Cardiovascular Sciences

Cardiovascular haemodynamic changes in women who develop gestational diabetes

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Cardiovascular haemodynamic changes in women diagnosed with gestational diabetes: A longitudinal case control study

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Declaration

I declare that this thesis is the result of my own work based on research that was undertaken at the Academic division of Obstetrics and Gynaecology, Leicester Royal Infirmary, University Hospitals of Leicester during the period of January 2015 to January 2018

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Publications and presentations

Publications

1. Longitudinal study to assess changes in arterial stiffness and cardiac output parameters among low-risk pregnant women.
   ***Osman M.W**, Nath M, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
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2. Diurnal variation and repeatability of arterial stiffness and cardiac output measurements in the third trimester of uncomplicated pregnancy.
   ***Osman M.W**, Leone F, Nath M, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
   Journal of Hypertension, 2017 Dec;35:12:2436-2442 doi:
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3. Association between arterial stiffness and wave reflection with subsequent development of placental-mediated diseases during pregnancy: findings of a systematic review and meta-analysis.
   ***Osman M.W**, Nath M, Breslin E, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
   Journal of Hypertension, 2018, May;36(5):1005-1014.
   DOI: 10.1097/HJH.000000000001664

4. Haemodynamic differences between women screened for gestational diabetes and low risk healthy women.
   ***Osman M.W**, Nath M, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
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5. The effects of metformin on maternal haemodynamics in gestational diabetes mellitus: A pilot study.
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Presented abstracts

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Mohamed Waseem Osman, Sharan Sambhwani, Asma Khalil, David Webb, Thompson G. Robinson, Hatem Mousa.

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1. The use of arterial stiffness measurements as a predictor of placenta-mediated disease: ISUOG 2016
Mohamed Waseem Osman, Mintu Nath, Asma Khalil, David Webb, Thompson G. Robinson, Hatem Mousa.

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1. Haemodynamic changes amongst women who were screened for gestational diabetes in comparison to health controls
Mohamed Waseem Osman, Mintu Nath, Asma Khalil, David Webb, Thompson G. Robinson, Hatem Mousa

2. The effects of metformin on maternal haemodynamics in gestational diabetes mellitus: A pilot study
Mohamed Waseem Osman, Mintu Nath, Asma Khalil, David Webb, Thompson G. Robinson, Hatem Mousa

3. Does one-size-fit all? A cross-sectional study to assess arterial stiffness among pregnant women with high body mass index ≥35 kg/m^2
Leone FMT, **Osman MW**, Webb D, Mousa H

4. Unique maternal haemodynamic changes in pregnant women with high body mass index≥35 kg/m^2
   Leone FMT, **Osman MW**, Webb D, Mousa H

5. Central systolic blood pressure in pregnant women with high BMI: a new therapeutic target?
   Leone FMT, **Osman MW**, Webb D, Mousa H

The International working group for Maternal Hemodynamics congress 2018

1. Longitudinal changes in maternal hemodynamics in pregnancy and postpartum
   Mohamed Waseem Osman, Mintu Nath, Asma Khalil, David Webb, Thompson G. Robinson, Hatem Mousa.

2. Maternal Hemodynamic changes amongst women who were screened for gestational diabetes in comparison to health controls
   Mohamed Waseem Osman, Mintu Nath, Asma Khalil, David Webb, Thompson G. Robinson, Hatem Mousa

3. A pilot study of the effects of metformin on maternal hemodynamics in gestational diabetes mellitus
   Mohamed Waseem Osman, Mintu Nath, Asma Khalil, David Webb, Thompson G. Robinson, Hatem Mousa

ABSTRACT

Title

Cardiovascular haemodynamic changes in women diagnosed with gestational diabetes: A longitudinal case control study

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Background

Pregnancy can be considered a predictor of future medical risk. Certain conditions that develop in pregnancy, such as, pregnancy induced hypertension and GDM evolve into chronic forms of the condition in subsequent years. GDM is observed in about 5% of pregnancies, is increasing in prevalence, and is associated with complications to the pregnancy and a long-term risk of diabetes in both mother and offspring. More than 60% of women with GDM develop type 2 diabetes mellitus (T2DM) within the following 15 years.

Arterial stiffness is an independent predictor of CV mortality and morbidity, both in low and high risk populations. The link between arterial stiffness and GDM is unclear and debatable with only a handful of small case-controlled studies having investigated arterial stiffness in women with GDM in late pregnancy and in the immediate postpartum period

Aim

The overall aims of the work to be presented in this thesis are to examine maternal cardiovascular changes among women diagnosed with Gestational diabetes mellitus (GDM).

In order to examine the current hypothesis additional studies were performed to assess the diurnal changes and intra-observer repeatability and reproducibility in central cardiovascular haemodynamics during normal pregnancy among low-risk pregnant women. This thesis also explored the longitudinal changes in maternal haemodynamics among low-risk healthy pregnant women and then looked at the haemodynamic changes amongst women who were screened for GDM in comparison to low-risk healthy controls and finally demonstrated the maternal haemodynamics among pregnant women diagnosed with GDM and commenced on metformin in comparison to women diagnosed with GDM remaining on diet modification only.

Conclusion

The null hypothesis that there was no difference in the maternal cardiovascular changes during pregnancy between pregnant women diagnosed with GDM, pregnant women at risk of developing GDM and low-risk healthy pregnant women was robustly rejected

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List of abbreviations

AASI: Ambulatory arterial stiffness index

AC: Alternating current

AIx: Augmentation index

AIx-75: Augmentation index adjusted to heart rate of 75bpm

Ao AIx: Aortic augmentation index

Ao PWV: Aortic pulse wave velocity

AN: antenatal

AS: Arterial stiffness

BMI: Body mass index

Br AIx: Brachial augmentation index

BSA: Body surface area

C1: Large artery compliance

C2: Small artery compliance

Cf-PWV: carotid-femoral pulse wave velocity

CI: Cardiac Index;

CO: Cardiac output

CdBP: Central diastolic blood pressure

CsBP: Central systolic blood pressure

CV: Cardiovascular

CVD: Cardiovascular disease

ECG: electrocardiogram

FGR: fetal growth restriction

GA: gestational age

GDM: Gestational diabetes mellitus

GDM-D: Gestational diabetes mellitus treated with diet modification

GDM-M: Gestational diabetes mellitus treated with metformin

GTF: Generalised transfer function

HAPO: Hyperglycaemia and adverse pregnancy outcome

HBA_1c: Glycosylated haemoglobin

HCG: Human chorionic gonadotrophin

HPL: Human placental lactogen

HR: Heart rate

ICC: Intra-class correlation

IUGR: Intrauterine growth restriction

IUFD: Intrauterine fetal death

Jugsy: Jugular sternal distance

LGA: Large for gestational age

MAP: Mean arterial pressure

MESH: Medical subject headings

MiG: Metformin in gestational diabetes

MoM: multiples of the median

MOOSE: Meta-Analysis of Observational Studies in Epidemiology

MOP: Metformin in obese pregnant women

MRI: Magnetic resonance imaging

NICOM: Non-invasive cardiac output monitor

NO: Nitric oxide

OGTT: Oral glucose tolerance test

OGTT 1: fasting blood glucose value

OGTT 2: Blood sugar value 2hrs after oral intake of 75gm of glucose

PAC: pulmonary artery cathether

PCOS: Polycystic ovarian syndrome

PET: pre-eclampsia

PGI_2: Prostaglandins

PN: postnatal

PRISMA: Preferred Reporting items for systematic reviews and meta-analyses

PWA: pulse wave analysis

PWV: Pulse wave velocity

PWVao: Pulse wave velocity of the aorta

PT/TT ratio: Peak time divided by total time

RDS: respiratory distress syndrome

RT: Return time

SGA: small for gestational age

sBPao: systolic blood pressure of the aorta

SMD: Standardised mean difference

SD: standard deviation

SV: stroke volume

SVI: Stroke volume index

SVR: systemic vascular resistance

TEB: Thorasic bioimpedence

T1DM: Type 1 diabetes mellitus

T2DM: Type 2 diabetes mellitus

TPR: total peripheral resistance

TPRI: total peripheral resistance index

TVR: total vascular resistance

UKPDS: United Kingdom prospective diabetes study

CHAPTER 1:
INTRODUCTION

1. INTRODUCTION

Pregnancy can be considered a predictor of future medical risk. Certain conditions that develop in pregnancy, such as, pregnancy induced hypertension and gestational diabetes mellitus (GDM) evolve into chronic forms of the condition in subsequent years. GDM is observed in about 3-5% of pregnancies, is increasing in prevalence, and is associated with complications to the pregnancy and a long-term risk of diabetes in both mother and offspring(6, 7). More than 60% of women with GDM develop type 2 diabetes mellitus (T2DM) within the following 15 years(8, 9). Diabetes is the most common metabolic disorder affecting pregnancy, and reflects recent global trends in obesity(10). There is a great burden associated with the maternal and fetal complications of diabetes, including, the propensity to becoming obese; hyperglycaemia, pre eclampsia(11), operative deliveries, a higher risk for T2DM, birth trauma, and increased risk of future cardiovascular disease(10). Fetuses of diabetic mothers are at a greater risk of being macrosomic, which is associated with an increased risk of birth trauma such as shoulder dystocia. Furthermore, these infants have a higher risk of suffering from electrolyte imbalances, respiratory disease, obesity and T2DM(10). To reduce the myriad of maternal and fetal complications caused by any degree of glucose intolerance, clinicians need to be able to recognise and treat diabetes in a timely and effective manner(10)

Results from several observational and randomised trials over the course of the last decade demonstrated that the use of metformin in pregnancy is safe, with no evidence of an increase in birth defects or other pregnancy related complications(12-15). In a randomised controlled trial(16), metformin alone or with supplemental insulin, was noted to be as effective and safe as insulin for women with GDM who met the criteria for insulin. Evidence then began to grow implicating blood sugar control with pregnancy complications. The hyperglycaemia and adverse pregnancy outcome (HAPO) study (17) found that there is a continuous association of maternal glucose levels below those diagnostic of diabetes with an adverse outcome with an increased risk of maternal complications like pre-eclampsia (PET). Savvidou(18) and

Hausvater(19) et al then went on to demonstrate that arterial stiffness is increased in diabetic and PET pregnancies, respectively. In a cross sectional study , Savvidou(18) and colleagues showed that arterial stiffness indices were higher in women with established T2DM and GDM, but rather surprisingly, not in type 1 diabetes mellitus (T1DM)(20). Furthermore, the studies showed that there was an association between Augmentation index (AIx) and the duration of diabetes with no association of arterial stiffness indices to diabetes control. The conflicting results could possibly be due to women with T1DM being studied at 20 weeks gestation in contrast to the others at 32 weeks gestation. However, glucose control gauged by the mean glycosylated haemoglobin (HbA_{1c}) was worse in the T1DM group than in either the GDM or T2DM groups (6.4%, 5,7% and 5.8%, respectively). In light of the growing evidence exploring the associations between glycaemic control, the role of oral diabetes therapy, pregnancy complications and future cardiovascular disease, and the recommendation that longitudinal studies were to be undertaken, it was reasoned to be important to explore these interactions closely and therefore led to the work of this thesis.

1.1 NORMAL PREGNANCY

1.1.1 Maternal haemodynamics

The cardiovascular system undergoes physiological adaptations during pregnancy from as early as six weeks gestation. These include an increase in intravascular volume, cardiac output (CO), and heart rate, together with a decrease in vascular resistance and mean arterial pressure (MAP) (21-23).

1.1.1.1 Circulating blood volume

Maternal blood volume increases during pregnancy. The increase in circulating volume begins at week 6 of pregnancy and reaches approximately 50% more than in the pre-pregnant state towards the end of pregnancy. This is due to an increase in plasma, red and white cell volumes(24). The plasma volume increase (40-50%) is proportionately greater than the increase in the red cell volume (15-20%)(24) (Figure 1.1). This causes haemodilution leading to the state described as “physiological anaemia of pregnancy”(24).

There is inconsistency among studies pertaining to the magnitude and timing of this increase. Studies concur that blood volume progressively increases until mid-pregnancy; others describe a plateau in the third trimester(25, 26), however, one study reported a continual increase until term(27).

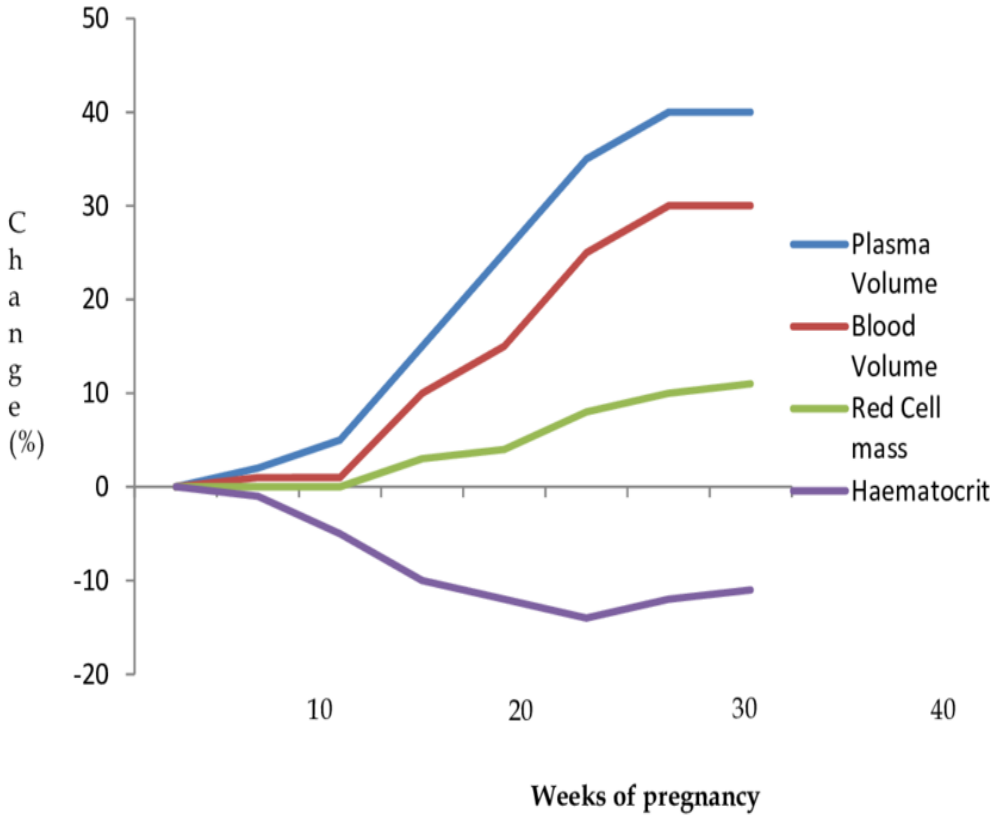


Figure 1.1. Graphical representation of haematological changes in pregnancy(1) (reproduced with permission)

1.1.1.2 Peripheral vascular compliance and resistance

Cardiac afterload refers to the tension developed by the myocardium during ventricular systolic ejection, it is the resistance the heart must overcome to open the aortic valve and propel the blood volume into the systemic circulation. The resistance offered by the systemic circulation is called systemic vascular resistance (SVR) or may be called either total peripheral resistance (TPR) or total vascular resistance (TVR). Systemic ventricular afterload may be determined by either measuring the arterial systolic pressure or calculating the systemic vascular resistance. From the 5th week of pregnancy, there is a decline in SVR, which reaches a nadir between weeks 20 and 32 weeks(22, 23). There is then a gradual increase in SVR from 32 weeks until term(22,

23). The pattern of a reduction in SVR is due to changes in resistance and flow in multiple vascular beds, such as the utero-placental unit(23) and mirrors the pattern of arterial stiffness changes in pregnancy.

1.1.1.3. Anatomical changes during pregnancy

Along with the alterations in preload (expansion of cardiac myocytes prior to contraction), there is remodelling of the atria and ventricles. The left atrial dimensions undergo a gradual increase from as early as the 5th week of gestation, reaching a dimension 15% greater than the preconception measurements(28). Similarly, left ventricular wall thickness progressively increases by about 15% to 20%, however this increase starts at around 12 weeks of gestation(28). Left ventricular mass demonstrates the greatest and most protracted increase. It increases by around 50% in the last trimester of pregnancy(28). These adaptations reduce wall stress, sustain myocardial oxygenation and maintain cardiac performance over the course of pregnancy(29).

1.1.1.4. Myocardial function and contractility

Over the first two trimesters of pregnancy, CO gradually increases with the greatest increase occurring by 16 weeks of gestation(30-32). The increase in the heart rate and stroke volume (SV) contribute to this increase in CO(21). The rise in CO typically plateaus after 20 weeks of gestation but remains at that elevated level until term(31, 33), whereas heart rate continues to increase until around 32 weeks of pregnancy(21). Overall CO increases by 40%, whereas heart rate only increases by around 10%(32, 33). Cardiac function indices remain relatively stable in the third trimester(32, 33). These changes are necessary to allow maternal adaptation to the gravid uterus, and promote the delivery of oxygen and nutrients to the fetus(32). A graphical representation of the changes in cardiac function in pregnancy and postpartum are detailed in figure 1.2.

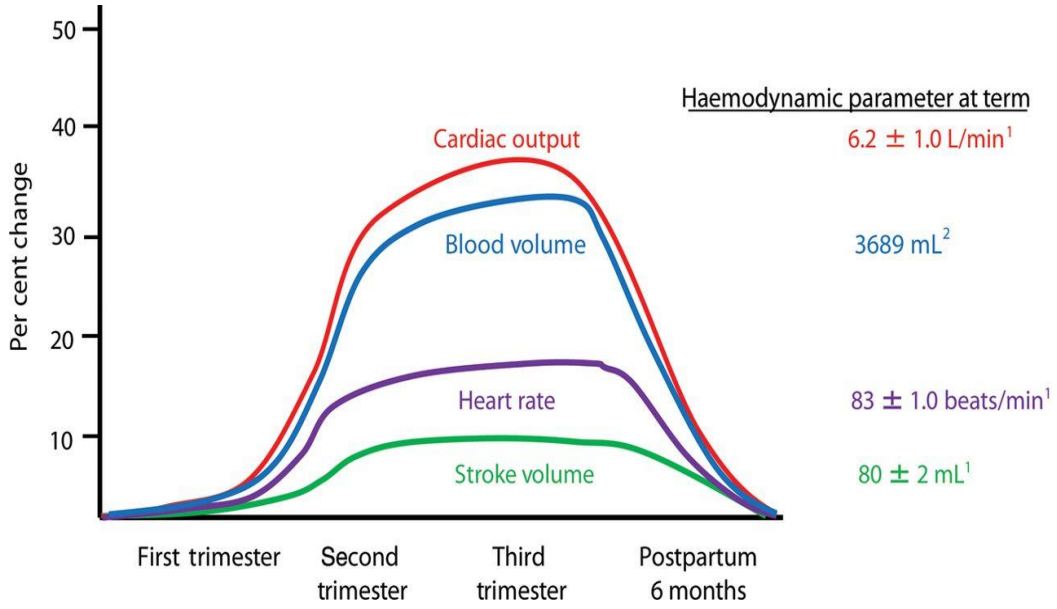


Figure 1.2. Haemodynamics changes during pregnancy. Cardiac output, heart rate, stroke volume, and blood volume all increase between 5 and 8 weeks of gestation, peak by mid-pregnancy, and increases sustained until the end of pregnancy. These parameters are reversed by 6 months postpartum.(2, 3)

1.1.1.5. Neurohormonal factors

A neurohormonal effect is also associated with haemodynamic changes in pregnancy. Vasodilators such as Nitric oxide (NO) and prostaglandins may be responsible for the observed drop in peripheral resistance as well as the changes in uterine and renal blood flow(34). An initiation of baroreceptor –mediated neurohormonal events, such as the activation of the renin-angiotensin-aldosterone and sympathetic nervous systems occurs secondary to these haemodynamic changes(35). The renin-angiotensin system regulates salt and water homeostasis within the body, and both renin and angiotensin are increased during pregnancy(35). The reduced peripheral vascular resistance and arterial pressure leads to the activation of the sympathetic nervous

system, however, the increased plasma volume during pregnancy suppresses catecholamine levels(35). Both these opposing influences are active during pregnancy, and findings within the literature differ with regard to the extent and nature of the net sympathetic activation during normal pregnancy(30, 36).

The natriuretic peptides, atrial (ANP) and brain natriuretic peptide (BNP) are released in response to atrial and ventricular distension, respectively(30, 37). They are involved in integration of cardiovascular states and are released as a response to volume overload(30, 37). Imbalances in the levels of autonomic nervous and renin-angiotensin systems, and impairment in production and activity of vasodilators have all been implicated in the pathogenesis of disease states such as PET(38).

1.1.1.6. Changes in the postpartum period

Haemodynamic parameters undergo a slow return to their pre-pregnancy levels, however full resolution may take as long as 6 months post-delivery. Within the first two weeks postpartum, systemic vascular resistance increases by 30%, conversely, heart rate decreases to its baseline levels within this time(39). Cardiac output demonstrates a sharp increase in the initial first 24 hours postpartum but then slowly returns to pre-pregnancy levels over the next 3 months, in a similar pattern to stroke volume(39). There is a regression of myocardial mass over the 3 months postpartum (40).

1.2 GESTATIONAL DIABETES

1.2.1 Glycaemic control during normal pregnancy

Maternal metabolism, by means of alterations in the hormonal milieu, undergoes significant changes during the course of pregnancy and postpartum; from an initial anabolic state (increase in insulin sensitivity) in the first trimester of pregnancy to a catabolic state (insulin resistance) in the third(41, 42). The purpose of this adaptation is to ensure a continuous supply of nutrients to the fetus despite periodic food intake, and to store nutrients in early pregnancy in order to meet the fetal and maternal demands of late pregnancy and lactation(41, 42). These adaptations are driven by hormones released from the feto-placental unit such as human chorionic

gonadotrophin (HCG), human placental lactogen (HPL), progesterone, cortisol and prolactin, as well as an alteration in β cell responsiveness leading to insulin resistance(41-44). The insulin resistance leads to an increase in maternal plasma glucose and free fatty acid concentrations, allowing for additional substrate availability for supply to the fetus(41). The fetal brain is dependent solely on glucose for its energy supply. In humans, insulin does not cross the placenta in biologically active amounts in either direction(44). Glucose, conversely, does cross the placenta freely by facilitated diffusion. Fetal plasma glucose levels mirror maternal plasma glucose levels(10, 42-44). Unfortunately, the fetus is not able to protect itself from excess glucose, leading to diabetic fetopathy with complications antenatally, intrapartum and in the neonatal period.

1.2.2. Definition and diagnosis of gestational diabetes

Diabetes mellitus is a historic disease, first reported in the Egyptian Ebers papyrus around 1500BC. It was only in 1824 that the first description of diabetes in pregnancy was produced by Bennewitz in Germany(45). Work continued in the quest to determine a link between large babies born from mothers who presented with intensive thirst and recurrent glycosuria. It was only in the 1950's that the term GDM was accepted (46-48). GDM is a form of diabetes that develops during pregnancy and ceases to exist once the baby is born. Pregnancy is a carbohydrate-intolerant state with GDM resulting from both insulin resistance and impaired insulin secretion(49). The prevalence seems to be growing in direct proportion to the increasing incidence of obesity(10) as well as the increasing number of women choosing to fall pregnant at a later age(10, 50).

1.2.3. Screening for gestational diabetes mellitus during pregnancy

With the association between maternal hyperglycaemia and adverse neonatal outcome becoming more apparent, screening programs have been proposed for the early detection of GDM. It is diagnosed by means of a screening test performed in women classified as being at high risk for developing diabetes mellitus. The

International Association of Diabetes and Pregnancy Study Group Consensus Panel recommend that all women should have a 75-gram oral glucose tolerance test (OGTT) between 24 and 28 weeks(51). However, in the UK, after a cost-benefit analysis, the National Institute for Health and Care excellence (NICE) recommend testing for GDM in women who have certain risk factors rather than blanket testing of all pregnant women(50). Further details of the women screened are described in Chapter 2 of this thesis. An OGTT entails testing the plasma glucose of an individual, before and 2 hours after ingesting 75grams of glucose. The body's response to this glucose challenge is then compared and analysed. GDM is diagnosed if the fasting plasma glucose level is 5.6mmol/litre or above, or if a two-hour plasma glucose level is 7.8mmol/litre or above(50).

1.2.4. Prevalence

Overall, up to 5% of pregnant women in the UK have either pre-existing diabetes or GDM(6, 7, 50). The majority (87.5%) have GDM, 7.5 % have type 1 diabetes and the remaining 5 % have type 2 diabetes mellitus(50). We conducted an audit at the University Hospitals of Leicester NHS Trust to assess distribution of ethnicity among pregnant women diagnosed with GDM(52). Ethnicities were divided into three main groups: Caucasian, Asian (India, Pakistan, Bangladesh and East Asia), and African and Caribbean. Data were compared at three time periods including 2003, 2013, and 2015. Overall, there has been a declining trend in the number of pregnant women with GDM in the Caucasian and Asian groups between 2003 and 2013. However, rather disappointingly, both groups then demonstrated an increase in the number of women with GDM in the years from 2013 to 2015. The greatest change in women with GDM was noted in the African-Caribbean group, as the number of cases nearly halved in the two years from 2013 to 2015. Overall, rather worryingly, the results demonstrate significant increase in the incidence of GDM within the Asian population (Figure 1.3, Table 1.1).

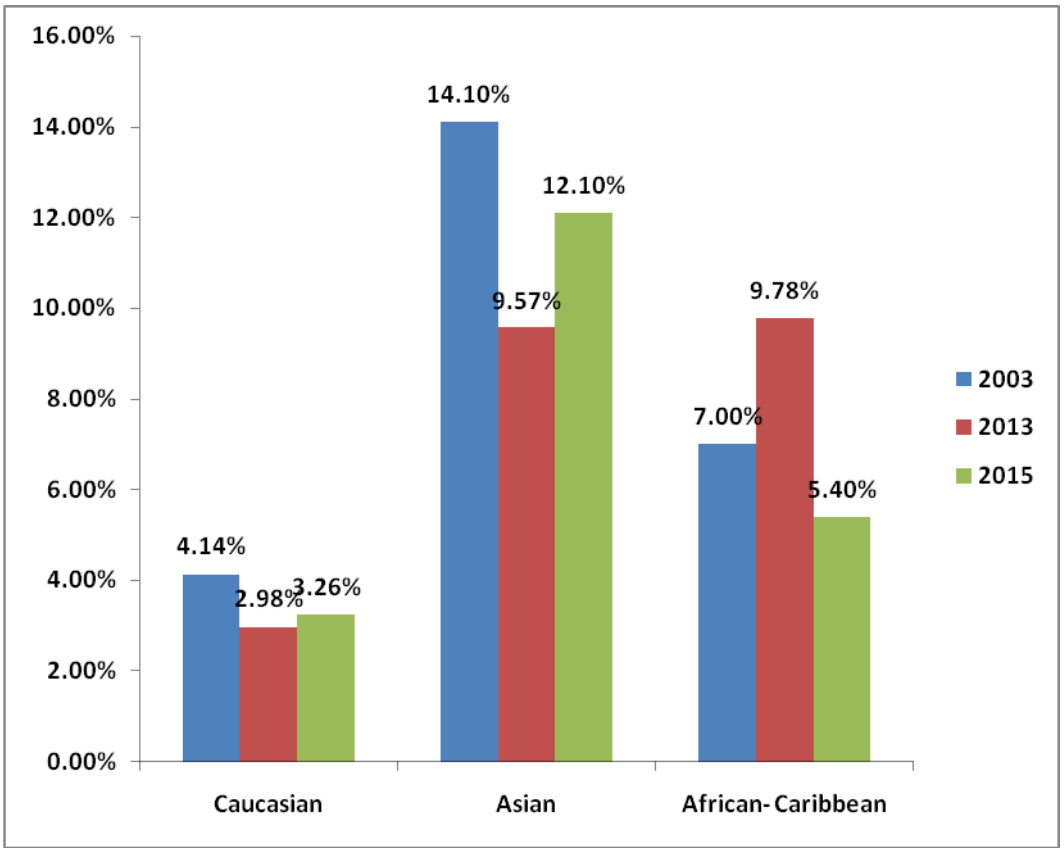


Figure 1.3: Ethnic distribution of Gestational Diabetes mellitus among pregnant women attending diabetic clinic within the University Hospitals of Leicester (2003 to 2015).

Table 1.1: Prevalence of gestational diabetes mellitus according to ethnicity.

Year	Caucasian		Asian		African- Caribbean	
	Total Births	Number of GDM	Total Births	Number of GDM	Total births	Number of GDM
2003	6106	253 (4.14%)	1896	268 (14.1%)	327	23(7%)
2013	7534	225 (2.98%)	2600	249 (9.57%)	511	50(9.78%)
2015	7106	232 (3.26%)	2641	321(12.1%)	477	26(5.4%)

The significant increase of GDM in the Asian population is likely to result in a rising incidence of T2DM in the longer term. The results identify a need for education within the Asian population in order to address lifestyle and dietary causes with emphasis of the immediate and long-term implications of GDM and Diabetes mellitus.

1.2.5. Treatment

Until recently, diabetes in pregnancy was only treated with dietary adjustments and or insulin, as previously oral treatment with Metformin was considered unsafe as the drug crosses the placenta, posing a potential threat to the fetus. However, results from several observational and randomised trials over the past decade demonstrate that the use of metformin in pregnancy is safe, with no evidence of an increase in birth defects or other pregnancy related complications(12-15). Therefore, GDM is now treated with dietary and lifestyle adjustments, metformin or occasionally insulin(50). Metformin is used (unlicensed) as an oral glucose lowering agent in pregnant women with GDM. Metformin, which belongs to the biguanide class of drugs, is the only drug from this class currently in use. Others such as Phenformin were removed from the market due to toxic effects such as a lactic acidosis. Metformin exerts its effect by suppressing glucose production from the liver and increasing peripheral utilisation of glucose by activating AMP kinase(16). Oral metformin has proven to be more acceptable than insulin treatment(16). Insulin therapy requires patient education for the safe administration of the drug(16). Furthermore, use of insulin carries the risk of hypoglycaemia and weight gain, which metformin therapy offers some protection against(16).

Confidence in the use of metformin was reinforced with the results of the Metformin in gestational diabetes trial (MiG)(16), which demonstrated that metformin alone or with supplemental insulin, is an effective and safe treatment option for women with GDM who meet the usual criteria for starting insulin, and that metformin is more acceptable to women with GDM than insulin alone(16). Metformin use in pregnancy was further endorsed by the findings of a systematic review in 2009(53). Nicholson et

al(53) demonstrated that studies have shown that there is no increase in the risk of harm associated with the use of metformin in pregnancy for the treatment of GDM. The Metformin in Obese non-diabetic pregnant women (MOP) trial has further demonstrated the promising effects of metformin(54). This multicentre, placebo controlled trial concluded that among women without diabetes, with a body mass index (BMI) greater than 35, the antenatal administration of metformin reduces maternal weight gain but not neonatal birth weight (4.6 kg [interquartile range, 1.3 to 7.2] vs. 6.3 kg [interquartile range, 2.9 to 9.2], P<0.001)(54). The results also demonstrated a significant reduction in the frequency of PET but not the rate of GDM. The effect of metformin on PET was consistent with the results of a previous meta-analysis reported by Feng and Yang(55)

In addition, the UK prospective Diabetes study (UKPDS) demonstrated that metformin use in obese patients with T2DM is associated with beneficial effects on cardiovascular disease outcomes, with a 36% relative risk reduction in all-cause mortality and a 39% relative risk reduction in myocardial infarction(56). In a randomised, placebo controlled trial, short-term metformin therapy was found to improve arterial stiffness and endothelial function in young women with polycystic ovarian syndrome (PCOS)(57). Findings from a systematic review in 2013 demonstrated the promising effects of metformin in women with PCOS(58). The authors found that metformin use throughout pregnancy in women with PCOS reduced the rates of early pregnancy loss(59-63), preterm labour(64) and potentially protects against fetal growth restriction (FGR)(65).

1.2.6. Effect of GDM on maternal and fetal disease and outcome

It is difficult to categorically differentiate the risks between maternal and fetal health since some of the complications pose a continuous risk to either mother or offspring. The potential maternal and fetal-neonatal risks are tabulated in Table 1.2. Notably, even the slightest level of hyperglycaemia during pregnancy can have an adverse

impact on maternal health which in turn has a direct influence on fetal outcome and neonatal health.

1.2.6.1. Maternal complications: Short term

1.2.6.1.1. Risk of developing hypertensive disease

The Hyperglycaemia and adverse pregnancy outcome (HAPO) study demonstrated a continuous association between maternal glucose levels (even below those diagnostic of diabetes) and adverse outcomes, with an increased risk of maternal complications such as PET(17). There is also work to show that there is an independent and significant association between GDM and PET(11), with the rate of PET being influenced by the level of glycaemic control(66), and the severity of GDM and pre-pregnancy BMI(66). Furthermore, several studies have illustrated that an association with GDM is true for the entire spectrum of hypertensive disorders(67-69). Results from secondary analysis of the Calcium for Pre-eclampsia Prevention multicentre trial demonstrated that the relative risk of developing any form of hypertensive disease in pregnancy reached statistical significance in women who had screened positive for GDM, OR 1.54 (1.28-2.11)(70). More recently, results from a systematic review identified a positive and statistically significant association between GDM and PET (pooled RR = 1.69, 95% CI 1.31-2.18; p< 0.001)(71).

1.2.6.1.2. Mode of delivery

Women with GDM have 1.5 times greater chance of having a caesarean section compared to women without GDM(72), with results from a systematic review demonstrating a consistent association with GDM and caesarean delivery (RR = 1.55; 95% CI 1.24 - 1.51; p < 0.001)(71).

As fetuses of diabetic mothers may have a trunk mass larger than their head, termed macrosomia, vaginal delivery exposes the mother to a greater risk of operative vaginal delivery, episiotomy and perineal trauma(73).

1.2.6.2. Maternal complications: Long term

1.2.6.2.1. Metabolic syndrome, Obesity and Type 2 Diabetes

The majority of women with GDM return to normal glucose tolerance 6-8 weeks postpartum. Nevertheless, the diagnosis of GDM brings a seven fold increased risk of developing T2DM over their lifetime(8). Findings from a systematic review demonstrated the conversion rate to T2DM ranged from 2.6 to 70%, over a period of 6 weeks to 28 years postpartum(74). The method of glucose control during pregnancy also plays a role in the likelihood of developing T2DM, with insulin use carrying a 3 to 5 fold increase in risk, denoting a more severe level of disease(75, 76). Furthermore, studies have shown that being diagnosed with GDM denotes a definitive risk factor, and predictor of the metabolic syndrome(77-80).

1.2.6.2.2. Hypertension and Cardiovascular disease

It is believed that having GDM may lead to a subclinical vasculopathy predisposing women to hypertension and atherosclerotic vascular disease(81, 82).There is also evidence demonstrating that cardiac function is impaired post GDM pregnancy. Heitritter et al evaluated maternal haemodynamics in women with prior GDM and compared them to controls(83). The authors found that women with prior GDM had greater vascular resistance with reduced CO and SV as well as elevated markers of inflammation. The effects of GDM was highlighted by Carr et al, who found that these women not only had an increased risk of cardiovascular disease (CVD) risk factors, including metabolic syndrome and T2DM, but they also experienced cardiovascular disease events at a younger age compared to controls without a history of GDM(84).

1.2.6.3. Fetal and neonatal complications: Short term

1.2.6.3.1. Fetal macrosomia

Growth during the initial phases of embryogenesis is controlled by the genome. Once organogenesis is complete, control of growth is influenced by several factors, such as nutrients and the materno-fetal environment as well as aberrant metabolic states,

such as diabetes(85). There is an increased placental transfer of glucose and nutrients from a mother with GDM to her fetus. This surplus of available nutrients leads to increased growth of the fetus and a typical fetal growth pattern leading to macrosomia. Macrosomic babies have birth weights in excess of 4000 grams which are likely to be above the 95th percentile of gestational age for the population(86). These fetuses are at a greater risk of death, premature birth, birth trauma and respiratory distress syndrome (RDS). Even though these fetuses appear to be "well grown", they are less resilient to a hostile intrauterine environment and respond poorly to intrapartum insults.

1.2.6.3.2. Shoulder Dystocia and Brachial plexus injury

Shoulder dystocia is an unexpected condition whereby the fetal shoulders get trapped under the pubic symphysis of the mother and fails to deliver after an already delivered head. Even though 50% of cases of shoulder dystocia occur in neonates weighing less than 4000 grams, it is much more common in fetuses born to diabetic mothers, due to the fetus being macrosomic (87). Even when managed appropriately, shoulder dystocia can result in significant perinatal morbidity and mortality(88). Brachial plexus injury is one of the most important complications and affects up to 16% of such deliveries(89, 90). Permanent brachial plexus injuries were more likely in macrosomic babies(91, 92). Furthermore, morbidity is increased to the mother when shoulder dystocia occurs as there is a greater incidence of postpartum haemorrhage (11%) and severe perineal tears (3.8%).

1.2.6.3.3. Respiratory distress syndrome and preterm delivery

RDS, also known as hyaline membrane disease, is a breathing disorder of premature newborns in which the alveoli fail to remain patent due to the absence of or inadequate production of surfactant. It is more common in premature neonates, however, RDS is more prevalent in full term neonates born to women with GDM. Evidence suggests that hyperglycaemia may delay fetal lung maturity(93). Interestingly, women with good glycaemic control have fetal lung maturation comparable to non-diabetic women at similar gestation, suggesting glucose control is

an important determinant of fetal RDS(94). Even though RDS may be treated successfully, sequelae are severe and far reaching with long term morbidity. There is conflicting evidence regarding the increased risk of spontaneous preterm delivery in GDM(95), however, there is a greater chance of iatrogenic preterm delivery(96). Figure 1.4 demonstrates findings from a population based study which concluded that fetal outcomes such as Erb's palsy, large for gestational age (LGA), birth weight greater than 4.5kg, prematurity and major malformations are more common among pregnant women with GDM than in non-GDM pregnancies(4).

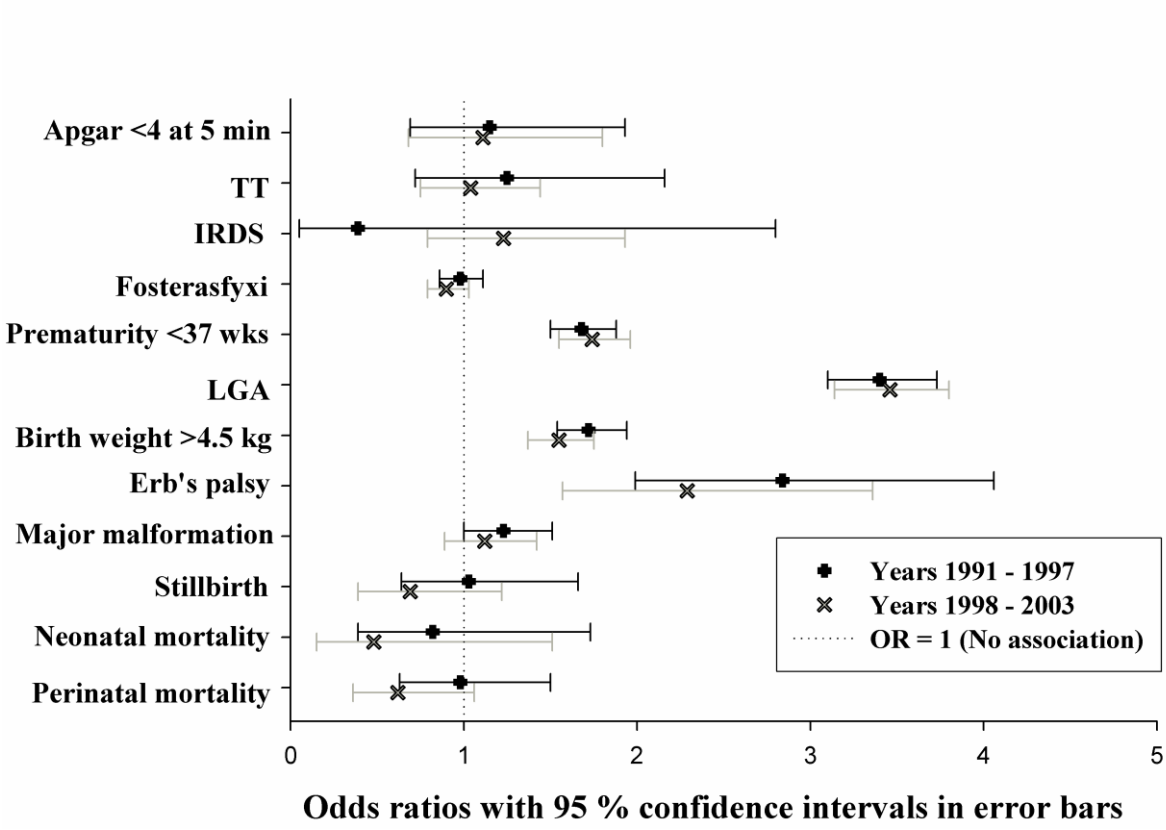


Figure 1.4. Relative risk of different outcomes for child in women with gestational diabetes mellitus (GDM) and the general obstetric population. Maternal and neonatal outcomes and time trends of gestational diabetes mellitus in Sweden from 1991 to 2003 (n = 1,260,297 women), split into 1991–1997 and 1998–2003. (Reproduced with permission(4).)

1.2.6.4. Fetal and neonatal complications: Long-term

1.2.6.4.1. Metabolic syndrome, obesity and Type 2 diabetes

Obesity with its subsequent risk of diabetes and affiliation with metabolic syndrome may develop in utero with macrosomic growth(77). Evidence suggests that offspring of diabetic mothers are at a greater risk of developing childhood obesity or metabolic syndrome(97). This leads to insulin resistance and increased risk of diabetes(97, 98). Evidence remains inconclusive on the risk of cardiovascular disease(99, 100); however, cardiovascular disease is a known and accepted potential consequence of obesity and T2DM.

Table 1.2. Maternal and fetal disease risks divided into short and long term risk.

	Maternal	**Fetal**
Short term	Propensity to weight gain Risk of developing Hypertensive disease Increased risk for Caesarean section and instrumental delivery	Fetal macrosomia Shoulder dystocia and brachial plexus injury Respiratory distress syndrome Neonatal Hypoglycaemia Hyperbilirubinaemia and jaundice Polycythaemia Calcium and magnesium abnormalities Preterm delivery
Long term	Metabolic syndrome Obesity Type 2 Diabetes Hypertension Cardiovascular disease	Metabolic syndrome Obesity Type 2 Diabetes Cardiovascular risk

1.2.7. Pathogenesis linking diabetes and hypertension

There are two theories exploring the link between diabetes and hypertension. Firstly, insulin resistance with secondary hyperinsulinaemia; it being postulated that the hypertensive effect of hyperinsulinaemia is due to weight gain, renal sodium retention leading to extracellular fluid volume expansion, and increased sympathetic activity(101). Secondly, the presence of low-grade inflammation considered secondary to obesity. High adiposity and hyperlipidaemia are associated with obesity and it is understood that inflammation mediated by adipokines and cytokines(102), especially tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)(102-104), may modulate insulin resistance in GDM.

As well as an impact of GDM on the spectrum of hypertensive disorders in pregnancy, there may also be adverse effects on arterial stiffness. In the next section, the definition and measurement of arterial stiffness will be discussed, before a review of the current knowledge of the effects of GDM on vascular stiffness.

