (50).

**Discussion**

This prospective cohort study found that the novel Samsung™ HS70A ultrasound technique did not agree with the Vicorder™ technique for Aix measurement. There was a significant difference between their Aix measurements.

**Comparison of oscillometry and ultrasound techniques**

A moderately high bias, wide LOAs and percentage error >30% was found between the two techniques of Aix measurement, suggesting that they are not agreeable. Therefore, our
The Vicorder™ measures Aix at a brachial arterial site whilst the Samsung™ HS70A assesses the carotid artery. These two techniques assess different arterial sites, which may have different levels of arterial stiffness, likely resulting in the differing Aix measurements obtained by the two techniques. Similarly, Dhindsa et al. found that assessment of Aix at different arterial sites cannot be used interchangeably (18). Furthermore, the significant difference between the measurements obtained by the two techniques may reflect the different methodological approaches used to calculate Aix. Therefore, before the Samsung™ HS70A technique can be utilised in clinical practice, a reference range specific to this device must be determined.

Comparison of technical methods

The Vicorder™ uses an oscillometry technique to obtain Aix, which requires participants to remain in a lying position while obtaining measurements. A meta-analysis by Hausvater et al. highlighted the lack of standardization of participant positioning between studies (1). Although our study ensured participant positioning was kept uniform, some pregnant women found it difficult and uncomfortable to lie in a left lateral position for the duration required with Vicorder™ measurements. This raises concern over the suitability and practicality of this device in an antenatal clinic setting. The semi-automated ultrasound technique on the
other hand, requires the participant to be in a semi-recumbent position and was found to cause minimum discomfort.

The Vicorder™ is fully automated, reducing inter-observer variability and improving reproducibility. Contrastingly, the novel ultrasound software is semi-automated in Aix measurement, it requires operator input which provides opportunity for human errors to occur and operator bias\(^{(18)}\). In this study, a large degree of variability was noted in repeated Aix-S measurements, reducing the reproducibility of this technique for measuring Aix-S. Thus, the Vicorder™ is superior to the semi-automated ultrasound technique in its reproducibility.

Some of the ultrasound cine-loops recorded in this study were obtained by an operator with no previous sonography experience, highlighting the minimal training needed to operate this device. The carotid artery is easily palpable and superficial in its anatomy allowing for ease of identification and therefore imaging. Obtaining cine-loops and arterial analysis typically took 2-3 minutes. In comparison the oscillometry technique, the ultrasound technique offered a quicker Aix assessment.

**Augmentation index measurements**

**Adjusting for Heart Rate**

In previous literature, Aix has been found to have an inverse relationship with HR\(^{(19)}\). It is argued that increasing HR does not reduce arterial stiffness, but affects the mathematical determination of Aix. As with an increased HR, the duration of systole is reduced; this decreases the contribution of reflected waves to the augmentation pressure in systole and instead causes them to contribute to the pulse wave in diastole. Wilkinson et al.
identified that this adjustment in timing of the reflected wave caused this inverse relationship between HR and Aix (19). Thus, adjusting Aix to HR is considered important to correctly assess arterial stiffness. As with the oscillometry technique, HR was measured simultaneously with Aix and therefore Aix was adjusted for HR. However, the ultrasound technique did not measure HR and therefore Aix-S could not be manually adjusted for HR. Furthermore, we were unable to determine whether the inbuilt formula of the novel ultrasound package did adjust for HR. If it was confirmed that Aix-S was not automatically adjusted for HR by the software, a separate device would need to be used to record HR to enable further manual adjustment. Further clarification of the algorithm used by the novel ultrasound package would be needed, before considering its use clinically.

**Aix and GA**

There is conflicting evidence regarding the association between Aix and GA. In corroboration with the findings of Khalil et al. which measured Aix in 541 women in all trimesters, our study depicted no correlation between Aix and advancing GA. Contrastingly, when analysing only longitudinal data of 45 women in the same study, Khalil et al. found Aix decreased until mid-pregnancy and increased thereafter till term (5)(20), as supported by the findings of Macedo et al. (14). It is clear, further longitudinal studies are necessary to confirm the relationship between Aix and GA, in order to determine if reference ranges specific to gestational age epochs are necessary before use in the clinical setting to assess pathological cases.

**Pulse wave velocity**
In this study, PWV positively correlated with advancing GA. Franz et al. conducted a longitudinal study which also found PWV varied with GA. However, they noted PWV increased between 3 to 6 months gestation and decreased thereafter till term in normotensive pregnancies (21). Longitudinal studies provide a better insight into the temporal changes of arterial stiffness during pregnancy. Therefore, more longitudinal studies are needed to establish the relationship between PWV and GA in normotensive pregnancies.