1.3. ARTERIAL STIFFNESS

"The pulse ranks the first among our guides, no surgeon can despise its counsel, no physician can shut his ear to its appeal"

Mahomed (1874)(105)

Civilisations of the past were aware of the importance of assessing the character of the pulse, but did not fully appreciate it. "The Yellow Emperor's Classic of Internal Medicine", described that hardening of the pulse may suggest disease of the kidney(106). A full grasp of the true function of the vasculature was lacking as was evident in the name of the prime vessels in the cardiovascular system, "artery", derived from aer and terein, meaning air-duct. This led to stagnation in the area for several years, up until the sphygmograph was developed by Marey and modified over time by several physicians(107). It was to be a medical student who would make the major contribution. Frederic Akbar Mahomed used the sphygmograph to analyse pulse waveforms on the radial artery of individuals with Bright's disease, now considered to be part of a spectrum of conditions within glomerulonephritis. He found a difference in waveforms between healthy subjects and individuals with Bright's disease but was unable to quantify it due to a deficiency in knowledge (105). Because of this inability and the difficulty in using this mechanical instrument, the sphygmograph became obsolete and paved the way for the development and rapid acceptance of the sphygmomanometer(108). There has now been a substantial increase in research during the 21st century(108), leading to a revival in methods exploring the pulse wave form, with advancing techniques for the assessment of the mechanical properties of arteries. Over the past three decades, studies of the pulse waveform have established that arterial stiffness increases with ageing and also in certain conditions such as hypertension, diabetes mellitus, hypercholesterolemia and end-stage renal failure(109). These are all associated with an increased risk of cardiovascular disease (CVD). A report from an evaluation of global mortality and disease burden estimated that 30% of global deaths in 2011 were due to CVD. They forecast that approximately 23 million people will die of CVD by 2030, a 40% increase from the 17 million dying in

2011, to remain the single leading cause of death.(110) The latest European Society of Hypertension and European Society of Cardiology guidelines emphasise the importance of utilising Pulse wave velocity (PWV) in stratifying total CV risk(111). It was also found that aortic PWV is associated with the presence and extent of atherosclerosis.(112).More recently, obstetricians have explored the possibility that the arterial waveform in pregnancy may be important as the maternal haemodynamic system requires a substantial adaptation to support pregnancy. Failures of vascular adaptation to the requirements of pregnancy have been implicated in placental mediated diseases. Studies have shown that arterial stiffness increases in pregnancies affected by PET, growth restriction and diabetes mellitus(19, 113, 114). Furthermore, research has shown that BMI and changes in BMI are strongly associated with stiffness progression(115).

Arterial stiffness is a general term for deviations in elasticity (or compliance) of arteries. Stiffening of the central arteries (arteriosclerosis) is associated with adverse cardiovascular outcomes in various patient groups(116), as well as in the general population(117). Stiffer arteries increase the load placed on the heart as demonstrated by increased left ventricular oxygen and perfusion demands. Propagation of the pressure wave along the arterial tree, PWV, is related to the intrinsic elasticity of the arterial wall. PWV is increased in stiffer arteries and when measured over the aorta, is an independent predictor of cardiovascular morbidity and mortality(118-121). Given the predictive power of PWV, identifying strategies that prevent or reduce stiffening may be important in prevention of future cardiovascular events.

Augmentation index (AIx), a measure of systemic vascular resistance, is a ratio derived from the blood pressure waveform and is a measure of wave reflection and arterial stiffness. AIx reveals the early changes of arterial stiffness, as the changes are more prevalent in younger individuals (age < 50 years). Whereas, PWV may reflect the later or chronic changes in arterial stiffness as age related changes are more marked in individuals over the age of 50(122). AIx has proven to be a predictor of adverse cardiovascular events in a variety of patient populations, i.e., a raised AIx indicating

target organ damage,(123) and was also found to increase with the duration of having diabetes(20).

1.3.1. Arterial stiffness and normal pregnancy

There are a limited number of studies(124-129) evaluating the longitudinal pattern of arterial stiffness during normal pregnancy. They conclude that PWV decreases mid-pregnancy(124, 128), remains low or increases slightly in the third trimester(124, 125, 128, 130), and returns to baseline in the postpartum period(131-133). AIx demonstrates a similar trend in all studies(124-129); decreasing significantly in the first trimester up to mid-pregnancy and then gradually increasing during the third trimester. However, only three studies(126, 127, 129) evaluated the same group of women longitudinally through pregnancy. Two of these studies(126, 129) adopted applanation tonometry, while one study(127) used an oscillometric method to evaluate maternal haemodynamic parameters. The remaining studies recruited case-matched controls at various gestations in pregnancy(125, 128, 130, 134).

Guidelines from the 2007 European Society of Hypertension propose that in arterial hypertension, PWV over 12m/s suggests sub-clinical organ damage(135). Normal limits for PWV in pregnancy have not been reported. However, in healthy non-pregnant women of similar age it is in the range of 10m/s(136). It has been reported that maternal weight and age increase PWV but not parity, ethnicity or smoking status(126, 137). AIx increases with mean arterial pressure (MAP) and has an inverse relationship with heart rate and body height.

The pattern of an initial reduction of arterial stiffness in the first trimester of normal pregnancy is understood to be due to the alterations of vaso-active substances such as Nitric oxide (NO)(34, 138), progesterone, relaxin, as well as the volume expansion of pregnancy(126). The subsequent rise from the mid-trimester of pregnancy to term is believed to be due to the inhibition of NO by the physiological elevation of Asymetric dimetylarginine (ADMA) (139-141), an increase in Cardiac output (CO)(142) and increased circulatory volume(142).

1.3.2. Arterial stiffness and pregnancy complications

In a systematic review of 23 studies, Hausvater and colleagues examined changes in arterial stiffness among pregnant women who developed PET(19). They observed a significant increase in parameters of arterial stiffness among pregnant women with PET compared to those with gestational hypertension. This suggests that arterial stiffness measurements may play a role in predicting PET, with arterial stiffness per se playing a role in the increased risk of future cardiovascular complications seen in women with a history of PET. The key findings were that PWV and AIx in PET pregnancies were significantly increased compared to that of normotensive pregnancies with a weighted mean difference for PWV (m/s) of 1.04 [95% Confidence Intervals (CI) (0.34 - 1.74)], and for AIx (%) of 15.10 (95%CI, 5.08 – 25.11); consistent with a significant increase in arterial stiffness indices in PET compared to normotensive women(19).

Work has also been done exploring arterial stiffness and fetal growth. In normal pregnancy, a relationship between PWV in the third trimester and birth weight has been reported; an increase of 1m/s in PWV is associated with a reduction in birth weight centiles by 17.6%(143). Tomimatsu et al demonstrated that AIx was significantly higher in mothers of small for gestational age (SGA) babies(114), while Khalil et al(144) reported no difference in PWV and heart rate-corrected to 75 beats per minute augmentation index (AIx-75) between the SGA and healthy groups. Nonetheless, in the SGA group with PET, AIx-75 was increased in comparison to the healthy population. The authors hypothesised that in pregnancies with impaired placentation, one of the determinants of whether there will be the development of PET, or SGA without PET, is a pre-existing susceptibility to CVD reflected in increased AIx-75. These studies suggest that measures of arterial stiffness may be used to detect a high-risk fetus in both low (without PET) and high-risk (with PET) mothers.

As hypertensive disorders of pregnancy account for 10 to 15% of maternal and perinatal morbidity worldwide(145), and the recent knowledge that changes in arterial stiffness measurements may be associated with PET(19), and possibly Intrauterine

Growth Restriction (IUGR)(114, 144), further work will be required to evaluate the correlation between changes in PWV and pregnancy outcome amongst women with pregnancy complications.

1.3.3. Assessment of arterial stiffness:

There are several methods of assessing arterial stiffness with terms such as compliance, distensibility and elasticity used interchangeably with resultant blurring of their true meaning (Figure 1.5)(5). Arterial compliance is defined as the change in volume for a given pressure change, this is demonstrated as a change in artery diameter caused by left ventricular ejection. Distensibility is the relative volume or diameter change for a given pressure change. Compliance and distensibility provide information about the elasticity of the artery as a hollow cylinder. A reduction in arterial elasticity results in reduced arterial compliance and distensibility. Table 1.3 summarises the various measures available for assessing arterial stiffness with the advantages and limitations of each one.

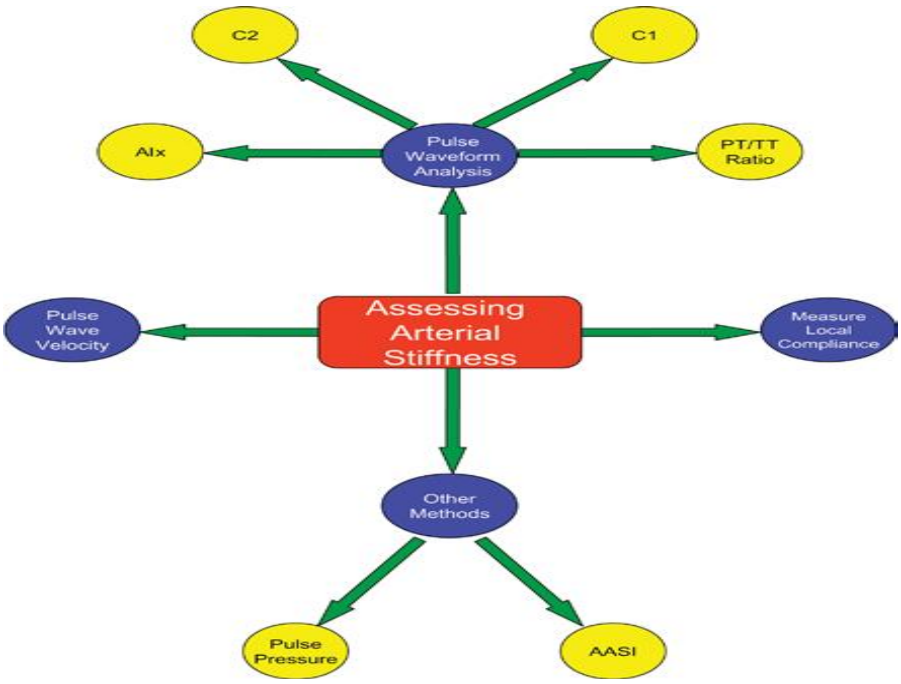


Figure 1.5 Summary of the methods available for assessing arterial stiffness. (Reproduced by kind permission of Hamilton et al(5))

Abbreviations:

C1: Large artery compliance

C2: Small artery compliance

AIx: Augmentation index

PT/TT ratio: Peak time divided by total time

AASI: Ambulatory arterial stiffness index

Table 1.3 Summary of the various measures available for assessing arterial stiffness with the advantages and limitations

Arterial stiffness indices	**Features**	**Calculation**	**Attributes**	**Limitations**
Pulse pressure	Simplest surrogate measure of arterial stiffness Quantifies the impairment of the buffering capacity of the larger arteries	Pulse pressure is calculated by subtracting the diastolic BP from the systolic BP	Predictor of CVD in: • general population • healthy individual • untreated hypertensive patients • treated hypertensive patients • type 2 diabetes mellitus • type 1 diabetes mellitus	Affected by: • number of physiological factors • aortic valve insufficiency • arteriovenous fistulae
Pulse wave velocity	Carotid-femoral PWV is considered the "gold standard" measurement of arterial stiffness	Calculated by measuring the time taken for a pulse wave to travel over a specified distance.	PWV is associated with the presence and extent of arteriosclerosis, end-stage renal disease, and predictive of primary CV events and mortality.	PWV can be influenced by several confounders. <u>PWV is increased by:</u> • smoking • acute and chronic caffeine intake • acute mental stress. <u>PWV is decreased by</u>: • moderate alcohol consumption
AASI: ambulatory arterial stiffness index	• As a single number describes the dynamic relationship between diastolic and systolic BP over a 24hr period.	Calculated by plotting a scatter diagram of systolic BP readings against diastolic BP readings. A regression line is then drawn, and AASI is calculated as 1- the gradient of this line.	Used to predict cardiovascular mortality.	• Lower predictive power for cardiac mortality when adjusted for pulse pressure • Retains predictive power for fatal stroke after adjusted for pulse pressure

	• Reflects the mechanical properties of small arteries			• Predictive value of AASI is lower than the predictive value of PWV • Time consuming and uncomfortable for patients.
AIx: Augmentation index	AIx is derived from the aortic pressure waveform and is expressed as a percentage of the aortic pulse pressure	Equals to the difference between the second and first systolic peaks as a percentage of pulse pressure	Increased in: • hypercholesterolaemia • type 1 diabetes mellitus • endothelial dysfunction • renal disease	Affected by several factors: • alterations in heart rate • left ventricular ejection • PWV • timing of reflection wave • arterial tone • structure at peripheral reflecting sites • BP • age • sex • height
C1: Large artery compliance	Using the Windkessel model, employs computer analysis of the diastolic decay part of the arterial pressure waveform.	Calculated by pulse waveform analysis. Relationship between pressure and volume change in the arteries during the exponential component of diastolic pressure decay	• Large (C1) arterial stiffness has been suggested to parallel endothelial reactivity • Able to determine changes prior to any complication developing	• Doubts about reliability of pulse contour analysis to accurately measure arterial stiffness

C2: Small artery compliance	Using the Windkessel model, employs computer analysis of the diastolic decay part of the arterial pressure waveform. Compliance may either be reflective or oscillatory.	Calculated by pulse waveform analysis. Relationship between oscillating pressure and oscillating volume change around the exponential pressure decay during diastole	• small (C2) arterial stiffness has been suggested to parallel endothelial reactivity • able to determine changes prior to any complication developing • predictor of CV events	Doubts about reliability of pulse contour analysis to accurately measure arterial stiffness
PT/TT ratio: Peak time divided by total time	Used to provide insight into the mechanical properties of small vessels	Calculated by dividing the peak time by the total time	Studied in the microcirculation of patients with Raynaud's phenomenon	Prone to error as PT/TT ratio in digital arteries cannot distinguish between arterial stiffness and early wave reflections

Abbreviations:

C1: Large artery compliance

C2: Small artery compliance

AIx: Augmentation index

PT/TT ratio: Peak time divided by total time

AASI: Ambulatory arterial stiffness index

1.3.4. Methods for the assessment of arterial stiffness

There are several methods of measuring arterial stiffness; each with its own merits and limitations. The European Expert Consensus document on arterial stiffness states that the "gold standard" for measurement of arterial stiffness is the carotid-femoral PWV (cf-PWV). This is due to it being validated with clinical and epidemiological studies in different populations with published reference values(116). Validation means establishing by objective evidence that a process consistently produces a result meeting its predetermined specifications. Carotid-femoral PWV is also known as aortic PWV. The decision to use aortic PWV as the gold standard measure of arterial stiffness was based on several factors, such as its relative ease of use(116), being non-invasive(135), cost-effectiveness, heritage, reproducibility(116, 146) and ability to predict outcome(116). The methods can firstly be divided into either invasive or non-invasive, and secondly into either regional or local(147, 148) (Table 1.4).

Table 1.4: Summary of the various methods of assessment of arterial stiffness divided into level of invasiveness

		Methods	Measurement
Non-invasive	Regional	Applanation tonometry +/- integrated ECG	PWV, PWA and AIx
		Piezo-electronic technology	PWV and AIx
		Oscillometric method	PWV and AIx
		Ultrasound	PWV, distensibility, compliance
	Local	Magnetic resonance imaging	PWV, distensibility, compliance and stiffness index
		Ultrasound	PWV, distensibility, compliance
Invasive	Local	Angiography	PWV, distensibility and compliance

1.34.1. Applanation tonometry

Applanation tonometry utilises Pulse Wave Analysis (PWA) to record arterial pressure waveforms by means of a hand-held tonometer(149). The device resembles a pencil with a sensor at the tip. When it is pressed against an artery, it flattens but does not occlude the artery. It is due to the flattening of this cylindrical surface of either the

radial or carotid artery that the sensor is able to detect the intramural pressure(150). Values of peripheral waveforms are recorded giving pressures at a peripheral artery. Central pressures carry a greater prognostic value, therefore a mathematical (generalised) transfer function is applied to the peripheral pressure waveform to determine the central pressure waveform and thereby determine the central pressures and stiffness(150). This method of determining aortic AIx is called PWA. PWV is the result of the pulse wave transit distance divided by the pulse wave transit time between two sites. Tonometry of the radial artery is superior and more accurate than at the brachial or carotid artery as it is easier to achieve flattening of the radial artery against the bone(151, 152). It was also found that subcutaneous adiposity hinder access to the carotid artery, making location and recording of waves difficult(149). Limitations of this technique are that the predicted central BP values are distorted by the margin of error related to non-invasive measurement of brachial BP(153), multiple measurements may be required and probe placement is critical(149).

1.3.4.2. Piezo-electronic technology +/- ECG

The piezoelectric effect is the ability of certain materials to generate an electric charge in response to applied mechanical stress. To determine aortic PWV, piezoelectric mechanotransducers are used to record the carotid and femoral pulse, with some devices requiring signals to be synchronized to the same electrocardiogram (ECG) R wave. The advantage of this method is that it can determine PWV from several pathways; carotid-femoral, carotid-brachial or femoral-dorsalis pedis. As it is evaluating peripheral waves, a generalised transfer function may be required to determine the central pressures and stiffness. Limitations of this technique are that the predicted central BP values are distorted by the margin of error related to non-invasive measurement of brachial BP(153), the digitized waveform may cause difficulty in distinguishing the arrival time of the wave and the transducer's placement in the femoral region are poorly tolerated as well as reliability of determining the distance travelled(149).

1.3.4.3. Oscillometric method

PWV estimation is obtained by recording oscillometric pressure curves due to changes in volume within the vessel in question by means of plethysmography. Plethysmography is the determination of changes in volume resulting from fluctuations in the amount of blood or air that is contained within the organ or the whole body. Devices register the pulsatile pressure changes in an artery on the upper arm. As the cuff is deflated, the oscillations are increased and reach a peak at mean arterial pressure. Arterial stiffness has an influence on the pattern of the oscillations, and, by linking this to a computer algorithm, arterial stiffness can be calculated. This technique also requires a generalised transfer function to reveal the aortic arterial stiffness. The benefit of this technique is that the exact location of the artery need not be located. Patients do find that the pressure cuff becomes uncomfortable(149) when inflated to supra-systolic BP (systolic BP + 35mmHg)(136). The Arteriograph® is an example of an oscillometric method of determining arterial stiffness.

1.34.4. Ultrasound

Arterial stiffness may also be determined by ultrasound. The technique is restricted to measuring arterial distensibility and compliance of the large accessible arteries (brachial, femoral, carotid and abdominal aorta)(108). PWV may also be determined by estimating the time delay between the diameter waveforms recorded simultaneously at two close positions along the vessels(147). PWV is then determined by the ratio of the temporal and longitudinal diameter gradients. However, it is not possible to analyse the carotid and femoral waves simultaneously, they need to be normalised separately with ECG gating. Although this technique has the advantage of being non-invasive, it does require expensive, minimally mobile equipment which has a steep learning curve and is operator dependent(108). Furthermore, detection of very small changes in vessel diameter may prove to be difficult if the resolution is poor(108, 154). Historic measures to reduce operator dependency have been considered: such as fixing the ultrasound transducer within a robotic arm as well as immobilising the

subject's arm with a brace(108). Newer machines are more accurate and in B-mode, the image produced is more precise and therefore increases the accuracy of the measurements(148). Ultrasound offers simultaneous evaluation of other pathologies such as plaques and blockages on the vessels measured(148).

1.3.4.5. Magnetic resonance imaging

The use of magnetic resonance imaging (MRI) allows direct imaging of the entire aorta without the use of geometric assumptions(147). The use of MRI has continued to increase in modern clinical practice, but its role in being the forerunner for arterial stiffness analysis is yet to be determined. Several arterial stiffness parameters can be assessed with MRI: PWV, elasticity, distensibility, compliance and stiffness index(155). MRI has been used for absolute PWV calibration(156). However, it remains expensive, immobile, time consuming, requires highly trained staff and can only be applied to large arteries(108). Therefore, MRI utilisation for arterial stiffness analysis remains limited to well-equipped research settings.

1.3.4.6. Invasive measures

Undoubtedly, the most accurate method of measuring PWV of the aorta is via the use of flow meters or catheter-based pressure probes via a peripheral artery. The pressure and flow waveforms within the vasculature are used to determine the superimposed pulse wave(157). The high level of accuracy is overshadowed by the invasiveness and risks associated, such as; arterial damage, haemorrhage, emboli and infection. Therefore, this method would not succeed as a routine measure of arterial stiffness except during indicated clinical procedures(158).

1.4. CARDIAC OUTPUT MONITORING

CO is the volume of blood pumped by the heart per minute (ml blood/min). CO is the product of stroke volume (SV) and heart rate and can therefore be manipulated by alteration in heart rate or rhythm, preload, contractility and afterload. CO gives important information about tissue perfusion and oxygen delivery. There are several direct and indirect techniques to measure cardiac output. Methods are largely classified into three groups(159) (Table 1.5).

Invasive methods of measuring CO by means of a pulmonary artery catheter (PAC) are considered to be the “gold standard” technique(160). Unfortunately, these methods have been associated with several iatrogenic complications including pneumothorax, arrhythmia, infection, pulmonary artery rupture, valve injury, knotting and thrombosis leading to embolism(159, 161). It was due to these complications and several technical errors that led to the research and development of less invasive methods for CO monitoring. Newer methods of CO monitoring are easier to use, have fewer complications and have been validated against the ‘gold standard’ method(159). It is for these reasons that non-invasive cardiac output monitor assessments were used in the proposed studies.

Table 1.5 Methods of cardiac output monitoring with advantages and disadvantages

Level of invasiveness	Method	Advantage	Disadvantage
Invasive	Pulmonary artery catheter	Gold standard	Iatrogenic complications Invasive Expertise needed Expensive
Minimally invasive	Lithium dilution CO (LiDCO)	Requires only one arterial line Potential for continuous CO monitoring	Calibration needed Contra-indicated for patients on lithium therapy Contra-indicated in the first trimester of pregnancy with uncertainty regarding safety later in pregnancy May be inaccurate during haemorrhage
	Pulse contour analysis CO (PiCCO and FloTrac)	Continuous CO monitoring Accurate during haemodynamic instability	Requires frequent calibration Requires a central venous line and an arterial line
	Oesophageal Doppler (OD)	Ease of use once trained Reliable	Lack of patient tolerance in pregnancy Bulky equipment Assumptions of the aortic size may be inaccurate
	Transoesophageal echocardiography (TOE)	Clearer image to TTE Good views of atria and atrial septum	Expensive Expertise required Requires transducer to be placed within the oesophagus: requires sedation or general anaesthesia

Non-invasive	Partial gas rebreathing	Ease of use Continuous CO measurement	Clinical experience is limited
	Thoracic bioimpedance	Continuous CO measurement Ease of use	Accuracy affected by: patient movement/positioning, electrocautery, electrode placement and arrhythmia Pulmonary oedema and changes in peripheral vascular resistance may affect reliability
	Thoracic bioreactance	Continuous CO measurement Ease of use in all clinical areas More flexible with sensor placement More reliable in obesity and pleural effusions	Expensive Requires maintenance of skill
	Endotracheal cardiac output monitor	Direct monitoring of impedance changes from the ascending aorta Eliminates any potential interference or anomalous signals from structures in the thorax Continuous CO measurement	Expensive Affected by electrocautery Still not adequately validated in humans
	Portable Doppler device	Completely noninvasive Ease of use Mobile	Concern over inter-observer variability and length of learning curve More research needed
	Photoelectric plethysmography.	Completely noninvasive Ease of use	More research needed Does not meet the required level of clinical interchangeability
	Transthorasic echocardiography	May be performed at various anatomical locations such as the left ventricular outflow tract, the ascending aorta, the main pulmonary artery, the right ventricular outflow tract, the mitral valve and the tricuspid valve More suitable in obstetrics	Less clear images compared to TOE Requires training
	MRI		Not tested in pregnant women

1.5. ARTERIAL STIFFNESS AND GDM

The link between arterial stiffness and GDM is unclear and debatable with only a handful of small case-controlled studies having investigated arterial stiffness in women with GDM in late pregnancy(18, 162, 163)(Table 1.6) and in the immediate postpartum period(164). Savidou et al(18) found that in patients with GDM, mean AIx (a measure of arterial wave reflection) was significantly higher compared to healthy controls, (13.1 ± 8.9% vs 0.7 ± 11.4%; $P < 0.001$), and the mean PWV was marginally increased (6.0 ± 1.5 vs 5.4 ± 0.6 m/s; $P = 0.07$). Results from this cross-sectional study concluded that pregnancies complicated by GDM and T2DM are associated with increased maternal arterial stiffness. When all groups were considered together, there was a significant trend of increasing AIx ($P = 0.001$) and PWV ($P <0.001$) from controls to those with GDM toT2DM. This is in contrast to the two other studies(162, 163) which found that there was no significant difference in PWV and AIx between the GDM and control groups. Savvidou et al(18) had a GDM population that had a significantly higher BP than control subjects, and this may have influenced the results within the GDM group, as it is understood that BP is an accepted determinant of aortic PWV(116, 165). Equally, the control group in the study of Bulzico et al(162) had a higher prevalence of T2DM and cardiovascular disease in their first degree relatives(166), which may be associated with higher aortic stiffness(166).

Importantly, there is increasing evidence that GDM is associated with chronic effects on vascular stiffness and outcomes. For example, it has also been observed that women with a history of GDM have some degree of endothelial dysfunction(164, 167). In addition, Ueland et al have demonstrated that a history of GDM results in the individual having a higher risk of cardiovascular disease at 5-year follow-up as indicated by an increase in PWV and hyperlipidaemic profile, even after adjusting for known risk factors(168). Likewise, it was found that vascular function is influenced by the persistence of subclinical and clinical hyperglycaemia(164, 169).

From the research done to date, the HAPO(17) study demonstrated the continuous association between maternal glucose levels and adverse pregnancy outcome, including an increased risk of PET. While the findings of the MiG(16) and MOP(54) trials highlighted the desired health benefits of metformin use in pregnancy, and the initial and encouraging work done exploring the association between arterial stiffness and GDM(18, 162, 163), as well as other pregnancy complications(19) (Figure 1.6) . However, there was an obvious gap in research within this theme as concluded from our published systematic review(170) which also revealed that there is a significant increase in arterial stiffness and wave reflection parameters among pregnant women who subsequently developed PET and SGA fetuses. However, there were only a small number of studies on arterial stiffness and diabetes in pregnancy, therefore, further research exploring this with a longitudinal study investigating the pattern of arterial stiffness in pregnancy and postpartum, as well as metformin's effects on pregnancies affected by GDM was considered necessary. In the following chapters, normal maternal haemodynamic values in pregnancy are described as well as diurnal and repeatability of these measurements in uncomplicated third trimester of pregnancy is explored. Additionally, differences in maternal haemodynamics between women at risk of GDM and low-risk healthy women are investigated, and the effect of metformin treatment in women with GDM in comparison to women on diet control only and low-risk healthy pregnancy are assessed. The overall aims and objectives of these studies are described in the following chapter.

Table 1.6: List of studies investigating the relation between arterial stiffness and GDM

Title	Author	Year	Study number	Design	Gestational age Mean(SD)	Results
Assessment of arterial stiffness in women with gestational diabetes.	Bulzico et al.	2012	24 women with GDM, with 27 matched controls	Case control study	GDM: 30.2 (5) Control: 31.3 (6.4)	Women with GDM had aortic pulse wave velocity comparable with control subjects: 7.2 ± 0.9 vs. 7.3 ± 1.2 m⁄s (P = 0.79
Arterial stiffness, inflammatory and pro-atherogenic markers in gestational diabetes mellitus	Salmi et al.	2012	22 women with GDM, 31 without GDM	Cross sectional study	GDM: 29 (2.43) Control: 29.6 (1.54)	PWV (8.28 ± 1.48 vs. 7.97 ± 1.12) and AIx (16.73 ± 10.98 vs. 16.13 ± 9.64 %) were not significantly different between the two groups
Maternal arterial stiffness in pregnancies complicated by gestational and type 2 diabetes mellitus	Savvidou et al.	2010	34 women with GDM, 34 controls	Cross sectional study	GDM: 31.5 (1.2) Control: 32 (3.02)	In patients with GDM, compared to their controls, mean AIx was higher (13.1 ± 8.9% vs 0.7 ± 11.4%; *P* <0.001) and mean PWV was marginally increased (6.0 ± 1.5 vs 5.4 ± 0.6 m/s; *P* = 0.07).

ABREVIATIONS:

SD: standard deviation

GDM: gestational diabetes mellitus

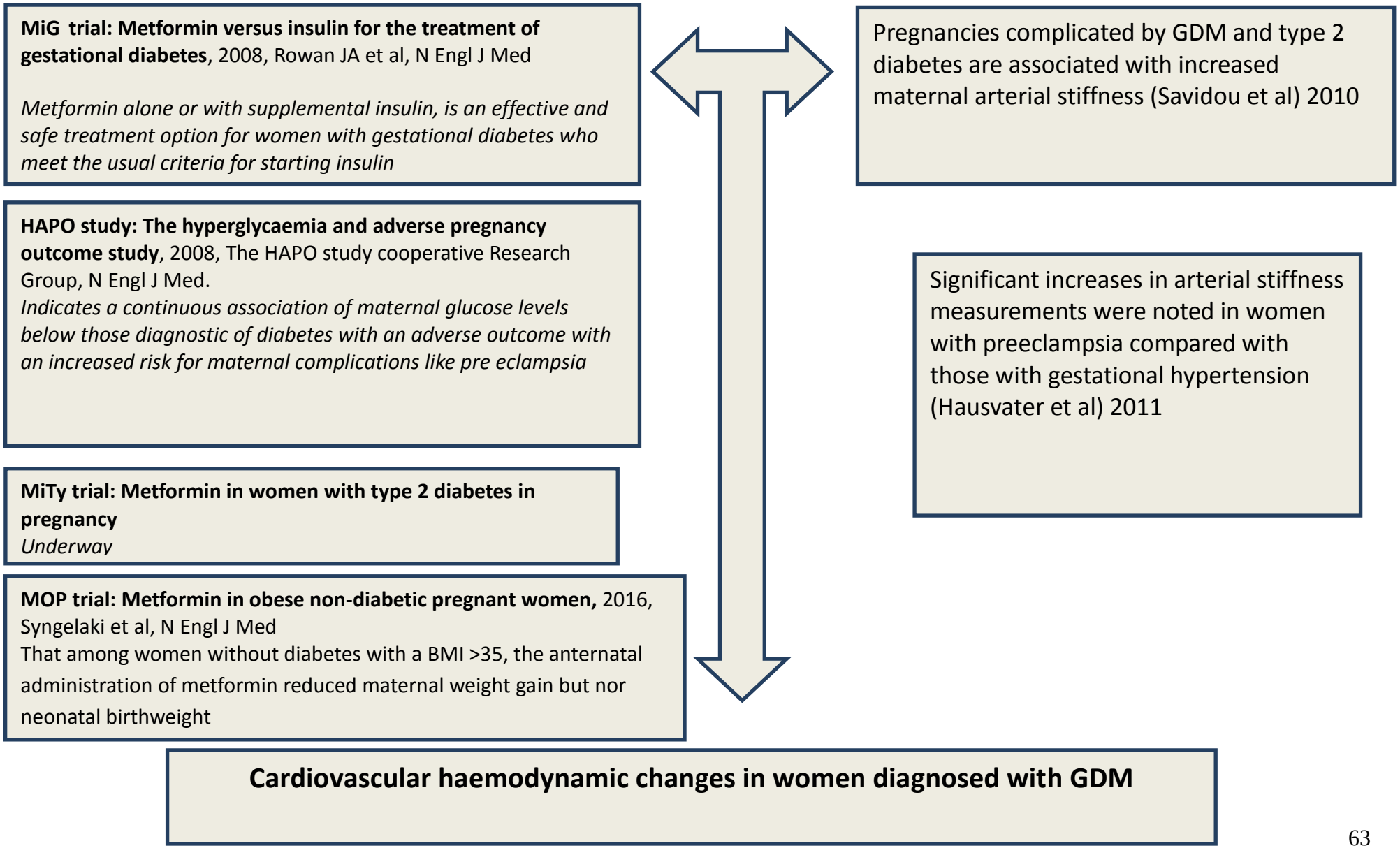


Figure 1.6: Link between current research and thesis

CHAPTER 2:

AIMS AND METHODOLOGY

2. AIMS AND METHODOLOGY

2.1 Aims

The overall aims of the work to be presented in this thesis are to examine maternal cardiovascular changes among women diagnosed with GDM.

The null hypothesis is that there is no difference in maternal cardiovascular changes during pregnancy between pregnant women diagnosed with GDM, pregnant women at risk of developing GDM and low-risk healthy pregnant women.

In order to examine the current hypothesis additional studies were performed to assess**:**

- Diurnal changes in central cardiovascular haemodynamics during normal pregnancy among low-risk pregnant women, which are described in Chapter 4.
- Intra-observer repeatability and reproducibility of maternal haemodynamic measurements in pregnancy by repeated measurements during the same visit, which are described in Chapter 4.
- Longitudinal changes in maternal haemodynamics among low-risk healthy pregnant women, which are described in Chapter 5.
- Haemodynamic changes amongst women who were screened for GDM in comparison to low-risk healthy controls, which are described in Chapter 6.
- Maternal haemodynamics among pregnant women diagnosed with GDM and commenced on metformin in comparison to women diagnosed with GDM remaining on diet modification only, which are described in Chapter 7.

2.2. Ethical approval

Ethical approval was obtained from the NRES Committee London- Stanmore (Reference number: 12/LO/0810) and the University Hospitals of Leicester Research and Innovation Department (UHL ethics reference: 11310) prior to commencing the

study. The study was conducted in accordance with “Good Clinical Practice” and the declaration of Helsinki(171).

2.3. Subjects

2.3.1. Setting

Eligible participants were recruited between May 2015 and March 2016 from the University Hospitals of Leicester NHS Trust (Leicester Royal Infirmary) Obstetrics service. The unit undertakes over 7,000 deliveries per year. The patient population within this tertiary hospital consists of a diverse ethnic mix with high risk pregnant women. There are specialist multidisciplinary antenatal clinics, e.g. diabetes clinic where women were recruited and followed up.

After a full explanation of the investigative procedures aided by a lay language participant information leaflet and a minimum period of 1 hour to consider the decision, written informed consent was obtained. Women were recruited from the antenatal ultrasound clinic, glucose tolerance testing clinic and antenatal diabetes clinic.

2.3.2. Study groups

In order to assess the maternal cardiovascular changes in pregnant women with GDM, participants were recruited into three defined cohorts:

1. Control group: classified as low-risk healthy pregnant women with a body mass index (BMI)>18 and <25kg/m^2
2. At risk screened group: pregnant women deemed at risk of GDM according to the NICE screening criteria,
3. Gestational Diabetic Group: Women deemed to be at risk of GDM according to the NICE screening criteria, and testing positive on OGTT. They are then subdivided according to method of control of diabetes into:
    a) Gestational diabetic under diet control (GDM-D).
    b) Gestational diabetic under Metformin control (GDM-M).

NICE recommends screening of women originating from families with a high prevalence of diabetes from the following countries:

- South Asian (specifically women whose country of family origin is India, Pakistan or Bangladesh)
- Black Caribbean
- Middle Eastern (specifically women whose country of family origin is Saudi Arabia, United Arab Emirates, Iraq, Jordan, Syria, Oman, Qatar, Kuwait, Lebanon or Egypt).

Women are also tested for GDM if they have a BMI greater than 30kg/m^2, have had a previous macrosomic baby weighing 4.5kg or more, previous GDM or have a family history of diabetes (first degree relative).
Screening is offered at 24-28 weeks of gestation and diagnosis of GDM is made if the woman has either:

- A fasting plasma glucose level of 5.6 mmol/litre or above OR
- A 2-hour plasma glucose level of 7.8 mmol/litre or above(50).

Women attending for screening for GDM were invited to participate in the study after meeting rigorous inclusion criteria. Pregnant women screening positive for GDM in their OGTT were placed into the GDM study group. Women screening negative, were placed into the OGTT normal/ GDM-at risk population arm of the study after meeting the rigorous inclusion criteria. For the purposes of this study we have excluded women with pre-existing diabetes mellitus

2.3.3 Study population

Three groups of pregnant women were recruited to the studies described in this thesis. The control group was made up of low-risk healthy women with no medical conditions when booking for pregnancy care and had a normal pregnancy outcome. Their routine antenatal care would be carried out by the midwives, usually in the community. Hospital attendance would usually be for their routine antenatal fetal

dating and anomaly scans. Thirty women were recruited at their first trimester booking/dating scan and 30 women were recruited from the midwife-led Rhesus negative clinic according to the inclusion criteria to provide baseline assessment of the maternal cardiovascular changes in normal pregnancy in comparison to women who develop GDM and those at risk of GDM. A further 21 women were recruited as inpatients in their third trimester as part of the diurnal variation study.

The second group of participants comprised of pregnant women who were deemed to be at risk of developing GDM according to NICE screening criteria. A total of 120 women were recruited at screening for GDM and make up the study population. They were recruited from the OGTT screening clinic. The total screened population recruited to this study (n=120) and they are described and analysed in comparison to the control group in Chapter 6.

Sixty of these women had a normal OGTT value and the remaining 60 had a value meeting the diagnostic criteria for GDM. The latter, made up the third group. The GDM population (n=60) was further separated and analysed according to management of GDM: diet modification (GDM-D) vs metformin therapy(GDM-M) in Chapter 7.

2.3.4 Inclusion criteria

Control group:

1. Aged between 16 and 45 at booking during pregnancy
2. BMI >18 and < 25 kg/m^2
3. Women not classified at risk of developing GDM
4. Women categorised as suitable for midwife-led care
5. Women willing and able to give informed consent for participation in the study.
6. Currently pregnant at time of entry into study
7. Singleton pregnancy
8. Ability to speak and read English

At risk screened group

They included pregnant women identified at risk of developing GDM, and therefore requiring screening with an OGTT. There was no restriction on BMI.

1. Aged between 16 and 45 at booking during pregnancy
2. Women willing and able to give informed consent for participation in the study
3. Currently pregnant at time of entry into the study
4. Singleton pregnancy
5. Ability to speak and read English

Gestational diabetic group

They included pregnant women with a confirmed diagnosis of GDM. There was no restriction on BMI.

1. Aged between 16 and 45 at booking during pregnancy
2. Women willing and able to give informed consent for participation in the study
3. Currently pregnant at time of entry into the study
4. Singleton pregnancy
5. Ability to speak and read English

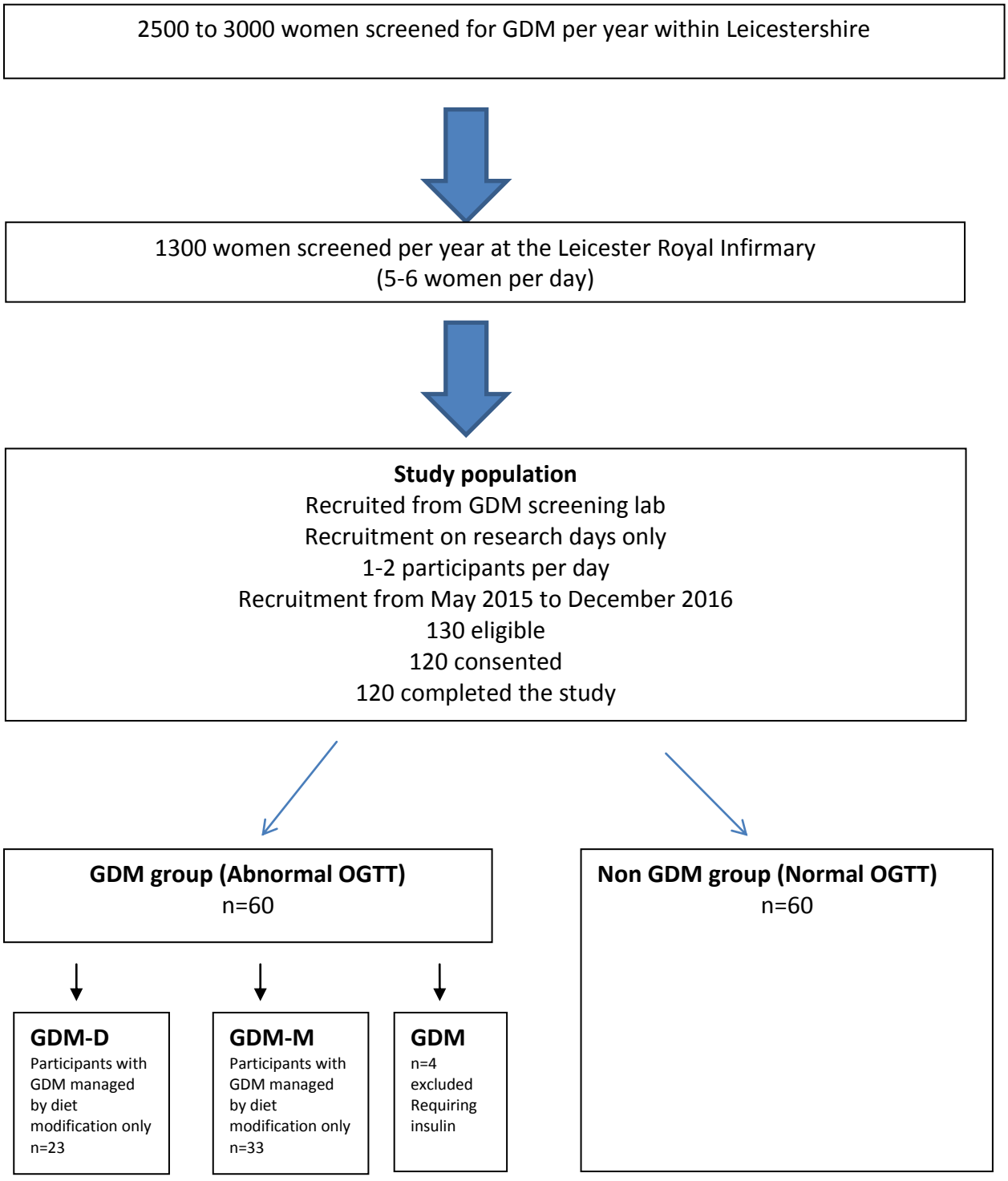
2.3.5 Exclusion criteria:

For all groups in the study

1. Medical conditions that may affect cardiovascular function, e.g: hypertension, hyperthyroidism
2. Pre-existing diabetes mellitus (Type 1 or 2)
3. Medication use which may alter maternal haemodynamics
4. Fetus affected with major congenital malformation or aneuploidy
5. Prisoners
6. Any others deemed to belong to a vulnerable group.

2.4. Consort diagram

2.4.1. Recruitment: Screened population



2.4.2. Consort diagram of recruitment: Control group

7000 Maternities registered at the Leicester Royal Infirmary

15 dating scans performed per day at the Leicester Royal Infirmary
-15 notes screened per day for eligible participants
-3 of 15 women were eligible for recruitment into normal group
- 12 to 15 women did not meet entry criteria

Control group
Recruited from antenatal dating scan clinic
-3 eligible women per day
-Recruitment from December 2015 to March 2016

62 eligible (2 declined participation)
61 consented (1 participant dropped out due to time constraints)
60 completed the study

Measurements:
The first 30 participants had measurements performed at six time points:
13, 20, 28, 34 and 37 weeks gestation and postnatally

The last 30 participants had measurements performed at four time points:
28, 34 and 37 weeks gestation and postnatally

2.5. Data collection

The demographic and clinical data including maternal age, booking and recruitment BMI, body surface area (BSA), ethnicity, parity, OGTT results, BP and gestational age were recorded for all women recruited. Gestational age was established on the basis of the dating ultrasound scan in the first trimester of pregnancy.

Participants were assessed in a temperature-controlled room (22°) in a semi recumbent position. Participants rested for a minimum of ten minutes prior to non-invasive haemodynamic examination and did not speak or move when the measurements where being performed. Arterial stiffness measurements (aortic PWV and AIx were obtained with an Arteriograph® (Tensiomed Ltd, Hungary) and cardiac output analysis was performed using a Non-invasive cardiac output monitor (NICOM®, Cheetah medical, Portland, Oregon). The recordings were made by professional single researcher who received appropriate training according to the standard operating procedures on the use of both the Arteriograph® and the NICOM® devices.