Additionally, Franz et al. found that pre-eclampsia had a greater effect on Aix than PWV (21). This suggests that Aix could be a more sensitive predictor of pre-eclampsia than PWV. Perhaps, this is because pre-eclampsia is associated with a greater change in vasoconstriction than arterial stiffness, and vasoconstriction has a greater impact on Aix than PWV (21). However more studies are imperative to identify the superior measure for use in a predictive test for pre-eclampsia.

**Strengths and Limitations**

This study is novel, as it is the first to use the Samsung™ HS70A ultrasound technique of Aix measurement. We were able to prospectively recruit across all gestational age epochs required for the study, permitting a good representation of mid-late pregnancy. Furthermore, all ultrasound measurements were taken by a single operator, ensuring standardisation and consistency in the technique and analysis. Close liaison with the representative from the manufacturer of the novel ultrasound technique, ensured the novel technique was used as per recommended instructions.
A limitation of this study is the lack of diversity amongst the participants recruited. Anand et al. demonstrated that the extent of atherosclerosis can vary with ethnicity (22). Most participants were of Caucasian ethnicity (n=28) and therefore the parameters suggested by this study may not be reflective of other ethnicities.

A further limitation of this study, included potential confounding factors that were not controlled for. It could not be guaranteed that participants refrained from consuming caffeine for an appropriate time prior to being assessed. This may have affected their cardiovascular measurements, such as BP and HR. However, it is thought that arterial stiffness variation occurs over a longer period of time, therefore the effect of this on the main aims of the study is minimal.

**Conclusion**

The novel semi-automated ultrasound technique was unable to successfully reproduce Aix values obtained by the oscillometry technique. These two techniques cannot be used interchangeably which may reflect the different methodologies adopted. Therefore, a normal Aix reference range specific to this novel device must be established before use in the clinical setting to assess pathological cases.
Table 1: Participant demographics. Means and Standard deviations (SD) are included where stated. Values with asterisks (*) are median values with interquartile range in parantheses to represent non-normally distributed data.

Abbreviations: Body Mass Index at time of study participation (BMI), Mean Arterial Pressure (MAP), Gestational age (GA), augmentation index measured by Vicrodertm adjusted for heart rate (Aix-V), augmentation index measured by novel semi-automated ultrasound technique (Aix-S).
Table 2: Arterial assessment parameters in normotensive pregnancy between 24 to 40 weeks of gestation. The mean measurements of participants at each gestational age epoch is shown. Standard deviations are stated in parentheses.

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>N</th>
<th>Aix-V</th>
<th>Aix-S</th>
<th>PWV</th>
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<tr>
<td>24<em>0-27</em>6</td>
<td>11</td>
<td>13.73 (10.45)</td>
<td>20.52 (8.64)</td>
<td>6.19 (1.05)</td>
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<td>21.09 (10.89)</td>
<td>7.15 (1.89)</td>
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<td>7.10 (1.09)</td>
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<td>36*0-40</td>
<td>8</td>
<td>11.48 (11.50)</td>
<td>20.18 (8.26)</td>
<td>8.36 (4.10)</td>
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<tr>
<td>Overall: 24-40</td>
<td>61</td>
<td>16.18 (10.36)</td>
<td>22.34 (10.12)</td>
<td>7.13 (2.11)</td>
</tr>
</tbody>
</table>
Figure 1: Pulse wave trace at a single arterial site during a cardiac cycle. As the heart enters systole (red) a pulse wave spreads through the arterial system some of which is reflected. Reflected pulse waves increase the pulse pressure (P2). Augmentation index is calculated as $\frac{\text{Augmentation pressure}}{\text{Pulse pressure}}$. In stiffer arteries, the reflected wave is greater and thus the augmentation pressure and augmentation index is increased. Adapted from ‘Maternal Wave Reflections and Arterial Stiffness in Normal Pregnancy as Assessed by Applantation Tonometry’ (14).
Figure 2: Measuring augmentation index using Samsung™ HS70A Ultrasound echotracking.

A. Contour lines were plotted by the operator along the luminal border of the adventitia layer on the near and far wall of the longitudinal section of the right common carotid artery.

B. A magnified image depicting the contouring of the adventitia layer.

C. Automated carotid wave graph. Minimum diameter value (yellow), interception point (orange) and maximum diameter value (green) were determined by operator.
Figure 3: Correlation between the two different techniques of Augmentation index measurement. A simple scatter plot showing the correlation between Augmentation index measured by SamsungTM HS70A (Aix-S) and Augmentation index measured by VicorderTM (Aix-V) adjusted for heart rate. Regression line is displayed and Pearson correlation coefficient and corresponding p value is specified.
Figure 4: A Bland-Altman plot to compare the oscillometry and novel semi-automated ultrasound technique to measure augmentation index (Aix). The solid orange line displays the bias (6.20%). The dashed lines display limits of agreement (-13.92, 26.32).
Appendix 3 Research documents

Appendix 3a Ethical approval

06/02/2015

Mr Christoph Lees
Centre for Fetal Care
Queen Charlotte’s Hospital
DuCane Road
London, W12 0HS

Dear Mr Lees

RE: A longitudinal cohort study into maternal cardiovascular and metabolic changes in fetal growth restriction with or without preeclampsia in pregnancy (PRECEPT Study)

Joint Research Compliance Office Reference number: 15-H-2516

This is to confirm that the above named research project utilises human participants, their organs, tissue and/or data as defined under the sponsorship requirements of the Research Governance Framework for Health and Social Care 2005, incorporating the Medicines for Human Use (Clinical Trials) Regulations 2004.

On behalf of Imperial College Healthcare NHS Trust, we undertake to act as the identified Research Sponsor for this project.

This letter confirms:

- The research proposal has been discussed, assessed and registered with the Joint Research Compliance Office of Imperial College Academic Health Science Centre, Imperial College Healthcare NHS Trust and provisional sponsor approval granted.
- The Chief Investigator has undergone a process of scientific critique commensurate with the scale of the project.
- Imperial College Healthcare NHS Trust will indemnify the project under standard NHS Hospital Indemnity for negligent or wrongful harm this will be provided under clinical negligence scheme for Trusts clinical risk management NHS Litigation Authority (NHSLA) for NHS Trusts in England.
- Resources and support are available to the research team to aid delivery of the research as proposed.
- Management, monitoring and reporting responsibilities for the research have been approved.
- Imperial College Healthcare NHS Trust will undertake and enforce those sponsor duties set out in the NHS Research Governance Framework for Health and Social Care.

Imperial College Healthcare NHS Trust Sponsorship is conditional on the project receiving applicable ethical and regulatory approval for all research related aspects of its conduct. It is also conditional on successful contract and agreement negotiations and sign off via the Joint Research Office of Imperial College Academic Health Science Centre, where relevant, and before the study commences.

A copy of the ethics approval letter must be sent to the Research Governance Manager prior to the study commencing.

Sponsorship is dependent on obtaining R&D Office approval for all NHS sites where the research is being conducted.

Yours sincerely

[Imperial College Healthcare NHS Trust logo]

[Imperial College Healthcare NHS Trust address]

[Imperial College Healthcare NHS Trust contact information]

[Imperial College Healthcare NHS Trust letterhead]

[Imperial College Healthcare NHS Trust logo]

[Imperial College Healthcare NHS Trust address]

[Imperial College Healthcare NHS Trust contact information]
Mr Christoph Lees  
Centre for Fetal Care  
Queen Charlotte's Hospital  
Du Cane Road  
London W12 0HS  

Dear Dr Lees  

RE: JRCO Study Approval  

**Project Title:** A longitudinal cohort study into material cardiovascular and metabolic changes in fetal growth restriction with or without pre-eclampsia in pregnancy  

**Short Title:** PRECEPT Study  

**Joint Research Compliance Office Reference number:** 15HH2516  

**Ethics reference number:** 15/LO/0341  

**Principal Investigator:** Mr Christoph Lees  

Strickland  

I confirm that this project has now been approved by the Joint Research Compliance Office. The project may now start at Imperial College Healthcare NHS Trust sites. Please note that the start date of the project is the date of this letter and the duration is the same as that provided in your application form.  

The list of documents reviewed and approved by the Joint Research Compliance Office under requirements of the Research Governance Framework are as follows:  

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHS HRA NRES Committee London – Riverside favourable ethical opinion</td>
<td>REC Reference: 15/LO/0341</td>
<td>09 April 2015</td>
</tr>
<tr>
<td>NHS REC Application Form</td>
<td>Version 3.5</td>
<td>06 February 2015</td>
</tr>
<tr>
<td>NHS NRES Sites Specific Information Form</td>
<td>Version 3.5</td>
<td>02 February 2015</td>
</tr>
<tr>
<td>Investigator CV – Mr Lees</td>
<td>7</td>
<td>05 February 2015</td>
</tr>
<tr>
<td>Study Protocol</td>
<td>2</td>
<td>Jan 2015</td>
</tr>
<tr>
<td>Advertisement material</td>
<td>1</td>
<td>28 January 2015</td>
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<tr>
<td>Non-validated Questionnaire – Interview Sheet</td>
<td>1</td>
<td>01 January 2016</td>
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<tr>
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<td>4</td>
<td>24 March 2015</td>
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<td>Participant Information Sheet (PIS) - Control</td>
<td>4</td>
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<tr>
<td>Participant Information Sheet (PIS) - Pathology</td>
<td>4</td>
<td>19 March 2015</td>
</tr>
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</table>

Before you commence your research, please note that you must be aware of your obligations to comply with the minimum requirements for compliance with the Research Governance indicators 17 (Data Protection), 25 (Health and Safety) and 22 (Financial Probity). Details of the requirements to be met can be found in the Research Governance Framework available on www.dh.gov.uk.