2.6. Arterial stiffness measurements

Arterial stiffness measurements were obtained using a commercially available, non-invasive, validated platform that utilises established methodology, tried and tested in clinical populations. A brief description of these methodologies is detailed below. The device used is called the Arteriograph® (Figure 2.2; TensioMed Ltd, Budapest, Hungary). It falls under the non-invasive, regional category of devices used to determine arterial stiffness(147) , and is fully automated, and has been validated against invasive and non-invasive measurements(172, 173), in non-pregnant population. Even though there are no direct validation studies of the Arteriograph® in pregnancy, it has been used on a very large scale in pregnancy research(137, 172, 174). When compared to conventional tonometric and piezo-electric platforms, measurements from the Arteriograph® had a highly significant correlation (136). Estimates for the variance within one session was lowest for the Arteriograph®

(0.18m^2/s^2, n=219); for the piezo-electic device it was 0.312m^2/s^2 (n=282) and for the tonometric device 0.363m^2/s^2 (n=296)(136). The reproducibility between two sessions was also lowest for the Arteriograph® (1.18m2/s2); as compared to the piezo-electric (1.55m^2/s^2) and tonometric devices (1.67m^2/s^2)(136). The study demonstrated that variability and reproducibility were the best for the Arteriograph® in comparison to the other two devices(136). Furthermore, the Arteriograph faired extremely well when compared to invasive measurements of arterial stiffness during cardiac catherisation(173). The results showed that the AIx (r = 0.9, P< 0.001), SBPAo (r=0.95, P< 0.01) (central systolic blood pressure) and PWV showed strong correlation to the invasively obtained values(173). The PWV values were 9.41 ± 1.8 m/s and 9.46 ± 1.8 m/s (mean ± SD), at cardiac catherisation and by the Arteriograph®, respectively(173). Furthermore, the Pearson's correlation was 0.91 (P < 0.001) with agreed limits of 11.4% for AIx and 1.69m/s for PWV(173).

The Arteriograph® determines the PWV and AIx by analysing oscillometric pressure curves based on plethysmography(175). Plethysmography is the determination of changes in volume within an organ or body part by means of a plethysmograph. The device registers the pressure curves from the brachial artery by its piezo-electric sensor located within the cuff, which resembles a basic blood pressure cuff(176). The system calibrates automatically when it measures systolic and diastolic BP. The main benefit of this method is that the exact position of the brachial artery need not be located. One of the drawbacks of the Arteriograph® is that participants sometimes experience the cuff to feel uncomfortable when inflated to suprasystolic BP. During measurement, it also requires the arm of the participant to remain completely motionless.

Measurement of arterial stiffness entails the placement of a BP cuff over the brachial artery. The cuff inflates to measure the actual systolic and diastolic BP oscillometrically, and then the device decompresses the cuff completely. It then inflates, first to the actual diastolic pressure, then the suprasystolic (systolic BP +

35mmHG) pressure, and records the signals for 8 seconds at both cuff pressure levels. The signals received by the device are transmitted to a laptop computer. The laptop computer has inbuilt software designed to calculate the relevant parameters. In young, healthy individuals, central systolic BP (SBP) is significantly lower than peripheral SBP, whereas DBP remains stable throughout the arterial tree. This phenomenon of an alteration in central and peripheral SBP is known as pulse pressure (PP) amplification(177). Due to arterial stiffening in the older population PP amplification is diminished and therefore central BP may not be representative of brachial BP(178). As central BP has a direct effect on target organ function, it is recommended that central values be optimally obtained and this requires a generalised transfer function (GTF) to the brachial artery waveform to reconstruct approximate central values(116). The GTF mathematically transforms the radial to aortic pressure waveform, and uses the ratio in amplitude and phase of radial and aortic pressure harmonics(116). Controversy does exist with regard to the validity of this approach; however, the estimation of central values continues to be used(179, 180). An example of a GTF with site A being the aorta and site B being the radial artery is:

$$H_{(A-B)} = P_B(\omega)/P_A(\omega)$$

where $P_A(\omega)$ and $P_B(\omega)$ represent the frequency domain of the pressure wave at sites A and B, respectively and ω is the angular frequency(181).

The device takes three minutes to measure the following parameters: peripheral (brachial) BP (systolic BP, diastolic BP, mean arterial pressure, pulse pressure, heart rate), central BP (aortic) (sBPao, dBPao), AIx (aortic and brachial), and aortic PWV. Ideally, the AIx should be measured at the ascending aorta as that is the most accurate representation of the ventricular afterload imposed by central large artery walls. Due to the obvious difficulty in obtaining direct measurements on central arteries, AIx was estimated from the brachial artery waveforms.

The AIx is determined by using the formula:

$$AIx\ (\%) = \frac{P2 - P1}{PP} \times 100$$

P1 is the amplitude of the first (direct) wave, P2 is the amplitude of the later (reflected) systolic wave and PP is the pulse pressure (figure 2.1).

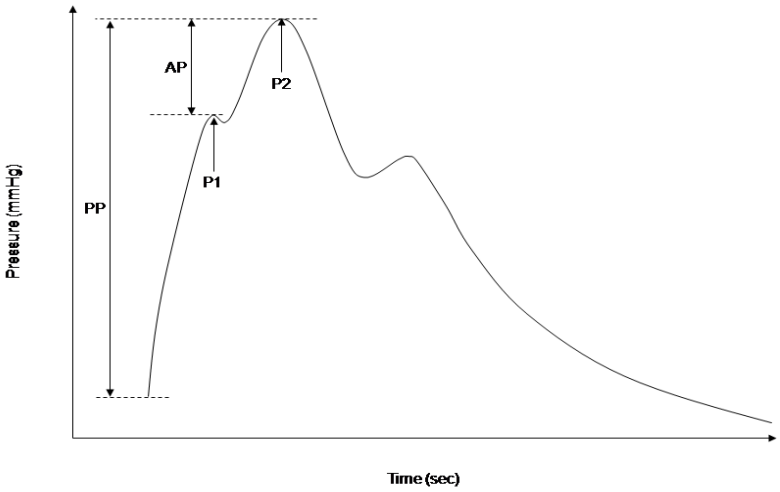


Figure 2.1. The aortic waveform. The first systolic peak (P1) is the maximum pressure created by the advancing pressure wave. The second systolic peak (P2) is a composite of the advancing and reflected waveforms. AIx is Augmentation pressure (AP) expressed as a percentage of aortic pulse pressure.

The Arteriograph® uses the physiological pattern of the wave reflection. The pattern of the ejected direct (first systolic) pulse wave is reflected back mostly from the aortic bifurcation. The time interval between the peaks of the direct (first) and reflected (late) systolic wave (return time) is measured by the device(182). Aortic PWV is considered the 'gold standard' measure of arterial stiffness as the thoracic aorta has the largest contribution to the arterial tree(116) .For aortic PWV (PWVAo) to be calculated, the true aortic length is estimated with the Jugulum-symphysis distance (Jugsy). The Jugsy measurement is the distance between the jugular-sternal notch and

the superior aspect of the pubic symphysis. In essence, PWV is the distance covered by the pulse wave in meters divided by the time required in seconds.

The PWVao is calculated using the formula:

$$PWVao\left(\frac{m}{s}\right) = \frac{JugSy\ (m)}{\frac{RT}{2}(s)}$$

A disadvantage of this method of regional PWV measurement with reliance on the Jugsy measurement for the true aortic length is the margin of error with measuring the Jugsy distance(108). The margin of error could possibly have been greater within this pregnant population given the gravid uterus. Therefore, a means of limiting error was sought. The device manufacturer shared an algorithm via personal communication. This unpublished data on work done by a single examiner comparing the Jugsy measurement to height (n=26,695) on the same individual offered a more accurate determination of the true aortic length. This formula (encrypted) was shared with the author via an excel programme to use on all recruited subjects to limit the chance of error on this key aspect in PWV measurement.

Abbreviations:

PWV: Pulse wave velocity

Jugsy: Jugular sternal distance

RT: Return time

Figure 2.2: The Arteriograph with blood pressure cuff and laptop computer (images courtesy of Tensiomed Ltd)

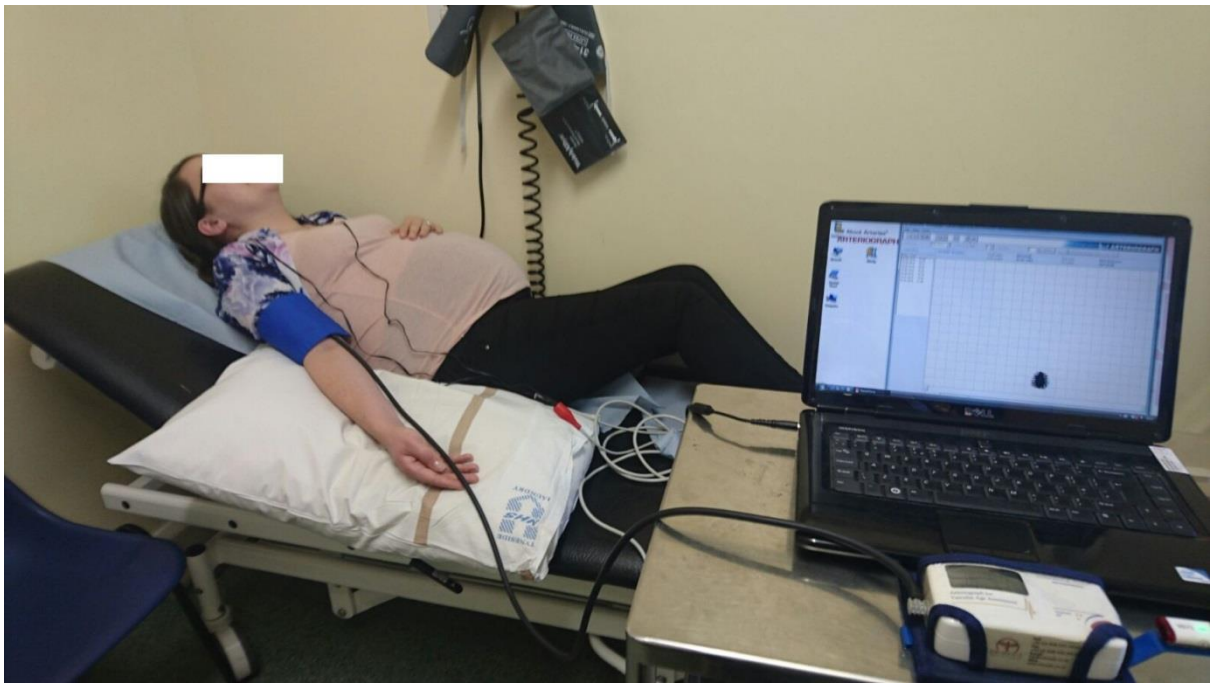


Figure 2.3: Demonstration of setup with pregnant patient in the research assessment room with the Arteriograph® on the bottom right

2.7. Non-invasive cardiac output monitoring

To determine the cardiac output, the Non-Invasive Cardiac Output Monitor (Figure 2.4; NICOM®, Cheetah medical, Portland, Oregon) was used. This is an operator-independent device that uses thoracic bioreactance, which applies the principle by which an Alternating Current (AC) is applied to the thorax, the pulsatile blood flow

taking place in the large thoracic arteries causes the amplitude of the applied thoracic voltage to change. The passing AC also causes a time delay or phase shift between the applied current and the measured voltage. The NICOM® device is a modification of the Thoracic bioimpedance (TEB) method of cardiac output monitoring(159). The TEB method had major limitations due to interference with electrocautery, proper electrode placement, patient movements and arrhythmia affecting the accuracy of results obtained. The NICOM® device is less affected by electrocautery and is more patient and clinician friendly. A small drawback of NICOM is the ongoing cost of the single use skin surface electrodes.

The device requires the placement of two-dual electrodes on either side of the thorax. Sine-wave high frequency (75kHZ) current is transmitted into the body through one electrode (outer sensor), the inner sensor records the signal passing between the inner sensors(159). The final value is the mean to the two readings(159, 183).

In addition to values for cardiac output including heart rate and SV, the NICOM device calculates the stroke volume index (SVI), cardiac output index (COI), the total peripheral resistance/index and BP. The index for each NICOM parameter is the measurement, i.e stroke volume divided by the body surface area (BSA) in square metres. This allows direct comparison of the parameter in question between large and small patients.

There was minimal variation when the NICOM® device was compared to the Pulmonary Artery Catheter (PAC) in a validation study(184). The mean CO for the study sample was 5.17 and 5.18 l/min as measured by the NICOM® system and by the PAC, respectively(184). The NICOM® device has recently been validated against echocardiographic assessment in pregnancy and demonstrated good repeatability and reproducibility, respectively, (ICC=0.953, 95% CI 0.927-0.969)(185).

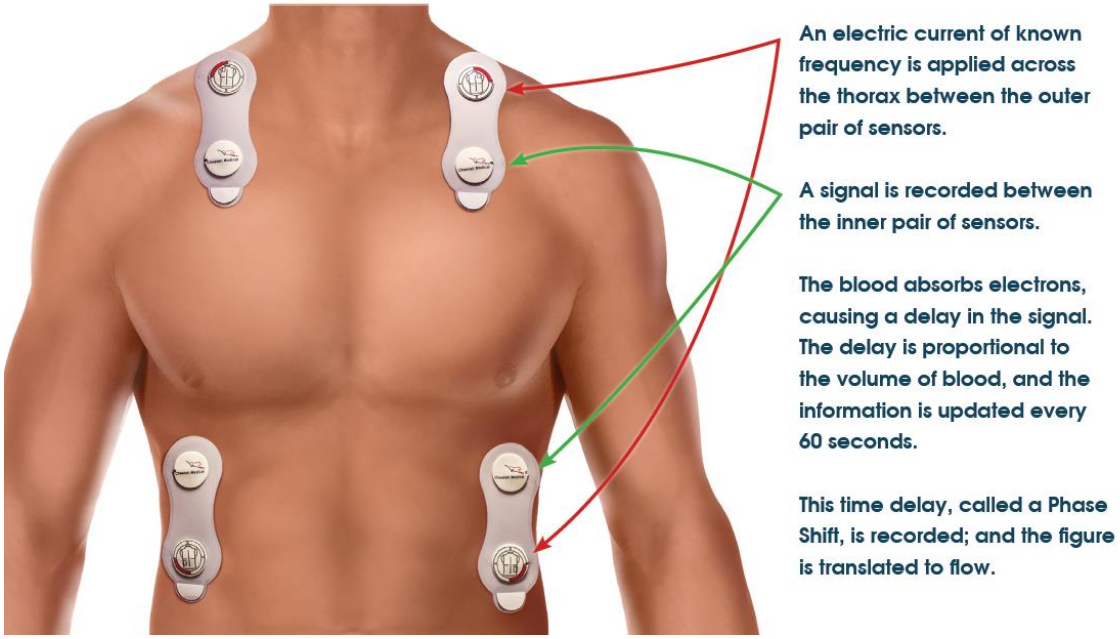


Figure 2.4: Electrode placement over the thorax with annotation for NICOM assessment. In pregnancy, the probes are placed at the same level, but on the back (images courtesy of Cheetah medical)

2.8. Statistical analysis

2.8.1. Sample size

There has been limited data regarding longitudinal changes in normal pregnancy and it was therefore decided to base the power calculation on a similar maternal haemodynamic study undertaken as a MD thesis by one of the named supervisors(186). Recognising that this calculation was based on limited data, it was determined that the study would need 27 women in each group (3 x27) to have a 90% power to detect a difference of 1.5m/s in PWV at the 5% significance level.

The ratio of women in the GDM-diagnosed group requiring treatment in the form of dietary and lifestyle control to metformin is 1:0.9(52). In the GDM-diagnosed group, the study would need 27 participants, in order to detect a difference of 1.5m/s in PWV before and after treatment assuming a SD of 1.5 m/s. A standard deviation of 1.5m/s

was chosen as published work on GDM in pregnancy found that women with GDM had a mean PWV of 6 +/- 1.5 m/s(18).

For the longitudinal study, a small number of longitudinal studies of maternal haemodynamics in pregnancy were identified(126, 131-133), which had a mean (SD) sample size of 51(4.1). Longitudinal studies of this nature are expected to have up to 15% to 20% of participants declining to continue in the study, missing appointments and/or have preterm delivery per time-point. It was therefore concluded that a final sample of 60 participants would be adequately powered and allow for the abovementioned issues. Furthermore, the primary objective of the study was met and therefore suggests that the sample size was adequate.

CHAPTER 3:

ASSOCIATION BETWEEN ARTERIAL STIFFNESS AND WAVE REFLECTION WITH SUBSEQUENT DEVELOPMENT OF PLACENTAL MEDIATED DISEASES DURING PREGNANCY: FINDINGS OF A SYSTEMATIC REVIEW AND META-ANALYSIS

This chapter was published in the Journal of Hypertension

Association between arterial stiffness and wave reflection with subsequent development of placental-mediated diseases during pregnancy: findings of a systematic review and meta-analysis.
***Osman M.W**, Nath M, Breslin E, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
Journal of Hypertension, 2018, In press
DOI: 10.1097/HJH.0000000000001664

The topic of the systematic review was driven by Dr H.A Mousa. Once agreement on the topic was reached, I designed the project, conducted the literature search (with the support of medical librarians: Sarah Sutton and Stuart Glover). Dr E Breslin and I screened the literature separately and reviewed the necessary studies. Dr Nath Mintu conducted the meta-analysis after I collated all the data for him. After interpreting the analysis, I then wrote the manuscript and received academic editorial input from all supervisors. Upon submission, the reviewers' comments were addressed by myself and my response was approved by all supervisors prior to re-submission

3.1 ABSTRACT

Objective

A comprehensive systematic review of published literature was examined to determine whether arterial stiffness and wave reflection measurements during pregnancy differed between healthy low risk women and participants who developed placental-mediated diseases including PET, small for gestational age (SGA), fetal death, and placental abruption, and a quantitative assessment of the findings using a meta-analysis approach.

Methods

We searched MEDLINE, EMBASE and The Cochrane Library for studies of arterial stiffness in pregnancy, analysed pregnancy outcomes and conducted a meta-analysis of data evaluated by trimesters of pregnancy. Haemodynamic parameters evaluated included: PWV, AIx and Augmentation index-75 (AIx-75).

Results

We screened 2806 citations, reviewed 36 studies and included 9 (n=15,923) studies for further quantitative assessment. Compared to healthy pregnancy, measures of arterial stiffness and wave reflection were consistently increased among pregnant women who subsequently developed PET during all trimesters. In the first trimester, mean AIx-75 (%) in the PET group was significantly higher with estimated standardised mean difference (SMD) of 0.90 [95% confidence intervals (95% CI): 0.07-1.73; p=0.034]. In the second trimester, the PET group had significantly higher PWV (m/s) with estimated SMD of 1.26 (95% CI: 0.22-2.30; p=0.018). Concerning the SGA group, mean AIx (%) was greater during the second trimester only: 65.5 (standard deviation 15.6) vs. 57.0 (11.2), p<0.01.

Conclusion

There is significant increase in arterial stiffness and wave reflection parameters among pregnant women who subsequently developed PET and SGA fetuses. Larger studies

with consistent methodological designs are required to evaluate the role and usefulness of arterial stiffness and wave reflection measurements as a screening tool for placental mediated diseases during pregnancy.

3.2 INTRODUCTION

PET and fetal growth restriction (FGR) affects around 3% of all pregnancies(187, 188), while placental abruption complicates up to 1% of all pregnancies(189), and are associated with increased maternal and fetal morbidity and/or mortality worldwide. Of the 30 million growth restricted infants born worldwide each year, 15% (4.5 million) are associated with PET(190) and an estimated half a million die due to PET(190). Therefore, it would be ideal to screen women in early pregnancy to determine if the pregnancy is at risk of placental-mediated disease and individualise antenatal surveillance and timely delivery accordingly.

Arterial stiffness measurements have proven to be an important predictor of cardiovascular risk and great interest has been shown in the role of arterial stiffness in the prediction of pregnancy complications and CVD during pregnancy(19). PWV is regarded as a direct marker of arterial stiffness, whereas AIx is regarded as an indirect marker for arterial stiffness and a direct measure of wave reflection(136).

Most studies evaluating arterial stiffness in pregnancy examine changes that are associated with PET. Hausvater and colleagues examined changes in arterial stiffness among pregnant women who developed PET(19). They observed a significant increase in parameters of arterial stiffness among pregnant women with PET compared to those with gestational hypertension. This suggests that arterial stiffness measurements may play a role in predicting PET, with arterial stiffness per se playing a role in the increased risk of future cardiovascular complications seen in women with a history of PET. The key findings from the systematic review of Hausvater and colleagues were that carotid-femoral PWV (cf-PWV) and AIx in PET pregnancies were significantly increased compared to that of normotensive pregnancies with a weighted mean difference for cf-PWV (m/s) of 1.04, [95% Confidence Intervals (CI) (0.34 - 1.74)], and for AIx (%) of 15.10 (95%CI, 5.08 – 25.11); consistent with a significant increase in arterial stiffness indices in PET compared to normotensive women(19).

In our systematic review, we proposed to examine changes in non-invasive arterial stiffness indices among pregnant women who subsequently developed placental-

mediated diseases during pregnancy. We have focused on the most commonly used arterial stiffness indices studied in the literature (PWV, AIx, AIx-75). In addition, we proposed to investigate whether there was an influence of trimester of pregnancy on these indices.

3.3 METHODS

3.3.1 Data sources and study selection

The study was performed and reported according to the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) statement(191) Table 3.1) and in accordance with the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines(192) (Table 3.2). We searched MEDLINE, EMBASE and the Cochrane Library for relevant citations from database inception to March 2015. In addition, we contacted authors who published abstracts or presented at conferences. The search strategy with medical subject headings (MESH) and keywords are detailed in Table 3.3.

We included studies that evaluated arterial stiffness in normal pregnancies at recruitment and analysed pregnancy complications including PET, FGR, SGA, placental abruption and/or intrauterine fetal death (IUFD), irrespective of the machine or method used for arterial stiffness measurements. We have excluded: a) studies measuring only umbilical and uterine arteries Doppler as a measure of vascular compliance exclusively; b) studies that evaluated pre-pregnancy arterial stiffness and tried to link to pregnancy outcome; c) studies that were carried out during pregnancy but did not address or report placental mediated diseases; and d) studies that examined changes in arterial stiffness among pregnant women following the development of placental mediated diseases such as PET, placental abruption, SGA, intrauterine growth restriction (IUGR), or IUFD.

Based on our search terms and no language restriction, we retrieved 757 papers from MEDLINE, 2049 papers from EMBASE, but we did not retrieve any papers from the Cochrane Library. We evaluated 36 full-text papers and nine studies were included in

the quantitative analysis (Figure 3.1) and excluded 27 (Table 3.4). The flow-chart in Figure 3.1 demonstrates the search strategy and included studies in the systematic review and meta-analysis. Screening was carried out by two independent researchers Dr M.W. Osman (MWO) and Dr E. Breslin (EB); differences were resolved by a third adjudicating reviewer, Dr H.A. Mousa (HAM). We analysed the most commonly used vessel haemodynamic measurements: PWV, AIx and AIx-75; extracted data being analysed by each of the three trimesters.

Table 3.1 Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Pages 3-4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Pages 5-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Pages 5-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 6-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Pages 6-7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it	Pages 6-7

		could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 7-9
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Pages 7-9
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Pages 9
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Page 8

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page 8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Pages 10-12
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Pages 10-12
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 12
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Pages 8-13
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Pages 8-13
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Pages 12-13
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Pages 12-13
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Pages 14-18

Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Pages 14-18
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Pages 17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Table 3.2 Meta-Analysis of Observational Studies in Epidemiology (MOOSE) Checklist

Reporting of background should include	
Problem definition	Introduction, paragraph 4
Hypothesis statement	Introduction, paragraph 4
Description of study outcome(s)	Methods, "Data sources and Study selection"
Type of exposure or intervention used	Methods, "Data sources and Study selection"
Type of study designs used	Methods, "Data sources and Study selection"
Study population	Methods, "Data sources and Study selection"
Reporting of search strategy should include	
Qualifications of searchers (e.g. librarians and investigators)	Methods, "Data sources and study selection"
Search strategy, including time period included in the synthesis and keywords	Methods, "Data sources and study selection"
Effort to include all available studies	Methods, "Data sources and study selection"
Databases and registries searched	Methods, "Data sources and study selection"
Search software used, name and version, including special features used (e.g. explosion)	Methods, "Data sources and study selection"
Use of hand searching (e.g. reference lists of obtained articles)	Methods, "Data sources and study selection"
List of citations located and those excluded, including justification	Supplementary Table 4
Method of addressing articles published in languages other than English	Methods, "S Data sources and study selection"
Method of handling abstracts and unpublished studies	No abstracts were identified that indicated unpublished studies.
Description of any contact with authors	No publications were identified that required contact with authors
Reporting of methods should include	
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Introduction, Results, Table 1

Rationale for the selection and coding of data (e.g. sound clinical principles or convenience)	Methods, "Statistical analysis"
Documentation of how data were classified and coded (e.g. multiple raters, blinding, and interrater reliability)	Methods, "Statistical analysis"
Assessment of confounding (e.g., comparability of cases and controls in studies where appropriate)	Methods, "Statistical analysis"
Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Methods, "Data extraction and quality assessment"; "Statistical analysis"
Assessment of heterogeneity	Methods, "Statistical analysis"
Description of statistical methods (e.g. complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	Methods, "Statistical analysis"
Provision of appropriate tables and graphics	Supplementary table 5, 6, 7, 8
Reporting of results should include	
Graphic summarizing individual study estimates and overall estimate	Supplementary table 6
Table giving descriptive information for each study included	Table 1
Results of sensitivity testing (e.g. subgroup analysis)	Results
Indication of statistical uncertainty of findings	Results
Reporting of discussion should include	
Quantitative assessment of bias (e.g., publication bias)	Results
Justification for exclusion (e.g. exclusion of non–English-language citations)	Supplementary Table 4
Assessment of quality of included studies	Discussion
Reporting of conclusions should include	
Consideration of alternative explanations for observed results	Discussion,
Generalization of the conclusions (i.e. appropriate for the data presented and within the domain of the literature review)	Discussion,
Guidelines for future research	Discussion,
Disclosure of funding source	No funding was obtained to do this study.

Table 3.3: Search strategy

ARTERIAL STIFFNESS	PREGNANCY	COMPLICATIONS/ OUTCOME
"arterial stiffness".	pregnan*.	"IUGR".
"vascular stiffness".	exp PREGNANCY/;	intrauterine growth retardation
"augmentation index".	exp TOXEMIC PREGNANCY/;	intrauterine growth retardation
"central blood pressure".	exp PREGNANCY COMPLICATION	intrauterine growth restriction
"aortic blood pressure".	exp PREGNANCY OUTCOME	intra uter* growth restriction
"aortic pressure".		intra uter* growth retardation
"arterial pressure".		fetal growth retardation
PWV.		foetal growth retardation
"pulse wave velocity".		PIH
"blood vessel compliance".		"pre eclamps*".
"pulse wave analy*".		albuminurea
"blood vessel analy*".		albuminuria
"arter* complianc*".		proteinurea
"Maternal haemodynamics		proteinuria
"maternal hemodynamic*".		(placent* adj2 abrup*).
(maternal adj2 hemodynamic*).		; (soluti* adj2 placent*).
(maternal adj2 haemodynamic*).		"IUFD".
(mother adj2 hemodynamic*).		"intra uter* fetal death".
(mother adj2 haemodynamic*).		“intra uter* fetal demise”
exp ARTERIAL STIFFNESS/;		"intra uter* foetal death".
exp AUGMENTATION INDEX/;		"intra uter* foetal demise".
exp ARTERIAL PRESSURE/;		"foetal death".
exp BLOOD VESSEL COMPLIANCE/;		"fetal death".
exp ARTERY COMPLIANCE/;		"foetal demise".
		"fetal demise".
		"stillbirth".
		"foetal mortality".
		"fetal mortality".
		; "small for gestational age".
		; "toxaemia".
		"toxemia".
		"toxaemic".
		; exp INTRAUTERINE GROWTH RETARDATION
		; (pregnancy AND induced AND hypertension).
		exp MATERNAL

		HYPERTENSION/;
		exp PREECLAMPSIA/;
		exp ALBUMINURIA/;
		exp PROTEINURIA/;
		exp SOLUTIO PLACENTAE/;
		exp FETUS DEATH/;
		exp TOXEMIA/;
		exp TOXEMIA GRAVIDUM
		exp TOXEMIA,PREECLAMPTIC/

The search strategy with MESH and keywords were developed in conjunction with a senior medical librarian from the University Hospitals of Leicester NHS Trust. We used a combination of MESH and keywords to generate three subsets of citations; one indexing all the forms of arterial stiffness measurements; the second indexing pregnancy and the third indexing pregnancy outcome/complications. The three subsets were then combined with “AND” to generate a subset of citations relevant to the research questions. No limits were applied to the search and duplicates were removed during the process of assessing the full-text articles for eligibility.

Figure 3.1: Flow-chart of search strategy and included studies in the systematic review and meta-analysis

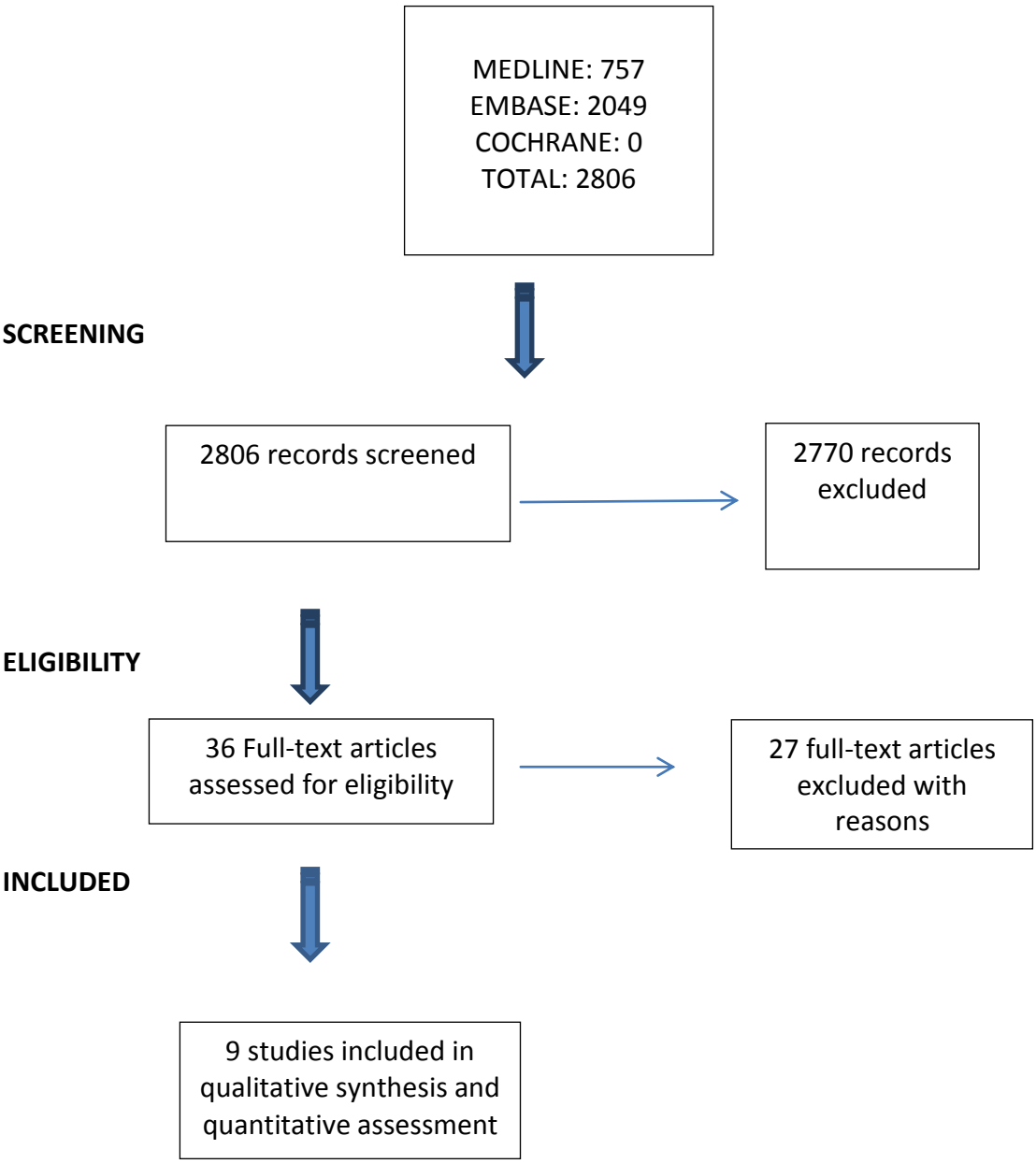


Table 3.4: Details of excluded studies

	Title	Journal	Author	Reason for exclusion
1	**Prediction of iatrogenic preterm delivery in women with chronic vascular disease and/or previous early onset preeclampsia**	Pregnancy Hypertension January 2015, vol./is. 5/1(140), 2210-7789 (January 2015)	Cockerill Cockerill R.; Shawkat E.; Horn J.; Chmiel C.; Bernatavicius G.; Jonhstone E.; Crocker I.;Myers J.E.	**Women had pre-existing medical conditions (chronic hypertension and diabetes)**
2	**Pulse wave analysis and the risk of early-onset preeclampsia**	Pregnancy Hypertension January 2015, vol./is. 5/1(26), 2210-7789 (January 2015	Lan P.; Hyett J.; Gillin A.	**Population group consisted of women categorised as high risk for pre-eclampsia**
3	**Pulse wave velocity and copeptin: Prediction and the possible early etiology of preeclampsia**	American Journal of Obstetrics and Gynecology, January 2015, vol./is. 212/1 SUPPL. 1(S258), 0002-9378 (January 2015	Santillan M.; Santillan D.; Scroggins S.; Min J.; Leslie K.; Hunter S.; Zamba G.; Grobe J.; Haynes W.; Pierce G.	**The objective of this study was to determine if 1st trimester CPP correlates with early changes in CFPWV as an indicator of early vascular dysfunction, excluded as not looking at pregnancy outcome**
4	**Measurement of aortic augmentation index in pregnant women with raised blood pressure and subsequent outcomes: A preliminary prospective cohort study**	Hypertension in Pregnancy, November 2014, vol./is. 33/4(476-487), 1064-1955;1525-6065 (01 Nov 2014)	Fullerton G.; Crilly M.A.; Bhattacharya S.; Danielian P.J.	**Women recruited to the study were attending antenatal triage for hypertension**
5	**The assessment of arterial stiffness in pre-eclamptic patients**	Clinical and Experimental Hypertension, December 2014, vol./is. 36/8(603),	Oylumlu M.; Yildiz A.; Yuksel M.	**Women recruited into the study had hypertension**

		1064-1963;1525-6006 (01 Dec 2014)		
6	**Cardiovascular variability before and after delivery: recovery from arterial stiffness in women with preeclampsia 4 days postpartum**	Hypertension in Pregnancy, February 2014, vol./is. 33/1(1-14), 1064-1955;1525-6065 (February 2014)	Walther T.; Voss A.; Baumert M.; Truebner S.; Till H.; Stepan H.; Wessel N.; Faber R.	**Not evaluating pregnancy outcome**
7	**Ambulatory arterial stiffness index and nocturnal blood pressure dipping in pregnancies complicated with hypertension**	Clinical Physiology and Functional Imaging, January 2014, vol./is. 34/1(39-46), 1475-0961;1475-097X (January 2014)	Karkkainen H.; Saarelainen H.; Heiskanen N.; Valtonen P.; Laitinen T.; Vanninen E.; Heinonen	**Not evaluating birth outcome**
8	**Noninvasive assessment of endothelila dysfunction in preeclampsia**	Clinical Chemistry and Laboratory Medicine, September 2012, vol./is. 50/9(A192), 1434-6621 (September 2012)	): Suhadolc K.; Osredkar J.	**Evaluating changes within a population affected with preeclampsia**
9	**Individual common carotid artery wall layer dimensions, but nor carotid intima-media thickness, indicate increased cardiovascular risk in women with preeclampsia: an investigation using non-invasive high frequency ultrasound**	Circulation: Cardiovascular Imaging, September 2013, vol./is. 6/5(762-768), 1941-9651;1942-0080 (September 2013)	Akhter T.; Wikstrom A.-K.; Larsson M.; Naessen T.	**Analysis in women with preeclampsia**
10	**Can pulse wave analysis predict poor**	BJOG: An International Journal	Cockerill R.; Chmiel	**Population have hypertension**

	obstetric outcome in pregnant women with hypertension	of Obstetrics and Gynaecology, June 2013, vol./is. 120/(128), 1470-0328 (June 2013)	C.; Crocker I.; Myers J.	
11	**Change in pulse wave velocity throughout normal pregnancy and its value in predicting pregnancy induced hypertension: a longitudinal study**	Am J Obstet Gynecol. 2006 Aug;195(2):464-9. Epub 2006 May 2.	Oyama-Kato M, Ohmichi M, Takahashi K, Suzuki S, Henmi N, Yokoyama Y, Kurachi H.	**Predicting pregnancy induced hypertension**
12	**Augmentation index and pulse wave velocity in normotensive and pre-eclamptic pregnancies**	Acta Obstetricia et Gynecologica Scandinavica, August 2013, vol./is. 92/8(960-966), 0001-6349;1600-0412 (August 2013) .	Franz M.B.; Burgmann M.; Neubauer A.; Zeisler H.; Sanani R.; Gottsauner-Wolf M.; Schiessl B.; Andreas M	**Not pregnancy outcome Population already have pre eclampsia**
13	**Central arterial wall dynamic properties in women with previous preeclampsia**	Journal of Clinical Hypertension, April 2012, vol./is. 14/, 1524-6175 (April 2012)	Polonia J.; Olival C.; Ribeiro S.; Silva J.A.; Barbosa L.	**Not pregnant**
14	**Persistent increments in proximal arterial stiffness and peripheral resistance following preeclampsia might contribute to cardiovascular**	European Heart Journal, August 2012, vol./is. 33/(301), 0195-668X (August 2012) .	Estensen M.-E.; Grindheim G.; Remme E.W.; Swillens A.;	**Was at term and postpartum. Not predicting pregnancy outcome**

	risk in future life		Smiseth O.A.; Segers P.; Henriksen T.; Aakhus S	
15	**carotid remodelling in preeclampsia by analysis with echo tracking system**	Journal of the American Society of Echocardiography, June 2012, vol./is. 25/6(B59), 0894-7317 (June 2012)	): Yuan L.-J.; Duan Y.-Y.; Xue D.; Yang H.-G.; Cao T.-S.; Zhou N	**In preeclamptic populations**
16	**Comparison of vascular function in pre eclamptic and normotensive pregnant women in the rural eastern Cape province in South Africa**	Pregnancy Hypertension, July 2012, vol./is. 2/3(250-251), 2210-7789 (July 2012)	Namugowa A.V.; Meeme A.	**Not predicting pregnancy outcome In preeclamptic population**
17	**Hemodynamic assessment by applanation in women with early and late preeclampsia**	Pregnancy Hypertension, July 2012, vol./is. 2/3(231), 2210-7789 (July 2012)	Poiati J.R.; Peracoli J.C.; Magalhaes C.G.; Costa R.A.A.; Oliveira A.P.; Borges V.T.M.	**In preeclamptic population**
18	**The association between preeclampsia and arterial stiffness**	Journal of Hypertension, January 2012, vol./is. 30/1(17-33), 0263-6352;1473-5598 (January 2012)	Hausvater A.; Giannone T.; Sandoval Y.-H.G.; Doonan R.J.; Antonopoulos C.N.; Matsoukis I.L.; Petridou E.T.; Daskalopoulou S.S.	**Systematic review**
19	**Association of augmentation index with uterine artery Doppler and risk**	Pregnancy Hypertension, July 2011, vol./is. 1/3-4(290-291),	Everett T.R.; Mahendru A.A.;	**In high risk population- abnormal Uterine artery dopplers**

	of preeclampsia	2210-7789 (July-October 2011)	Wilkinson I.B.; Lees C.C.	
20	**Association of aortic stiffness with uterine artery Doppler pulsatility index and risk of preeclampsia**	Pregnancy Hypertension, July 2011, vol./is. 1/3-4(289), 2210-7789 (July-October 2011)	Everett T.R.; Mahendru A.A.; Wilkinson I.B.; Lees C.C	**In high risk population- abnormal Uterine artery dopplers**
21	**Non-invasive assessment of hemodynamics in early pregnancy**	Pregnancy Hypertension, July 2011, vol./is. 1/3-4(264), 2210-7789 (July-October 2011)	Van Der Graaf A.M.; Zeeman G.G.; Groen H.; Lumbers E.R.; Roberts C.; Dekker G.A	**Looked at association between maternal hemodynamic characteristics and maternal demographics. Did not look at birth outcome**
22	**Pre-pregnancy to early pregnancy changes in maternal cardiovascular physiology**	Pregnancy Hypertension, July 2011, vol./is. 1/3-4(262-263), 2210-7789 (July-October 2011)	Mahendru A.A.; Everett T.R.; McEniery C.M.; Wilkinson I.B.; Lees C.C.	**Looked at the longitudinal changes from pre-pregnancy to early pregnancy**
23	**Aortic stiffness in normal and hypertensive pregnancy**	Blood Pressure, 2010, vol./is. 19/1(11-15), 0803-7051;1651-1999 (2010)	Avni B.; Frenkel G.; Shahar L.; Golik A.; Sherman D.; Dishy V.	**Looked at women who had pre-existing chronic hypertension**
24	**Maternal arterial stiffness in pregnancies affected by preeclampsia**	American Journal of Physiology - Heart and Circulatory Physiology, August 2009, vol./is. 297/2(H759-H764), 0363-6135;1522-1539 (August 2009)	Kaihura C.; Savvidou M.D.; Anderson J.M.; McEniery C.M.; Nicolaides K.H	**In patients with pre-eclampsia**

25	**Raised uterine artery impedence is associated with increased maternal arterial stiffness in the late second trimester**	Placenta, Jul 2012, vol. 33, no. 7, p. 572-577 (July 2012)	Everett, T R; Mahendru, A A; McEniery, C M; Wilkinson, I B; Lees, C C	**Women where high risk recruited from obstetric ultrasound clinic**
26	**Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: insights into future vascular risk**	Circulation, Nov 2010, vol. 122, no. 18, p. 1846-1853 (November 2, 2010)	Yinon, Yoav; Kingdom, John C P; Odutayo, Ayodele; Moineddin, Rahim; Drewlo, Sascha; Lai, Vesta; Cherney, David Z I; Hladunewich, Michelle A	**Undertaken on women who were postpartum**
27	**Prepregnancy vascular dysfunction in women who subsequently develop hypertension during pregnancy**	Pregnancy Hypertens. 2013 April 1; 3(2): 140–145. doi:10.1016/j.preghy.2013.01.006.	**Hale S.A**, **Gary J. Badger G.J**, **McBride C**, **Magness R**,and **Bernstein I.M**,	**Methods used to determine PWV was unsound.**

3.3.2 Data extraction and quality assessment

MWO and EB extracted data independently from the included studies onto a standard spreadsheet (Microsoft Excel®) template for systematic review and meta-analysis data collection. We assessed heterogeneity between studies using Cochrans Q and Higgins I^2 tests, as well as appraised heterogeneity and publication bias using the asymmetry of the funnel plots and Egger's test. We also assessed the methodological quality of all articles that met the selection criteria.

Quality was defined as the confidence that the study design, conduct and analysis minimised bias in the estimation of test accuracy, based on existing check-lists(193). Quality assessment involved scrutinising study design and relevant features of the population, test and outcomes of the study. A study was considered to be of good quality if it used a prospective design, consecutive enrolment, full verification of the test result with the reference standard, had adequate test description and had appropriate analyses to minimise the bias in the estimation of test accuracy. All studies were found to be of good quality according to the aforementioned criteria.

3.3.3 Meta-analysis

Meta-analyses were undertaken where data were obtained from two or more studies (Table 3.5). Estimates of mean and standard deviation (SD) were obtained from the relevant studies. For studies where the estimates were reported as the median and inter-quartile range, approximate estimates of mean and SD were calculated using the available estimates of the median, minimum and maximum(194). Estimated standardised mean difference (SMD) and corresponding 95% confidence intervals (lower, upper) were used for presentation of data. Forest plots were used for presenting the meta-analysis based on pooled estimates of SMD, as the average difference in PWV, AIx, and AIx-75.