Under the Research Governance regulations, Serious Adverse Event Reports, Adverse Reactions and amendments to the protocol or other supporting documents must be forwarded to the Joint Research Compliance Office and Ethics Committee.

In accordance with the Research Governance Framework, research projects carried out in the Trust will be randomly chosen by the Joint Research Compliance Office for auditing. Please see the attached checklist for documentation that will be required during the audit.

I wish you well in your research.

Yours sincerely

Ms Becky Ward
Research Governance Manager
### Joint Research Compliance Office Audit Checklist

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<td></td>
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<tr>
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<td>Investigator's brochure</td>
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<tr>
<td>4</td>
<td>Protocol and amendment if any</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Any revision to consent form</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Patient information sheet</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Signed informed consent</td>
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</tr>
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<td>8</td>
<td>Subject screening log</td>
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<tr>
<td>9</td>
<td>Financial agreement document</td>
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<tr>
<td>10</td>
<td>Insurance statements, subjects' compensation where required</td>
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<tr>
<td>11</td>
<td>Sponsorship agreement</td>
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<td>12</td>
<td>Relevant communications other than site visits</td>
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<td>13</td>
<td>Regulatory authority(ies) authorisation / approval</td>
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<tr>
<td>14</td>
<td>Curriculum vitae and/or other relevant documents evidencing qualifications of investigator(s) and/or supporting staff in the study</td>
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<td>Sample label(s) attached to investigational medicinal product container(s)</td>
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<tr>
<td>18</td>
<td>Instructions for handling of investigational medicinal product(s) and trial related materials</td>
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<td>19</td>
<td>Distribution records for investigational medicinal product(s) and trial related materials</td>
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</tr>
<tr>
<td>20</td>
<td>Master randomisation list</td>
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</table>
Appendix 3b Patient information sheet and consent

Centre For Fetal Care
Queen Charlotte’s & Chelsea Hospital
Du Cane Road London W12 0HS

Tel: 020 8383 1000
Fax: 020 8383 3588

www.imperial.nhs.uk

Participant’s Information Sheet

Information Sheet for Research Participants - Controls

Study title:

A longitudinal cohort study into maternal cardiovascular and metabolic changes in fetal growth restriction with or without pre-eclampsia in pregnancy (PRECEPT)

A study into heart function and blood profile changes in mums with healthy pregnancy and those with pre-eclampsia and/or small babies

REC Number: 15/LO/0341

Principal Investigator:

Mr Christoph Lees, MD MRCOG

You are being invited to take part in the PRECEPT study. Before you decide to participate, it is important for you to understand why this study is being conducted and what it will involve on your part. Please take time to read the following information carefully. Please contact us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to be involved in the study.

If you do decide to take part, please let us know beforehand if you have been recruited to any other research studies in the last year. If you decide not to take part or to withdraw at any other time without explanation, your future care will not be affected by your decision. Thank you for reading this.

Who we are

The Centre for Fetal Care at Queen Charlotte’s & Chelsea Hospital is a nationally renowned specialist unit that looks after women who have high risk pregnancies and fetal abnormalities. The hospital is also an integrated research center in partnership with Imperial College London, and
undertakes internationally rated clinical research to improve patient care and experience.

**What is the purpose of the study?**

When a woman becomes pregnant, her body undergoes changes to optimise conditions for the growth of her baby. These adaptations are crucial to a healthy pregnancy, and involve vast alterations to a mother’s blood pressure and heart function. We think abnormalities in these adaptations are linked to the development of raised blood pressure (preeclampsia) or restricted growth of the baby.

We would like to recruit pregnant women with healthy ongoing pregnancies to compare their heart function with those women who have developed complications in their pregnancies. We will carry out heart function and blood pressure assessment every 4 weeks until after you deliver. All tests are non-invasive and not harmful to the pregnancy. We will also collect blood and urine samples during study visits. We may carry out genetic analysis on the blood samples you provide. Participation in the study will not interfere with any routine maternity care.

**Do I have to take part?**

Your participation is voluntary. If you wish to be part of the study, we would require you to complete a consent form, and your GP will be informed. Travel cost to attend study visits will not be reimbursed. However, where possible, we will time your study visits to coincide with routine maternity care appointments to avoid additional travel costs. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive in the future. If for some reason you lose the capacity to consent once you are enrolled onto the study, data and tissue already collected with consent will be retained, but no further research procedures or sample collections will be carried out.

**What do I have to do?**

During our initial contact with you, we will ask you whether you would like to participate in the study, and may ask you initial screening questions to ensure your suitability in participating in the study. If so, we will invite you for a first consultation.

**What will happen to me if I take part?**

The first appointment will last approximately one hour. At the first consultation, we will ask you to complete a questionnaire about your pregnancy history, general health and your lifestyle.

We will also give you our contact details, so that you can call or email if you have any questions or want further advice after the consultation. All telephone contact will be with a midwife or an obstetrician working on the study.

At every visit, we will measure your height and weight and perform cardiovascular tests (all non-invasive) The cardiovascular tests will involve

- Blood pressure cuffs around your right arm and right thigh with a further neck sensor. These will inflate and deflate similar to the blood pressure machines used in your GP surgery
- Inhaling and exhaling an inert gas via a mouthpiece attached to a special machine to detect how efficiently your heart is pumping (done lying down and standing upright on all visits)

We perform ultrasound scans of your baby at 28, 32 and 36 weeks of pregnancy. These scans are usually abdominal (not internal) and last approximately 20 minutes.
• With each ultrasound scan appointment, we will ask you for a 20 ml or a 4-teaspoons sample of blood, as well as a urine sample. We will also ask you to fill in a questionnaire about your medication intake. You will have cardiovascular tests performed as you did during your first study visit. These will take approximately 45 minutes.

• When you have delivered, we will follow the outcome of your pregnancy. We will see you again 6 weeks afterwards, to carry out cardiovascular tests and obtain a 20ml (4 teaspoons) of blood and a urine sample.

You can opt out of the study at any stage. All tests and scans for the study are carried out at Queen Charlotte’s & Chelsea Hospital. In addition to the scans we perform, we will also obtain the reports of your routine antenatal scans, either from your maternity hand-held notes, or your booking hospital.

**What are the possible disadvantages and risks of taking part?**

None of the tests will cause harm to you, your baby or your pregnancy. The blood test is similar to one that you may have at your GP. Ultrasound scans are routinely carried out in pregnancy, and there is no evidence that it causes any harm to yourself or your baby. There may be an additional demand on your time as you will be expected to attend study visits lasting approximately an hour (to complete both ultrasound scans and cardiovascular tests)

**What are the possible benefits of taking part?**

Taking part in the study will not affect your pregnancy care. By taking part in the study, you will benefit from receiving pregnancy scans to check on the wellbeing of your baby. We hope that women in the future will benefit from your participation and the information we gain from this study.

**What if a problem is detected?**

If a problem is detected, we will refer you to the appropriate clinical team and the condition will be followed-up and managed according to standard practice. We would explain our findings, counsel you with regards to implications on your pregnancy and provide appropriate support.

**Will my taking part in this study be kept confidential?**

All information that is collected about you during the course of the research will be kept strictly confidential and anonymous. Your consent form will be stored securely on hospital premises. Your name and address will be removed from the information when it is shown to other medical staff outside the study.

**What happens if I withdraw?**

You can decide to withdraw at any time without explanation. If you do so, your future care will not be affected by your decision. The data that is already collected will be used in the study unless you ask us not to do so.
Who is organising the research?
This study is being conducted by the staff of the Department of Obstetrics & Gynaecology at Imperial College NHS Trust and Imperial College London. Findings from the PRECEPT study may form part of doctorate projects based at Imperial College London.

Who has reviewed the study?
This study was given a favorable ethical opinion for conduct in the NHS by xxx Research Ethics Committee

What if something goes wrong?
Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College Healthcare NHS Trust is at fault. This does not affect your legal rights to seek compensation. If you are harmed due to someone’s negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator. The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

Contact for further information:

For more information or if you wish to lodge a complaint, you can phone: Dr Jasmine Tay on 02083837316 or Mr Christoph Lees on 0208 383 1000,

Or write to either:

PRECEPT Investigators,
Centre for Fetal Care, Queen Charlotte’s & Chelsea Hospital, Du Cane Road, W12 0BZ, London

Or

Ref: PRECEPT project
Women’s Health Research Centre, c/o Imperial College London, IRDB, Ground Floor, Du Cane Road, W12 0NN, London

Tel: 02033135281, Fax: 02033135284, email: whrcenquiries@imperial.ac.uk
Information Sheet for Research Participants

Study title:

A longitudinal cohort study into maternal cardiovascular and metabolic changes in fetal growth restriction with or without pre-eclampsia in pregnancy (PRECEPT)

A study into heart function and blood profile changes in mums with healthy pregnancy and those with pre-eclampsia and/or small babies

REC Number: 15/LO/0341

Principal Investigator:

Mr Christoph Lees, MD MRCOG

You are being invited to take part in the PRECEPT study. Before you decide to participate, it is important for you to understand why this study is being conducted and what it will involve on your part. Please take time to read the following information carefully. Please contact us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to be involved in the study.

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Who we are
The Centre for Fetal Care at Queen Charlotte’s & Chelsea Hospital is a nationally renowned specialist unit that looks after women who have high risk pregnancies and fetal abnormalities. The hospital is also an integrated research center in partnership with Imperial College London, and undertakes internationally rated clinical research to improve patient care and experience.