Two(114, 144) relevant studies with the primary outcome measure of SGA fetuses were included in the results. Meta-analysis was not performed on these two studies as they were evaluating women in two different trimesters and pooling of results could not be performed.

We used a step-ladder approach to help with presentation of data:

a) Type of placental-mediated disease (PET, SGA, placental abruption, and IUFD).
b) Time of assessment of arterial stiffness assessment during pregnancy (first, second, or third trimester),
c) Method of arterial stiffness assessment used (PWV, AIx, and AIx-75).

Table 3.5: Sample size of Normal and Disease groups for each trimester in studies included in the meta-analyses

Study		**Trimester 1**		**Trimester 2**		**Trimester 3**	
Author	**Year**	**Normal**	**Disease**	**Normal**	**Disease**	**Normal**	**Disease**
Khalil et al	2009	196	14				
Khalil et al	2010	210	42				
Savvidou et al	2011	70	29				
Khalil et al	2012	6766	181				
Khalil et al	2014	181	64	181	64	181	64
Carty et al	2013	123	17	123	17		
Katsipi et al	2014	97	21				
	Total	**7643**	**665**	**304**	**81**	**181**	**64**

3.3.4 Statistical analysis

We identified methodological heterogeneity between studies. We employed random effect models for meta-analyses of the data where at least two studies were available. We also presented the single-study outcome without conducting any meta-analysis. For the random effect model, we estimated the heterogeneity (τ^2) using restricted maximum likelihood method, and tested the statistical significance using Cochran's Q-test. We computed two other estimates of heterogeneity relative to total variability; the percentage of total heterogeneity to total variability (I^2), and the ratio of total variability and sampling variability (H^2). We estimated standardised residuals from the fitted model and evaluated if a particular study fitted the model appropriately. We obtained the funnel plot as a diagnosis of heterogeneity and publication bias, and assessed the asymmetry of the funnel plot by Egger's test. For sensitivity analysis, we computed adjusted standard errors of the estimated coefficients using Hartung-Knapp-Sidik-Jonkman and modified Knapp-Hartung methods(195) We also estimated different influential diagnostics statistics namely Cook's distance, covariance ratio, dffit and dfbeta to identify an influential study. The influence of an individual study on all estimates of parameters was also assessed by excluding each study. All statistical tests were two-sided and statistical significance was considered where $p<0.05$. All meta-analyses models were fitted using the metafor (196)package (version 2.0) in R 3.4 environment(197).

3.4 RESULTS

Nine relevant studies (n=15,923) of placental mediated diseases were identified and fulfilled our inclusion and exclusion criteria (Table 3.6). Mean arterial stiffness measurements were compared between pregnant women who developed placental-mediated diseases and those who did not develop them. The characteristics of participants from included studies are presented in table 3.7.

Table 3.6: Results of included studies

Study	Author	Year	Study design	Comparison	Sample size	Outcome	Method used
First-trimester markers for the prediction of PET in women with a-priori high risk	Khalil et al	2010	Nested case control	Investigating the predictive value of the combination of first trimester serum placental protein 13 (PP13), uterine artery Doppler pulsatility index (PI) and pulse wave analysis (AIX-75)	n=252	Women who developed PET had a higher AIX-75 ($p<0.001$)	Applanation tonometry- radial waveform
Longitudinal changes in maternal haemodynamics in a population at risk for PET.	Khalil et al	2014	Prospective longitudinal study	Investigating longitudinal changes in maternal haemodynamics in pregnancies that develop PET and gestational hypertension	n=245	AIX, PWV and aortic systolic blood pressure (sBPao) were significantly higher in the preterm PET group but not in the term PET group.	Tensiomed arteriography- brachial pressure cuff- Measured AIX and PWV
Maternal arterial stiffness in women who subsequently develop PET.	Savvidou et al	2011	Cross sectional study	Assessing whether alterations in maternal arterial stiffness precede the onset of PET in at risk women	n=99	AIx was similar between the 2 groups. PWV was higher in the PET group compared to the normal group: (1.10+/- 0.14 MOM vs 0.99+/- 0.11) $P<0.01$)	Applanation tonometry- radial artery
Maternal haemodynamics at 11-13 weeks'	Khalil et al	2012	Longitudinal	Examining the potential value of assessment of PWV and AIX at 11-13	n=6947	PET group had a higher AIX-75 (1.13 vs. 1.00 MOM, $P<0.0001$) and	Tensiomed arteriography- brachial pressure

gestation and risk of PET				weeks gestation in identifying women who subsequently develop PET		PWV (1.06 vs. 1.00 MOM P<0.0001).	cuff- Measured AIX and PWV
Pulse wave analysis: a preliminary study of a novel technique for the prediction of PET	Khalil et al	2009	Prospective screening study	Prediction of PET by AIx-75	n=210	For a false positive rate of 11%, AIx-75 had a detection rate of 79% for all cases of PET and 88% for early onset PET	Applanation tonometry- radial artery
Pulse wave analysis for the prediction of preeclampsia.	Carty et al	2014	Longitudinal study	Investigating if PWA can predict PET before the onset on clinically detectable disease	n=140	No significant difference in PWA characteristics between 16 and 28 weeks	Applanation tonometry-radial artery
The use of PWV in predicting PET in high-risk women.	Katsipi et al	2014	Longitudinal	Evaluate the diagnostic utility of PWV in predicting PET	n=118	PWV higher in PET women vs non-PET women (10.2+/- 1.9 vs. 7.2 +/- 1.1 m/s) P<0.001	Pulse wave forms were obtained at two different sites by Doppler ultrasound using a 5MHz transducer and compared to the R wave of the ECG
Maternal arterial stiffness in normotensive pregnant women who subsequently	Tomimatsu et al	2012	Longitudinal	Assessing the association between maternal arterial stiffness and delivery of a baby that is SGA in normotensive pregnant	n=151	Aix and Aix-75 were significantly higher in the SGA group compared with the normal group. Mean AIx for the SGA	Applanation tonometry with SphgmoCor

delivered babies that are SGA				women		group was 65.5 (SD 15.6) compared to 57.0 (SD 11.2) in the healthy group	
Maternal haemodynamics at 11-13 weeks of gestation in pregnancies delivering SGA neonates	Khalil et al	2012	Longitudinal	Examining PWV, Aix and Aix-75 at 11-13 weeks gestation in pregnancies delivering SGA neonates with and without PET	n=6814	In the SGA group without PE, compared to the unaffected controls, there was no difference in Aix-75 (1.03 vs 1.00 MoM) and PWV (0.98 vs 1.00 MoM)	Tensiomed arteriography- brachial pressure cuff- Measured AIX and PWV

Abbreviations:
PWV: pulse wave velocity
AIX: augmentation index
PWA: pulse wave analysis
sBPao: systolic blood pressure of the aorta
Aix-75: augmentation index (adjusted to a heart rate of 75)
MoM: multiples of the median
SGA: small for gestational age
SD: standard deviation
PET: pre-eclampsia
ECG: electrocardiogram

Table 3.7: Maternal characteristics amongst participants within the included studies

Author	Year	Sample size	Maternal age (years)		BMI (kg/m$^{2)}$		Parous		Smoking		Heart rate			Blood pressure		Systolic Blood Pressure		Diastolic blood Pressure		Mean arterial blood pressure	
			CON	PET	CON	PET	CON (%)	PET (%)	CON (%)	PET (%)	CON	PET	P value	Trimester of BP measurement	BP differences between the two groups	CON (range)	PET (range)	CON (range)	PET (range)	CON (range)	PET (range)
Khalil et al	2010	n=252	30.1 ± 5.84	30.0 ±5.00	26.6 ±4.25	27.6 ± 3.34	97 (46.2)	22 (42.4)	23 (11)	1 (2.4)	NA	NA	NA	First	Not significant	110 (83-250)	115 (95-138)	69.5 (50-120)	69 (56-85)	NA	NA
Khalil et al	2014	n=245	33.5 (29.5-36.5) Median (IQR)	28.5 (27.5-35.5) Median (IQR)	25.71 (23.44-28.01) Median (IQR)	26.89(25.32 – 20.04) Median (IQR)	53 (29.3)	8(36.4)	8 (4.4)	0	NA	NA	Significant	NA	NA	#	#	#	#	#	#
Savvidou et al	2011	n=99	30.8 ±6.3	29.4 ±5.7	26.7 ± 4.1	29.4± 4.4	51 (45.9)	15 (51.7)	21(18.9)	7 (24.1)	NA	NA	NA	Second	P<0.01	94.9± 8.6	104.3±11.1	64.0± 6	72.4 ± 9.1	NA	NA
Khalil et al	2012	n=6947	32 (28-35.4) Median (IQR)	32.8 (27.9-37.1) Median (IQR)	23.5(21.3-26.5) Median (IQR)	26.4 (23.5-29.7) Median (IQR)	3101(45.8)	72(39.8)	413(6.1)	11(6.1)	NA	NA	NA	First trimester	Not significant	108 (101-117)	122 (113-133)	NA	NA	NA	NA
Khalil et al	2009	n=210	30.4 +/- 6.3	32.3 +/- 6	26.7 +/- 5.2	28.3 +/- 5	110 (56.1)	8(47.1)	8 (4.1)	0	83± 11	85± 11	Not significant	First trimester	Not significant	NA	NA	NA	NA	85.1 ±13.2	86.1± 6.1
Carty et al	2013	n=140	32.7 +/- 5.3)	30.5 +/- 5.6	27.6 +/- 5.4	30.7 +/- 6.3	1(5.8)	8(6.5)	56 (46)	1 (6)	77± 12	80± 9	Not significant	Second trimester	Not significant	120 ± 12.7	125 ± 11	73 +/- 8.8	79 +/- 7	NA	NA
Katsipi	201	n=118	27.6	29.1	25.5 +/-	29.5 +/-	52	13(61.	37	8	NA	NA	NA	Second	P<0.01	111.2	122.5	66.8	73.6	81.6	90.0

et al	4		+/- 6	+/- 5.2	6.1	4	(43.4)	9)	(38.1)	(38.1)				trimester		±12.8	±11.1	±10.7	±9.2	±10.6	±8.7
Small for gestational age study			**CON**	**SGA**	**CON**	**SGA**	**CON**	**SGA**	**CON**	**SAG**	**CON**	**SG A**	**P value**	**Trimester of BP measurem ent**	**BP differen ces betwee n the two groups**	**CON (%)**	**SGA**	**CON (%)**	**SGA**	**CON (%)**	**SGA**
Tomima tsu et al	201 2	n=151	33.5 +/- 5.4	32.2 +/- 5.4	23 +/- 3.3	23.2 +/- 3.6	61 (54.95)	6 (15)	7(6)	6 (15)	79.5 ±9.4	73. 1 ±8. 8	Signific ant P<0.01	2nd and 3rd trimester	Not significa nt	106.7 ±7.1	108.3 ±9.3	66.4 ±6.5	68.2 ±7.8	NA	NA
Khalil et al	201 2	n=681 4	32 (28.2-35.4) Medi an (IQR)	31.1 (25.5-34.7) Medi an (IQR)	22.6 (20.7-25.3) Median (IQR)	23.5 (21.4-26.4) Median (IQR)	2960 (46)	114 (33.8)	360 (5.6)	53 (15.7)	NA	NA	NA	First trimester	p = 0.019	108 (101-117)	108(101 -116)	NA	NA	NA	NA

#: Systolic BP did not differ significantly between the normotensive and hypertensive group (p=0.052). Values not provided

Con: control
PET: pre-eclampsia group
SGA: small for gestational age

3.4.1 ARTERIAL STIFFNESS AND WAVE REFLECTIONS AND RELATION TO PET

3.4.1.1 Study population

Seven studies, with a total of 8,958 participants, evaluated arterial stiffness and the risk of PET (Table 3.6). Six of the seven included studies carried out arterial stiffness measurements in the first trimester. A total of 8,308 participants were evaluated in the first trimester; Khalil et al(137) had the largest study population (n=6,947). Fewer participants were identified in the second and third trimester cohort studies with 385 and 265 women, respectively. Table 3.8 summarises the use of PWV, AIx, and AIx-75 in each trimester across included studies.

Table 3.8: Studies included in the meta-analyses of arterial stiffness measurements of Pulse Wave Velocity (PWV), Augmentation index (AIX) and Augmentation index corrected to the heart rate of 75 beats per minute (AIX-75) at three trimesters

Study		**1st Trimester**			**2nd Trimester**			**3rd Trimester**		
Author	Year	PWV	AIX	AIX-75	PWV	AIX	AIX-75	PWV	AIX	AIX-75
Khalil et al	2009			✓						
Khalil et al	2010			✓						
Savvidou et al	2011			✓						
Khalil et al	2012	✓	✓							
Carty et al	2013		✓	✓		✓	✓			
Khalil et al	2014	✓	✓		✓	✓		✓ *	✓ *	
Katsipi et al	2014				✓					
	Total	**2**	**3**	**4**	**2**	**2**	**1**	**1**	**1**	**0**

✓ : Arterial stiffness indices performed
Blank box: Arterial stiffness indices not performed
*: meta-analysis not performed

3.4.1.2 Device used

The studies used three different methods to undertake arterial wave analysis. Four studies used Applanation tonometry with either the SphygmoCor(198, 199, 199, 200, 200, 201, 201). (Atcor Medical, Sydney, Australia) ; two(130, 130, 137) used an oscillometric brachial pressure cuff device, the Arteriograph (Tensiomed Ltd, Budapest, Hungary); one study used Pulse Trace 6000 (5 MHz transducer)(202).

3.4.1.3 Arterial stiffness and wave reflection measurements by trimester

The results of arterial stiffness parameters by trimester of pregnancy are presented in Table 3.9. Six(130, 137, 198-201) of the seven studies undertook arterial stiffness measurements in the first trimester, with 7643 women in the normal group and 665 women in the PET group. Overall, SMD in PWV (m/s) between PET and healthy groups was only significant in the second (1.26, 95%CI: 0.22, 2.30; p=0.018) and third (0.49, 95%CI: 0.20, 0.78; p<0.001) trimesters (Figures 3.2 A-C). The estimated mean AIx-75 (%) was significantly higher in the PET compared with the healthy group in the first trimester with SMD of 0.90 (95%CI: 0.07, 1.73; p=0.034) (Figures 3.3 A-B). The estimated mean AIx (%) was higher in the PET compared to healthy groups in the third trimester only with SMD of 0.48 (95%CI: 0.20, 0.77; p=0.001) (Figures 3.4A-C).Khalil(130) (2014) was the only study to measure PWV and AIx in the third trimester, therefore the data are discussed without conducting any meta-analysis.

Table 3.9: Estimated standardised mean difference (SMD) and corresponding 95% confidence intervals (lower, upper) between the pre-eclampsia (PET) and healthy groups for arterial stiffness measurements (Pulse wave velocity, PWV; Augmentation index, AIx; Augmentation index 75, AIx-75) at three trimesters

Parameter	**1st Trimester**	**2nd Trimester**	**3rd Trimester**
PWV (m/s)	0.85 (-1.07, 2.78)	1.26 (0.22, 2.30)* p=0.018	0.49 (0.20, 0.78)* p<0.001
AIx (%)	0.38 (-0.18, 0.93)	0.19 (-0.06, 0.44)	0.48 (0.20, 0.77)* p=0.001
AIx-75 (%)	0.90 (0.07, 1.73)* p=0.034	0.05 (-0.46, 0.56)	No study

*: significant

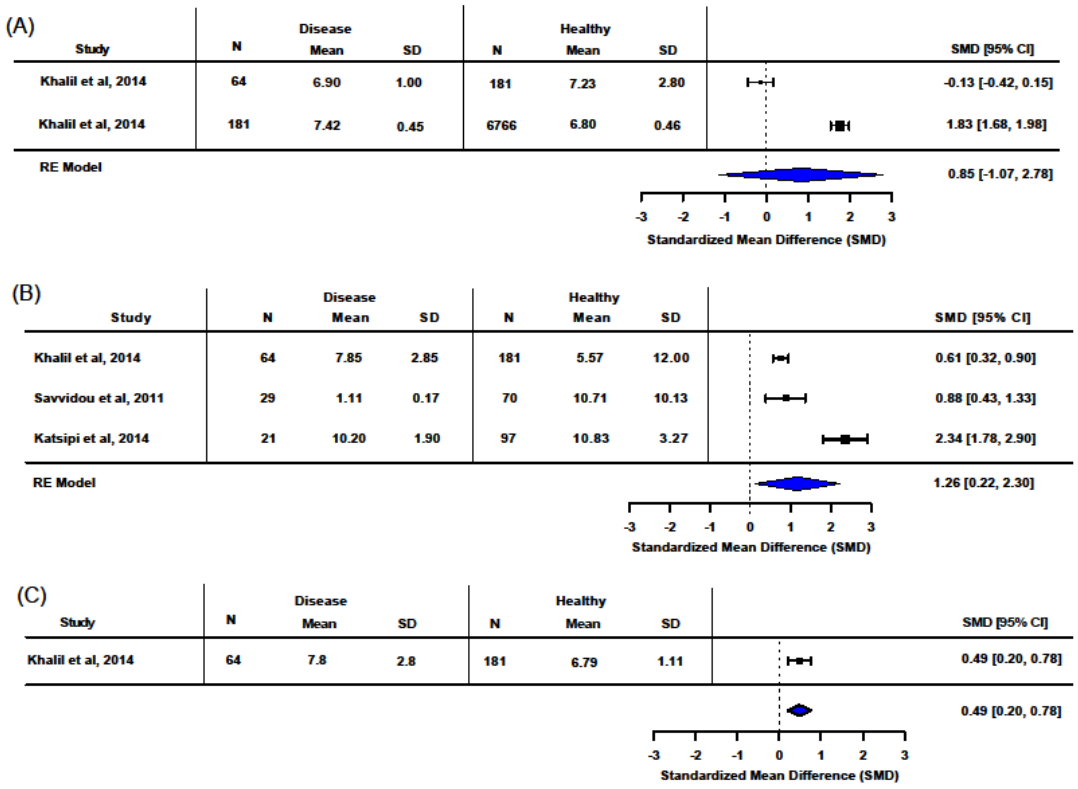


Figure 3.2: Forest plots showing the standardised mean differences (SMD) in pulse wave velocity (PWV) between the pre-eclampsia (PET) and healthy groups in the [A] first, [B] second and [C] third trimester of pregnancy. The estimated SMD in each study is presented as a square (drawn proportional to the precision of the estimate) and the corresponding 95% confidence interval presented as extended lines. The pooled estimates of SMD and corresponding 95% confidence interval, estimated from the random effect (RE) model, are depicted as a diamond.

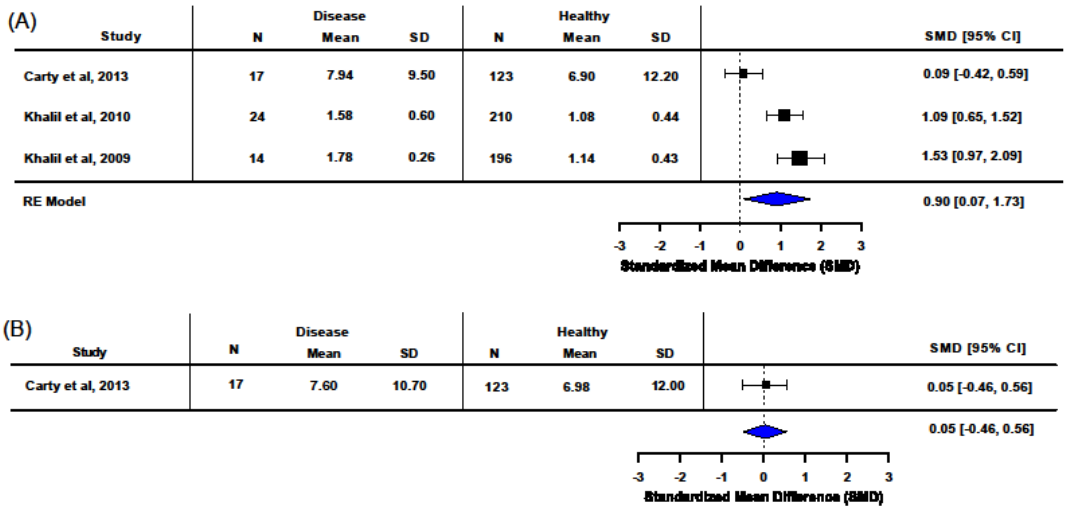


Figure 3.3: Forest plots showing the standardised mean differences (SMD) in augmentation index 75 (AIX-75) between the pre-eclampsia (PET) and healthy groups in the [A] first and [B] second trimester of pregnancy. The estimated SMD in each study is presented as a square (drawn proportional to the precision of the estimate) and the corresponding 95% confidence interval presented as extended lines. The pooled estimates of SMD and corresponding 95% confidence interval, estimated from the random effect (RE) model, are depicted as a diamond.

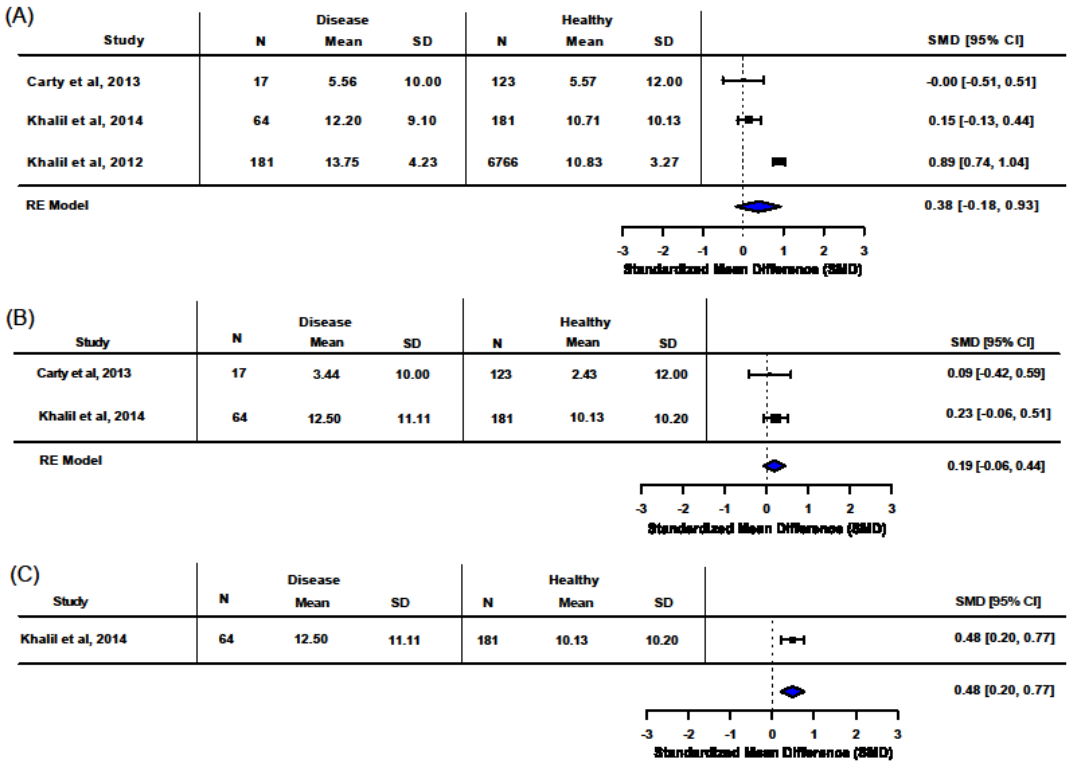


Figure 3.4: Forest plots showing the standardised mean differences (SMD) in augmentation index (AIx) between the pre-eclampsia (PET) and healthy groups in the [A] first, [B] second and [C] third trimesters of pregnancy. The estimated SMD in each study is presented as a square (drawn proportional to the precision of the estimate) and the corresponding 95% confidence interval presented as extended lines. The pooled estimates of SMD and corresponding 95% confidence interval, estimated from the random effect (RE) model, are depicted as a diamond.

3.4.2 ARTERIAL STIFFNESS AND SGA FETUSES

Two studies(114, 144) including 6,965 participants evaluated arterial stiffness and the risk for SGA (Table 3.10). Khalil et al(144) carried out arterial stiffness measurements in the first trimester and had a greater study population (n= 6,814), and Tomimatsu et al(114) (n=151) carried out measurements in the second trimester. Stiffness indices parameters by trimester of pregnancy are presented in Table 3.11.

Khalil et al(144) demonstrated in the SGA group without PET when compared with unaffected controls that there was no significant difference in PWV (0.98 vs.1.00)

multiples of the median (MoM) within these groups when measured in the first trimester, (p=0.599). Tomimatsu et al(114) did not measure PWV**.**

Both of the above studies also measured AIx-75. Khalil *et al*(144) found that there was no significant difference in MoM between the two groups (1.03 vs. 1.00; p=0.998). However, Tomimatsu(114) established that mean AIx-75 was significantly higher in the SGA compared to control group (64.9 (14.8) vs. 59.0% (10.8); p=0.01) in the second trimester. AIx-75 was higher in the SGA groups for both studies.

With regard to AIx, Khalil et al(144) found that there was no significant difference in MoM between the two groups (means 1.03 vs. 1.00; p=0.998). Tomimatsu(114) in the second trimester determined that mean AIx was significantly higher in the SGA compared to control group (65.5(15.6) vs. 57.0% (11.2); p<0.01).

Table 3.10: Sample size of Normal and Disease groups for each trimester in studies not included in the meta-analyses

Study		**Trimester 1**		**Trimester 2**		**Trimester 3**	
Author	**Year**	**Normal**	**Disease**	**Normal**	**Disease**	**Normal**	**Disease**
Khalil et al	2012	6429	48				
Tomimatsu et al	2012			111	40		
	Total	**6429**	**48**	**111**	**40**		

Table 3.11: Studies not included in the meta-analyses of arterial stiffness measurements of Pulse Wave Velocity (PWV), Augmentation index (AIX) and Augmentation index corrected to the heart rate of 75 beats per minute (AIX-75) at three trimesters for small for gestational age pregnancy

Study		**1st Trimester**			**2nd Trimester**			**3rd Trimester**		
Author	Year	PWV	AIX	AIX-75	PWV	AIX	AIX-75	PWV	AIX	AIX-75
Khalil et al	2012	✓	✓	✓						
Tomimatsu et al	2012				✓		✓			
	Total	**1**	**1**	**1**	**1**	**0**	**1**	**0**	**0**	**0**

3.4.2.1 Device used

Khalil et al(144) used the oscillometric brachial pressure cuff device, the Arteriograph (Tensiomed Ltd, Budapest, Hungary), and Tomimatsu et al used Applanation tonometry with SphygmoCor (Atcor Medical, Sydney, Australia)(114).

3.4.3 ARTERIAL STIFFNESS AND OTHER PLACENTAL MEDIATED DISEASES

No studies were identified that addressed arterial stiffness and wave reflection measurements and placental abruption or IUFD.

3.5 PUBLICATION BIAS

Egger's test to check the asymmetry of Forest plot did not show evidence of asymmetry (p-values ranged from 0.061 to 0.831) suggesting no publication bias. However, it should be noted that we conducted Egger's test only where the total number of studies included was at least three.

3.5.1 Sensitivity analysis

We modelled the data using a random effect model, whenever possible, thus accounting for the heterogeneity among true effects, and providing an unconditional inference about a larger set of studies. This approach is more reasonable compared with the fixed-effect model as the fixed effect model restricts inferences about the effect size to the set of studies included in the meta-analysis. For all random effect models, we observed the presence of statistically significant estimates of τ^2 suggesting considerable heterogeneity among the true effects. To investigate this further, we conducted sensitivity analysis and computed adjusted standard errors of the estimated coefficients, particularly those relevant to a small number of studies, for example, Hartung-Knapp-Sidik-Jonkman and modified Knapp-Hartung methods(195). These adjusted estimates suggested that there was no evidence that different measures of arterial stiffness differed between PET and healthy groups. This could happen due to the inclusion of highly influential studies (discussed below). Although the modified estimates may still fail to provide meaningful coverage in the presence of highly influential studies, it is important that we account for this additional

information while considering the exact point estimates of the effect size and corresponding confidence interval.

We estimated the standardised residual, dffit and Cook's distance; higher estimates of these parameters suggest a strong influence on the model fit. Amongst the second trimester studies of PWV, the study by Katsipi et al(202) had a strong positive influence on the fit of the model characterised by higher estimates of standardised residual and Cook's distance. The fitted model, after excluding the study, showed no evidence of heterogeneity (p=0.315), and the estimate of SMD between disease and healthy patients reduced to 0.69 (95% CI: 0.44, 0.94; p<0.001). Similarly, for the AIx-75 data at first trimester, the studies by Khalil et al(201) (positive) and Carty et al(199) (negative) were most influential. The exclusion of the study by Carty et al(199) resulted in a statistically non-significant estimate of τ^2 (p=0.219) as well as an increased estimate of SMD (1.27; 95% CI: 0.84, 1.70; p<0.001) between disease and normal patients. For the AIx data at the first semester, Khalil et al(144) had a large influence on the model fit during the first trimester. When the study was excluded, the fitted model resulted in a non-significant (p=0.610) estimate of τ^2, although the estimate of SMD was still statistically not significant (p=0.363).

3.6 DISCUSSION

3.6.1 Summary of findings

Based on this up-to-date comprehensive systematic review and meta-analysis of approximately 9,000 pregnant women from seven studies, we observed that women who developed PET were noted to have increased AIx-75 in the first trimester, increased PWV in the second trimester, and increased PWV and AIx in the third trimester of pregnancy. Only two studies evaluated SGA fetuses, with a disparity in the findings of the influence of vascular stiffness on this placental-mediated disease pregnancy outcome. We did not identify studies that investigated a possible association between vascular stiffness and either placental abruption or IUFD. Therefore, whilst the current evidence is limited, it appears that arterial stiffness and wave reflection analysis may potentially have a role in predicting PET and possibly SGA

babies, though this is dependent on the modality used to define arterial stiffness and the gestational age.

3.6.2 Interpretation of findings

The ability to predict an at-risk PET population at the earliest opportunity is important, and therefore AIx-75, the only parameter that was significantly different between PET and healthy groups in the first trimester, appears a suitable prognostic marker. Importantly, Khalil et al(172) demonstrated that this difference was seen in both women who were already deemed at high risk for developing PET (warranting potential prevention and enhanced surveillance) and those deemed low risk during the assessment at the antenatal booking visit. Khalil et al(130) also assessed longitudinal changes to predict PET, identifying changes in arterial stiffness measurements in women with preterm and term PET. The second and third trimester measurements were performed before the onset of PET. Preterm PET has a higher rate of perinatal interventions and less favourable perinatal outcomes than term PET(203, 204), therefore identifying those women who may develop preterm PET may identify a higher risk group that warrants further investigation of preventive strategies and pharmacological interventions that could improve pregnancy outcomes. It is important to stress that AIx is an index of wave reflection, which is influenced by factors such as: peripheral vascular resistance, heart rate, stroke volume and arterial stiffness. It is also believed that AIx may reflect the early changes of arterial stiffness, as the changes are more prevalent in younger individuals (age<50years), whereas PWV may reflect the late changes in arterial stiffness as age related changes are more marked in individuals over the age of 50(122). To limit the impact of heart rate of AIx, we consider AIx-75 may be a better parameter for comparisons within and across arterial stiffness studies during pregnancy. As a potential pathophysiological explanation for our findings, it is established that in normal pregnancy, a lower peripheral vascular resistance is an adaptation to maintain BP within a normal range, despite a profound increase in blood volume (205). However, AIx appears to be reduced in the first two trimesters (137, 200) and then increases in the third trimester, reaching a peak in the postpartum (132, 205). Whilst this may be secondary to physiological changes of pregnancy associated with alterations of heart rate, this cannot be determined from these studies. The

pattern of AIx in this systematic review demonstrates that in women who subsequently develop PET, there is an impaired vascular adaptation in early pregnancy in the form of an impaired reduction in peripheral vascular resistance. Furthermore, this impairment is continued into the third trimester with a greater increase in peripheral vascular resistance.

Several studies have identified significant changes in arterial stiffness measurements during the third trimester among pregnant women with pregnancy complications(19, 130). Our observations were in keeping with the systematic review findings of Hausvater et al(19) that there is a significant increase in the PWV indices in women with PET compared to women with normotensive pregnancies. Whilst an interesting observation, substantiating the link between arterial stiffness and PET. The application of arterial stiffness measurements is yet to be examined as a screening tool in clinical practice. PWV is known to increase with age and BP and are the major determinants of PWV(206). Therefore, it could be argued that the findings of an increase in PWV in the latter two trimesters are a result of an increase in BP in the women who developed PET. However, the study by Khalil et al demonstrated that BP did not differ significantly between the normal group and the PET group(130). Future non-invasive PWV assessment studies should use techniques with high validity and reproducibility data.

Only one study reported that markers of vascular stiffness, AIx and AIx-75, were significantly higher in mothers of SGA babies(114). The authors demonstrated that there was an inverse correlation of birth weight to AIx and AIx-75, suggesting that increased arterial wave reflections may affect fetal growth in pregnant women even in the absence of hypertension. Khalil et al(144), however, reported no difference in PWV and AIx-75 between the SGA and healthy groups. Nonetheless, in the SGA group with PET, AIx-75 was increased in comparison to the healthy population. The authors hypothesized that in pregnancies with impaired placentation, one of the determinants of whether there will be the development of PET, or SGA without PET, is a pre-existing susceptibility to cardiovascular disease reflected in increased AIx-75. These studies suggest that measures of arterial stiffness and wave reflection may be used to detect the fetuses at risk in both low (without PET) and high-risk (with PET) mothers.

3.6.3 Strengths and weaknesses

This is the first systematic review to examine the association between maternal arterial stiffness and all disorders of placental origin. We undertook a detailed search without language restrictions to identify all relevant publications. Our review differs from Hausvater et al(19) in that we assessed haemodynamic parameters among pregnant women before development of placental mediated diseases and the influence of gestational age at time of assessment. The quality of individual studies was assessed, and variations in the definition of disease and in assessment and reporting of associations were taken into account using a standardised approach to data extraction. Potential biases in meta-analyses, such as the design of original studies and sample sizes, were explored where possible. There were several important methodological differences across the studies, which is characterised by higher estimates of heterogeneity among true effects due to influential studies. Several other factors, including time of day of the recording and study conditions (e.g. food and drink intake, patient positioning), were not standardised or reported across all studies. Some older studies used different definitions of PET, although newer studies used the widely accepted International Society of the Study of Hypertension in Pregnancy definition(207). Furthermore, the exclusion of women with pre-existing vascular disease varied across the studies, so it is uncertain whether abnormalities in vascular stiffness were a marker of pre-existing disease or PET. Most studies focused on women who were deemed at high risk of developing PET. The results presented may also not be reproducible in low-risk women and should be interpreted with caution due to the observed heterogeneity. Furthermore, there is no agreement about the normal cut-off levels for PWV and AIx.

3.6.4 Clinical and research implications

The review demonstrates and strengthens the supposition that women who develop PET may show an increased arterial stiffness early during pregnancy before the onset of the disease. Large studies with consistent methodological designs are required to examine the application of arterial stiffness assessment, in the first and/or second trimester, as a screening tool for placental mediated diseases particularly PET and IUGR. Furthermore, future work is required to critically evaluate available machines,

provide reliability and repeatability data, provide normal reference ranges and guide clinicians for optimum time of screening. In particular, attention needs to be paid to establishing what is the best non-invasive device to assess arterial stiffness during pregnancy. In our current systematic review several devices were used including SphygmoCor(198-201)(Atcor Medical, Sydney, Australia), Arteriograph (Tensiomed Ltd, Budapest, Hungary), Pulse Trace 6000 (5 MHz transducer)(202), and Doppler ultrasound[15] [Doppler (10 MHz transducer)]. Salvi and colleagues(208) previously assessed three major non-invasive methods for assessing large artery stiffness against standard methodology in 50 subjects (25 men and 25 women) aged 20–84 years. They observed that the Complior (Artec-medical, Pantin, France) and the PulsePen (DiaTecne s.r.l., Milan Italy) devices were more reliable than PulseTrace (Micro Medical Ltd, Rochester, UK). There is a research need to investigate devices that will allow reliable and reproducible PWV assessment. Finally, future studies will need to examine changes in maternal haemodynamics during pregnancy among high risk groups and associated changes in vascular bio-markers.

CHAPTER 4:

REPEATABILITY AND DIURNAL VARIATION OF ARTERIAL STIFFNESS AND CARDIAC OUTPUT MEASUREMENTS IN THE THIRD TRIMESTER OF UNCOMPLICATED PREGNANCY

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Diurnal variation and repeatability of arterial stiffness and cardiac output measurements in the third trimester of uncomplicated pregnancy.
Osman M.W*, Leone F, Nath M, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
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PMID: 28719470

I recruited all participants to this study, performed maternal haemodynamic measurements, collated, analysed and interpreted the data. I wrote the manuscript and received academic editorial input from all supervisors. Upon submission, the reviewers' comments were addressed by myself and my response was approved by all supervisors prior to re-submission

4.1 ABSTRACT

Aim

To investigate same day repeated measures and diurnal variation of arterial stiffness, CO, SV and TPR during the third trimester among low-risk pregnant women.

Methodology

PWV and AIx were recorded using the Arteriograph®, while CO, SV and TPR were recorded using NICOM®. The measurements were obtained in the third trimester of pregnancy from 21 low-risk healthy pregnant women at four time points (morning, afternoon, evening and midnight) over a 24hr period. Triplicate measurements of 67 women were obtained at five-minute intervals to assess repeatability between measurements within a subject.

Results

Diurnal measurements of arterial stiffness for brachial AIx, aortic AIx and PWV were not statistically significantly different at any of the four time points. Estimated means (standard deviation) for PWV at the four stated time points were 7.81 (2.05), 8.45 (1.68), 7.87 (1.74) and 7.64 m/s (1.15), respectively, (p=0.267). Estimates for AIx at those time points were 10.22 (15.62), 4.44 (10.07), 6.49 (10.92) and 8.40% (8.16), respectively, (p=0.295). Similarly, Mean Arterial Pressure (MAP), SV, Stroke Volume Index (SVI) and TPR did not show any evidence of diurnal variation. However, we observed that the mean CO, Cardiac Index (CI) and Heart Rate (HR) varied from morning to midnight; the mean CO, HR and CI increased significantly in the afternoon compared with the corresponding mean morning measurements in a similar fashion to HR. Mean (standard deviation) CO estimates at the four stated time points were 5.90 (1.33), 6.38 (1.49), 6.18 (1.43) and 5.80 L/min (1.19), respectively (p<0.001), while mean CI estimates were 3.65 (0.58), 3.93(0.68), 3.81 (0.65), and 3.57 (0.48), respectively (p<0.001) and mean HR estimates were 95 (12), 98 (13), 95 (12) and 88 (12.98), respectively (p<0.001).

Triplicate measurements of 67 women in our repeatability study showed moderate to high correlation between observations on the same woman for all Arteriograph and NICOM variables (estimates of intra-class correlation ranged from 0.49 to 0.91).

Conclusion

With the exception of CO, CI and HR which showed a diurnal variation, measurements of most haemodynamic parameters did not change significantly from morning to midnight suggesting there was no evidence of systematic differences in the mean values of these variables at these time points. Multiple consecutive non-invasive measurements of vascular stiffness, CO, SV and TPR were highly correlated confirming repeatability of measurements in the third trimester of uncomplicated pregnancy, so these haemodynamic measurements do not need to be undertaken at a specific time period

4.2 INTRODUCTION

The cardiovascular system undergoes positive adaptations during pregnancy. In normal pregnancy, arterial stiffness decreases during the first trimester and remains low until the end of pregnancy due to either reduced smooth muscle tone or vessel wall remodelling(21, 209) . Furthermore, there is an increase in intravascular volume, CO, SV and HR, together with a decrease in vascular resistance and mean BP from as early as six weeks gestation(21, 23, 210). CO steadily increases from the first trimester of pregnancy to 45% above the non-pregnant level at 24 weeks of gestation, it then plateaus but remains elevated until term(21), and is a consequence of an increased SV, HR and decreased SVR. From the 5th week of pregnancy, there is a decline in SVR, which reaches a nadir between weeks 20 and 32 weeks(22, 23), and is due to changes in resistance and flow in multiple vascular beds, such as the utero-placental unit.(23) Thereafter, there is a gradual increase in SVR from 32 weeks until term(22, 23).

Increased systemic arterial stiffness has been reported among women with hypertensive disorders during pregnancy(19, 211, 212), is associated with fetal growth restriction(144), and may have a role as a potential screening tool in pregnancy(170). Central arterial stiffening is associated with adverse cardiovascular outcomes in various patient groups(116), as well as in the general population(117); associated increased PWV being an independent predictor of cardiovascular morbidity and mortality(118-121). European Society of Hypertension guidelines propose that in arterial hypertension, PWV over 12m/s suggests sub-clinical organ damage(135), though normal limits for PWV in pregnancy have not been reported. However, in healthy non-pregnant women the normal limit is 10m/s(136).

Furthermore, device manufacturers commonly state that it is necessary to standardise the time of the day when performing non-invasive cardiovascular assessments of arterial stiffness. However, previous studies of diurnal variability of arterial stiffness have been limited to healthy non-pregnant populations(213-215) with inconsistent methodologies. Only one small (n=15) study of diurnal variation in PWV used four time points and reported a lack of significant circadian rhythm changes (215). In addition, the repeatability of PWV has only been assessed in non-pregnant women(136, 214,

216). However, understanding the circadian pattern of maternal haemodynamics in normal pregnancy is crucial to both clinicians and researchers, as it may influence both the reliability and performance in screening for adverse pregnancy outcome. Therefore, the aim of the present study was to examine the repeatability of successive non-invasive cardiovascular measurements among pregnant women, and to assess diurnal haemodynamic changes in uncomplicated pregnancy.

4.3 METHODS

4.3.1Non-invasive Cardiovascular Assessment

The arterial stiffness measurements were obtained using a commercially available, validated platform that use established methodology, tried and tested in clinical populations, the Arteriograph® (Tensiomed Ltd, Hungary). CO measurements were obtained using the Non-Invasive Cardiac Output Monitor, NICOM (Cheetah Medical, Portland, Oregon). In addition to values for CO including HR and SV, the NICOM device calculates the SV, CI, TPR and BP.

Both devices were automated and this therefore reduced the risk of inter-observer variability. Both studies were approved by the Stanmore National Research Ethics committee. Written informed consent was obtained from all participating women before their enrolment.

4.3.2 Repeatability study

Sixty-seven women were recruited. As previously described in the methods section (chapter 2, section 2.3), we have excluded participants who had one or more of the following conditions: a BMI >25kg/m^2 at booking, multiple pregnancy, fetal anomalies, essential hypertension, pregnancy-induced hypertension, PET, pregnancy complicated with FGR or SGA, thyroid disease requiring medication, renal disease, diabetes mellitus or on any medication that could affect BP. Participants were assessed in a temperature-controlled room (22°C) in a semi-recumbent position on a hospital bed at the antenatal clinic. Participants rested for a minimum of ten minutes prior to non-invasive haemodynamic examination and were free from distractions, including

speaking and moving, during the assessments. Three repeated non-invasive cardiovascular measurements at five-minute intervals were recorded.

4.3.3 Diurnal Variation

Twenty-one low-risk pregnant women were recruited with singleton viable intra-uterine pregnancy. The same exclusion criteria as those mentioned above were applied. Participants were inpatients at the hospital for the duration of the study. Participants were investigated at four time points during a 24-hour cycle:

a. morning (between 0900 to 1000h),
b. afternoon (1400-1500h),
c. evening (2000-2100h),
d. midnight (0000-0100h).

Participants were assessed in a temperature-controlled room (22°C) in a semi-recumbent position on their hospital bed, more than 45 minutes after food intake, and having avoided caffeine and alcohol for 24 hours. Participants rested for a minimum of ten minutes prior to the non-invasive haemodynamic examination and were free from distractions, including speaking and moving, during the assessments.