What is the purpose of the study?
When a woman conceives a pregnancy, her body undergoes changes to optimise conditions for the growth of her baby. These adaptations are crucial to a healthy pregnancy, and involve vast alterations to a mother’s blood pressure and heart function. We think abnormalities in these adaptations are linked to the development of raised blood pressure (preeclampsia) or restricted growth of the baby. We would like to recruit pregnant women who have one or both of the above conditions. We will carry out heart function and blood pressure assessment upon your diagnosis, repeating them until your delivery and then see you once more after you deliver. All tests are non-invasive and not harmful to the pregnancy. We will also collect blood and urine samples during study visits. Where possible, we will time this with your clinical appointments. We may carry out genetic analysis on the blood samples you provide. Participation in the study will not interfere with any routine maternity care.

Do I have to take part?
Your participation is voluntary. If you wish to be part of the study, we would require you to complete a consent form, and your GP will be informed. Travel cost to attend study visits will not be reimbursed. However, where possible, we will time your study visits to coincide with routine maternity care appointments to avoid additional travel costs. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive in the future. If for some reason you lose the capacity to consent once you are enrolled onto the study, data and tissue already collected with consent will be retained, but no further research procedures or sample collections will be carried out.

What do I have to do?
During our initial contact with you, we will ask you whether you would like to participate in the study, and may ask you initial screening questions to ensure your suitability in participating in the study. If so, we will invite you for a first consultation.

What will happen to me if I take part?
The first appointment will last approximately one hour. At the first consultation, we will ask you to complete a questionnaire about your pregnancy history, general health and your lifestyle.

We will also give you our contact details, so that you can call or email if you have any questions or want further advice after the consultation. All telephone contact will be with a midwife or an obstetrician working on the study.

We will then measure your height and weight and perform cardiovascular tests (all non-invasive). The cardiovascular tests will involve

- Blood pressure cuffs around your right arm and right thigh with a further neck sensor. These will inflate and deflate similar to the blood pressure machines used in your GP surgery
Inhaling and exhaling an inert gas via a mouthpiece attached to a special machine to detect how efficiently your heart is pumping (done lying down and standing upright on all visits, and after step exercise test on one occasion)

- We perform scans of your baby every few days or weekly upon diagnosis, with additional scans as clinically appropriate. These scans are usually abdominal (not internal) and last approximately 20 minutes.

- At each weekly appointment, we will ask you for a 20 ml or a 4-teaspoons sample of blood, as well as a urine sample. We will also ask you to fill in a questionnaire about your medication intake. You will also have cardiovascular tests performed as you did during your first study visit. These will take approximately 45 minutes.

- When you have delivered, we will follow the outcome of your pregnancy. We will see you again 6 weeks afterwards, to carry out cardiovascular tests and obtain a 20ml (4 teaspoons) of blood and a urine sample.

You can opt out of the study at any stage. All tests and scans for the study are carried out at Queen Charlotte’s & Chelsea Hospital. In addition to the scans we perform, we will also obtain the reports of your routine antenatal scans from your maternity hand-held notes.

**What are the possible disadvantages and risks of taking part?**

None of the tests will cause harm to you, your baby or your pregnancy. The blood test is similar to one that you may have at your GP. Where possible, we will coordinate the timing of blood tests with those you are required to have as part of your clinical assessment. Ultrasound scans are routinely carried out in pregnancy, and there is no evidence that it causes any harm to yourself or your baby.

**What are the possible benefits of taking part?**

Taking part in the study will not affect your pregnancy care. By taking part in the study, you will benefit from receiving pregnancy scans to check on the wellbeing of your baby. We hope that women in the future will benefit from your participation and the information we gain from this study.

**What if a problem is detected?**

If a problem is detected, we will refer you to the appropriate clinical team and the condition will be followed-up and managed according to standard practice. We would explain our findings, counsel you with regards to implications on your pregnancy and provide appropriate support.

**Will my taking part in this study be kept confidential?**

All information that is collected about you during the course of the research will be kept strictly confidential and anonymous. Your consent form will be stored securely on hospital premises. Your name and address will be removed from the information when it is shown to other medical staff outside the study.

**What happens if I withdraw?**

You can decide to withdraw at any time without explanation. If you do so, your future care will not be affected by your decision. The data that is already collected will be used in the study unless you ask us not to do so.
Who is organising the research?
This study is being conducted by the staff of the Department of Obstetrics & Gynaecology at Imperial College NHS Trust and Imperial College London. Findings from the PRECEPT study may form part of doctorate projects based at Imperial College London.

Who has reviewed the study?
This study was given a favorable ethical opinion for conduct in the NHS by xxx Research Ethics Committee.

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OrRef: PRECEPT project
Women’s Health Research Centre, c/o Imperial College London, IRDB, Ground Floor, Du Cane Road, W12 0NN, London
Tel: 02033135281, Fax: 02033135284, email: whrcenquiries@imperial.ac.uk
Participant Consent Form

A longitudinal cohort study into maternal cardiovascular and metabolic changes in fetal growth restriction with or without pre-eclampsia in pregnancy (PRECEPT Study)

Version 4 Date: 24th March 2015 REC reference: 15/LO/0341

Principal Investigator:
Mr Christoph Lees MD, MRCOG.