4.3.4 Statistical analysis

Due to the limited data available in the literature, a pilot study was performed. After analysis of the results from the pilot study(208),which was the first study of its kind in pregnancy, using a linear mixed model in a simulation framework to determine a power calculation, it was determined that a similar prospective study with 12 or more patients would have an adequate power (approximately 80%) to detect a mean difference of 20% or more for AIx with a type 1 error of 5%. The aim of this work was to detect a 20% or greater difference in AIx and PWV from the baseline. It was determined that a sample of 12 would be inadequately powered to detect changes in PWV, and recruiting 20 women would give a power of 89%. Therefore it was decided to recruit 21 women into the final study with the additional one participant to cater for any missing data or drop-outs.

Data from the diurnal study on the different Arteriograph® and NICOM® variables, measured on 21 pregnant women during the third trimester of pregnancy at four different time points (morning, afternoon, evening and midnight), were analysed using a linear mixed model incorporating time as a fixed effect and individual patient as a random effect. The model assumes that errors were normally, independently and identically distributed with zero mean and constant variance. The Distribution assumptions of error were checked using standard residual plots (Histogram, QQ plot etc).

Data from the repeatability study (n=67) were analysed using a separate linear mixed model for each of the Arteriograph® and NICOM® variables. The model incorporated time as fixed effects and individual patient as a random effect. A sample size of 67 was determined by convenience during the time of recruitment as this was not the primary outcome measure.

To assess the correlation between observations on the same patient for both studies, we estimated the intra-class correlation (ICC) coefficient as the ratio of between patient variability to the overall variability and obtained 95% confidence interval of ICC by sampling the data using the bootstrap-based approach. Grading of ICC was performed as detailed in Table 4.1(218). A predictor was considered statistically significant if the two-sided type I error rate was less than 5% (i.e. $p<0.05$). All statistical analyses were carried out using the R software version 3.3 with appropriate R packages(219) (R Core Team, 2016).

Table: 4.1: Intra-class correlation grading

Intra-class correlation	**Grade**
Less than 0.40	Poor
Between 0.40 and 0.59	Fair
Between 0.60 and 0.74	Good
Between 0.75 and 1.00	Excellent

4.4. RESULTS

4.4.1 REPEATABILITY STUDY

Sixty-seven women, of mean (SD) age 31.57 (6.09) years at a mean gestational age of 27.70 (2.29) weeks participated in the study to assess the repeatability of successive measurements of the Arteriograph® and NICOM® variables. . Demographic details of the study population are summarised in Table 4.2.

Table 4.2: Estimates of mean (standard deviation) of maternal characteristics of participants in the repeatability study.

	Repeatability (n=67)
Maternal age (years)	31.57 (6.09)
Maternal body surface area (m^2)	1.86 (0.19)
Maternal height (cm)	161.42 (6.42)
Maternal weight (kg)	75.61 (18.68)
Maternal body mass index (kg/m^2)	28.68 (6.49)
Gestational age (weeks)	27.70 (2.29)

Outcomes from the linear mixed models showed no evidence ($p>0.14$) that mean values of Arteriograph (AIx and PWV) and NICOM (CO, CI, SV, SVI) differed at three successive measurements taken five minutes apart as evidenced by Table 4.3. Most variables showed approximately 0.2 to 1.9% changes in consecutive measurements.

ICC estimates between measurements on the same subject showed excellent correlation for NICOM variables, with the exception of stroke volume variation, where correlation was good. For the Arteriograph® variables, aortic PWV exhibited excellent ICC, and aortic AIx good while brachial Aix demonstrated only fair ICC estimates (Table 4.3). A moderate to higher estimate of ICC suggests higher between-patient variability and lower within-patient variability

Triplicate measurements on each subject of Arteriograph® and NICOM® variables are presented graphically in Figure 4.1. Triplicate measurements on the same patient are joined by dotted lines to demonstrate the consistency in repeatable measurements for each patient. We also presented individual values along with box plots at each measurement points (Figure 4.2) illustrating similar central tendencies across three successive measurements (1, 2, 3) for each subject.

Table 4.3: Estimates of mean, standard deviation and intraclass correlation coefficients of arterial stiffness and cardiac output measurements at three replicates for 67 subjects.

Indices	**Cardiac Output**	**Cardiac Index**	**Stroke volume**	**Stroke Volume Index**	**Brachial AIx**	**Aortic AIx**	**Aortic PWV**
Unit	*mL /min*	*L/min/m^2*	*ml*	*ml/m^2*	*%*	*%*	*m/s*
(n=67)	7.37 (1.49)	3.97 (0.57)	82.95 (18.67)	44.27 (8.44)	-64.6 (20.85)	4.32 (8.40)	8.11 (1.56)
TIME							
Time 1 (0 min)	7.43 (1.50)	3.99 (0.59)	83.25 (18.98)	44.4 (7.99)	-65.78 (17.69)	4.09 (8.87)	8.14 (1.53)
Time 2 (5 min)	7.28 (1.61)	3.97 (0.58)	82.59 (19.03)	44.31 (8.36)	-66.84 (16.39)	3.80 (8.30)	8.09 (1.28)
Time 3 (10 min)	7.39 (1.35)	3.97 (0.56)	83 (18.27)	44.1 (9.07)	-60.85 (27.31)	5.15 (8.03)	8.11 (1.86)
P-value	0.756	0.708	0.818	0.686	0.136	0.450	0.638
ICC (95% lower, upper CI)	0.80 (0.62, 0.90)	0.78 (0.64, 0.85)	0.91 (0.85, 0.94)	0.80 (0.63, 0.89)	0.49 (0.23, 0.76)	0.67 (0.40, 0.83)	0.79 (0.58, 0.91)

*p value (f-test) represents the statistical significance of time obtained from the linear mixed model

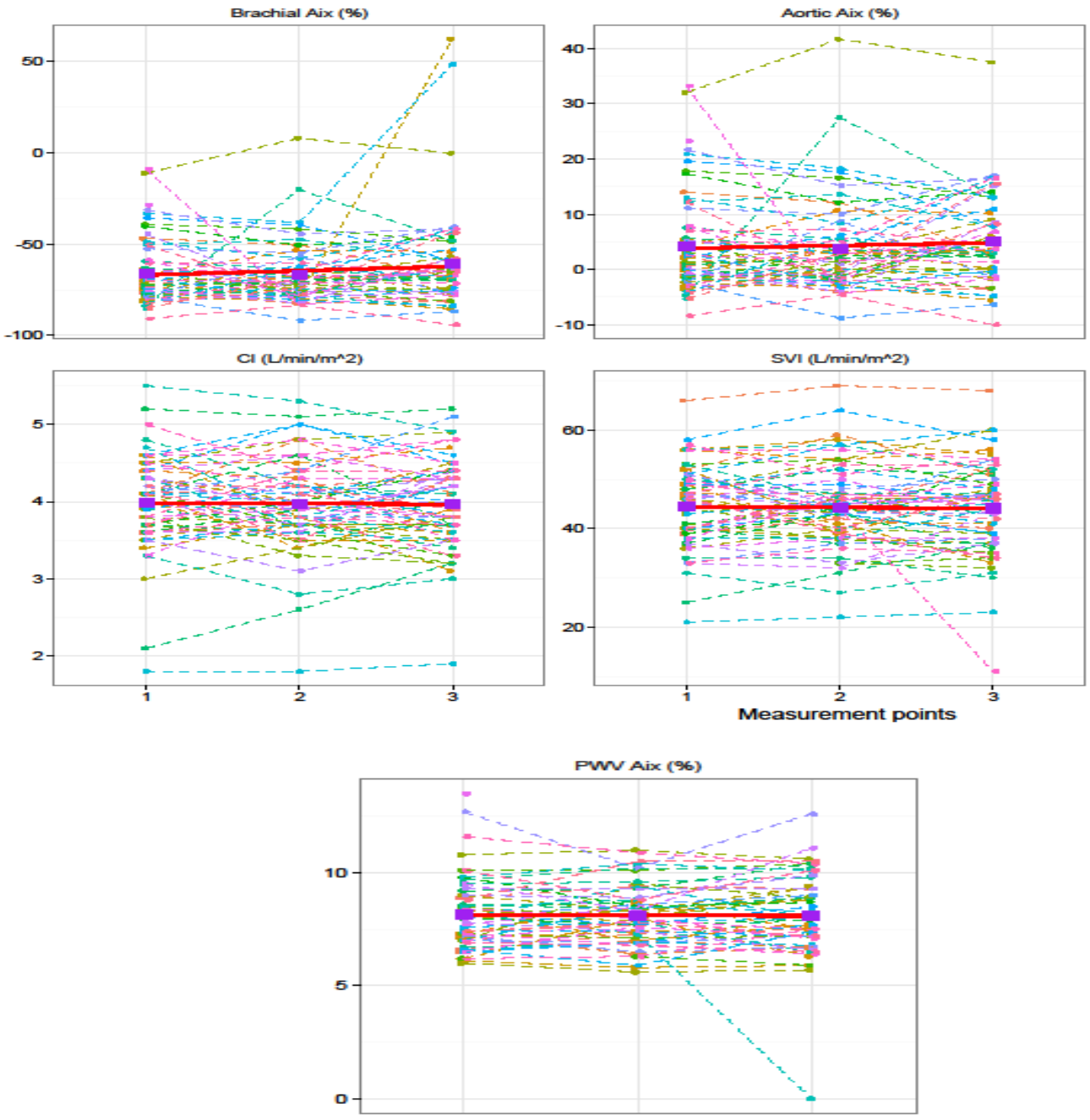

Figure 4.1: Measurements of Arteriograph® and NICOM® variables of all 67 patients (points) over three successive measurements (1, 2, 3) where triplicate measurements on the same patient are joined by dotted lines to demonstrate the consistency in repeatable measurements. The bold red line connects the mean values at each of the measurement points**.**
Points and lines with the same colour represent data from the same patient.

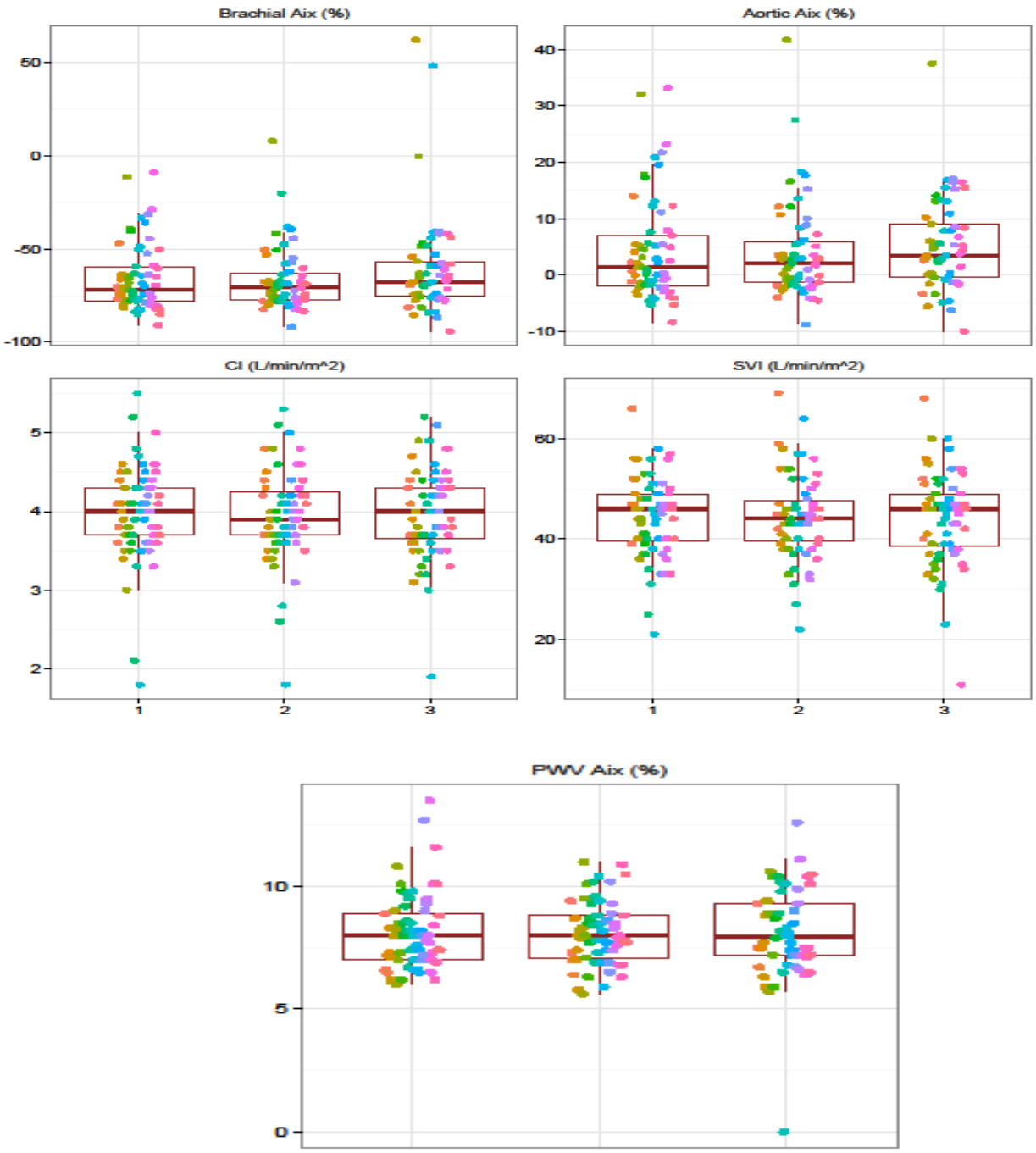

Figure 4.2: Measurements of Arteriograph® and NICOM ®variables of all 67 patients (points) over three successive measurements (1, 2, 3) along with the corresponding box plots showing the median and interquartile range
Br Aix: Brachial augmentation index; Ao Aix: Aortic augmentation index; Ao PWV: Aortic pulse wave velocity; CO: Cardiac output; CI: Cardiac Index; SVI: Stroke volume index.
Points with the same colour represent data from the same patient.

4.4.2 DIURNAL VARIATION

Twenty-one low-risk pregnant women of mean age 28.95 (SD=6.38) years with a mean gestational age of 34 (3.74) weeks fulfilled our inclusion and exclusion criteria and agreed to participate in the study. Demographic details of the study population are summarised in Table 4.4.

Table 4.4 Estimates of mean (standard deviation) of maternal characteristics of participants in the diurnal variation study.

	Diurnal variation (n=21)
Maternal age (years)	28.95 (6.38)
Maternal body surface area (m^2)	1.62 (0.18)
Maternal height (cm)	158.4 (7.75)
Maternal weight (kg)	57.41 (10.41)
Maternal body mass index (kg/m^2)	22.62 (2.91)
Gestational age (weeks)	34.14 (3.74)

4.4.1 Diurnal variation in arterial stiffness:

The mean values of arterial stiffness, at four time points (morning, afternoon, evening and midnight), are presented in Table 4.5, and individual measurements at these time points with the corresponding box plots are presented in Figure 4.3. There were non-significant reductions in brachial and aortic AIx values from the morning to evening with increases afterwards, though PWV values increased non-significantly in the afternoon 8.45 (1.68) before decreasing later to 7.64 (1.15) (Table 4.5).

4.4.2 Diurnal variation in cardiac output measurements

The mean values of non-invasive CO variables using NICOM® are presented in Table 4.6, with individual measurements in Figure 4.3. Among cardiac output variables measured, MAP, SV, SVI and TPR did not show any evidence of diurnal variation between four time points in pregnant women in their third trimester. However, we observed that the mean CO, CI and HR varied from morning to midnight; the mean CO and CI increased significantly, $p<0.001$, in the afternoon compared with the

corresponding mean morning measurements in a similar fashion to HR. CO and HR increased by 0.48ml/min and 3bpm from the morning to the afternoon measurement, respectively.

Table 4.5 Estimates of mean, standard deviation and intra-class correlation coefficients of arterial stiffness and cardiac output measurements at four time points (morning, afternoon, evening, midnight) for 21 low risk patients.

Time interval	**Brachial Aix** *%*	**Aortic Aix** *%*	**Aortic PWV** *m/s*
Morning (0900 to 1000h)	-46.96 (41.49)	10.22 (15.62)	7.81 (2.05)
Afternoon (1400 to 1500h)	-65.57 (19.88)	4.44 (10.07)	8.45 (1.68)
Evening (2000 to 2100h)	-61.54 (21.59)	6.49 (10.92)	7.87 (1.74)
Midnight (0000 to 0100h)	-57.75 (16.12)	8.40 (8.16)	7.64 (1.15)
P-value	**0.077**	**0.295**	**0.267**
ICC (95% lower, upper CI)	0.33 (0.20, 0.51)	0.34 (0.18, 0.54)	0.46 (0.25, 0.67)

*p value (f-test) represents the statistical significance of time obtained from the linear mixed model

Table 4.6 Estimates of mean, standard deviation and intraclass correlation coefficients of cardiac output measurements at four time points (morning, afternoon, evening, midnight) for 21 low risk patients.

Time interval	**Cardiac Output** *mL /min*	**Cardiac Index** *L/min/m²*	**Stroke volume**	**Stroke Volume Index** *ml/m²*	**Mean arterial blood pressure**	**Heart rate**	**Total peripheral resistance**
Morning (0900 to 1000h)	5.90 (1.33)	3.65 (0.58)	64.17 (16.10)	39.05 (7.29)	81.70 (5.47)	95 (12.20)	1167.62 (300.72)
Afternoon (1400 to 1500h)	6.38 (1.49)	3.93 (0.68)	66.52 (17.85)	40.25 (7.65)	84.65 (10.64)	98 (13.10)	1110.20 (268.75)
Evening (2000 to 2100h)	6.18 (1.43)	3.81 (0.65)	63.64 (15.85)	41.36 (7.1)	84.49 (7.8)	95 (12.50)	1161.93 (270.12)
Midnight (0000 to 0100h)	5.80 (1.19)	3.57 (0.48)	66.16 (15.54)	40.67 (6.57)	81.43 (10.06)	88 (12.98)	1170.85 (314.03)
P-value	**<0.001**	**<0.001**	**0.5063**	**0.582**	**0.071**	**<0.001**	**0.307**
ICC (95% lower, upper CI)	0.92 (0.87, 0.95)	0.84 (0.72, 0.91)	0.90 (0.82, 0.94)	0.71 (0.52, 0.83)	0.76 (0.60, 0.84)	0.76 (0.60,0.84)	0.87 (0.78, 0.92)

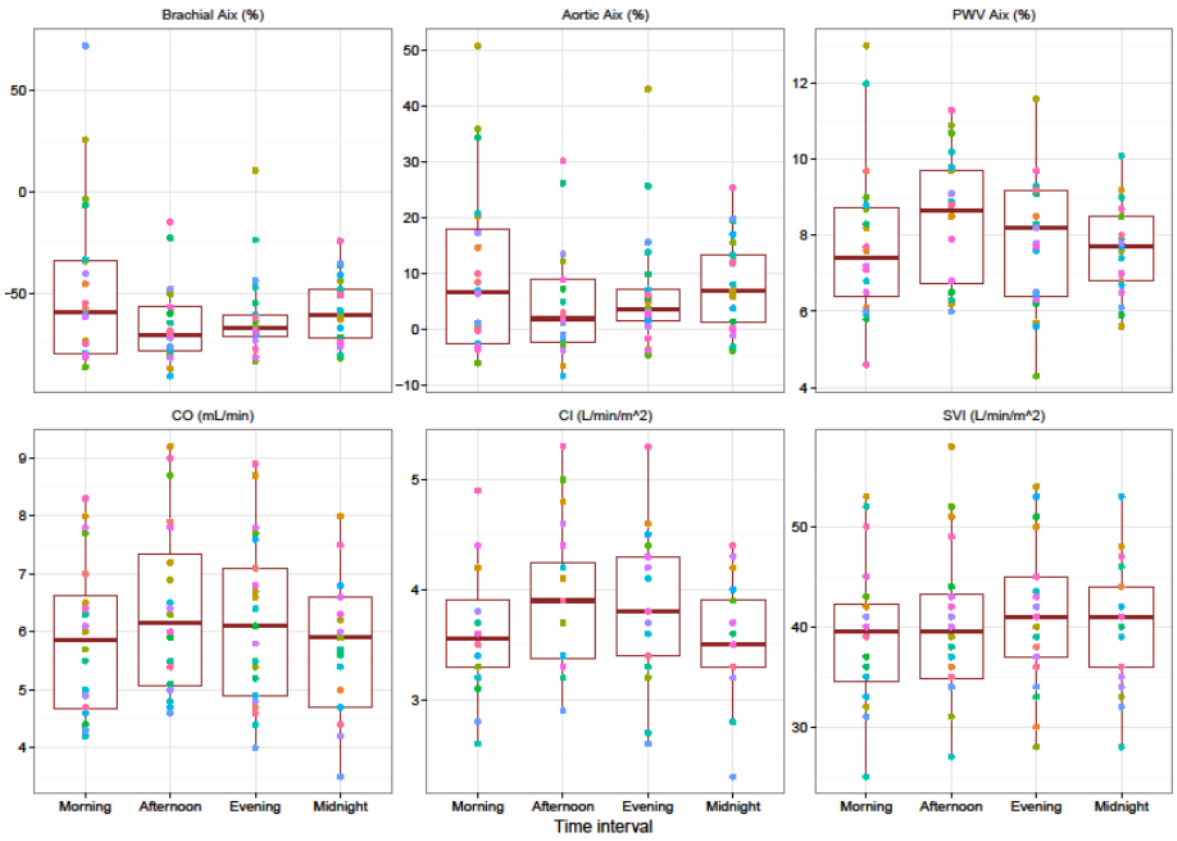

Figure 4.3: Measurements of Arteriograph and NICOM variables of 21 patients (points) at four time points (morning, afternoon, evening and midnight) with the corresponding box plots showing the median and interquartile range

Br Aix: Brachial augmentation index; Ao Aix: Aortic augmentation index; Ao PWV: Aortic pulse wave velocity; CO: Cardiac output; CI: Cardiac Index; SVI: Stroke volume index
**Points with the same colour represent data from the same patient.*

4.5 DISCUSSION

To the best of our knowledge, this is the first study to investigate the diurnal variation and repeatability of measurements of arterial stiffness and CO among women during their third trimester of pregnancy. There was no evidence of significant diurnal variability for the majority of arterial stiffness and CO parameters studied, with the exception of CO and CI. Furthermore, measurements of vascular stiffness and CO were repeatable, with fair to excellent ICC estimates.

Findings of non-significant diurnal variation of PWV and AIx reaffirm the previous work carried out in non-pregnant populations(213-215). Drager and colleagues reported no evidence of circadian rhythm in PWV at four time points (08h00, 12h00, 16h00 and 20h00), albeit in a small population (n=15)(215). Other groups studied diurnal variability at fewer time-points; Ter Avest et al(213) on two occasions (09h00 and 14h00) and Yanlei et al(214) (n=70) on three (09h00, 13h00 and 17h00), and reported no diurnal variation in PWV. For the non-invasive CO using the NICOM®, the statistically significant higher mean CO in the afternoon conforms to the normal expected physiological variation. The lack of diurnal variability in arterial stiffness parameters is an important outcome, as the lack of standardisation of measurements by time of day has been previously criticised (220-222). In addition, we can be confident of a lack of diurnal variability as measurements were performed at four time points in a day, compared to other studies which used longer intervals(213, 214). Also, the present study population for the diurnal variation study (n=21) was adequately powered to assess for any biologically important changes amongst this population group. Nonetheless, it is vital that all other determinants such as maternal age, HR and MAP(129) be carefully evaluated and adjusted for accordingly.

The repeatability study findings are also consistent with previous work carried out in non-pregnant women(214),(136),(216). Yanlei and colleagues examined changes in 7 subjects and reported that the coefficient of variation of the two repeated measurements of PWV,

5 minutes apart, was 6.1% with the absolute difference for the repeated measures being -0.151m/s (95% CI: -2.022 -1.720) (214). Baulman et al(136) validated measurements of arterial stiffness between an oscillometric (Arteriograph®), tonometric (SphygmoCor) and piezo-electronic methods (Complior®). The authors observed that the variance within one session was the lowest (0.18m/s) for the Arteriograph® compared with the Complior® (0.312m/s) and the SphygmoCor® (0.363m/s,). Importantly, we only looked at the repeatability of measurements within a single visit, whereas Baulman et al(136) evaluated the trend further by looking at the reproducibility of measurements between two sessions, undertaken one week apart. They found the measurement errors for the repeat measurements' were also the lowest for the Arteriograph® (1.18 m/s), as compared to the Complior® (1.55 m/s) and the SphygmoCor®(1.67 m/s). In the present study, we only assessed repeatability of measurements within a single visit, so as to reduce the possible bias of advancing gestation on maternal haemodynamics.

One potential limitation of the present study was that assessments were confined to the third trimester of pregnancy. Over the first two trimesters of pregnancy, CO gradually increases with the greatest increase occurring by 16 weeks of gestation. The rise in CO typically plateaus after 20 weeks of gestation but remains at that elevated level until the term(31). Therefore, we intentionally chose to evaluate the circadian rhythm of CO and arterial stiffness in women in their third trimester. In addition, our inclusion criteria aimed to reduce the influence of a maternal age range on the haemodynamic parameters as Khalil et al (172) have demonstrated that PWV and systolic BP have a directly proportional relationship to maternal age. It is also important to stress that diurnal variations reported in the present study pertained to young healthy low-risk pregnant women in their third trimester. Therefore, these conclusions may not be valid for women in the first two trimesters or patients with pregnancy complications or co-morbidity. Slightly lower estimates of ICC for Arteriograph measurements also suggest that a study with bigger sample size might be warranted to consider higher variabilities for these measurements.

4.6 CONCLUSION

With the exception of CO, diurnal measurements of these variables in young healthy low-risk pregnant women in their third trimester did not change significantly from morning to midnight. Multiple consecutive measurements of vascular stiffness and non-invasive CO measurements on the same woman in the third trimester of pregnancy were highly correlated, confirming excellent repeatability of measurements. Given the increase in the use of non-invasive haemodynamic monitors, results have great implications for clinical research and application in clinical practice. We propose that it is not mandatory to measure PWV and AIx at the same time of day. However, standardising the time of day for non-invasive cardiovascular assessment may be beneficial in longitudinal studies. Further work is required to evaluate the diurnal variation in high-risk pregnancies such as those complicated by diabetes, hypertension and other cardiac risk factors. Future work examining longitudinal changes among pregnant women during pregnancy was found to be required. This led to the next study, detailed in Chapter 5, which describes the longitudinal changes with weekly normograms of maternal haemodynamics in normal pregnancy.

CHAPTER 5:

Longitudinal study to assess changes in arterial stiffness and cardiac output parameters among low-risk pregnant women

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Longitudinal study to assess changes in arterial stiffness and cardiac output parameters among low-risk pregnant women.
Osman M.W*, Nath M, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
Pregnancy Hypertension, 2017 Oct;10:256-261 doi: 10.1016/j.preghy.2017.10.007.
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PMID: 29089251

I recruited all participants to this study, performed maternal haemodynamic measurements, collated , analysed and interpreted the data. I wrote the manuscript and received academic editorial input from all supervisors. Upon submission, the reviewers' comments were addressed by myself and my response was approved by all supervisors prior to re-submission

5. 1 ABSTRACT

Aim

A single-centre, prospective longitudinal study to assess changes in maternal arterial stiffness and CO parameters among low-risk healthy pregnant women.

Methodology

Thirty low-risk, healthy, pregnant women attending their routine antenatal dating ultrasound scan were recruited. Non-invasive assessment of arterial stiffness and CO were undertaken at five gestational windows from 11 to 40 weeks of pregnancy. Data were analysed using a linear mixed model incorporating time and other relevant predictors as fixed effects, and patient as a random effect.

Results

GA had a significant effect on all arterial stiffness parameters, including brachial AIx ($p=0.001$), AIx ($p=0.002$) and PWV ($p=0.002$). The aortic AIx reduced during pregnancy: the lowest mean (standard error, SE) was 4.07% (1.01) at 28 weeks before it increased to 7.04% (SE 1.64) at 40 weeks.

Similarly, non-invasive assessments of the CO ($p<0.001$), SV ($p=0.014$), HR ($p<0.001$) and TPR ($p<0.001$) demonstrated significant changes with GA. The mean CO (l/m) increased during pregnancy reaching a peak at 28 weeks' gestation [6.66L/min (SE 0.28)], but dropped thereafter to reach 5.71L/min (SE 0.25) at term.

Conclusion

The current study provides pregnancy normograms for gestational changes in arterial stiffness and CO parameters among low-risk, healthy pregnant women. Further work will be required to assess the risk of placental mediated diseases and pregnancy outcome among pregnant women with parameters outside the normal range.

5.2 INTRODUCTION

Pregnancy is associated with significant cardiovascular adaptations to support the pregnancy, ensuring adequate placental perfusion and fetal development. These changes however, differ between normal and pathological pregnancies, and do precede the onset of the clinical disorder(130, 144). Alterations in CO have been reported in hypertensive disorders of pregnancy, with an increased CO being detected prior to the onset of both PET and gestational hypertension.(223) Moreover, CO was noted to be reduced at the time of the clinical manifestation of pre-eclampsia(224).

Arterial stiffness is an independent predictor of CV mortality and morbidity, both in low and high risk populations(135). According to the European Society of Hypertension (ESH) and European Society of Cardiology guidelines, PWV is a useful parameter in the stratification of CV risk(111). Increased systemic arterial stiffness has been reported among women with hypertensive disorders during pregnancy(19, 211, 212). It is associated with FGR(144) and may have a role as a potential screening tool in pregnancy(170). Scientific and clinical interest continues to grow in evaluating the role of arterial stiffness and its association with pregnancy complications and cardiovascular disorders during pregnancy(19, 114, 144).

A number of studies(124-129, 131) have evaluated the longitudinal pattern of arterial stiffness during pregnancy. However, only four studies(126, 127, 129, 131) evaluated the same group of women longitudinally throughout pregnancy. Three studies(126, 129, 131) adopted applanation tonometry whilst one study(127) used an oscillometric method to evaluate maternal haemodynamic parameters. The remaining studies recruited case matched controls at various gestations in pregnancy. With this modest amount of information available, it has been identified that PWV decreases mid-pregnancy(124, 128) and then increases non-significantly in the third trimester(124, 125, 128, 130), albeit remaining within the normal non-pregnant range. However, the normal limits for PWV in pregnancy have not been reported. Recently, we have examined the repeatability and diurnal variation of maternal haemodynamics amongst healthy pregnant women in their third trimester(225).

Despite the wealth of literature, there remains a lack of agreement on the longitudinal pattern of CO in pregnancy. CO was reported to follow three different patterns of change throughout pregnancy; (1) a continued increase until term(33, 226, 227), (2) a steady increase, peaking in the latter half of pregnancy with a subsequent decrease to term(228, 229), (3) a steady increase, peaking in the latter half of pregnancy, with a plateau until term(22, 230, 231).

The aim of this longitudinal study was to assess, non-invasively, the changes in arterial stiffness and CO parameters among low-risk healthy pregnant women in order to improve the current understanding of normal cardiac adaptation in pregnant women and to provide normograms.

5.3 METHODS

This was a prospective longitudinal study of low-risk, healthy pregnant women with a singleton viable pregnancy. Consecutive participants were invited to join the study following attendance at their routine first trimester dating ultrasound scan at the University Hospitals of Leicester NHS Trust. The study received ethical approval by the Stanmore National Research Ethics Committee (Reference 12/LO/0810). Participants were excluded if they had BMI >25kg/m^2 at booking, multiple pregnancy, fetal anomalies, pre-pregnancy hypertension, pregnancy induced hypertension, PET, thyroid disease requiring medication, renal disease, liver disease, congenital or acquired cardiac condition, diabetes mellitus, were taking any medication that could affect the cardiovascular system or were current smokers.

Following informed written consent, the maternal demographics were recorded.
Non-invasive assessment of the arterial stiffness (Arteriograph®, Tensiomed Ltd, Hungary) and the cardiac output (NICOM®, Cheetah Medical, Portland, Oregon) were undertaken at five gestational windows between 11 to 40 weeks of pregnancy, in a temperature-controlled room (22°C), in a semi-recumbent position. Women were examined at the following gestational windows; 11-13, 20-22, 26-28, 32-34 and 37-40 weeks of pregnancy. Participants were rested for a minimum of ten minutes prior to non-invasive haemodynamic

examination and were free from distractions, including speaking and moving, during the assessments. Participants were advised to avoid caffeine intake on the day of assessment.

The arterial stiffness measurements, PWV and AIx, were obtained with an Arteriograph®. This non-invasive device, used to determine arterial stiffness(147) , is fully automated, and has been validated against invasive and non-invasive measurements(172, 173), in non-pregnant populations. Despite there being no validation studies of the Arteriograph® in pregnancy, the device has been used on a very large scale in pregnancy research(137, 172, 174). The accuracy of SBP, PWV and AIx determination has been validated against invasive monitoring(182). The Arteriograph® cuff was applied to the right arm over the brachial artery for the estimation of SBP (mmHg), PWV (m/s) and AIx (%) as previously described(172).

The NICOM® is an operator independent device that has recently been validated against echocardiographic assessment in pregnancy and has demonstrated excellent repeatability and reproducibility, (ICC=0.953, 95% CI 0.927-0.969, respectively)(185). After initial calibration, continuous values of SV, CO and TPR were recorded; SVI, CI and TPRI were determined by dividing each parameter by the body surface area.

All recordings were made by one researcher (MWO), who received appropriate training on use of the Arteriograph® and NICOM® devices.

5.3.1 Statistical analysis

Changes in each of the haemodynamic measurements, represented by the Arteriograph® (brachial and aortic AIx, PWV) and NICOM® (CO, CI, SV, SVI, HR) assessment, were modelled by separate linear mixed models incorporating the GA as a fixed effect. The linear mixed model accounts for the missing data based on the between and within subject level variability. Statistical significance ($p<0.05$) of linear, quadratic and cubic terms of GA with haemodynamic measurements were tested. If a term was statistically significant, the term in the model was retained in the model. Statistical significance was based on an f-test. The final models of the brachial and aortic AIx also included heart rate and MAP as additional fixed effects. All models included a random intercept of individual, and if statistically significant ($p<0.05$), a random time-specific slope for each individual. All continuous

variables were included in the model as a deviation from the population means. The final fitted model for each haemodynamic measurement was used to predict the means and the corresponding 95% confidence intervals, as well as different percentiles (5^{th}, 25^{th}, 50^{th}, 75^{th}, 95^{th}), across the different points of GA.

All fitted models were checked for their underlying model assumptions. The model selection within a set of candidate models was assessed by comparing the log-likelihood of the nested models along with Akaike information criterion and Schwarz Bayesian information criterion. All statistical tests were two-sided with type 1 error rate (p-value) of 0.05 to determine the statistical significance. All statistical analyses were carried out using the R software version 3.3 with appropriate R packages (nlme, multcomp, ggplot2) (R Core Team, 2016).

5.4 RESULTS

Thirty healthy low-risk pregnant women fulfilled the inclusion and exclusion criteria and were recruited to the study. Table 5.1 summarises the demographic details of our study population.

Table 5.1: Estimated mean (standard deviation) of different demographic variables of the study population

Demographic characteristics	**Mean (Standard deviation)**	
Maternal age, years	28.8 (4.2)	
Body Surface Area (m^2)	1.64 (0.13)	
Maternal height (cm)	163.2 (7.4)	
Maternal weight (kg)	59.9 (8.4)	
Maternal body mass index (kg/m^2)	22.1 (2.6)	
Parity	Nulliparous	14 (47%)
	Multiparous	16 (53%)
Ethnicity	Caucasian	27 (90%)
	Asian	2 (7%)
	Middle-eastern	1 (3%)

5.4.1 Arterial Stiffness

Significant changes in all the parameters of arterial stiffness were seen during healthy pregnancy (Table 5.2 and Table 5.3). Table 5.2 demonstrates the predicted mean (95% lower, upper confidence interval) of arterial stiffness measurements at five time points (13, 20, 28, 34 and 40 weeks) of GA. The table also presents the relationship of haemodynamic measurements with GA and the corresponding statistical significance (p-value). Table 5.3 demonstrates the estimates of mean, standard deviation and percentiles of Ao AIx and PWV at weekly time points from 13 to 40 weeks of gestation.

Both the brachial and aortic AIx reduced during early pregnancy, reached a nadir at 28 weeks, before increasing towards 40 weeks of gestation (Table 5.2, Figure 5.1). AIx reached its lowest point at mid-pregnancy (28 weeks) and then gradually increased towards term. Both demonstrated a quadratic relationship with GA. The mean arterial BP demonstrated a strong positive association ($p<0.001$), while HR showed a strong negative association ($p<0.001$) with AIx. For the brAIx ($p<0.001$): one unit increase in the HR value decreased the mean BrAIx by 0.49 unit while one unit increase in the CMAP value increased the mean BrAIx by 0.67 unit in women with otherwise identical conditions. Similarly, the AoAIx values decreased by 0.25 unit and increased by 0.36 units for one unit increase of HR and CMAP, respectively (Table 5.4). The estimated effects (standard error, SE) for the mean arterial BP were 0.36 (0.05) and 0.67 (0.10) for the aoAIx and the brAIx, respectively, while those for the HR -0.25 (0.05) and -0.49 (0.10) for the aortic and brachial AIx, respectively.

PWV also showed a significant variation during the pregnancy. The pattern was however more complex showing a cubic relationship with GA (Table 5.2, Figure 5.1). PWV reached its lowest value at 17 weeks of gestation and then increased, reaching a peak at 35 weeks gestation.

Table 5.2: The predicted mean (95% lower, upper confidence interval) of the arterial stiffness and cardiac output measurements at five time points (13, 20, 28, 34 and 40 weeks) of gestational age (GA) obtained from the fitted linear mixed model. The table also presents the relationship of haemodynamic measurements with GA and the corresponding statistical significance (p-value).

Gestational age	**Brachial Aix**	**Aortic Aix**	**Aortic PWV**	**Cardiac Output**	**Cardiac Index**	**Stroke volume**	**Total peripheral resistance**	**Heart rate**	**Mean Arterial Blood Pressure**
	%	*%*	*m/s*	*l/min*	*l/min/m^2*	*ml*	*dynes.sec/cm5*	*Beats/min*	*mmHg*
13 weeks	-54.57 (-59.92, -49.21)	9.90 (7.34, 12.46)	7.50 (7.07, 7.92)	6.34 (5.79, 6.89)	3.89 (3.58, 4.19)	76.74 (70.51, 82.97)	1146.57 (1046.28, 1256.46)	83.11 (78.67, 87.55)	58.27 (54.65, 62.12)
20 weeks	-63.05 (-66.96, -59.15)	5.83 (3.89, 7.77)	7.27 (6.88, 7.66)	6.39 (5.92, 6.85)	3.87 (3.62, 4.12)	74.08 (69.36, 78.80)	1091.08 (1025.98, 1160.31)	90.12 (86.47, 93.78)	57.65 (54.32, 61.18)
28 weeks	-66.5 (-70.35, -62.65)	4.07 (2.08, 6.06)	7.94 (7.6, 8.28)	6.66 (6.23, 7.09)	4.05 (3.82, 4.27)	71.05 (67.35, 74.75)	1110.75 (1044.5, 1181.21)	93.42 (89.57, 97.28)	57.39 (54.36, 60.59)
34 weeks	-64.71 (-68.51, -60.92)	4.76 (2.95, 6.58)	8.33 (7.97, 8.7)	6.53 (6.09, 6.96)	3.98 (3.75, 4.21)	68.77 (64.97, 72.57)	1186.06 (1120.91, 1255)	92.6 (88.93, 96.27)	62.85 (59.37. 66.53)
40 weeks	-59.17 (-65.88, -52.46)	7.19 (3.99, 10.4)	8.02 (7.48, 8.55)	5.71 (5.22, 6.20)	3.48 (3.21, 3.75)	66.5 (61.88, 71.12)	1324.42 (1233.22, 1422.36)	88.94 (83.84, 94.04)	80.28 (74.40, 86.62)
Relationship with GA	Quadratic	Quadratic	Cubic	Cubic	Cubic	Linear	Quadratic	Quadratic	Quadratic
p-value	0.001	0.002	0.002	<0.001	<0.001	0.013	0.011	<0.001	0.023

Augmentation index (AIx), pulse wave velocity (PWV). P values were obtained from the likelihood ratio test based on models with and without gestational age.

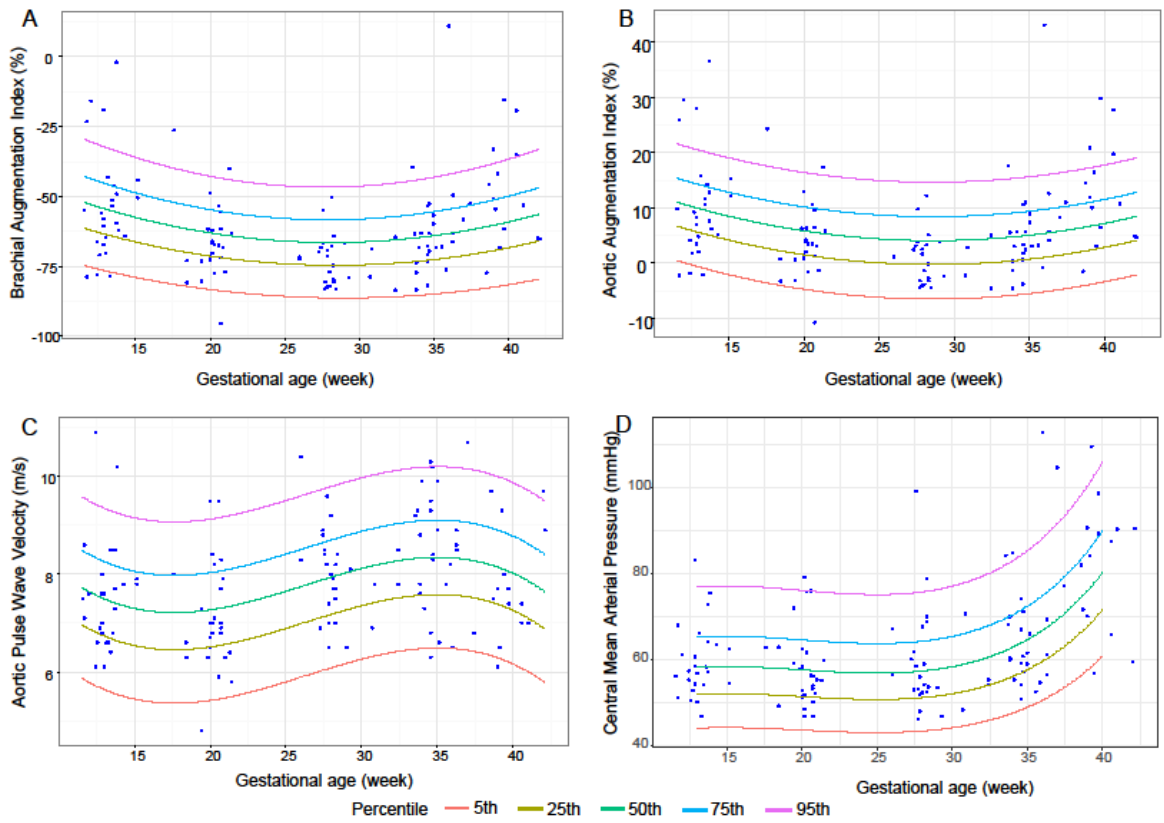

Figure 5.1: The relationship of gestational age with the brachial augmentation index (Br AIx), aortic augmentation index (Ao AIx), pulse wave velocity (PWV) and central mean arterial pressure (CMAP) measurements, based on the fitted linear mixed model. The lines represent 5th, 25th, 50th, 75th and 95th percentiles, and the points represent the observed data for each patient.

Table 5.3: The estimates of mean, standard deviation and percentiles of aortic augmentation index (Ao AIx) and pulse wave velocity (PWV) at weekly time points from 13 to 40 weeks of gestation

	Ao AIx								PWV						
GA	**Mean**	**SD**	**5th PC**	**25th PC**	**50th PC**	**75th PC**	**95th PC**		**Mean**	**SD**	**5th PC**	**25th PC**	**50th PC**	**75th PC**	**95th PC**
13	9.90	6.44	-0.69	5.56	9.90	14.25	20.50		7.50	1.13	5.64	6.73	7.50	8.26	9.35
14	9.18	6.44	-1.42	4.83	9.18	13.52	19.77		7.38	1.13	5.52	6.62	7.38	8.14	9.24
15	8.50	6.44	-2.10	4.15	8.50	12.84	19.09		7.30	1.13	5.44	6.54	7.30	8.06	9.16
16	7.87	6.44	-2.73	3.52	7.87	12.21	18.46		7.25	1.13	5.39	6.48	7.25	8.01	9.10
17	7.29	6.44	-3.31	2.94	7.29	11.63	17.88		7.22	1.13	5.36	6.46	7.22	7.98	9.08
18	6.75	6.44	-3.84	2.41	6.75	11.10	17.35		7.22	1.13	5.36	6.45	7.22	7.98	9.07
19	6.27	6.44	-4.33	1.92	6.27	10.61	16.86		7.23	1.13	5.38	6.47	7.23	8.00	9.09
20	5.83	6.44	-4.76	1.49	5.83	10.18	16.43		7.27	1.13	5.41	6.51	7.27	8.03	9.13
21	5.44	6.44	-5.15	1.10	5.44	9.79	16.04		7.32	1.13	5.47	6.56	7.32	8.09	9.18
22	5.10	6.44	-5.49	0.76	5.10	9.45	15.70		7.39	1.13	5.53	6.63	7.39	8.15	9.25
23	4.81	6.44	-5.79	0.47	4.81	9.15	15.40		7.47	1.13	5.61	6.71	7.47	8.23	9.33
24	4.56	6.44	-6.03	0.22	4.56	8.91	15.16		7.56	1.13	5.70	6.80	7.56	8.32	9.41
25	4.37	6.44	-6.23	0.02	4.37	8.71	14.96		7.65	1.13	5.79	6.89	7.65	8.41	9.51
26	4.22	6.44	-6.37	-0.12	4.22	8.56	14.81		7.75	1.13	5.89	6.99	7.75	8.51	9.60
27	4.12	6.44	-6.48	-0.22	4.12	8.46	14.71		7.84	1.13	5.99	7.08	7.84	8.61	9.70
28	4.07	6.44	-6.53	-0.28	4.07	8.41	14.66		7.94	1.13	6.08	7.18	7.94	8.70	9.80
29	4.06	6.44	-6.53	-0.28	4.06	8.41	14.66		8.03	1.13	6.17	7.27	8.03	8.79	9.89
30	4.11	6.44	-6.49	-0.24	4.11	8.45	14.70		8.12	1.13	6.26	7.35	8.12	8.88	9.97
31	4.20	6.44	-6.40	-0.15	4.20	8.54	14.79		8.19	1.13	6.33	7.43	8.19	8.95	10.05
32	4.34	6.44	-6.26	0.00	4.34	8.68	14.93		8.25	1.13	6.40	7.49	8.25	9.02	10.11
33	4.53	6.44	-6.07	0.18	4.53	8.87	15.12		8.30	1.13	6.45	7.54	8.30	9.06	10.16
34	4.76	6.44	-5.83	0.42	4.76	9.11	15.36		8.33	1.13	6.48	7.57	8.33	9.10	10.19
35	5.05	6.44	-5.55	0.70	5.05	9.39	15.64		8.35	1.13	6.49	7.58	8.35	9.11	10.20
36	5.38	6.44	-5.21	1.04	5.38	9.73	15.98		8.34	1.13	6.48	7.57	8.34	9.10	10.19
37	5.76	6.44	-4.83	1.42	5.76	10.11	16.36		8.30	1.13	6.44	7.54	8.30	9.06	10.16
38	6.19	6.44	-4.40	1.85	6.19	10.54	16.79		8.24	1.13	6.38	7.48	8.24	9.00	10.10
39	6.67	6.44	-3.93	2.32	6.67	11.01	17.26		8.14	1.13	6.29	7.38	8.14	8.91	10.00
40	7.19	6.44	-3.40	2.85	7.19	11.54	17.79		8.02	1.13	6.16	7.26	8.02	8.78	9.88

GA: gestational age

Table 5.4: The normograms representing the estimated means and standard deviations of the arterial stiffness measurements, brachial augmentation index (BrAIx), aortic augmentation index (AoAIx), and pulse wave velocity (PWV), at weekly time intervals from 13 to 40 weeks of gestational age (GA).

	BrAIX		AoAIX		PWV	
GA (weeks)	Mean (%)	SD	Mean (%)	SD	Mean (m/s)	SD
13	-54.57	13.44	9.90	6.44	7.50	1.13
14	-56.09	13.24	9.35	6.44	7.38	1.13
15	-57.51	13.05	8.66	6.44	7.30	1.13
16	-58.83	12.88	8.02	6.44	7.25	1.13
17	-60.04	12.72	7.43	6.44	7.22	1.13
18	-61.15	12.57	6.88	6.44	7.22	1.13
19	-62.15	12.44	6.39	6.44	7.23	1.13
20	-63.05	12.33	5.94	6.44	7.27	1.13
21	-63.85	12.24	5.54	6.44	7.32	1.13
22	-64.54	12.16	5.18	6.44	7.39	1.13
23	-65.13	12.09	4.88	6.44	7.47	1.13
24	-65.61	12.05	4.62	6.44	7.56	1.13
25	-65.99	12.02	4.41	6.44	7.65	1.13
26	-66.27	12.01	4.25	6.44	7.75	1.13
27	-66.44	12.02	4.14	6.44	7.84	1.13
28	-66.50	12.05	4.08	6.44	7.94	1.13
29	-66.47	12.09	4.06	6.44	8.03	1.13
30	-66.32	12.16	4.09	6.44	8.12	1.13
31	-66.08	12.23	4.17	6.44	8.19	1.13
32	-65.73	12.33	4.30	6.44	8.25	1.13
33	-65.27	12.44	4.48	6.44	8.30	1.13
34	-64.71	12.57	4.70	6.44	8.33	1.13
35	-64.05	12.72	4.97	6.44	8.35	1.13
36	-63.28	12.88	5.29	6.44	8.34	1.13
37	-62.41	13.05	5.66	6.44	8.30	1.13
38	-61.44	13.24	6.08	6.44	8.24	1.13
39	-60.36	13.44	6.54	6.44	8.14	1.13
40	-59.17	13.65	7.06	6.44	8.02	1.13

5.4.2 Cardiac output parameters

The CO and CI demonstrated significant changes ($p<0.001$) across GA (Table 5.2, Table 5.5 and Figure 5.2). Table 5.2 demonstrated the predicted mean (95% lower, upper confidence interval) of CO measurements at five time points (13, 20, 28, 34 and 40 weeks) of GA. The table also presents the relationship of haemodynamic measurements with GA and the corresponding statistical significance (p-value). Table 4.5 demonstrates the estimated means and standard deviations of cardiac output measurements: CO, SV and HR, at weekly time intervals from 13 to 40 weeks of GA. SV also showed a significant ($p=0.013$) change with GA; progressively reducing between weeks 13 and 40 of healthy pregnancy (Table 5.2, Figure 5.2, Table 5.6). A single unit increase in the GA decreased the mean SV by 0.68ml.

The mean TPR value also changed significantly with GA ($p=0.011$). The relationship of GA with TPR was quadratic. The mean TPR declined initially with advancing GA reaching the lowest value around 22 weeks, and then increased at subsequent time points (Figure 5.2).

The HR also showed a significant quadratic relationship with GA ($p<0.001$). The mean HR values initially increased with GA, reaching its highest value around the 30^{th} week, and thereafter decreased until term (Figure 5.2).

Table 5.5: The normograms representing the estimated means and standard deviations of cardiac output measurements, cardiac output, stroke volume and heart rate, at weekly time intervals from 13 to 40 weeks of gestational age (GA).

	Cardiac output		Stroke volume		Heart rate	
GA (weeks)	Mean (L/min)	SD	Mean (ml)	SD	Mean (BPM)	SD
13	6.34	1.51	76.74	10.49	83.11	12.36
14	6.31	1.48	76.36	10.41	84.35	12.36
15	6.30	1.45	75.98	10.33	85.51	12.36
16	6.30	1.43	75.60	10.25	86.59	12.36
17	6.31	1.40	75.22	10.17	87.59	12.36
18	6.33	1.38	74.84	10.09	88.51	12.36
19	6.35	1.37	74.46	10.01	89.36	12.36
20	6.39	1.35	74.08	9.93	90.12	12.36
21	6.42	1.34	73.70	9.84	90.81	12.36
22	6.46	1.32	73.33	9.76	91.42	12.36
23	6.50	1.31	72.95	9.68	91.95	12.36
24	6.54	1.30	72.57	9.59	92.40	12.36
25	6.58	1.29	72.19	9.50	92.78	12.36
26	6.61	1.28	71.81	9.42	93.07	12.36
27	6.64	1.27	71.43	9.33	93.29	12.36
28	6.66	1.26	71.05	9.24	93.42	12.36
29	6.67	1.25	70.67	9.15	93.48	12.36
30	6.67	1.24	70.29	9.06	93.46	12.36
31	6.66	1.24	69.91	8.97	93.36	12.36
32	6.63	1.23	69.53	8.88	93.19	12.36
33	6.59	1.22	69.15	8.78	92.93	12.36
34	6.53	1.22	68.77	8.69	92.60	12.36
35	6.45	1.21	68.39	8.59	92.18	12.36
36	6.35	1.21	68.01	8.50	91.69	12.36
37	6.23	1.20	67.64	8.40	91.12	12.36
38	6.08	1.20	67.26	8.30	90.47	12.36
39	5.91	1.19	66.88	8.20	89.75	12.36
40	5.71	1.19	66.50	8.10	88.94	12.36

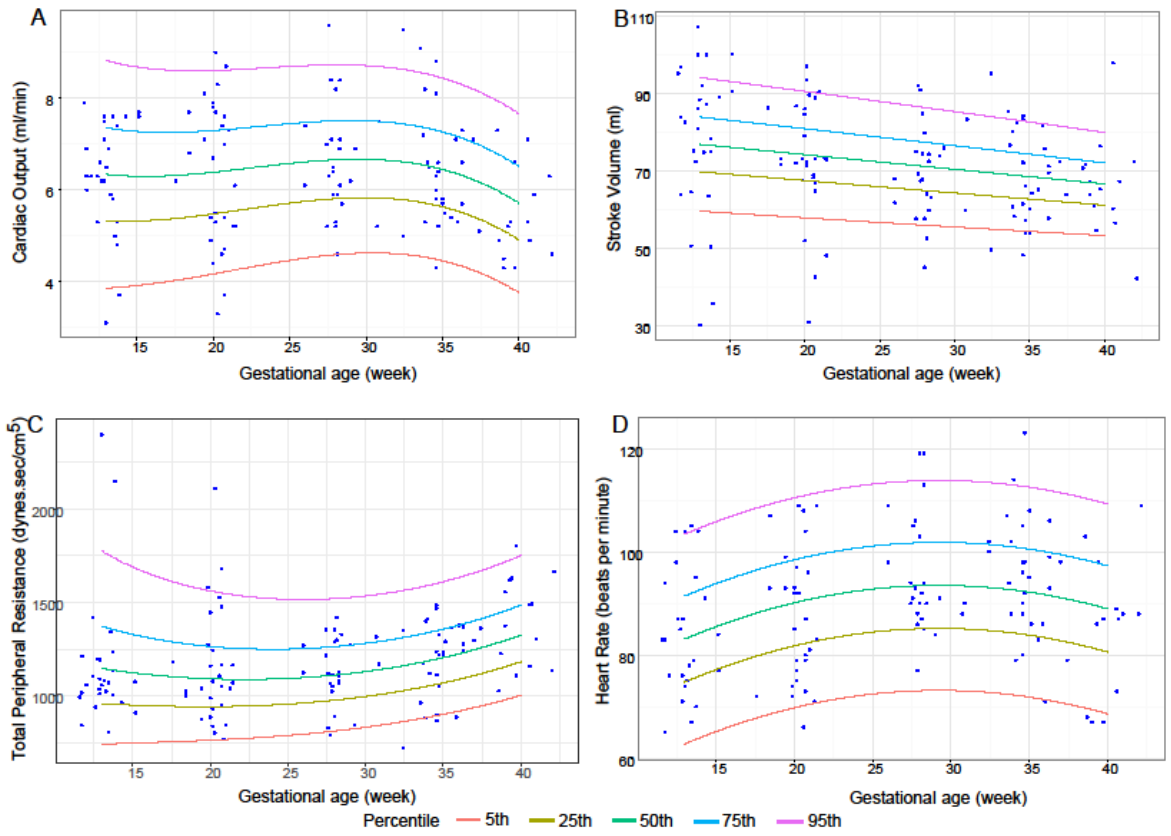

Figure 5.2: The relationship of gestational age with cardiac output (CO), stroke volume (SV), total peripheral resistance (TPR) and Heart rate (HR) measurements, based on the fitted linear mixed model. The lines represent the 5^{th}, 25^{th}, 50^{th}, 75^{th} and 95^{th} percentiles, and the points represent the observed data of each patient.

Table 5.6: The estimates of mean, standard deviation and percentiles of cardiac output (CO) and stroke volume (SV) at weekly time points from 13 to 40 weeks of gestation

Cardiac Output									Stroke volume						
GA	**Mean**	**SD**	**5th PC**	**25th PC**	**50th PC**	**75th PC**	**95th PC**		**Mean**	**SD**	**5th PC**	**25th PC**	**50th PC**	**75th PC**	**95th PC**
13	6.34	1.51	3.86	5.32	6.34	7.36	8.82		76.74	10.49	59.48	69.66	76.74	83.82	94.00
14	6.31	1.48	3.88	5.32	6.31	7.31	8.74		76.36	10.41	59.23	69.34	76.36	83.38	93.49
15	6.30	1.45	3.91	5.32	6.30	7.28	8.69		75.98	10.33	58.98	69.01	75.98	82.95	92.98
16	6.30	1.43	3.95	5.34	6.30	7.26	8.64		75.60	10.25	58.74	68.69	75.60	82.52	92.47
17	6.31	1.40	4.00	5.36	6.31	7.25	8.62		75.22	10.17	58.49	68.36	75.22	82.08	91.95
18	6.33	1.38	4.05	5.39	6.33	7.26	8.60		74.84	10.09	58.24	68.04	74.84	81.65	91.44
19	6.35	1.37	4.11	5.43	6.35	7.28	8.60		74.46	10.01	58.00	67.71	74.46	81.21	90.93
20	6.39	1.35	4.17	5.48	6.39	7.30	8.61		74.08	9.93	57.76	67.39	74.08	80.78	90.41
21	6.42	1.34	4.23	5.52	6.42	7.32	8.62		73.70	9.84	57.51	67.07	73.70	80.34	89.90
22	6.46	1.32	4.29	5.57	6.46	7.35	8.64		73.33	9.76	57.27	66.74	73.33	79.91	89.38
23	6.50	1.31	4.35	5.62	6.50	7.39	8.66		72.95	9.68	57.03	66.42	72.95	79.47	88.86
24	6.54	1.30	4.41	5.67	6.54	7.42	8.68		72.57	9.59	56.79	66.10	72.57	79.03	88.34
25	6.58	1.29	4.46	5.71	6.58	7.45	8.70		72.19	9.50	56.56	65.78	72.19	78.60	87.82
26	6.61	1.28	4.51	5.75	6.61	7.47	8.71		71.81	9.42	56.32	65.46	71.81	78.16	87.30
27	6.64	1.27	4.55	5.78	6.64	7.49	8.72		71.43	9.33	56.08	65.14	71.43	77.72	86.77
28	6.66	1.26	4.59	5.81	6.66	7.51	8.73		71.05	9.24	55.85	64.82	71.05	77.28	86.25
29	6.67	1.25	4.61	5.82	6.67	7.51	8.73		70.67	9.15	55.62	64.50	70.67	76.84	85.72
30	6.67	1.24	4.62	5.83	6.67	7.51	8.71		70.29	9.06	55.39	64.18	70.29	76.40	85.19
31	6.66	1.24	4.62	5.82	6.66	7.49	8.69		69.91	8.97	55.16	63.86	69.91	75.96	84.66
32	6.63	1.23	4.61	5.80	6.63	7.46	8.65		69.53	8.88	54.93	63.54	69.53	75.52	84.13
33	6.59	1.22	4.57	5.76	6.59	7.41	8.60		69.15	8.78	54.70	63.23	69.15	75.08	83.60
34	6.53	1.22	4.52	5.71	6.53	7.35	8.53		68.77	8.69	54.48	62.91	68.77	74.63	83.07
35	6.45	1.21	4.45	5.63	6.45	7.27	8.44		68.39	8.59	54.26	62.60	68.39	74.19	82.53
36	6.35	1.21	4.36	5.53	6.35	7.16	8.33		68.01	8.50	54.04	62.28	68.01	73.75	81.99
37	6.23	1.20	4.25	5.41	6.23	7.04	8.20		67.64	8.40	53.82	61.97	67.64	73.30	81.45
38	6.08	1.20	4.11	5.27	6.08	6.89	8.05		67.26	8.30	53.60	61.66	67.26	72.86	80.91
39	5.91	1.19	3.95	5.10	5.91	6.71	7.87		66.88	8.20	53.38	61.34	66.88	72.41	80.37
40	5.71	1.19	3.75	4.91	5.71	6.51	7.66		66.50	8.10	53.17	61.03	66.50	71.96	79.82

5.5 DISCUSSION

This study has demonstrated that normal pregnancy is associated with significant alterations in the maternal cardiovascular system, as demonstrated by the pattern of arterial stiffness and NICOM measurements, with GA having a significant effect on maternal haemodynamics. Using the linear mixed modelling framework, we were able to provide normograms for arterial stiffness and non-invasive CO parameters in healthy low-risk women, which have not been previously reported.

Our study establishes that in normal pregnancy, the AIx demonstrates a gradual decline in early pregnancy, reaching its lowest value at around 28 weeks of gestation, and then increases with advancing GA to term. This is in agreement with previous reports(126, 129, 132). The pattern of AIx in the present study suggests that the maternal circulatory adaptation is completed after the first trimester and remains constant through the second trimester.

The PWV demonstrated a more complex pattern, grossly resembling a sine wave. There was an initial decline to 17 weeks of gestation, increasing up to 35 weeks and subsequently declining again. This is similar to studies that have identified that PWV decreases mid-pregnancy(124, 128) and then increases non-significantly in the third trimester(124, 125, 128, 130) . However, it differs from Macedo and colleagues who observed that the PWV (carotid-radial and carotid-femoral) did not change significantly with gestation and was marginally different between pregnant and non-pregnant women(129). The normal limits of PWV in pregnancy have not been established. However, in healthy non-pregnant women the normal limit is 10m/s(136). Our overall mean PWV of 7.81 m/s is significantly lower than that expected in non-pregnant women. Several investigators proposed different mechanisms to explain the drop of PWV in the first trimester of pregnancy. It may be due to the alterations of the vaso-active substances such as NO(34, 138), progesterone, relaxin, as well as related to volume expansion in pregnancy(126). The subsequent rise from the mid-trimester of pregnancy to term could be due to the inhibition of NO(139, 140), increase in CO(142) and increased circulatory volume(142).

Similarly, the NICOM parameters also demonstrated a significant variation over the duration of pregnancy. It is understood that over the first two trimesters of pregnancy, CO gradually increases with the greatest increase occurring by 16 weeks of gestation(30-32). The rise in CO is believed to plateau after 20 weeks of gestation but remains at that elevated level until term(31, 33). The increase in the heart rate and SV contribute to this increase in CO(21). However, we noted that the CO reached a peak at around 28 weeks of gestation and then declined to term. The changes in HR within our study population mirrored previous observations by others(21) and was the main influence in the pattern of CO.

The resistance offered by the systemic circulation known as either SVR or TPR. It is understood that from the 5th week of pregnancy, there is a decline in SVR/TPR, which reaches a nadir between weeks 20 and 32 weeks(22, 23). There is then a gradual increase in SVR from 32 weeks until term(22, 23). In this study the mean TPR declined initially with increased GA reaching the lowest value around 22 weeks, and then it increased as pregnancy advanced to term. The pattern of a reduction in SVR is due to changes in resistance and flow in multiple vascular beds, such as the utero-placental unit. These changes are necessary to allow the maternal adaptation to the gravid uterus and promote the delivery of oxygen and nutrients to the fetus(32).

Most studies explored the maternal haemodynamics in women affected with medical conditions in pregnancy. However, in this longitudinal study, the thirty participants were required to meet stringent selection criteria to ensure that all variables such as raised BMI, smoking or medical conditions(19, 129) (pre-eclampsia, diabetes) that may influence the maternal haemodynamic parameters were eliminated. Furthermore, the women remained low risk throughout pregnancy and did not develop any medical conditions that may influence the maternal haemodynamics. The arterial stiffness and NICOM measurements are usually performed in the supine position. However, in the present study, all the measurements were performed in the left lateral position to avoid vena caval compression by the uterus. Therefore, the results obtained in this study represent good benchmark for normal values in pregnancy. Every effort was made to reduce selection bias in our study as women were recruited when attending a dating scan, rather than from a specific clinic. This increased the likelihood of women from a wider population being recruited. A limitation of our study is the small number of participants (n=30). However, an attempt to overcome this

was made with the longitudinal design of this study in which the women were investigated on five separate occasions. Consideration should be given towards the individual variation in metrics as explored in the preceding chapter which demonstrated that of the Arteriograph® variables, aortic PWV exhibited excellent ICC, and aortic AIx good while brachial Aix demonstrated only fair ICC estimates.

The findings of this study offer a useful addition to the established knowledge of maternal haemodynamics. They provide a new insight into the maternal adaptation to pregnancy and may prove useful for future research as well as in clinical use. Further work will be required to assess the risk of placental mediated diseases and pregnancy outcome among pregnant women with abnormal maternal haemodynamics.

5.6 CONCLUSION

The current study provides pregnancy longitudinal normograms for gestational changes in the arterial stiffness and non-invasive CO parameters among low-risk, healthy pregnant women. It was found that further work was required to assess maternal haemodynamic parameters among pregnant women attending for screening for gestational diabetes and this led to the work of the following chapter.

CHAPTER 6:

Maternal haemodynamic changes amongst pregnant women who were attending for screening for gestational diabetes in comparison to low-risk healthy controls

This chapter was published in the Journal of Pregnancy Hypertension

Haemodynamic differences between women screened for gestational diabetes and low risk healthy women.
Osman M.W*, Nath M, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
Pregnancy Hypertension, 2018, in press

I recruited all participants to this study, performed maternal haemodynamic measurements, collated , analysed and interpreted the data. I wrote the manuscript and received academic editorial input from all supervisors. Upon submission, the reviewers' comments were addressed by myself and my response was approved by all supervisors prior to re-submission

6.1 ABSTRACT

Aim

To assess the changes in haemodynamics amongst pregnant women who were attending for screening for GDM in comparison to low-risk healthy pregnant controls.

Methodology

A total of 120 pregnant women of mean age 31.03 (5.41) years who attended their oral glucose tolerance test as part of the national screening for GDM (study), and 60 low-risk healthy pregnant women (control) of mean age 29.71 (5.33) years, were invited to participate in this study. All women included in the study booked at the University Hospitals of Leicester NHS Trust and fulfilled the relevant inclusion criteria. Non-invasive assessment of arterial stiffness and cardiac output were undertaken on participants between 26 and 28 weeks of pregnancy. The mean difference between the study and control group for each of the arterial stiffness and cardiac output measurements was assessed by a two-sample unpaired t-test.

Results

Significant differences were found between the study group and control group in brachial (-64.5 vs. -69.5, $p<0.04$) and aortic AIx (5.2 vs. 2.7, $p=0.04$), though there was no significant difference for PWV (8.3 vs. 8.1, $p=0.49$). CO (7.6 vs. 7.0, $p=0.011$), SV (84.4 vs. 76.9, $p=0.013$) and MAP (71 vs. 58, <0.001) were also significantly different between groups. However, no significant differences were reported for heart rate, systolic and diastolic blood pressure, or total peripheral resistance.

Conclusion

Pregnant women at risk of GDM between gestational weeks 26 and 28 had significantly increased measures of arterial stiffness, as assessed by brachial and aortic AIx, compared with low-risk healthy pregnancy. Whether these women are at greater long-term cardiovascular disease risk warrants further investigation.

6.2 INTRODUCTION

Diabetes is the most common metabolic disorder in pregnancy; up to 5% having either pre-existing diabetes or GDM(50). The majority have GDM, with 7.5 % of this total having T1DM and 5 % T2DM(50). There is a significant burden associated with the maternal and fetal complications of diabetes, including adverse effects on organs, PET(11), operative deliveries, birth trauma, and increased long-term risk of T2DM and CVD(10). In view of these adverse consequences, screening programmes have been implemented for the early detection of diabetes mellitus in pregnancy. GDM is diagnosed by means of a screening test performed in women during pregnancy. The International Association of Diabetes and Pregnancy Study Group Consensus Panel recommend that all pregnant women have a 75-gram oral glucose tolerance test (OGTT) between 24 and 28 weeks(51). However, in the UK, after a cost-benefit analysis, the National Institute for Health and Care excellence (NICE) has recommended testing for GDM in women who have certain risk factors rather than universal testing of all pregnant women(50).

Whilst pregnancy is associated with significant cardiovascular changes, a link between arterial stiffness and GDM is unclear. There have been few case-controlled studies investigating arterial stiffness in women with GDM, and only three undertaking assessments in late pregnancy(18, 162, 163) and the immediate postpartum period(164). These studies report increased arterial stiffness in women with GDM or pre-existing T2DM compared with non-diabetic controls. However, there may be predictive value in evaluating arterial stiffness throughout pregnancy and postpartum, as women who develop GDM may have increased arterial stiffness from the first trimester(174). Importantly, there is also growing evidence that GDM is associated with chronic effects on vascular stiffness and longer-term outcomes. It has been reported that women with a history of GDM display evidence of endothelial dysfunction, and are at increased risk of vascular complications independent of known risk factors(164, 167).

Therefore, the aim of this cross sectional study was to assess, non-invasively, changes in arterial stiffness and cardiac output parameters among women being screened for GDM in comparison to low-risk, healthy pregnant women in order to determine if maternal haemodynamics are altered in women at risk of GDM.

6.3 METHODS

One hundred and twenty consecutive pregnant women, who were classed as at risk for GDM, attending their routine screening for GDM (study) and a further 60 low-risk healthy pregnant women (control), with no medical conditions, booked at the University Hospitals of Leicester NHS Trust, and fulfilling the relevant inclusion criteria (section 2.4.1), were invited to participate in this study. Participants were excluded if they had: multiple pregnancy, fetal anomalies, pre-pregnancy or pregnancy-induced hypertension, pre-eclampsia, thyroid disease requiring medication, renal disease, known diabetes mellitus, taking any medication that could affect the cardiovascular system or were current smokers. In addition, participants with a BMI >25 at booking were excluded from the healthy control group.

Following informed written consent (Stanmore National Research Ethics Committee, Reference 12/LO/0810), maternal characteristics, including medical history, were obtained. Participants were assessed at 26 to 28 weeks of pregnancy, in a temperature-controlled room (22°C) in a semi recumbent position. Participants were rested for a minimum of ten minutes, and were free from distraction, including speaking and moving, during the assessments. Assessments were not carried out following a large meal or caffeine intake. Non-invasive arterial stiffness measurements, pulse wave velocity (PWV) and augmentation index (AIx), were obtained with an Arteriograph® (Tensiomed Ltd, Hungary), which has previously been validated against invasive and non-invasive measurements(172, 173) in a non-pregnant population. The Arteriograph® cuff was applied to the right arm over the brachial artery for an estimation of MAP, aortic PWV and AIx, as previously described(172). CO was assessed using a non-invasive monitor (NICOM®, Cheetah medical, Portland, Oregon). After initial calibration, continuous values of SV, CO and TPR were measured.

All recordings were made by one observer (MWO), who received appropriate training in the use of the Arteriograph® and NICOM® devices. Analysis in relation to blood sugar values were undertaken, OGTT 1 refers to the fasting blood sugar value and OGTT 2 refers to the blood sugar value at 2hrs after the 75gm oral glucose challenge.

6.3.1 Statistical analysis

To account for the increased variability with the mean, data on central sBP (CsBP), diastolic BP (CdBP) and mean arterial pressure (MAP) were logarithmically transformed where necessary. Mean differences between study and control groups for arterial stiffness and CO measurements were assessed by a two-sample unpaired t-test. All statistical tests were two-sided with type 1 error rate (p-value) of 0.05 to determine statistical significance. The underlying assumptions of unpaired t-test were assessed and appropriately addressed. The associations between OGTT1 and OGTT2 with different arterial stiffness variable at the baseline were assessed by separate linear models. The model assumes that errors were normally, independently and identically distributed with zero mean and constant variance. The distribution assumptions of error were checked using standard residual plots (Histogram, QQ plot etc). Changes in each of the haemodynamic measurements represented by the Arteriograph® (brachial and aortic AIx, PWV) and NICOM® (CO, CI, SV, SVI, HR) were modelled by separate linear mixed models incorporating the OGTT as a fixed effect.

6.4 RESULTS

The study group comprised 120 women of mean age 31.03 (5.41) years, and the control group 60 women of mean age 29.71 (5.33) years. Baseline characteristics are described in Table 6.1. At risk GDM women had significantly higher mean age, weight and BMI, and were more likely to be of non-Caucasian descent (Table 6.1).

Table 6.1: Baseline characteristics of the control and study groups at 26 to 28 weeks of gestation.

		Control group	Study group	P value
		(n=60)	(n=120)	
Age (years)		29.7 (5.3)	31.0(5.4)	0.14
Height (cm)		163.9 (7.4)	160.8 (16.0)	0.08
Weight (kg)		61.1 (7.9)	76.7 (19.6)	<0.001
BMI ($kg/m^{2)}$		22.25 (2.1)	29.20 (7.6)	<0.001
OGTT				Normal
Fasting (mmol/L)			4.57(0.66)	<5.6
2 hour (mmol/L)			7.00(1.77)	<7.8
Parity	P0	27 (45%)	50 (41.7%)	
	P1	26 (43.3%)	43 (35.8%)	
	P2	6 (10%)	15 (12.5%)	
	P3	0	4 (3.3%)	
	P4	1 (1.6%)	4 (3.3%)	
	P5	0	3 (2.5%)	
	P6	0	1 (0.8%)	
Ethnicity	Asian	2 (3.3%)	35 (29.2%)	
	Caucasian	53 (88.3%)	53 (44.2%)	
	African	4 (6.7%)	18 (15%)	
	Far east	0	8 (6.6%)	
	Middle East	1 (1.7%)	6 (5%)	

Arterial Stiffness

Brachial and aortic AIx measures of arterial stiffness were significantly higher in the study group (-69.5 and 2.7) as compared to the control group (-64.5, 5.2),p=0.04, both. However no significant difference was seen in aortic PWV (p=0.49) (Table 6.2, Figure 6.1). Br AIx, Ao AIx and PWV did not demonstrate a non-significant association with the OGTT 1 value, however, both, Br and Ao AIx had a significant association with the OGTT2 value, p<0.05 for both whereas PWV did not demonstrate a significant association, p=0.05, Table 6.3. Furthermore, the association for the OGTT2 values and BP and HR were significant, Table 6.3. A single unit increase in the OGTT 2 value was associated with an increase in the Br AIx by 3.0% and Ao AIx by 1.3 %. Similarly a single unit increase in the OGTT 2 value increased the HR and MAP by 1.2bpm and 1.6mmHg, respectively.

Table 6.2: Estimates of mean and standard deviation of the arterial stiffness measurements for the study and control groups

Group	**Heart Rate** *(bpm)*	**Brachial Aix** *(%)*	**Aortic Aix** *(%)*	**PWV** *(m/s)*	**Central sBP**		**Central dBP**		**Central MAP**	
					mmHg	*Log tf*	*mmHg*	*Log tf*	*mmHg*	*Log tf*
Study (n=120)	89 (12)	-64.5 (16.9)	5.2 (8.9)	8.3 (1.6)	107 (15)	4.7	37 (9)	3.6	71 (13)	4.3
Control (n=60)	90 (12)	-69.5 (13.2)	2.7 (6.4)	8.3 (1.4)	104 (12)	4.6	35 (9)	3.5	58 (10)	4.1
P value	0.33	0.04	0.04	0.49	0.35#		0.10#		<0.001#	

p-values are based on unpaired t-test on the log-transformed data

Table 6.3: Estimates of slope and standard error of the arterial stiffness measurements for the study group in comparison to the OGTT values.

	OGTT1	P value	OGTT2	P value
Heart Rate(bpm)	3.0 (1.5)	0.05	1.2 (0.6)	0.03
Brachial AIx (%)	4.5 (2.9)	0.13	3.0 (1.1)	0.007
Aortic AIx (%)	0.22 (1.23)	0.85	1.3 (0.4)	0.003
PWV (m/s)	0.4 (0.2)	0.09	0.2 (0.1)	0.05
Central sBP (mmHg)	4.3(2.1)	0.004	2.3 (0.8)	0.007
Central dBP (mmHg)	0.9(1.4)	<0.0001	0.9 (0.5)	0.001
Central MAP (mmHg)	4.5(1.3)	<0.0001	1.6 (0.5)	0.002

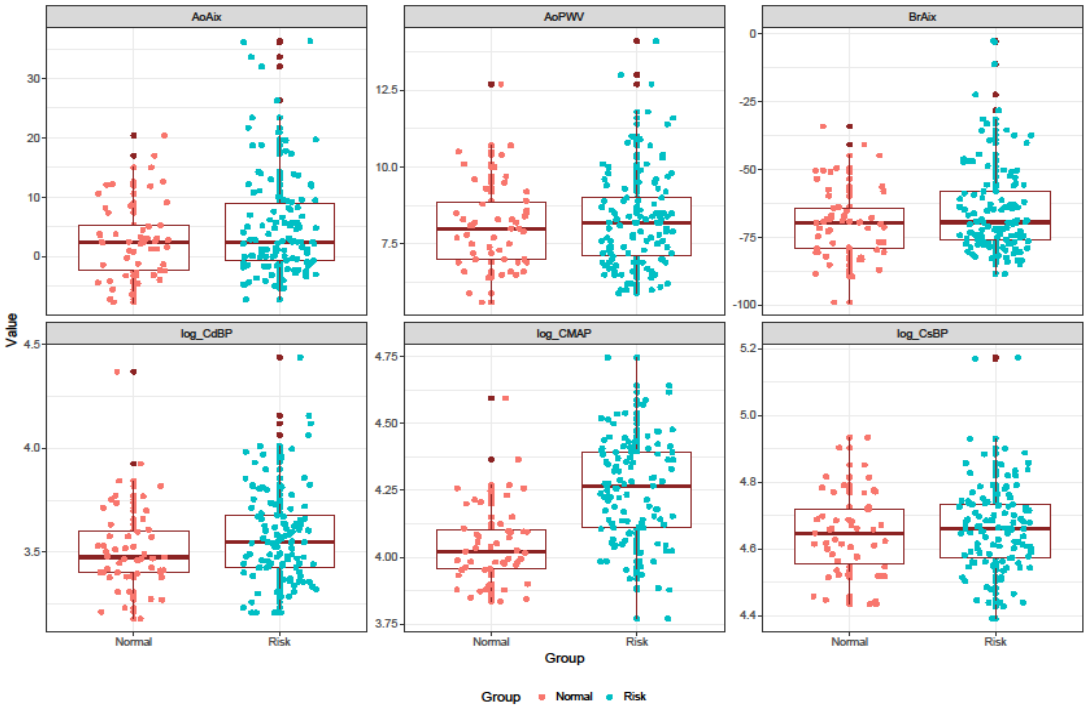

Figure 6.1: Measurements of Arteriograph variables between groups with the corresponding box plots showing the median and interquartile range

Non-invasive assessment of cardiac output parameters

There were also significant increases in CO, SV and central MAP within the study population, however, there were no differences in other central haemodynamic parameters (Table 6.4, Figure 6.2). CO, SV and TPR did not demonstrate a significant association with OGTT values whereas; BP and HR demonstrated a significant association with the OGTT2 , Table 6.5. A single unit increase in the OGTT 2 value increased the HR and MAP by 1.2bpm and 1.6mmHg, respectively.

On sub-group analysis of the study population, participants were divided into GDM+ (n=60) and GDM- (n=60) depending on the OGTT result (Table 6.6). GDM+ being women diagnosed with GDM from an above normal OGTT value as per NICE(50). It was found that women who went on to develop GDM, had a statistically significant difference in both the brachial and aortic AIx ($p<0.001$), Table 6.7. Additionally, the GDM group had higher blood pressures in comparison to the women who did not develop GDM, $p<0.01$. CO, SV and TPR did not demonstrate any difference between the two groups within the sub-group analysis.

Table 6.4 Non-invasive cardiac output measurements as mean(SD) between the study and control groups

Group	**Cardiac output** *(l/min)*	**Stroke volume** *(ml)*	**Total peripheral resistance** *(dynes.sec/cm$^{5)}$*	**Central SBP**		**Central DBP**		**Central MAP**	
				mmHg	*Log tf*	*mmHg*	*Log tf*	*mmHg*	*Log tf*
Study (n=120)	7.6 (1.5)	84.4 (19.1)	1013 (224)	107 (15)	4.7	37 (9)	3.6	71 (13)	4.3
Control (n=60)	7.0 (1.3)	76.9 (17.6)	1080 (204)	104 (12)	4.6	35 (9)	3.5	58 (10)	4.1
P value	0.01	0.01	0.05	0.35		0.10		<0.001	

Data are mean (standard deviation).
SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure;
Log tf: Log transformation;

Table 6.5: Estimates of slope and standard error of the NICOM measurements for the study group in comparison to the OGTT values.

	OGTT1	**P value**	**OGTT2**	**P value**
Cardiac output *(l/min)*	-0.1 (0.08)	0.96	0.05 (0.08)	0.56
Stroke volume *(ml)*	-2.1 (2.6)	0.43	-0.24 (0.1)	0.81
Total peripheral resistance *(dynes.sec/cm$^{5)}$*	49.7 (31.7)	0.05	4.9 (11.8)	0.67
Central sBP *(mmHg)*	4.3(2.1)	0.004	2.3 (0.8)	0.007
Central dBP *(mmHg)*	0.9(1.4)	<0.0001	0.9 (0.5)	0.001
Central MAP *(mmHg)*	4.5(1.3)	<0.0001	1.6 (0.5)	0.002

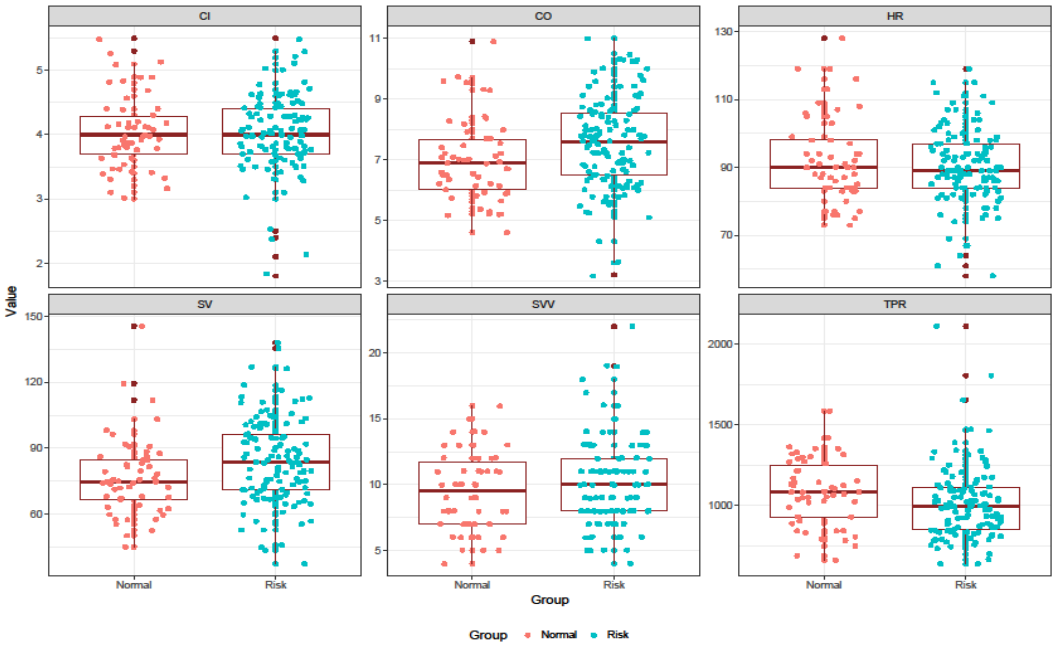

Figure 6. 2: Measurements of NICOM variables between groups with the corresponding box plots showing the median and interquartile range

Table 6.6: Baseline characteristics, in mean (SD) of the study group after sub-group analysis and division into GDM+ and GDM-at 26 to 28 weeks of pregnancy

		GDM- (OGTT normal)	GDM+ (OGTT raised)	P value
		(n=60)	(n=60)	
Age (years)		29.7(5.8)	32.3(5.4)	0.01
Height (cm)		159(21)	161(6)	0.54
Weight (kg)		74.6(19.8)	79(19.3)	0.03
BMI (kg/m$^{2)}$		27.8(6.4)	30.7(8.5)	0.03
OGTT				NORMAL VALUE
Fasting (mmol/L)		4.25(0.41)	4.9(0.72)	<5.6
2 hour (mmol/L)		5.60 (1.01)	8.40(1.12)	<7.8
Parity	P0	27 (45%)	24(40%)	
	P1	23 (38%)	21(35%)	
	P2	6 (10%)	9(15%)	
	P3	1(2%)	3(5%)	
	P4	3(5%)	1(2%)	
	P5	0	1(2%)	
	P6	0	1(2%)	
Ethnicity	Asian	19(31%)	16(27%)	
	Caucasian	33 (55%)	20(33.5%	
	African	7 (12%)	11(18%)	
	Far east	0	8(13%)	
	Middle East	1(2%)	5(8.5%)	

Table 6.7: Maternal haemodynamic measurements in mean (SD) of the study group after sub-group analysis and division into GDM+ and GDM- at 26 to 28 weeks of pregnancy

Group	**HR** *(bpm)*	**Cardiac output** *(l/min)*	**Stroke volume** *(ml)*	**Total peripheral resistance** *(dynes.sec/cm$^{5)}$*	**Central SBP**		**Central DBP**		**Central MAP**		**BrAix** *(%)*	**AoAix** *(%)*	**AoPWV** *(m/s)*
					mmHg	*Log tf*	*mmHg*	*Log tf*	*mmHg*	*Log tf*			
GDM- (OGTT normal)	90 (11)	7.54 (1.5)	85 (19)	993.9 (225.8)	102 (10.7)	4.62	34.6 (7.5)	3.52	57.2 (9)	4.37	-70.8 (11.5)	2.6 (7.2)	8.5 (1.7)
GDM+ (OGTT Raised)	91 (11)	7.58 (1.5)	84 (19)	1034.6 (223.4)	111.8 (18)	4.71	38.8 (10.4)	3.63	63.1 (12.2)	4.13	-58.2 (19)	7.9 (9.6)	8.1 (1.5)
P value	0.06	0.88	0.72	0.32	<0.001		<0.01		<0.001		<0.001	<0.001	0.15

Data are mean (standard deviation).

HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; Log tf: Log transformation; AoPWV: aortic pulse wave velocity; BrAIx: brachial augmentation index; Ao AIx: aortic augmentation index

6.5 DISCUSSION

This study has demonstrated that pregnant women at risk of GDM have significant alterations in haemodynamics compared to low-risk healthy women, when assessed at 26 to 28 weeks of gestation. In particular, measures of arterial stiffness (brachial and aortic AIx), CO, SV and MAP were all significantly higher.

Savidou et al(18) found that patients with GDM had significantly increased arterial stiffness compared to low-risk healthy pregnancy, as assessed by mean AIx. Our study, however, found a significant difference in AIx values in women at risk of GDM in comparison to normal pregnant women; brAIx, (p=0.039) and aoAix (p=0.040). In keeping with our study, Savidou et al(18) also reported no significant differences in PWV. This apparent discrepancy between different measures of arterial stiffness may reflect that AIx, as a measure of arterial wave reflection, reveals early changes of arterial stiffness; with changes more prevalent in individuals younger than 50 years of age, more typical of a pregnant population. Whereas PWV may reflect more chronic changes in arterial stiffness; being more likely to show changes in individuals over the age of 50 years(122).