Please initial each box

1. I confirm that I have read and understand the information sheet (Version 4.0, March 2015) for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by responsible individuals from the research staff, study sponsor, NHS Trust, or from regulatory authorities, where it is relevant to me taking part in this research. I give permission for these individuals to have access to my records.

4. I understand that information collected about me will be used to support other research in the future and may be shared anonymously with other researchers.

5. I agree to take part in the above study and have heart and circulation tests and ultrasound scans of my baby at the described points in my pregnancy.

6. I give permission for my samples to be stored for future ethically approved research projects.

7. I agree to my General practitioner being informed of my participation in this study.

8. I give permission for the research team to contact me via phone or email where my details has been disclosed to the research team.

Name of Participant ______________________ Date __________

Signature ______________________________

Investigator taking consent ______________________ Date __________

Signature ______________________________

Copy: Chief Investigator at ICHT Copy: Participant Copy: Hospital notes
Appendix 3c Data collection forms

Participant Interview- PRECEPT Study

To be completed by Clinical Research Fellow /Research Midwife at first study visit with participant

Version 4.0 – 19 March 2015

Date:

EDD:

Personal Details:

Participant number:
Address
Telephone number (home & mobile)
Email address
GP name
GP Practice Address
Ethnicity
Occupation

Partner details:

Name
Ethnicity
DOB
Occupation

Past medical & surgical history

Chronic hypertension ☐ (Age diagnosed )
Medication history including medication allergies

<table>
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<td>Aspirin</td>
<td></td>
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</table>

**Lifestyle:**

Do you smoke
- Yes [ ]
- No [ ]
- Ex-smoker [ ]

If Yes, how many a day?
- Light (<10) [ ]
- Moderate (10-20) [ ]
- Heavy (>30) [ ]

Highest educational attainment
- Postgrad [ ]
- Degree [ ]
- A Levels [ ]
- GCSE/O Levels [ ]
- No formal education [ ]

How much alcohol do you consume in a week?
- >14 units [ ]
- <14 units [ ]

Caffeine intake – average number of cups a day

**Family history of disease**

*Cardiovascular events*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Select</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Parents</td>
<td>(Age   )</td>
</tr>
<tr>
<td>Siblings</td>
<td>(Age   )</td>
</tr>
<tr>
<td>MI</td>
<td></td>
</tr>
<tr>
<td>Parents</td>
<td>(Age   )</td>
</tr>
<tr>
<td>Siblings</td>
<td>(Age   )</td>
</tr>
<tr>
<td>CVA</td>
<td></td>
</tr>
<tr>
<td>Parents</td>
<td>(Age   )</td>
</tr>
<tr>
<td>Siblings</td>
<td>(Age   )</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Parents</td>
<td>(Age   )</td>
</tr>
<tr>
<td>Siblings</td>
<td>(Age   )</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>(Age   )</td>
</tr>
<tr>
<td>Siblings</td>
<td>(Age   )</td>
</tr>
</tbody>
</table>
**Obstetric history:**

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Year</th>
<th>MOD</th>
<th>Birth weight</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**Anomaly USS**  
[ ]

**Booking BP:** (gestation )

**Booking BMI**

Height (cm)

Weight (kg)

**Participant anthropology:**

Weight (kg)

Body mass index
Pathology group only

PE □  FGR □  PE + FGR □

Gestation at Diagnosis

BP reading at diagnosis

Current medications

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Length of Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labetalol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td></td>
<td></td>
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<tr>
<td>Methyldopa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydralazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blocker ( )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ultrasound

AC

HC

BPD

FL

EFW

BW Centile

AFI

Doppler  PI  RI
Participant code:  
Date:  
Gestation:  
Visit:  
WEIGHT:  

Sitting
Peripheral  
1) BP  
Pulse  
2) BP  
Pulse

Lying (Left lateral)- Document length ( cm) sternal notch to mid leg cuff (lying STRAIGHT)

Vicorder - PWA
BP  
AoBP
PP  
AoPP  
Beats:
SEVR  
Augmentation pressure
HR  
Alx
MAP  
ESPI
ESP  
TPR
SV  
CI
CO

Vicorder - PWV
PPI  
TT
PWV  
HR

INNOCOR
CO  
SV
Cl  
HR
Vo2

Blood taken ( : ) Urine taken ( : )

Standing  
1) BP  
Pulse  
2) BP  
Pulse

INNOCOR
CO  
SV
Cl  
HR
Vo2

PRE:                                Visit:                     Date:
EDD:                                Gestation:                 Weight:

Sitting
Peripheral  1) BP                Pulse
2) BP                Pulse

Lying (Left lateral)- Document length (___ cm) sternal notch to mid leg cuff (lying STRAIGHT)

Vicorder - PWA
BP                              AoBP
PP                              AoPP
SEVR                             Augmentation pressure
HR                              Alx
MAP                              ESPI
ESP                              TPR
SV                              CI
CO

Vicorder - PWV
PPI                                TT
PWV                                HR

INNOCOR
CO                              SV
CI
Vo2

Take URINE SAMPLE (___ : ___) - take straight to lab if possible. Take blood sample (___ : ___)

Standing
Standing  1) BP                Pulse
2) BP                Pulse

INNOCOR
CO                              SV
CI
Vo2
REST, remain standing for 1 – 2 minutes

Exercise INNOCOR – 2.5 minutes then take measurement

<table>
<thead>
<tr>
<th>CO</th>
<th>SV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td></td>
</tr>
<tr>
<td>Vo2</td>
<td></td>
</tr>
</tbody>
</table>

Standing

1) BP       Pulse
2) BP       Pulse
Dear PRECEPT participant,

This questionnaire is to enable us to find out what medicinal products you have used recently, which is relevant when we analyse the blood and urine samples that you have provided today.

It is important that you record any medication that you have consumed, including prescribed medications, nutritional supplements, vitamin tablets and over-the-counter tablets. Please let us know if you have any questions.

With thanks,

PRECEPT Research Team

1) Please record any medications which you have taken over the last 24 hours, including nutritional supplements, vitamin tablets, or over-the-counter medications.

<table>
<thead>
<tr>
<th>MORNING</th>
<th>AFTERNOON</th>
<th>EVENING</th>
<th>NIGHT/EARLY DAWN</th>
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</thead>
<tbody>
<tr>
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</tbody>
</table>

2) Please record if you have had any antibiotics over the last 7 days, and how many days you have taken if for. If known, please state the name of the antibiotic.
<table>
<thead>
<tr>
<th>Study short title</th>
<th>PRECEPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&amp;D Study ref#</td>
<td></td>
</tr>
<tr>
<td>Principal investigator</td>
<td>Christoph Lees</td>
</tr>
<tr>
<td>Sponsor</td>
<td>Imperial College London</td>
</tr>
<tr>
<td>Site</td>
<td>QCCH</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>PRECEPT Number</th>
<th>Sample Type</th>
<th>Date of collection (DD/MM/YYYY)</th>
<th>Time of Collection (C) and Freezing (F)</th>
<th>Location (Freezer Number / Box Number)</th>
<th>Date of Transfer to IRDB*</th>
<th>Destruction Date (If applicable)</th>
<th>Print name &amp; Sign</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Blood</td>
<td>:</td>
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<td></td>
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<td></td>
<td>Urine</td>
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<td>B</td>
<td>Blood</td>
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<td>E</td>
<td>Blood</td>
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<td>Urine</td>
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</tr>
</tbody>
</table>

*All samples will be transferred to the IRDB for long term storage and analysis.*
**PRECEPT Delivery Information**

**Delivery date:**

**Time:**

**Onset of labour:**
- Induction
- Spontaneous
- Pre-labour

**Method of IOL:**
- Prostaglandin
- Syntocinon
- Other

**Mode of delivery:**
- SVD
- Forceps
- Ventouse
- ELCS
- EMCS

**Indication for CS/ instrumental:**

**Birth weight:**
- Male / Female

**(Name: )**

**Cord gases:**

**Apgars:**

**NNU:**

**Complications in labour:**

**Medications in labour:**

**Highest BP:**

**Epidural:**

**PET Protocol:**
- Yes
- No

**PET bloods:**

**Medications post-delivery:**

**Postnatal 6/52**

**Medications:**

**PET bloods:**

**Baby:**

**Complications:**
Appendix 3d Posters

Recruiting for PRECEPT Study

- Making a new diagnosis of Pre-Eclampsia?
- Reviewing scans with fetal growth restriction and abnormal dopplers?

Please contact Dr Jasmine Tay:
Ext: 37316 E: precept@imperial.nhs.uk  T: 07714051359 Blp: 9908

The PRECEPT study is a collaboration between Mr CC Lees and Professor P Bennett (Imperial College London) and Professor I Wilkinson and Dr Carmel McEniery (University of Cambridge)

Imperial College London
Imperial College Healthcare NHS Trust
UNIVERSITY OF CAMBRIDGE

PRECEPT Staff Study Poster- Version 1, Apr 2015
JOIN THE PRECEPT STUDY

We are trying to understand whether changes in your circulation in pregnancy are linked to problems in pregnancy. We’ll do simple, safe, non-invasive tests lasting approximately 1 hour and take blood & urine samples from you during and after pregnancy. And you’ll have extra ultrasound scans of your baby

Interested? contact Dr Jasmine Tay:
E: precept@imperial.nhs.uk  T: 07714051359

The PRECEPT study is a collaboration between Mr CC Lees and Professor P Bennett (Imperial College London) and Professor I Wilkinson and Dr Carmel McEniery (University of Cambridge)