In addition, the present study showed aberrations of central haemodynamics, with increased CO, SV and central MAP in the study group compared to the control women. Such changes are in keeping with the known physiological changes during pregnancy. CO increases from the first trimester of pregnancy and by eight weeks of gestation has increased by 20%(31, 33, 232). This is primarily driven by peripheral vasodilation resulting from endothelium-dependent factors such as NO, vasodilatory prostaglandins (PGI_2) and up-regulation of oestradiol(232). The peripheral vasodilation leads to a 25-30% drop in systemic vascular resistance requiring a compensatory increase in CO, around 40% by 28 weeks(232). The increase in CO is predominantly achieved by an increase in SV rather than an increase in HR(22, 23). The additional increase seen in at risk GDM pregnancy may relate to the significantly higher weight and BMI seen in these women. Obesity is known to increase total blood volume and

CO, in part due to the increased metabolic demand of the excess weight(233, 234). Furthermore, it is understood that obese individuals have greater CO and lower TPR than lean individuals(233, 234).

Importantly, mean OGTT values on the upper limit of normal, reflected in the study population, and associated changes in maternal haemodynamics may not be benign, both in the short- and long-term. The HAPO study demonstrated a continuous association between maternal glucose levels (even below those diagnostic of diabetes) and adverse outcomes, with an increased risk of maternal complications such as PET(17). There is also work to show that there is an independent and significant association between GDM and PET(11), with the rate of PET being influenced by the level of glycaemic control(66), and the severity of GDM and pre-pregnancy BMI(66). The findings of this study further supports this relationship as demonstrated in the association of OGTT values and AIx, HR and BP. Furthermore, several studies have illustrated that an association with GDM is true for the entire spectrum of hypertensive disorders(67-69). Results from secondary analysis of the Calcium for Pre-eclampsia Prevention trial demonstrated that the relative risk of developing any form of hypertensive disease in pregnancy reached statistical significance in women who had screened positive for GDM, odds ratio (OR) 1.54 (1.28-2.11)(70). More recently, results from a systematic review identified a positive and statistically significant association between GDM and PET (pooled RR = 1.69, 95% CI 1.31-2.18; p< 0.001)(71). Similarly, in our study, we found that there was a significant difference in CMAP (p,0.001) measurements between the two groups. Furthermore, on sub-group analysis of the study population, we found that women who went on to develop GDM (n=60), had a statistically significant difference in blood pressure in comparison to the women who did not develop GDM (n=60) from the OGTT screening, p<0.001, between the two groups. In addition, the women that went on to develop GDM had a statistically significant difference in mean OGTT (mmol/L) values of; 4.9(0.7) and 8.4(1.1) in comparison to 4.3(0.4) and 5.6(1.0) in the group that did not develop GDM (p<0.001).

NICOM variables such as CO and SV demonstrated a statistically significant difference between the two groups, p<0.05. Both CO and SV were higher in the study group and

this demonstrated an increase in cardiac workload possibly due to an inherent risk of metabolic disease within the study population. This is further corroborated from sub-group analysis of the study population whereby there was no significant difference in CO or SV in women who subsequently developed GDM in comparison to the women who remained non diabetic when compared at 26 – 28 weeks, p=0.88 and 0.72, respectively.

In our study, the one hundred and eighty participants were required to meet stringent eligibility criteria to ensure all potential confounding variables such as smoking or other medical conditions that may influence maternal haemodynamic parameters were removed. Our findings, owing to the large study population, strengthen the work done by Savidou et al(18) concluding that pregnancies complicated by GDM are associated with increased maternal arterial stiffness. Whilst our findings demonstrate the potentially significant effect hyperglycaemia has on the vascular wall, even over a short duration in pregnancy, as demonstrated by the alteration in AIx, further work in a larger population, at different gestational age and with other methods of haemodynamic analysis is required. Another potential limitation of our study is that there are no validation studies of the Arteriograph® in pregnancy, although it has been extensively used in pregnancy research(137, 172, 174). In addition, values of SBP, PWV and AIx determined with this device have been validated against invasive and non-invasive measurements(172, 173) in non-pregnant populations. The device used for non-invasive CO measurement has previously been validated against echocardiographic assessment in pregnancy; demonstrating excellent repeatability and reproducibility(185).

At the research site, University Hospitals of Leicester, we have observed a declining trend in the number of pregnant women diagnosed with GDM in the Caucasian and Asian groups between 2003 and 2013. That was followed by an increase in the number of women with GDM in the years from 2013 to 2015. The greatest change in women with GDM was noted in the African-Caribbean group, as the number of cases nearly halved in the two years from 2013 to 2015. Due to the patient population within the site, it was difficult to control for ethnicity within the control group.

6.6 CONCLUSION

Pregnant women at risk of GDM between gestational weeks 26 and 28, when compared with low-risk healthy pregnancy, had potentially increased measures of arterial stiffness, as assessed by brachial and aortic augmentation indices. However, PWV was not increased. Whether these women are at greater long-term cardiovascular disease risk warrants further investigation. Further work is required to assess longitudinal changes in haemodynamic parameters among pregnant diagnosed with GDM and commenced on Metformin. That will be the focus of the next chapter.

CHAPTER 7:

Longitudinal changes of maternal haemodynamics among pregnant women with GDM on metformin

This chapter was published in the Journal of Diabetes Research and Clinical Practice

The effects of metformin on maternal haemodynamics in gestational diabetes mellitus: A pilot study.
Osman M.W*, Nath M, Khalil A, Webb D.R, Robinson T.G, Mousa H.A*
Diabetes Res Clin Pract. 2018 May;139:170-178. doi: 10.1016/j.diabres.2018.03.003. Epub 2018 Mar 7.
PMID: 29524482

I recruited all participants to this study, performed maternal haemodynamic measurements, collated, analysed and interpreted the data. I wrote the manuscript and received academic editorial input from all supervisors. Upon submission, the reviewers' comments were addressed by

7.1 ABSTRACT

Aim

To assess longitudinal changes in maternal haemodynamics amongst pregnant women diagnosed with GDM requiring either metformin or dietary intervention in comparison to low-risk healthy controls, and to investigate the effects of metformin.

Methodology

Fifty-six pregnant women attending their first appointment at the GDM clinic and 60 low-risk healthy pregnant women (control) attending their routine antenatal clinics were recruited and assigned to three groups: GDM Metformin (GDM-M), GDM Diet (GDM-D) and Control. Non-invasive assessment of maternal haemodynamics, using recognised measures of arterial stiffness (Arteriograph®) and cardiac output (NICOM)®, were undertaken under controlled conditions within four gestational windows: antenatal (AN1) (26-28 weeks), AN2 (32-34 weeks) and AN3 (37-40 weeks), and postnatal (PN) (6-8 weeks after delivery). Data were analysed using a linear mixed model incorporating gestational age and other relevant predictors, including age, BP, baseline bodyweight and HR as fixed effects, and patient as a random effect.

Results

Fitted linear mixed models showed evidence of a two-way interaction effect between groups (GDM-D, GDM-M and Control) and stages of gestation (AN1, AN2, AN3 and PN) for maternal haemodynamic parameters: brachial AIx ($p=0.004$), aortic AIx ($p=0.008$), SV ($p=0.19$), TPR ($p=0.006$) and CsBP ($p=0.001$). However, differences in respect of aoPWV ($p=0.001$), CO ($p=0.01$) and HR ($p<0.001$) were only significant for gestational stage, but not between groups.

At AN2, metformin therapy had a potential beneficial effect on AIx, with a non-significant mean difference between GDM-M and control groups ($p=0.15$).

Conclusion

AIx and CsBP measures of arterial stiffness are adversely affected by GDM in comparison to controls during pregnancy. The potential beneficial effects of metformin therapy seen at 32 to 34 weeks of gestation require further exploration in a future intervention trial. Further work will be required to assess changes among women with GDM and compare the results to healthy controls.

7.2 INTRODUCTION

Pregnancy is associated with significant changes in maternal haemodynamics and measures of arterial stiffness across each trimester(235). In particular, significant increases in arterial stiffness and wave reflection parameters are noted amongst pregnant women who subsequently develop pre-eclampsia and small for gestational age fetuses(236), as well as those with known PET(19). Cross-sectional studies(18, 162, 163) conducted in late pregnancy or immediately post-partum have suggested an independent link between arterial stiffness and GDM, with increased PWV and AIx in GDM compared to women with normal glucose tolerance. Furthermore, GDM may be associated with chronic effects on vascular haemodynamics, impacting longer-term outcomes. Women with a history of GDM have evidence of endothelial dysfunction and are at increased risk of vascular complications independent of known risk factors(164, 167). However, these studies are limited by sample size and are cross-sectional in nature. A clearer understanding of changes in arterial stiffness throughout pregnancy and post-partum between GDM and healthy women may be of predictive value.

Pregnancy-associated diabetes has historically only been treated with dietary modification and/or insulin. Glyburide, a sulphonylurea, was the second line treatment after Insulin in the US for GDM. However, it has now superseded insulin as the most common treatment since 2007; being used in over 64.5% of women with GDM(9). Though this change was due to the perception that Glyburide does not cross the placenta(10), there have been reports of an increased rate of preeclampsia, neonatal jaundice requiring phototherapy, increased duration of stay in the neonatal unit, macrosomia and neonatal hypoglycaemia following its use(11). Results from a systematic review and meta-analysis established that glyburide is inferior to both metformin and insulin in the treatment of women with GDM(12). Metformin was considered unsafe, as the drug crosses the placenta, posing a potential threat to the fetus. However, results from several observational and randomised trials over the past decade have confirmed that metformin use in pregnancy is safe, with no evidence of increased birth defects or other pregnancy-related complications(12-15), though it

remains unlicensed for use in pregnancy. GDM is now treated with dietary and lifestyle modification, metformin and if needed insulin(50). Metformin may have cardiovascular benefits(237); the UK Prospective Diabetes Study (UKPDS) demonstrating that metformin use in obese patients with T2DM is associated with beneficial effects on cardiovascular disease outcomes, with a 36% and 39% relative risk reduction in all-cause mortality and myocardial infarction, respectively(56). In a randomised, placebo controlled trial, short-term metformin therapy was found to improve arterial stiffness and endothelial function in young women with PCOS(57). It is important to assess whether metformin use may be associated with potential benefits on vascular stiffness in GDM, as this may also be associated long-term cardiovascular benefit. Therefore, a pilot study was undertaken to assess;
i) longitudinal changes in maternal haemodynamics, including AIx and PWV parameters among pregnant women with GDM compared to healthy pregnancy; and
ii) to explore whether metformin compared to diet-only modification had beneficial effects on maternal haemodynamic assessments.

7.3 METHODS

Fifty-six consecutive women with a singleton viable pregnancy attending their first appointment at the GDM clinic and a further 60 low-risk healthy pregnant women attending their routine antenatal dating ultrasound scan at the University Hospitals of Leicester NHS Trust, were invited to participate in the study. Screening for women at risk of GDM is always offered to pregnant women with: BMI greater than 30kg/m^2, previous macrosomic baby weighing more than 4.5kg, previous history of GDM, family history of diabetes in a first degree relative, or of minority ethnic origin with a high prevalence of diabetes(50). Screening was offered at 24-28 weeks of gestation and diagnosis of GDM was made if the woman had either: a fasting plasma glucose level of 5.6 mmol/litre or above OR a 2-hour plasma glucose level of 7.8 mmol/litre or above(50).
Women screening positive for GDM were included into the GDM group.
Participants were excluded if they were current smokers, had a multiple pregnancy, fetal anomalies, pre-pregnancy or pregnancy-induced hypertension, PET, thyroid

disease requiring medication, renal disease, type1 or 2 diabetes mellitus, GDM requiring insulin or were taking any medication that could affect the cardiovascular system. In addition, eligibility into the control group required the participants to have a BMI between 18.5-24.9Kg/m^2 at booking and not to have diabetes mellitus. Following informed written consent (Stanmore National Research Ethics Committee, Reference 12/LO/0810), maternal characteristics, including medical history, were obtained. Participants were separated into two groups, GDM and control. The GDM group was further divided into two groups according to GDM management: diet modification (GDM-D) or metformin therapy (GDM-M). Upon diagnosis of GDM, women were reviewed in a multidisciplinary GDM clinic with a diabetes nurse and dietician, and were counselled on the diagnosis and dietary changes needed. They were then reviewed two weeks later at around 30 to 31 weeks of gestation to review the effects of dietary adjustments on blood sugar control. Women with poor control were then immediately started on metformin (500mg po bd) and therefore all women in the GDM-M group were on metformin during the AN2 measurement. Compliance was monitored by checking the electronic readings stored on the glucometer by the diabetic physician and women requiring insulin were excluded (n=4).

Participants were assessed at four gestational windows 26-28 [AN1], 32-34 [AN2] and 37-40 weeks [AN3]) and postpartum (at 6-8 weeks after delivery [PN]), in a temperature-controlled room (22°C) in a semi recumbent position. Participants were rested for a minimum of ten minutes, and were free from distraction, including speaking and moving, during the assessments. Assessments were not carried out following a large meal or caffeine intake. Arterial stiffness measurements of PWV and AIx were obtained with the Arteriograph® (Tensiomed Ltd, Hungary). The Arteriograph® cuff was applied to the right arm over the brachial artery for an estimation of MAP, aortic PWV and AIx, as previously described(172). CO was assessed using a non-invasive monitor (NICOM®, Cheetah medical, Portland, Oregon). After initial calibration, continuous values of SV, CO and TPR were measured. Recordings were made by one observer (MWO), who had received appropriate training in use of the Arteriograph®.

7.3.1 Statistical analysis

The ratio of women in the GDM-diagnosed group requiring treatment in the form of dietary and lifestyle control to metformin is 1:0.9(52). In the GDM-diagnosed group, the study would need 27 participants, in order to detect a difference of 1.5m/s in PWV before and after treatment assuming a SD of 1.5 m/s. A standard deviation of 1.5m/s was chosen as published work on GDM in pregnancy found that women with GDM had a mean PWV of 6 +/- 1.5 m/s(18). With this being a longitudinal study, it was anticipated that up to 15 to 20% of participants would either decline continuing in the study, miss appointments and/or have preterm delivery per time-point. We therefore concluded that a final sample of 60 participants would be adequately powered and allow for abovementioned issues. This was in keeping with the few longitudinal studies of maternal haemodynamics in pregnancy(126, 131-133), which had a mean sample size of 51.

We modelled the changes at gestational and post-natal stages for brachial and aortic AIx, and PWV, by separate linear mixed models incorporating group (three levels: Diet [GDM-D], Metformin [GDM-M] and Control), and gestational stage (four levels: AN1, AN2, AN3, PN), as fixed effects and individual participant as a random effect. If statistically significant ($p<0.05$), the final model also included the two-way interaction term of group and gestational stage. The final models for brachial and aortic Aix also included age, heart rate, central mean arterial pressure and baseline bodyweight, as additional fixed effects. Only included heart rate and baseline bodyweight were included for PWV, as height and age did not demonstrate a significant effect. We further investigated the two-way interaction effect for the different levels of group and gestational stage for AIx, SV, TPR and central systolic BP, by comparing mean differences for Control, GDM-D and GDM-M groups at each of the four gestational windows. Therefore, we compared 12 mean differences, and adjusted the estimated probabilities by Bonferroni correction to account for multiple comparisons. For variables where the two-way interaction effect was not statistically significant, we did not conduct any treatment group comparison at the predefined time points.

All statistical tests were two-sided with type 1 error rate (p-value) of 0.05 to determine statistical significance. All statistical analyses were carried out using the R software

version 3.3 with appropriate R packages (nlme, multcomp, ggplot2) (R Core Team, 2016).

7.4 RESULTS

A total of one hundred and sixteen women were recruited to the study; 56 women with GDM, of whom 33 of mean age 31.7 years (SD 5.4) were in the GDM-M group and 23 of mean age 33.1 years (4.7) were in the GDM-D group, and 60 women of mean age 29.71 years (SD 5.3) in the control group. Baseline characteristics are described in Table 7.1

Table 7.1: Baseline characteristics for all study participants

		Control	**GDM diet**	**GDM metformin**
		n=60	n=23	n=33
Age (years)		29.71 (5.33)	33.13(4.72)	31.76(5.43)
Height at booking (cm)		162.80 (7.09)	159.26(6.54)	163.33 (6.31)
Baseline body weight at booking(kg)		66.1(9.6)	71.87(16.86)	82.48 (19.99)
Body weight at recruitment (kg)		69.26 (16.79)	78.11(14.81)	88.00 (32.80)
Body surface area (BSA) at booking (m^2)		1.79(0.19)	1.80 (0.17)	1.93 (0.2)
BMI at booking (kg/m^2)		24.16(5.36)	27.96 (6.03)	32.13 (9.74)
BMI at recruitment (kg/m^2)		24.56(3.10)	30.76 (5.37)	32.80 (5.17)
Gestational age at recruitment (weeks + days)		28+2 (1.1)	28+3 (1.6)	27+2 (1.8)
Gestational age at OGTT (weeks + days)		N/A	28+3 (1.6)	27+2 (1.8)
	Normal OGTT			
OGTT fasting	≥5.6mmol/litre		4.53(0.62)	5.11(0.70)
OGTT 2hrs	≥7.8mmol/litre		8.07 (0.92)	8.54 (1.22)
Parity	P0	27 (45%)	8 (34.8%)	15 (45.4%)
	P1	26 (43.3%)	11(47.9)	9 (27.3%)
	P2	6 (10.0%)	2 (8.7%)	5 (15.3%)
	P3	0	1 (4.3%)	2 (6.0%)
	P4	1 (1.7%)	1 (4.3%)	0
	P5	0	0	2 (6%)
Ethnicity	Asian	2 (3.3%)	9 (39.1%)	7 (21.2%)
	Caucasian	53 (88.3%)	8 (34.8%)	15 (45.5%)
	African	4 (6.8%)	1 (4.4%)	8 (24.3%)
	Far East	0	3 (13%)	1 (3.0%)
	Middle East	1 (1.7%)	2 (8.7%)	2 (6.0%)

Continuos data are presented as mean (standard deviation) and categorical data are presented as count (% of totoal).
BSA: body surface area; BMI: body mass index. P: parity

7.4.1 ARTERIAL STIFFNESS MEASUREMENTS

7.4.1.1 Brachial Augmentation index

The fitted linear mixed model showed strong evidence of a two-way interaction effect between both groups (Control, GDM-D and GDM-M) and gestational stages (AN1, AN2, AN3 and PN) after adjusting for heart rate, BP, weight and age (p=0.004) (Tables 7.2 to 7.4, Figure 7.1). At AN1, significant differences were seen in the brAIx between the GDM-M and control groups, and GDM-D and control groups of 13.91% (p=0.02) and 8.33% (p=0.05), respectively, but not between the two GDM groups (p=0.817).). At AN1, the mean (±SE) brAIx (%) of GDM-M (-58.20±2.41) was significantly different (adjusted p = 0.020) from the control group (-68.15±1.78). However, the mean difference between brachial AIx (%) GDM-D (-59.02±2.65) although statistically significant (p=0.005), but the adjusted p-value (0.055) exceeded the pre-assigned type 1 error of 0.05

At AN2, only the mean difference between GDM-DD (-46.53±3.80) and control groups (-68.91±2.32) was significant (19.23%, p<0.0001).

No significant between group differences were seen at AN3.

Postnatally, again significant differences were seen in the brAIx between the GDM-M (-39.20±4.71) and control groups(-39.20±4.71), and GDM-D(-40.81±4.38) and control groups of 19.85% (p=0.03) and 19.89% (p=0.03), respectively.

7.4.1.2 Aortic augmentation index

Similar to brAIx , we also found strong evidence of a two-way interaction effect between both groups (Control, GDM-D and GDM-M) and gestational stages (AN1, AN2, AN3 and PN) after adjusting for HR, BP, weight and age (p=0.008) (Tables 7.2 to 7.4, Figure 7.2). At AN1, significant differences were seen in the aortic AIx between the GDM-M and control groups, and the GDM-D and control groups of mean difference of 6.75% (p=0.03) and 3.2% (p=0.02), respectively, but not between the two GDM groups. The mean different between aortic AIx (%) GDM-D (6.99±1.38) was statistically

significant (p=0.02), but following the adjustment of p-value by Bonferroni correction, the adjusted p-value (0.277) exceeded the pre-assigned type 1 error of 0.05.

At AN2, only the mean difference between GDM-D and control groups was significant (8.73%, p=0.0003, adjusted p<0.001),).

No significant between groups differences were seen at AN3.

Postnatally, again significant differences were seen in the aortic AIx between the GDM-M(17.53±2.39) and control groups(8.94±1.75), and the GDM-D(16.86±2.21) and control groups of 10.03% (p<0.05, adjusted p=0.036) and 10.05% (p<0.05, adjusted p=0.030), respectively, but not between the two GDM groups.

7.4.1.3 Pulse wave velocity

Only mean differences between gestational stages (p=0.001), but not between groups (p=0.511), were statistically significant for PWV, after adjusting for heart rate and weight (Table 7.2 to 7.4 and Figure 7.3). Height and age did not demonstrate a significant interaction with PWV (p=0.06 and p=0.38, respectively). The mean PWV values were significantly higher at AN2 (p=0.005) and PN (p=0.003) compared to the value at AN1, but the mean PWV at AN3 was not significantly different from the mean PWV at AN1 (p=0.458).

Table 7.2 Arterial stiffness measurements in healthy pregnant (control) and gestational diabetes mellitus populations managed by diet or metformin at three antenatal and one post-partum gestational time-points

	AN1 26 -28 weeks			#	AN2 32-34 weeks			#	AN3 37-40 weeks			#	PN 6-8 weeks			#	*Group: stage interaction*
	Control	GDM-D	GDM-M	P value	Control	GDM-M	GDM-D	P value	Control	GDM-D	GDM-M	P value	Control	GDM-D	GDM-M	P value	*Global P-value*
Central Systolic BP *mmHg*	104.72 (12.89)	107.04 (11.35)	114.88 (21.38)		111.49 (13.41)	111.23 (12.45)	117.39 (15.89)		111.79 (12.16)	108.40 (11.53)	116.21 (15.41)		110.33 (10.84)	118.16 (14.91)	120.29 (15.59)		*0.0006*
Central Diastolic BP *mmHg mmHg*	34.49 (8.72)	35.63 (7.73)	40.71 (12.31)		37.09 (7.77)	37.28 (8.58)	40.78 (12.31)		39.31 (9.58)	39.71 (5.89)	44.93 (12.11)		39.92 (8.52)	43.58 (9.75)	43.96 (8.93)		*NS*
Central Mean BP *mmHg*	57.90 (9.58)	59.53 (8.31)	65.43 (14.82)		37.09 (7.77)	62.69 (9.32)	66.32 (12.54)		63.47 (9.69)	62.61 (6.72)	68.69 (13.99)		63.38 (8.75)	68.44 (11.13)	69.40 (10.67)		*NS*
Brachial AIx %	-69.52 (13.16)	-61.19 (15.92)	-55.61 (21.80)	0.81	-65.49 (16.21)	-46.26 (29.99)	-56.63 (19.35)	0.15	-51.01 (17.93)	-41.04 (31.48)	-54.54 (22.85)	0.12	-44.10 (19.16)	-24.21 (27.59)	-24.25 (30.78)	1	0.004
Aortic AIx %	2.73 (6.39)	5.93 (7.98)	9.48 (11.02)	1	4.49 (8.21)	13.22 (15.89)	9.04 (9.83)	0.63	12.12 (9.92)	16.86 (15.94)	10.02 (11.57)	0.12	15.33 (9.69)	25.38 (13.97)	25.36 (15.59)	1	0.007
PWV m/s	8.13 (1.41)	8.05 (1.11)	8.93 (1.99)		8.58 (1.25)	9.23 (2.05)	9.27 (1.42)		8.13 (1.36)	8.39 (1.45)	8.72 (1.41)		8.19 (1.49)	8.58 (1.32)	8.80 (1.97)		*NS*

Data are mean (standard deviation). P value refers to group: gestational stage interaction for measurements at four time-points, $p<0.05$ indicating significance. NS: non-significant
BP: blood pressure; AIx: augmentation index; PWV: pulse wave velocity; AN: ante-natal; PN: post-natal; GDM-D: gestational diabetes, diet controlled; GDM-M: gestational diabetes, metformin controlled; NI: no interaction.

#: GDM-M to GDM- Interaction
The mean difference between GDM-M and GDM-D groups for AIx measurements was assessed by a two-sample unpaired t-test

Table 7.3: Arterial stiffness measurements in **healthy pregnant (control)** and **gestational diabetes mellitus managed by diet modification (GDM-D)** groups at three antenatal and one postpartum gestational time-points.

		AN1 26-28 weeks			AN2 32-34 weeks			AN3 37-40 weeks			PN 6-8 weeks			Group: stage interaction
	Units	**Normal**	**GDM-D**	**P value**	**Normal**	**GDM-D**	**P value**	**Normal**	**GDM-D**	**P value**	**Normal**	**GDM-D**	**P value**	**Global p value**
Heart rate	*Bpm*	90.89 (11.91)	90.14 (11.70)	*NS*	93.57 (11.20)	88.17 (11.71)	*NS*	86.21 (14.02)	79.94 (11.56)	*NS*	76.15 (9.35)	73.79 (10.21)	NS	*NS*
Central Systolic BP	*mmHg*	104.72 (12.89)	107.04 (11.35)	*1*	111.49 (13.41)	111.23 (12.45)	*1*	111.79 (12.16)	108.40 (11.53)	*0.43*	110.33 (10.84)	118.16 (14.91)	0.17	*0.0006*
Central Diastolic BP	*mmHg*	34.49 (8.72)	35.63 (7.73)	*NS*	37.09 (7.77)	37.28 (8.58)	*NS*	39.31 (9.58)	39.71 (5.89)	*NS*	39.92 (8.52)	43.58 (9.75)	NS	*NS*
Central Mean BP	*mmHg*	57.90 (9.58)	59.53 (8.31)	*NS*	37.09 (7.77)	62.69 (9.32)	*NS*	63.47 (9.69)	62.61 (6.72)	*NS*	63.38 (8.75)	68.44 (11.13)	NS	*NS*
Brachial AIx	**%**	-69.52 (13.16)	-61.19 (15.92)	*0.05*	-65.49 (16.21)	-46.26 (29.99)	*<0.0001*	-51.01 (17.93)	-41.04 (31.48)	*0.30*	-44.10 (19.16)	-24.21 (27.59)	0.03	*0.004*
Aortic AIx	**%**	2.73 (6.39)	5.93 (7.98)	*0.02*	4.49 (8.21)	13.22 (15.89)	*0.0003*	12.12 (9.92)	16.86 (15.94)	0.42	15.33 (9.69)	25.38 (13.97)	0.03	*0.007*
PWV	*m/s*	8.13 (1.41)	8.05 (1.11)	*NS*	8.58 (1.25)	9.23 (2.05)	*NS*	8.13 (1.36)	8.39 (1.45)	NS	8.19 (1.49)	8.58 (1.32)	NS	*NS*

Data are mean (standard deviation). P value refers to group interaction, $p<0.05$ indicating significance.
BP: blood pressure; AIx: augmentation index; PWV: pulse wave velocity; AN: antenatal; PN: postnatal; GDM-D: gestational diabetes diet controlled; NS: non-significant.

Table 7.4: Arterial stiffness measurements in **healthy pregnant (control)** and **gestational diabetes mellitus managed with metformin (GDM-M)** groups at three antenatal and one postpartum gestational time-points.

		AN1 26-28 weeks			AN2 32-34 weeks			AN3 37-40 weeks			PN 6-8 weeks			Group:stage interaction
	Units	**Normal**	**GDM-M**	***P value***	**Normal**	**GDM-M**	***P value***	**Normal**	**GDM-M**	***P value***	**Normal**	**GDM-M**	**P value**	***Global p value***
Heart rate	*Bpm*	90.89 (11.91)	92.19 (9.45)	*NS*	93.57 (11.20)	93.31 (11.50)	*NS*	86.21 (14.02)	85.28 (15.72)	*NS*	76.15 (9.35)	71.87 (11.73)	*NS*	*NS*
Central Systolic BP	*mmHg*	104.72 (12.89)	114.88 (21.38)	*0.12*	111.49 (13.41)	117.39 (15.89)	*1*	111.79 (12.16)	116.21 (15.41)	*1*	110.33 (10.84)	120.29 (15.59)	*0.28*	*0.0006*
Central Diastolic BP	*mmHg*	34.49 (8.72)	40.71 (12.31)	*NS*	37.09 (7.77)	40.78 (12.31)	*NS*	39.31 (9.58)	44.93 (12.11)	*NS*	39.92 (8.52)	43.96 (8.93)	*NS*	*NS*
Central mean BP	*mmHg*	57.90 (9.58)	65.43 (14.82)	*NS*	37.09 (7.77)	66.32 (12.54)	*NS*	63.47 (9.69)	68.69 (13.99)	*NS*	63.38 (8.75)	69.40 (10.67)	*NS*	*NS*
Brachial AIx	**%**	-69.52 (13.16)	-55.61 (21.80)	*0.02*	-65.49 (16.21)	-56.63 (19.35)	*0.15*	-51.01 (17.93)	-54.54 (22.85)	*1*	-44.10 (19.16)	-24.25 (30.78)	*0.02*	*0.04*
Aortic AIx	**%**	2.73 (6.39)	9.48 (11.02)	*0.03*	4.49 (8.21)	9.04 (9.83)	*0.21*	12.12 (9.92)	10.02 (11.57)	*1*	15.33 (9.69)	25.36 (15.59)	*0.03*	*0.007*
PWV	**m/s**	8.13 (1.41)	8.93 (1.99)	*NS*	8.58 (1.25)	9.27 (1.42)	*NS*	8.13 (1.36)	8.72 (1.41)	*NS*	8.19 (1.49)	8.80 (1.97)	*NS*	*NS*

Data are mean (standard deviation). P value refers to group: gestational stage interaction for measurements at four time-points, $p<0.05$ indicating significance.

BP: blood pressure; AIx: augmentation index; PWV: pulse wave velocity; AN: ante-natal; PN: post-natal; GDM-D: gestational diabetes, diet controlled; GDM-M: gestational diabetes, metformin controlled; NS: non-significant.

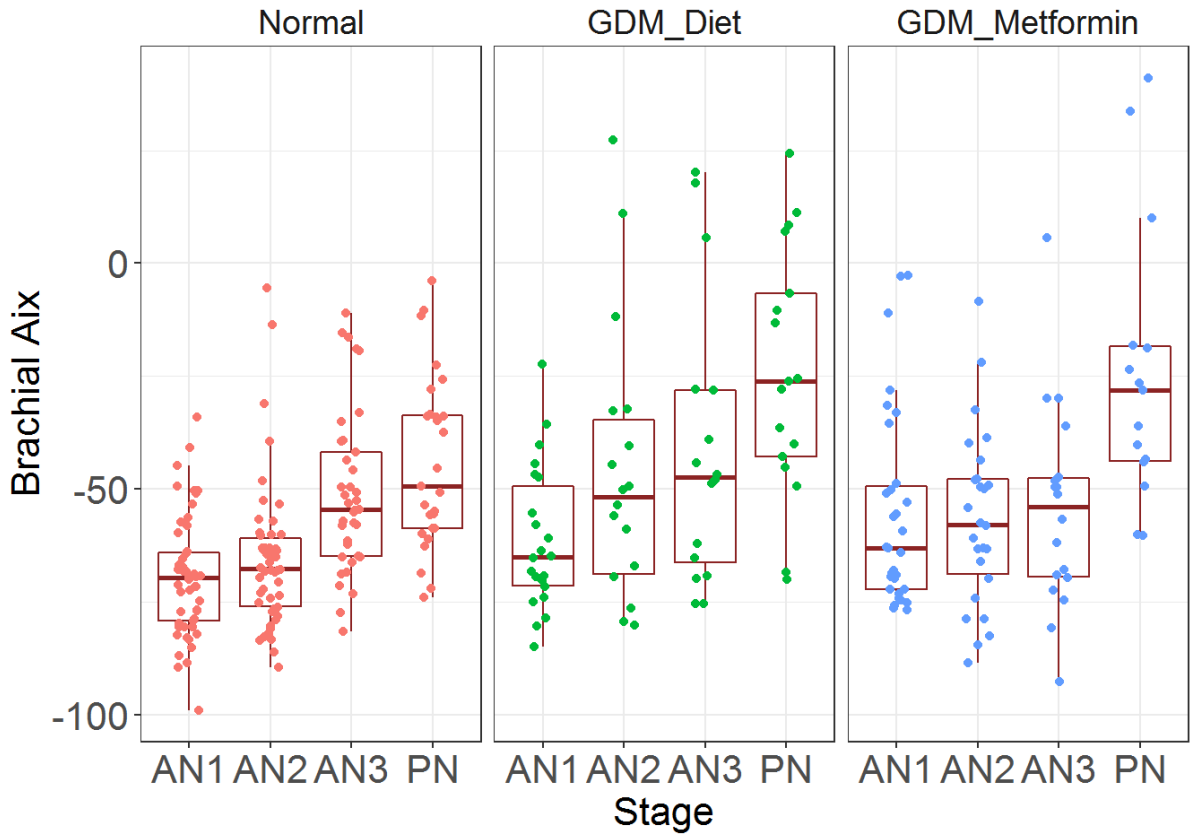

Figure 7.1: Measurements of Brachial AIx for participants in all three groups (points) at four time points (AN1, AN2, AN3, and PN) with the corresponding box plots showing the median and interquartile range.

AN1: 26-28 weeks: Control (n=52), GDM-D (n=22) and GDM-M (n=33)
AN2: 32-34 weeks: Control (n=51), GDM-D (n=18) and GDM-M (n=29)
AN3: 37-40 weeks: Control (n=38), GDM-D (n=17) and GDM-M (n=18)
PN: 6-8 weeks after delivery: Control (n=26), GDM-D (n=17) and GDM-M (n=15)

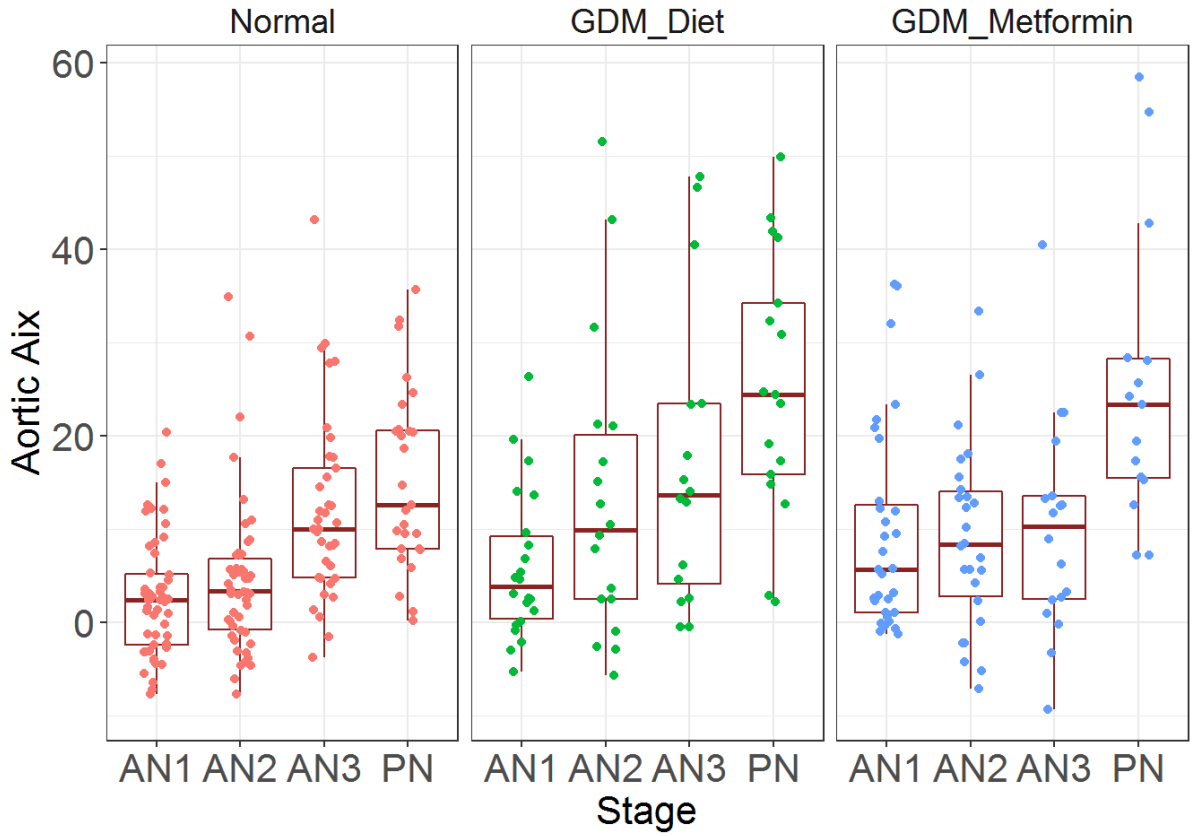

Figure 7.2: Measurements of Aortic AIx for participants in all three groups (points) at four time points (AN1, AN2, AN3, and PN) with the corresponding box plots showing the median and interquartile range.

AN1: 26-28 weeks: Control (n=52), GDM-D (n=22) and GDM-M (n=33)
AN2: 32-34 weeks: Control (n=51), GDM-D (n=18) and GDM-M (n=29)
AN3: 37-40 weeks: Control (n=38), GDM-D (n=17) and GDM-M (n=18)
PN: 6-8 weeks after delivery: Control (n=26), GDM-D (n=17) and GDM-M (n=15)

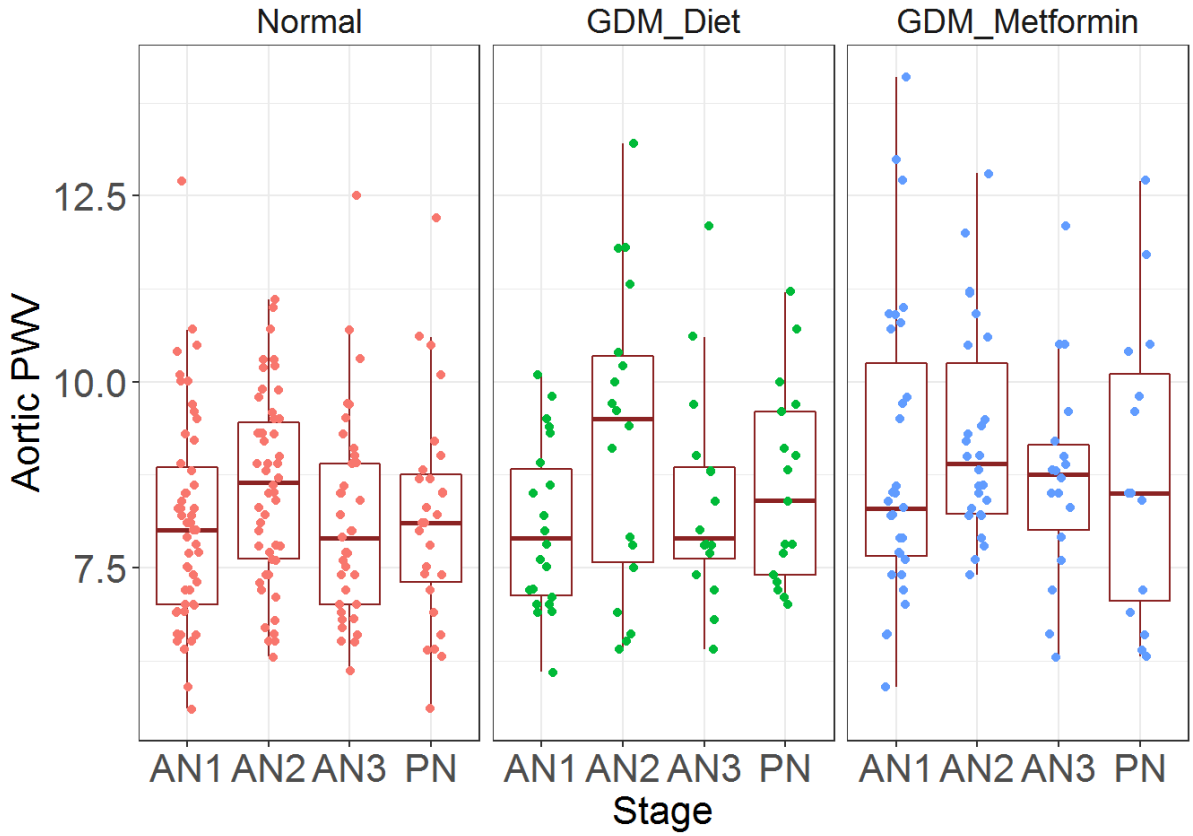

Figure 7.3: Measurements of PWV for participants in all three groups (points) at four time points (AN1, AN2, AN3, and PN) with the corresponding box plots showing the median and interquartile range.

AN1: 26-28 weeks: Control (n=52), GDM-D (n=22) and GDM-M (n=33)
AN2: 32-34 weeks: Control (n=51), GDM-D (n=18) and GDM-M (n=29)
AN3: 37-40 weeks: Control (n=38), GDM-D (n=17) and GDM-M (n=18)
PN: 6-8 weeks after delivery: Control (n=26), GDM-D (n=17) and GDM-M (n=15)

7.4.2 NICOM PARAMETERS

7.4.2.1Cardiac output and stroke volume

There was a significant difference in CO (p=0.011) and SV (p=0.005) over gestational stages (Tables 7.5 to 7.7 and Figures 7.3 and 7.4), but CO did not demonstrate any significant difference between groups. Group stage interaction for SV demonstrated that at AN3, there was a significant difference in SV between the GDM-M group in comparison to the control group; mean difference 13.9ml (p=0.01).

7.4.2.2 Pulse and Blood pressure

The mean differences for HR between gestational stages differed significantly (p<0.001), but not between groups (p=0.19), CdBP and MAP demonstrated a significant mean difference between the groups as well as gestational stages, (p <0.05) and (p=0.01), respectively. This is demonstrated in tables 7.5 to 7.7.

The gestational stages had a statistically significant effect on both CdBP and MAP, p<0.001, both. Both the CdBP and MAP increased incrementally and significantly with increasing gestation, p<0.05.

The CsBP values differed significantly between the GDM-M and the control group at AN1, GDM-D and control at AN3 and both GDM-D and GDM-M vs control at AN3. However, as we accounted for the multiple comparisons (Bonferroni correction), the adjusted probabilities for both assessments were greater than the acceptable type 1 error rate (0.05).

The mean differences in the MAP values between stages AN1, AN2, AN3 and PN were; 0.05, 0.07, 0.09mmHg (log transformation), p=0.02, p=0.0002, p<0001, respectively. The comparison between groups demonstrated a statistically significant difference between the GDM-M and the control group, mean difference of 0.08mmHg (log transformation), p=0.006. For MAP the change was significantly different at PN in comparison to AN1 (P<0.001). The mean differences between the groups were not statistically significant (P=0.124 and P=0.098, respectively).

7.4.2.3 Total peripheral resistance

TPR demonstrated a two-way interaction effect between both groups and the stages of gestation (p=0.006), (Tables 7.5 to 7.7).

At PN significant differences were seen in the TPR between both GDM-D and GDM-M in comparison to the control, p= 0.01 and p=0.02, respectively. However, as we accounted for the multiple comparisons (Bonferroni correction), the adjusted probabilities for both assessments were greater than the acceptable type 1 error rate (0.05).

Table 7.5: Cardiac output measurements in healthy pregnant (control) and gestational diabetes mellitus populations managed by diet or metformin at three antenatal and one post-partum gestational time-points

		AN1 26-28 weeks			**AN2** 32-34 weeks			**AN3** 37-40 weeks			**PN** 6-8 weeks			**Group:stage interaction**
	Units	**Control**	**GDM-D**	**GDM-M**	**Control**	**GDM-D**	**GDM-M**	**Control**	**GDM-D**	**GDM-M**	**Control**	**GDM-D**	**GDM-M**	**Global p value**
Heart rate	*Bpm*	90.89 (11.91)	90.14 (11.70)	92.19 (9.45)	93.57 (11.20)	88.17 (11.71)	93.31 (11.50)	86.21 (14.02)	79.94 (11.56)	85.28 (15.72)	76.15 (9.35)	73.79 (10.21)	71.87 (11.73)	*NS*
Cardiac output	l/min	6.97 (1.34)	7.09 (1.31)	7.70 (1.55)	6.86 (1.39)	7.45 (1.05)	7.60 (1.31)	6.29 (1.43)	7.23 (1.99)	7.52 (1.47)	6.86 (1.23)	6.63 (1.21)	7.53 (1.13)	*NS*
Stroke Volume	ml	76.88 (17.60)	80.57 (18.16)	83.23 (18.27)	72.85 (15.20)	82.01 (13.94)	81.44 (15.95)	73.24 (15.95)	86.40 (24.94)	86.83 (14.64)	87.53 (15.19)	85.38 (16.39)	96.66 (12.88)	*0.01*
Total Peripheral resistance	dynes. sec/cm^5	1080.56 (204.76)	1057.67 (174.40)	1041.90 (259.81)	1146.90 (230.24)	1028.12 (193.56)	1069.67 (261.23)	1266.00 (350.22)	1110.64 (295.08)	1029.15 (208.01)	1086.88 (191.97)	1187.94 (305.95)	1111.36 (243.59)	*0.05*
Central Systolic BP	*mmHg*	104.72 (12.89)	107.04 (11.35)	114.88 (21.38)	111.49 (13.41)	111.23 (12.45)	117.39 (15.89)	111.79 (12.16)	108.40 (11.53)	116.21 (15.41)	110.33 (10.84)	118.16 (14.91)	120.29 (15.59)	*0.0006*
Central Diastolic BP	*mmHg*	34.49 (8.72)	35.63 (7.73)	40.71 (12.31)	37.09 (7.77)	37.28 (8.58)	40.78 (12.31)	39.31 (9.58)	39.71 (5.89)	44.93 (12.11)	39.92 (8.52)	43.58 (9.75)	43.96 (8.93)	*NS*
Central Mean BP	*mmHg*	57.90 (9.58)	59.53 (8.31)	65.43 (14.82)	37.09 (7.77)	62.69 (9.32)	66.32 (12.54)	63.47 (9.69)	62.61 (6.72)	68.69 (13.99)	63.38 (8.75)	68.44 (11.13)	69.40 (10.67)	*NS*

Data are mean (standard deviation). P value refers to group: gestational stage interaction for measurements at four time-points, $p<0.05$ indicating significance.
BP: blood pressure; AIx: augmentation index; PWV: pulse wave velocity; AN: ante-natal; PN: post-natal; GDM-D: gestational diabetes, diet controlled; GDM-M: gestational diabetes, metformin controlled; NS: non-significant

Table 7.6: Cardiac output measurements in **healthy pregnant (control)** and **gestational diabetes mellitus managed by diet modification (GDM-D)** groups at three antenatal and one postpartum gestational time-points.

		AN1 26-28 weeks			AN2 32-34 weeks			AN3 37-40 weeks			PN 6-8 weeks			Group: stage interaction
	Units	**Control**	**GDM-D**	**P value**	**Control**	**GDM-D**	**P value**	**Control**	**GDM-D**	**P value**	**Control**	**GDM-D**	**P value**	**Global p value**
Heart rate	*Bpm*	90.89 (11.91)	90.14 (11.70)	*NS*	93.57 (11.20)	88.17 (11.71)	*NS*	86.21 (14.02)	79.94 (11.56)	*NS*	76.15 (9.35)	73.79 (10.21)	NS	*NS*
Cardiac output	l/min	6.97 (1.34)	7.09 (1.31)	*NS*	6.86 (1.39)	7.45 (1.05)	*NS*	6.29 (1.43)	7.23 (1.99)	*NS*	6.86 (1.23)	6.63 (1.21)	NS	*NS*
Stroke Volume	ml	76.88 (17.60)	80.57 (18.16)	*1*	72.85 (15.20)	82.01 (13.94)	*1*	73.24 (15.95)	86.40 (24.94)	*0.01*	87.53 (15.19)	85.38 (16.39)	1	*0.01*
Total Peripheral resistance	dynes. sec/cm^5	1080.56 (204.76)	1057.67 (174.40)	*1*	1146.90 (230.24)	1028.12 (193.56)	*1*	1266.00 (350.22)	1110.64 (295.08)	*1*	1086.88 (191.97)	1187.94 (305.95)	0.12	*0.05*
Central Systolic BP	*mmHg*	104.72 (12.89)	107.04 (11.35)	*1*	111.49 (13.41)	111.23 (12.45)	*1*	111.79 (12.16)	108.40 (11.53)	*0.43*	110.33 (10.84)	118.16 (14.91)	0.17	*0.0006*
Central Diastolic BP	*mmHg*	34.49 (8.72)	35.63 (7.73)	*NS*	37.09 (7.77)	37.28 (8.58)	*NS*	39.31 (9.58)	39.71 (5.89)	*NS*	39.92 (8.52)	43.58 (9.75)	NS	*NS*
Central Mean BP	*mmHg*	57.90 (9.58)	59.53 (8.31)	*NS*	37.09 (7.77)	62.69 (9.32)	*NS*	63.47 (9.69)	62.61 (6.72)	*NS*	63.38 (8.75)	68.44 (11.13)	NS	*NS*

Data are mean (standard deviation). P value refers to group interaction, $p<0.05$ indicating significance.
BP: blood pressure; AIx: augmentation index; PWV: pulse wave velocity; AN: antenatal; PN: postnatal; GDM-D: gestational diabetes diet controlled; Non-significant

Table 7.7: Cardiac output measurements in **healthy pregnant (control)** and **gestational diabetes mellitus managed with metformin (GDM-M)** groups at three antenatal and one postpartum gestational time-points.

		AN1 26-28 weeks			AN2 32-34 weeks			AN3 37-40 weeks			PN 6-8 weeks			Group:stage interaction
	Units	**Normal**	**GDM-M**	***P value***	**Normal**	**GDM-M**	***P value***	**Normal**	**GDM-M**	***P value***	**Normal**	**GDM-M**	**P value**	***Global p value***
Heart rate	*Bpm*	90.89 (11.91)	92.19 (9.45)	*NS*	93.57 (11.20)	93.31 (11.50)	*NS*	86.21 (14.02)	85.28 (15.72)	*NS*	76.15 (9.35)	71.87 (11.73)	*NS*	*NS*
Cardiac output	l/min	6.97 (1.34)	7.70 (1.55)	*NS*	6.86 (1.39)	7.60 (1.31)	*NS*	6.29 (1.43)	7.52 (1.47)	*NS*	6.86 (1.23)	7.53 (1.13)	*NS*	*NS*
Stroke Volume	ml	76.88 (17.60)	83.23 (18.27)	*NS*	72.85 (15.20)	81.44 (15.95)	*NS*	73.24 (15.95)	86.83 (14.64)	*0.55*	87.53 (15.19)	96.66 (12.88)	*NS*	*0.01*
Total Peripheral resistance	dynes. sec/cm^5	1080.56 (204.76)	1041.90 (259.81)	*NS*	1146.90 (230.24)	1069.67 (261.23)	*NS*	1266.00 (350.22)	1029.15 (208.01)	*NS*	1086.88 (191.97)	1111.36 (243.59)	*0.12*	*0.05*
Central Systolic BP	*mmHg*	104.72 (12.89)	114.88 (21.38)	*0.12*	111.49 (13.41)	117.39 (15.89)	*NS*	111.79 (12.16)	116.21 (15.41)	*NS*	110.33 (10.84)	120.29 (15.59)	*0.28*	*0.0006*
Central Diastolic BP	*mmHg*	34.49 (8.72)	40.71 (12.31)	*NS*	37.09 (7.77)	40.78 (12.31)	*NS*	39.31 (9.58)	44.93 (12.11)	*NS*	39.92 (8.52)	43.96 (8.93)	*NS*	*NS*
Central mean BP	*mmHg*	57.90 (9.58)	65.43 (14.82)	*NS*	37.09 (7.77)	66.32 (12.54)	*NS*	63.47 (9.69)	68.69 (13.99)	*NS*	63.38 (8.75)	69.40 (10.67)	*NS*	*NS*

Data are mean (standard deviation). P value refers to group: gestational stage interaction for measurements at four time-points, $p<0.05$ indicating significance.
BP: blood pressure; AIx: augmentation index; PWV: pulse wave velocity; AN: ante-natal; PN: post-natal; GDM-D: gestational diabetes, diet controlled; GDM-M: gestational diabetes, metformin controlled; Non-significant

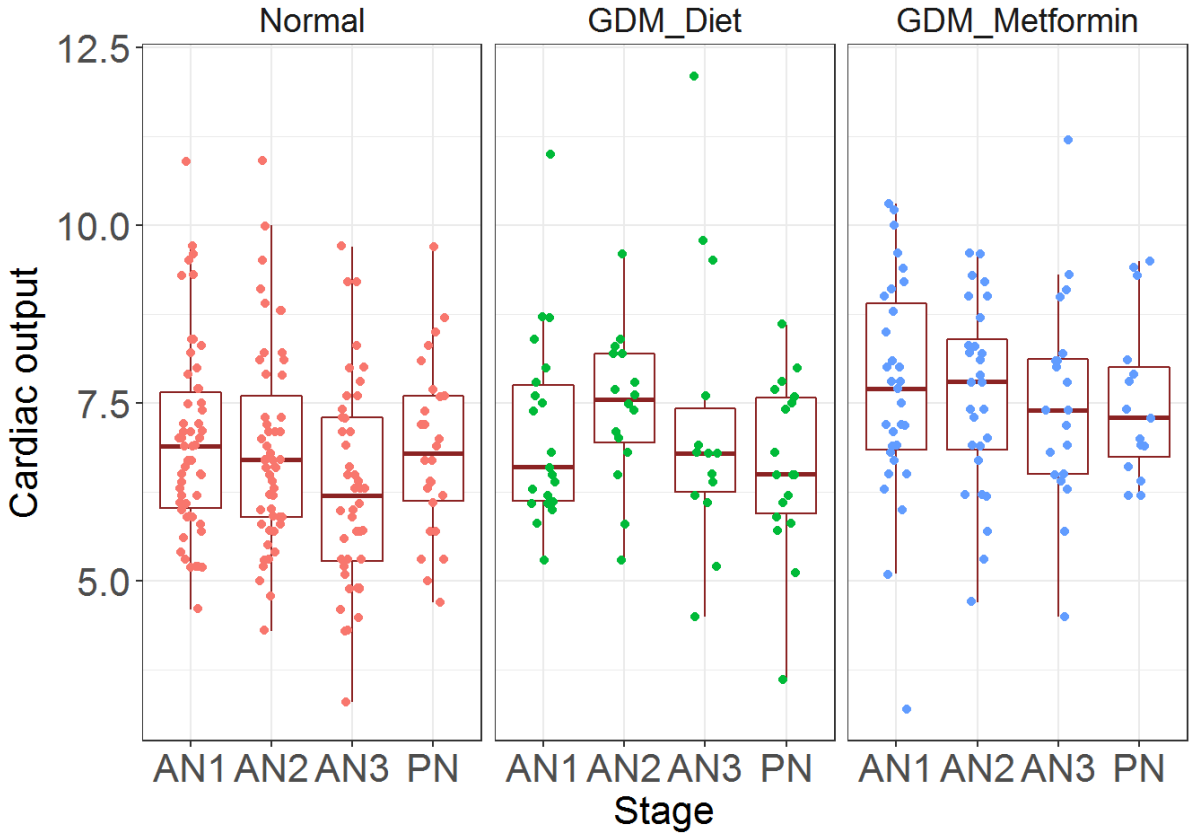

Figure 7.4: Measurements of Cardiac output for participants in all three groups (points) at four time points (AN1, AN2, AN3, and PN) with the corresponding box plots showing the median and interquartile range**.**
AN1: 26-28 weeks
AN2: 32-34 weeks
AN3: 37-40 weeks
PN: 6-8 weeks after delivery

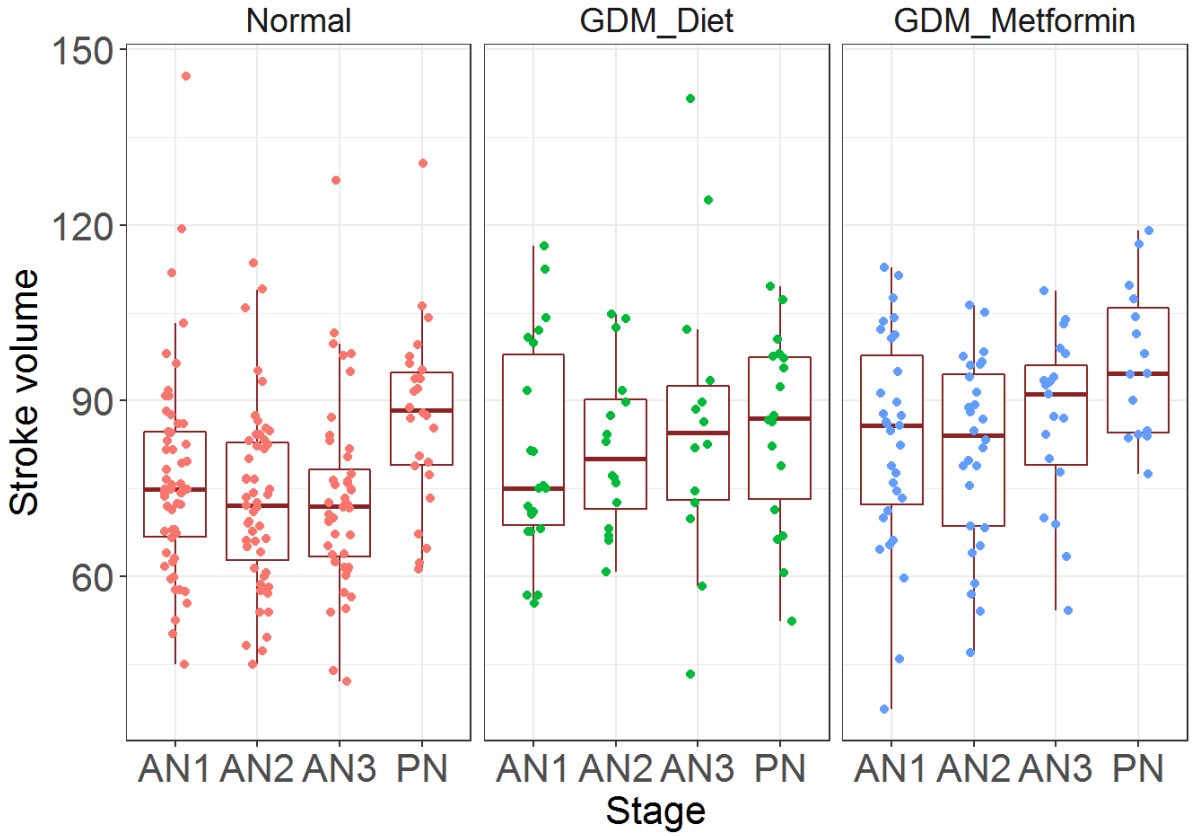

Figure 7.5: Measurements of Stroke volume for participants in all three groups (points) at four time points (AN1, AN2, AN3, and PN) with the corresponding box plots showing the median and interquartile range please.

AN1: 26-28 weeks
AN2: 32-34 weeks
AN3: 37-40 weeks
PN: 6-8 weeks after delivery

7.5 DISCUSSION

This longitudinal pilot study has demonstrated that pregnancies affected by GDM may be associated with significant alterations in maternal haemodynamics, as demonstrated by temporal changes in AIx, BP and PWV. Exploring the effect of metformin on maternal haemodynamics, we observed a potential interaction effect suggesting that metformin may attenuate the GDM associated AIx rise during the second trimester. This is evident between 32-34 weeks of pregnancy.

Our study describes differences in arterial stiffness measurements over the course of GDM and normal glucose tolerant pregnancies. However, apart from AIx, there was no significant difference in arterial stiffness parameters between the GDM and control groups in pregnancy or postpartum. Further merit is given to this finding when the comparison is performed at PN. The GDM-M group would now not be on any therapy, as metformin is stopped on the day of delivery, yet we found a significant mean difference between the GDM-D and GDM-M vs the control group, with a marked increase in AIx in the GDM metformin group at PN. This trend could mean that metformin may offer a protective effect on the vasculature and once stopped, the protection ceases. This is in keeping with the understanding that AIx may reflect the early changes of arterial stiffness, as the changes are more prevalent in younger individuals (age<50years), whereas, PWV may reflect the later or chronic changes in arterial stiffness as age related changes are more marked in individuals over the age of 50(122). It is understood that endothelial cells are more sensitive than smooth muscle cells to mitochondrial reactive oxygen species (mROS). Mitochondria act as a sensor within the endothelium(238, 239). These mitochondria are very sensitive to changes within the endothelial milieu and conditions such as hyperlipidaemia and hyperglycaemia are known to damage the mitochondria(238, 239). When these mitochondria are damaged they release reactive oxygen species which then damage the endothelium(238, 239). It is now believed that metformin may have an effect in reducing the hyperglycaemic levels around the mitochondria and thereby reduce the

release of reactive oxygen species which when released, cause endothelial damage(238, 239). In addition, our results are in keeping with previous work demonstrating that AIx, increases with advancing gestational from 28 weeks of gestation to term(126, 129, 132). The pattern of AIx in this study, however, demonstrates a unique alteration in the expected pattern, i.e., the metformin group had an improvement in the AIx at AN3. This potential atypical trend was only present during the time of metformin treatment and reverted to the expected pattern at PN when metformin was stopped. This further strengthens the proposed effects of metformin in this population group.

Savidou et al(18) found that in patients with GDM, mean AIx (a measure of arterial wave reflection) was significantly higher compared to healthy controls, (13.1 ± 8.9% vs 0.7 ± 11.4%; p<0.001). Similarly, this study found a significant difference in the AIx values in women with GDM managed with metformin or diet modification in comparison to the control group . Our study found that at AN1, mean AIx was higher in the GDM-M group, 4.90% (11.02), in comparison to controls.

Savidou et al(18) also found that in patients with GDM the mean PWV was marginally increased compared to healthy controls (6.0 ± 1.5 vs 5.4 ± 0.6 m/s; p =0.070). Similarly, in our study we found that the mean PWV is higher in women with GDM, with the mean value in the GDM-D group being 8.54 m/s (1.51) and in the GDM-M group being 8.97 m/s (1.71), in comparison to 8.28m/s in the healthy control group. However, the differences were not statistically significant, p=0.494 (Figure2). This re-affirms the findings from two previous studies(162, 163) which found that there was no significant difference in PWV between GDM and control groups. Additionally, we found that the mean difference between the stages of pregnancy was significant and observed that the pattern of PWV in the diet and healthy control group followed a pattern, grossly resembling a sine wave, similar to the findings of other studies of longitudinal changes of PWV during pregnancy(125, 130). However, the metformin group did not demonstrate any such pattern. The PWV in the metformin group did not exhibit the characteristic reduction in the third trimester of pregnancy, remaining higher at AN3 and PN.

Maternal haemodynamic variables assessed by NICOM® such as CO and HR did show a significant change over the stages of pregnancy but not between groups. Stroke volume, nevertheless, demonstrated a significant difference between stages and between groups. However, it did not demonstrate the characteristic decline towards term(232) in the GDM group despite the adjustment in heart rate demonstrating an underlying increase in cardiac workload. The pattern of these parameters behave as expected in the latter half of pregnancy, as cardiac output reaches a peak at 30 weeks of gestation and then declines to term, reaching to below first trimester levels at term. HR increased up to 30 weeks of gestation and this followed a similar pattern to previous work published(21, 232) and was the main influence in the pattern of CO.

We found that TPR was the lowest in the Metformin group in comparison to the GDM-D and control group (p=0.006). In addition, we found that there was a significant difference between BMI in the three groups, p<0.0001, with the BMI being greatest in the GDM-M group. Obesity is known to increase the total blood volume and CO, in part due to the increased metabolic demand of the excess weight(233, 234). Furthermore, it is understood that obese individuals have a greater CO and a lower TPR than lean individuals(233, 234).

The current study has several strengths. We have used a well-defined inclusion and exclusion criteria to limit the effect of any maternal condition on haemodynamic parameters. We have longitudinal follow-up to make sure that changes are directly related to the same group. In comparison , Savvidou et al(18) however, had a GDM population that had a significantly higher blood pressure than the control subjects, and this may have influenced the results within the GDM group, as it is understood that PWV is known to increase with BP, and BP is a recognised determinant of aortic PWV(116, 165, 206). Equally, the control group in the study of Bulzico et al(162) had a higher prevalence of T2DM and cardiovascular disease in their first degree relatives(166), which may be associated with higher aortic stiffness(166). Throughout our study, BP in both groups over all the four time points remained within the normal range, however Central mean BP was significantly higher in the GDM group in comparison to the healthy control, 67.02mmHG (13.25) vs 61.25mmHG (9.57), p=0.02. Furthermore, CdBP and MAP were significantly higher in the GDM metformin group

compared to the healthy control group, p=0.01 and p=0.06, respectively. Therefore, it could be argued that the findings of an increased PWV in the GDM groups are a result of an increase in BP and possibly age.

We cannot fully explain the cause of this interesting observation which may allude to Metformin having pleiotrophic actions yet unknown to us, therefore more research is necessary. Even though we did not find that metformin had a significant effect on all maternal haemodynamic parameters in women with GDM, the mean difference between the GDM-M and GDM-D groups for aortic AIx at AN3 was statistically significant (p=0.033), the AIx in the GDM-M group changed more than in the GDM-D group, -54.54%(22.85) vs -41.04 (31.48), respectively. This may be attributed to a potential beneficial effect of metformin on the vascular walls of arteries. Further work with larger studies are required to explore this pattern more closely.

The study has certain limitations. Firstly, the small number of participants (n=56). However, an attempt to overcome this was made with the longitudinal assessment of women on four separate occasions. Unfortunately, loss to follow-up is an understood weakness of a longitudinal study, and the authors found a higher loss to follow up in the postnatal period. This was attributed to the practical difficulties to a new mother in the puerperium. We employed the mixed effects model to accommodate this loss of data. Ideally, a randomised controlled trial having defined arterial stiffness outcomes with a larger population and consistent methodological designs are required to further explore these findings. One could argue that the current observation may only be applicable to this population with a distinct ethnic mix. It was also noted that the GDM-M group had a greater means fasting and 2hr OGTT plasma glucose level in comparison to the GDM-D group, (4.53[0.62] vs. 5.11[0.70]) and (8.07[0.92] vs. 8.54[1.22]), respectively. These findings demonstrate the potentially significant effect hyperglycaemia has on the vascular wall, even over a short duration in pregnancy, and highlighting the beneficial effects of metformin. This study did not evaluate women prior to 26 weeks of pregnancy and therefore women in the GDM group may have prior undiagnosed diabetes. However, as much as there is a case for glycaemic

memory(32) influencing the haemodynamic measurements for the worse, there is a stronger case displaying the early and beneficial effects of metformin. There are numerous studies(33-36) recommending that early intensive control of hyperglycaemia is able to reduce the risk of diabetic micro- and macro-vascular complications, therefore, the work done demonstrating the pleiotrophic effects of metformin are welcome. It is important to stress that the current study did not examine the risk of placental mediated diseases. It is generally understood that arterial stiffness increases with age(206); however, our GDM-M group were younger than the GDM-D group and demonstrated an improvement in their AIx after metformin therapy.

Even though there are no validation studies of the Arteriograph® in pregnancy, it has been extensively used in pregnancy research(137, 172, 174). The accuracy of CsBP, PWV and AIx determination have been validated against invasive and non-invasive measurements(172, 173), in non-pregnant populations. Furthermore, triplicate measurements in a previous repeatability study performed by the authors showed moderate-to-high correlation between observations on the same woman for all Arteriograph variables (estimates of intra-class correlation ranged from 0.49 to 0.91)(225). The NICOM®, (Cheetah medical, Portland, Oregon), has been validated against echocardiographic assessment in pregnancy; demonstrated good intra-observer repeatability and reproducibility(185).

7.6 CONCLUSION

In conclusion, our study documented that AIx and central systolic BP measures are adversely affected by GDM in comparison to controls during pregnancy. Metformin intake may influence changes in AIx over the course of pregnancy. The potential beneficial effects of metformin therapy seen at 32 to 34 weeks of gestation require further exploration in a future intervention trial.

CHAPTER 8:

Conclusion

8.1 THE MAIN FINDINGS OF THE STUDIES

In this thesis, I have described normal maternal haemodynamic values in pregnancy, and explored the diurnal and repeatability of these measurements in uncomplicated third trimester of pregnancy. Furthermore, I have investigated the maternal haemodynamics in women at risk of GDM compared with low-risk healthy women and finally, I explored the effect of metformin treatment on the maternal haemodynamics in women with GDM in comparison to those with GDM which was controlled by diet modification, as well as those with a low-risk healthy pregnancy.

The systematic review I undertook to determine whether arterial stiffness and wave reflection measurements during pregnancy differed between healthy women and those with placental-mediated diseases including pre-eclampsia (PET), small for gestational age (SGA), stillbirth, and placental abruption(236). This was the first systematic review to examine the association between maternal arterial stiffness and all disorders of placental origin. It was found that compared to healthy pregnancy, some measures of arterial stiffness and wave reflection were impaired among pregnant women who subsequently developed PET during all trimesters. In the first trimester, the mean AIx-75 (%) in the PET group was significantly higher with estimated standardised mean difference (SMD) of 0.90 [95% confidence intervals (95% CI): 0.07-1.73; p=0.034]. In the second trimester, the PET group had significantly higher PWV (m/s) with estimated SMD of 1.26 (95% CI: 0.22-2.30; p=0.018). Concerning the SGA group, the mean AIx (%) was higher during the second trimester only: 65.5 (standard deviation 15.6) vs. 57.0 (11.2), p<0.01.

The longitudinal study in low-risk healthy pregnant women presented in Chapter 4 demonstrated that GA had a significant effect on all arterial stiffness parameters, including brachial AIx (p=0.001), aortic AIx (p=0.002) and PWV (p=0.002). Non-invasive assessment of the cardiac output (p<0.001), stroke volume (p=0.014), heart rate (p<0.001) and total peripheral resistance (p<0.001) also demonstrated significant changes with GA. Moreover, the study provided pregnancy normograms for

gestational changes in arterial stiffness and cardiac output parameters among low-risk, healthy pregnant women. Normal limits for arterial stiffness measurements in pregnancy have not been fully reported, and therefore the novel findings presented in this study provide new insight into the maternal adaptation to pregnancy. These findings are therefore useful for future research, as well as in clinical use.

The study, presented in Chapter 5, explored the diurnal variation and repeatability of arterial stiffness and cardiac output measurements in the third trimester of uncomplicated pregnancy. With the exception of CO, CI and HR, which showed a diurnal variation, the majority of haemodynamic parameters assessed did not change significantly from morning to midnight. The estimated means (standard deviation) of PWV at the four stated time points were 7.81 (2.05), 8.45 (1.68), 7.87 (1.74) and 7.64 m/s (1.15), respectively, (p=0.267). The estimates of AIx at those time points were 10.22 (15.62), 4.44 (10.07), 6.49 (10.92) and 8.40% (8.16), respectively, (p=0.295). Triplicate measurements of 67 women in our repeatability study showed moderate to high correlation between observations on the same woman for all Arteriograph® and NICOM® variables (estimates of intraclass correlation ranged from 0.49 to 0.91), thus confirming the repeatability of measurements, albeit in the third trimester of uncomplicated pregnancy only. However, this does indicate that the majority of haemodynamic measurements do not need to be undertaken at a specific time in the day. This is in contrast to the device manufacturers' advice about standardising the time of the day when non-invasive cardiovascular assessments are undertaken, and is relevant for easier application in clinical practice.

Chapter 6 described the haemodynamic changes amongst women who were screened for GDM in comparison to low-risk healthy controls. Significant differences were found between the at-risk GDM and normal groups in brachial (-64.5 vs. -69.5, p<0.04) and aortic AIx (5.2 vs. 2.7, p=0.04), though there was no significant difference in PWV (8.3 vs. 8.1, p=0.49). Cardiac output (7.6 vs. 7.0, p=0.011), stroke volume (84.4 vs. 76.9, p=0.013) and central mean arterial pressure (71 vs. 58, p<0.001) also demonstrated a significant different between the two groups. This led to the conclusion that women at

risk of GDM had increased measures of arterial stiffness in comparison to low-risk healthy controls. This may be an earlier marker of poorer long-term cardiovascular prognosis, and warrants further research and correlation to the pregnancy outcome. The final results chapter, Chapter 7, which explored the longitudinal changes in the maternal haemodynamics amongst pregnant women diagnosed with GDM requiring either metformin or diet modification in comparison to low-risk healthy controls, and to investigate the cardiovascular effects of metformin in this group. The study found that there was significant differences between the three groups as well as longitudinally for both brachial AIx (p=0.004) and aortic AIx (p=0.008), as well as for central systolic BP (p=0.001). Furthermore, it seems that metformin therapy could have a potential beneficial effect on AIx, with a non-significant mean difference between the GDM-metformin and control groups (p=0.15). The results demonstrate that the AIx and central systolic BP measures of arterial stiffness are adversely affected by GDM in comparison to the healthy controls during pregnancy. The potential beneficial effects of metformin therapy seen at 32 to 34 weeks of gestation require further exploration in a future intervention trial.

8.2 STRENGTHS

In this work we have used strict exclusion criteria to ensure that all variables such as a raised BMI, BP, smoking or medical conditions(19, 129) (pre-eclampsia, diabetes) that may influence the maternal haemodynamic parameters were accounted for. Furthermore, women in the longitudinal study of normal women were assessed on all their visits to ensure that they remained low risk throughout pregnancy and did not develop any medical conditions that may influence maternal haemodynamics. If they did, they would be excluded. Similarly, women recruited into the GDM and at risk of GDM groups were required to meet certain inclusion criteria such as; being non-smokers, not having hypertensive or renal disease, and not having type 1 or 2 diabetes mellitus, in order to reduce the potential variables that may influence maternal haemodynamics.

The longitudinal nature of the studies with repeat assessment of the same individual has given the opportunity to identify within subject changes occurring during normal and abnormal pregnancy.
The potential for inter-observer bias was significantly reduced because all patients were reviewed by a single operator responsible for undertaking all the maternal haemodynamic assessments. Overall, 1250 separate measurements (yielding over 16,000 values) were performed to complete the above studies, and therefore I have become highly skilled and competent in the execution of the study methodologies.
The measurements of arterial stiffness conformed with the European Expert Consensus document on arterial stiffness(111), which also states that the "gold standard" for the measurement of arterial stiffness is the carotid-femoral PWV (cf-PWV).

For studies in GDM, patients were fastidiously categorised into groups according to National guidance for diagnosing GDM(50). The GDM study which explored the longitudinal changes in the maternal haemodynamics amongst pregnant women diagnosed with GDM requiring either metformin or diet modification in comparison to low-risk healthy controls was original and revealed key information laying down the foundation for further work exploring the effects of metformin in the management of GDM.
Therefore, the null hypothesis that there was no difference in the maternal cardiovascular changes during pregnancy between pregnant women diagnosed with GDM, pregnant women at risk of developing GDM and low-risk healthy pregnant women can be firmly rejected.

8.3 LIMITATIONS

It is important to recognise that there are a number of limitations to the work presented in this thesis.

Firstly, there have been no validation studies of the Arteriograph® in pregnancy, despite its extensive use in pregnancy research(137, 172, 174). However, the accuracy of SBP, PWV and AIx determination have been validated against invasive and non-invasive measurements(172, 173) in non-pregnant populations. Invasive validation in pregnant women is likely to be very difficult, both ethically and logistically. Therefore, invasive validation studies are very scarce.

Secondly, the assessment of regional PWV relied on the Jug-sy measurement for true aortic length(108). The margin of error associated with this calculation of true aortic length could possibly have been greater within this pregnant population. Unpublished data by the device manufacturer compared the Jug-sy measurement to height in 26,695 subjects; devising an algorithm to facilitate a more accurate determination of the true aortic length. This formula (encrypted) was shared with our research team, and used to limit the margin of error on this key aspect in PWV measurement by using height rather than attempting to determine the Jug-sy measurement over a gravid uterus.

The indices derived from the Arteriograph® such as, aortic PWV exhibited excellent ICC, with aortic AIx demonstrating good ICC, however, brachial Aix demonstrated only fair ICC estimates. The fair ICC estimates for Br AIx is a potential limitation for the reliability of central AIx values, as central AIx values are a function of the brachial AIx. This is a known limitation of peripheral measurements and GTF's are expected to correct for this.

The "at-risk" population that may develop gestational diabetes was noted to be of Asian ethnic background in Leicestershire. It would therefore be difficult to identify if ethnicity has an effect on the arterial stiffness. However, studies have reported no effect of ethnicity on the arterial stiffness in pregnancy (126). Finally, the high ethnic

minority population recruited to the studies presented in this thesis, whilst representative of the Leicester population, may limit the generalisability of the results. The assessment of women at risk of GDM was carried out from 22-28 weeks when they attended for their glucose tolerance test. It was not possible to identify the changes that occurred in that group before pregnancy and in the early stage of pregnancy prior to recruitment. However, we have used strict inclusion and exclusion criteria to limit the impact of maternal medical conditions or medication on cardiovascular assessment.

8.4 FUTURE WORK

Large prospective studies using consistent methodological designs are required to examine the application of arterial stiffness assessment throughout pregnancy, including all trimesters, and importantly linking findings to the risk of developing placental mediated disorders and adverse pregnancy outcome. Whilst important data has been gathered with respect to the reliability and repeatability, and normal reference ranges, it is important to undertake further studies to guide clinicians for the optimum time for screening studies. Furthermore, larger studies will be required to evaluate the impact of using Metformin in the treatment of gestational diabetes together with its effects on pregnancy and the maternal long term cardiovascular outcomes. Future research should also examine women prior to pregnancy, at all stages during pregnancy, and postpartum to help determine the link to pre-existing risk factors and the changes that occur during pregnancy between normal and at risk pregnancies, and the pregnancy outcome. This will help us to identify those patients that are most at risk of developing pregnancy complications and future cardiovascular co-morbidity. Early identification will allow targeted health promotion strategies to reduce the CVD risk and minimise serious morbidity and mortality in young mothers. This will have a reciprocal and longstanding effect on prudent utilisation of finite NHS resource.

APPENDIX
Research documents

Consent form

University Hospitals of Leicester NHS
NHS Trust

Fetal Medicine
Ground Floor
Antenatal Assessment
Kensington
Tel: 0116 258 6106

Leicester Royal Infirmary
Infirmary Square
Leicester
LE1 5WW

Version 2
22nd June 2012
Name of Principal Investigator: Dr Waseem Osman
Project ID number: 12/LO/0810

CONSENT FORM

Title of project: Cardiovascular changes in pregnancy
Name of Chief Investigator: Dr Mohamed Waseem Osman

Please initial the boxes below

1.	I confirm that I have read and understood the information sheet dated 22nd June 2012 (Version 2) about this study and have had the opportunity to ask questions.	
2.	I confirm that I have had enough time to consider whether or not I want to take part in the study	
3.	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.	
4.	I understand that sections of my medical notes may be looked at by responsible individuals involved in the research or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	
5.	I agree for the researchers to conduct non-invasive studies on my heart and blood vessels.	
6.	I agree to take part in this study.	

_____	_____	_____
Name of patient	Date	Signature

_____ _____ _____

Person taking consent Date Signature

Researcher to be contacted if there are any queries: Dr Waseem Osman on 0116 258 5895

If you have any comments or concerns, please discuss them with the investigator. If you wish to complain further about any aspect of the way you have been approached or treated during the course of the study, you should contact the Patient Information and Liaison Service on 0808 178 8337 or Molly Patterson (Research Midwife) on 0116 258 5589.

Participant information leaflet

University Hospitals of Leicester NHS
NHS Trust

Antenatal clinic
Fetal and Maternal Medicine
Ground Floor
Antenatal Assessment
Kensington
Tel: 0116 258 6106

Leicester Royal Infirmary
Infirmary Square
Leicester
LE1 5WW

Name of Principal Investigator: Dr M.W. Osman
Project ID number: 12/LO/0810
Contact telephone number: 0116 258 5895

Information Leaflet:

Cardiovascular changes in pregnancy (CVP)

We are inviting women to take part in a research study on the changes in the heart and blood vessels in pregnancy. Before you decide whether to take part, it is important for you to understand why the research is being done and what it involves. Please read the information below and discuss it with others, if you wish. Ask us about anything that is not clear and please take your time deciding whether you wish to be involved.

Why is this study important?
Several changes in the mother's heart and blood vessels are necessary for a successful pregnancy. These changes can be studied by simple non-invasive (no needles) tests such as measuring blood pressure and using ultrasound scans, which are safe in pregnancy. Changes in the heart and blood vessels can start before the onset of many of the pregnancy complications such as preeclampsia, which causes high blood pressure, protein in the urine and swelling of ankles and hands, is a serious condition for both mother and baby.

What is the purpose of the study?
The purpose of our study is to establish what heart and blood vessels changes occur in women who had normal pregnancies and those affected by complications, both during and after the pregnancy.

Why have I been chosen?
We are inviting all pregnant women to take part in this research.

What will happen if I agree to participate?
If you agree to take part, we will perform tests to assess your heart and blood vessels; these tests are simple and non-invasive (similar to ultrasound scans and using a cuff on your upper arm similar to a blood pressure cuff). We will ask you to give an extra blood sample (20mL, i.e. approximately two teaspoons). These tests will measure levels of substances made by the blood vessels (one is known as soluble endoglin, for example) which can be abnormal in women with preeclampsia. Your measurements will be tested anonymously (so there will be no way of identifying these measurements as yours).

Confidentiality
Any information will be used only by the research team as part of the study and will not be divulged to any third party.

Do I have to take part?
No. It is up to you whether or not to take part. If you do, we will ask you to sign a form confirming your consent. You may withdraw at any time without giving a reason. Not taking part will not affect your care or your baby's care.

What do I have to do?
You do not have to do anything different as part of this study.

What are the benefits of participating?
The study is not designed to help you directly now. We hope that the study will give us information which will improve the care we can give to women and their babies. It is unlikely that the results will be available during your current pregnancy. We hope that the results will benefit mothers and babies in future; this may include you.

Are there any risks?
All the tests we perform are safe for your health and that of your baby. The safety of ultrasound in pregnancy has been extensively studied and these scans have been shown to be safe for mothers and their babies.

What are the arrangements for compensation?
In the event that something goes wrong and you are harmed during the research, and this is due to someone's negligence, then you may have grounds for legal action against University Hospitals of Leicester, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you. You can also contact The Patient Advice Liaison Service (Phone: 0808 178 8337).

Whom can I contact?
Please contact Dr M.W. Osman (Leicester Royal Infirmary 0116 258 5895) to discuss any concerns or clarify any points. If you have any complaints about the way the study

is being conducted, please contact the Patient Information and Liaison Service on 0808 178 8337 or Molly Patterson (Research Midwife) on 0116 258 5589.

Who will have access to the information?

Only the researchers involved in the study will have access to the information. We will also need to contact your GP to inform him or her that you are taking part in this study. The data are also legally safeguarded under the 1998 Data Protection Act. For further information on the Act, you can contact the hospital's data protection officer, via the University Hospitals of Leicester switchboard (0300 303 1573). We hope to make our overall findings known to other healthcare professionals involved in caring for pregnant women and their babies, via meetings, presentations and journal publications. None of this presented information could allow individuals to be identified in any way.

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the NRES Committee London - Stanmore

Thank you for taking part in this study.

Dr Mohamed Waseem Osman
Clinical Research Fellow- Obstetrics and Gynaecology
University Hospitals of Leicester

Ethical clearance: NRES Committee London

NRES Committee London - Stanmore
Skipton House
Ground Floor
NRES/HRA
80 London Road
London SE1 6LH

Telephone: 020 7972 2554
Facsimile: 020 7972 2592

30 July 2012

Dr Asma Khalil
Consultant in Obstetrics and Fetal Medicine
St George's Healthcare NHS Trust
Fetal Medicine Unit
Blackshaw Road
London SW17 0QT

Dear Dr Khalil

Study title: Cardiovascular Changes in Pregnancy (CVP)
REC reference: 12/LO/0810

Thank you for your letter of 23 July 2012, responding to the Committee's request for further information on the above research and submitting revised documentation. The further information was considered in correspondence by a sub-committee of the REC. A list of the sub-committee members is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Participant information leaflet	2	22 June 2012
Participant consent form	2	22 June 2012
Protocol	2	22 June 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/LO/0810 Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Mrs Rosemary Hill
Chair
Email: NRESCommittee.London-Stanmore@nhs.net

NRES Committee London - Stanmore

Attendance at Sub-Committee of the REC meeting on 25 July 2012

Written comments received from:

Name	Position
Mrs Rosemary Hill - Chair	Statistician
Dr Geraldine Edge	Consultant Anaesthetist
Dr Anthony Gilbert	Senior Study Physician

Ethical clearance: Research and Development office: Leicester

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28/08/2014

Tommy Mousa
University Hospitals of Leicester NHS Trust
Robert Kilpatrick Clinical Science Building
Leicester Royal Infirmary
Infirmary Square
Leicester

Dear Tommy Mousa

Ref: UHL 11310
Title: Cardiovascular Changes in Pregnancy (CVP)
Project Status: Project Approved
End Date: 17/06/2016

Date of Valid Application: 28/08/2014
Days remaining to recruit first patient: 67 Days

I am pleased to confirm that with effect from the date of this letter, the above study has Trust Research & Development permission to commence at University

Hospitals of Leicester NHS Trust. The research must be conducted in line with the Protocol and fulfil any contractual obligations agreed between UHL & the Sponsor. If you identify any issues during the course of your research that are likely to affect these obligations you must contact the R&D Office.

In order for the UHL Trust to comply with targets set by the Department of Health through the 'Plan for Growth', there is an expectation that the first patient will be recruited within 70 days of receipt of a Valid Application. The date that a Valid application was received is detailed above, along with the days remaining to recruit your first patient. It is essential that you notify the UHL Data Management Team as soon as you have recruited your first patient to the study either by email to RDData@uhl-tr.nhs.uk or by phone 0116 258 4573.

If we have not heard from you within the specified time period we will contact you not only to collect the data, but also to record any issues that may have arisen to prevent you from achieving this target. It is essential that you get in touch with us if there is likely to be a problem in achieving this target so that we can discuss potential solutions. The Trust is contractually obliged to meet the 70 day target and if an adequate reason acceptable to the NIHR has not been submitted to explain the issues preventing the recruitment of your first participant, the Trust will be financially penalised.

In addition, we are required to publish the Title, REC Reference number, local target recruitment and actual recruitment as well as 70 days data for this study on a quarterly basis on the UHL public accessed website.

All documents received by this office have been reviewed and form part of the approval. The documents received and approved are as follows:
Please note: No activity can take place until a fully executed agreed contract is in place with University Hospitals of Leicester and St Georges NHS Health care Trust

Description	Version
Participant information leaflet	V2 Dated: 22 June 2012
Participant consent form	V2 Dated: 22 June 2012
Protocol	V2 Dated: 22 June 2012

Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments should be followed. Failure to do so may invalidate the approval of the study at this trust.

Undertaking research in the NHS comes with a range of regulatory responsibilities. Please ensure that you and your research team are familiar with, and understand the roles and responsibilities both collectively and individually.

Documents listing the roles and responsibilities for all individuals involved in research can be found on the R&D pages of the Public Website. It is important that you familiarise yourself with the Standard Operating Procedures, Policies and all other relevant documents which can be located by visiting

www.leicestershospitals.nhs.uk/aboutus/education-and-research

The R&D Office is keen to support and facilitate research where ever possible. If you have any questions regarding this or other research you wish to undertake in the Trust, please contact this office. Our contact details are provided on the attached sheet.

We wish you every success with your research.

Yours sincerely

David Hetmanski
R&D Assistant Director

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