From THE DEPARTMENT OF CLINICAL SCIENCES,
DANDERYD HOSPITAL
Karolinska Institutet, Stockholm, Sweden

CARDIOVASCULAR FUNCTION IN NORMAL PREGNANCY AND FETAL GROWTH

Charlotte Iacobaeus

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Cardiovascular function in normal pregnancy and fetal growth
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By

Charlotte Iacobaeus, M.D.

Principal Supervisor:
Ellika Andolf, M.D, Professor
Karolinska Institutet
Department of Clinical Sciences,
Danderyd Hospital
Division of Obstetrics and Gynecology

Co-supervisor):
Thomas Kahan, M.D, Professor
Karolinska Institutet
Department of Clinical Sciences,
Danderyd Hospital
Division of Cardiovascular Medicine

Katarina Bremme, M.D, Associate Professor
Karolinska Institutet
Department of Women's and Children's Health
Division of Obstetrics and Gynecology

Eva Östlund M.D, PhD
Karolinska Institutet
Department of Clinical Science and Education
Södersjukhuset
Division of Obstetrics and Gynecology

Opponent:
Christoph Lees, M.D, Professor
Imperial College, London
Faculty of Medicine
Department of Surgery and Cancer

Examination Board:
Anna-Karin Edstedt Bonamy, M.D, Associate Professor
Karolinska Institutet
Department of Medicine, Solna
Division of Clinical Epidemiology

Tord Naessén, M.D, Professor
Uppsala Universitet
Department of Women's and Children's Health
Division of Obstetrics and Gynecology

Maria Schaufelberger, M.D, Associate Professor
Göteborgs Universitet
Department of Molecular and Clinical Medicine
Division of Medicine

To my parents, Margaritha and Ulf Iacobaeus

ABSTRACT

Background: Pregnancy is a challenge to a woman's cardiovascular system, and extensive haemodynamic changes are required for optimal utero-placental circulation and fetal growth. The aim of this thesis was firstly to examine different aspects of vascular function in the same women longitudinally during normal pregnancy, and secondly to explore whether there is an association between maternal cardiovascular function, placental function, and fetal growth.

Methods and Main Results: Study I and **II** were prospective cohort studies investigating cardiovascular structure and function in 52 healthy women longitudinally at 14, 24 and 34 weeks gestation, and nine months postpartum. In **Study I**, brachial and central blood pressure, pulse-wave velocity, augmentation index, common carotid artery structure and endothelial function in the brachial artery (FMD) and in the forearm skin microcirculation was assessed. The study found that brachial and central blood pressure initially decreased and then increased. FMD and endothelium dependent microvascular reactivity increased during pregnancy. Pulse wave velocity and augmentation index decreased, reaching a nadir in the second trimester. In **Study II**, cardiac function, myocardial mechano-energetic efficiency (MEE), and ventricular-arterial coupling (VAC) were measured. Left ventricular (LV) mass and cardiac output (CO) increased during pregnancy. LV diastolic function decreased but MEE and VAC maintained during pregnancy. There was a positive correlation between first and second trimester heart rate, and birth weight centile. **Study III** was a cross-sectional study (n=56) investigating the association between first trimester vascular function and birth weight centile. Maternal first trimester vascular reactivity in the brachial artery, and in the forearm skin microcirculation, related positively to birth weight centile. **Study IV** was a cross-sectional study (n=53) investigating the association between levels of circulating maternal pregnancy-associated plasma protein A (PAPP-A), and first trimester maternal vascular function. PAPP-A correlated positively to first trimester maternal skin microvascular endothelial function index.

Conclusions: During normal pregnancy**,** there were profound structural and functional changes throughout the cardiovascular tree. These changes are probably necessary for sufficient fetal growth, which is supported by the observed relation between maternal heart rate, vascular vasodilator capacity, and fetal growth. Given that first trimester PAPP-A levels relate to placental function, our results suggest that placental function can be reflected by first trimester endothelium dependent skin microvascular reactivity.

LIST OF SCIENTIFIC PAPERS

I. Iacobaeus C, Andolf E, Thorsell M, Bremme K, Jörneskog G, Östlund E, Kahan T. Longitudinal study of vascular structure and function during normal pregnancy. Ultrasound Obstet Gynecol 2017; 49 (1):46-53

II. Iacobaeus C, Andolf E, Thorsell M, Bremme K, Östlund E, Kahan T. Cardiac function, myocardial mechano-energetic efficiency, and ventricular-arterial coupling in normal pregnancy. J Hypertens, in press 2017

III. Iacobaeus C, Kahan T, Jörneskog G, Bremme K, Thorsell M, Andolf E. Fetal growth is associated with first trimester maternal vascular function. Ultrasound Obstet Gynecol 2016; 48: (4):483-490

IV. Iacobaeus C, Kahan T, Jörneskog G, Bremme K, Andolf E, Thorsell M. Pregnancy-associated plasma protein A is positively correlated to first trimester skin microvascular reactivity. Ultrasound Obstet Gynecol 2017 doi: 10.1002/uog.17486. [Epub ahead of print]

CONTENTS

LIST OF ABBREVIATIONS

A	Late mitral flow velocity
a´	Late mitral annular diastolic velocity
AIx	Augmentation index
ACh	Acetylcholine
AU	Arbitrary units
BMI	Body mass index
BP	Blood pressure
BPD	Biparietal diameter
BSA	Body surface area
CCA	Common carotid artery
CO	Cardiac output
C dBP	Central diastolic blood pressure
C sBP	Central systolic blood pressure
E	Early mitral flow velocity
e´	early mitral annular diastolic velocity
EFW	Estmated fetal weight
FGR	Fetal growth restriction
FMD	Flow mediated vasodilatation
GA	Gestational age
GTN	Glyceryl trinitrate
hs-CRP	High sensitivity C-reactive protein
IMT	Intima media thickness
IVS	Interventricular septum
IUGR	Intrauterine growth restriction
LA	Left atrial
LDF	Laser Doppler fluxmetri
LV	Left ventricular
LVDdes	Left ventricular dimension in end-systole
LVDed	Left ventricular dimension in end-diastole
LVM	Left ventricular mass
LVOT	Left ventricular outflow tract
MAP	Mean arterial pressure

MME	Myocardial mechano-energetic efficiency
MVO2	Myocardial oxygen consumption
NO	Nitrate oxid
PAPP-A	Pregnancy-associated plasma protein A
PE	Preeclampsia
PP	Pulse pressure
PWT	Posterior wall thickness
PWV	Pulse wave velocity
RV	Right ventricular
RWT	Relative wall thickness
s´	systolic mitral annular velocity
SNP	Sodium nitroprusside
UtA-PI	Uterine artery pulsatility index
VAC	Ventricular-arterial coupling

1 INTRODUCTION AND RATIONALE

Pregnancy is a challenge to a woman's cardiovascular system, and extensive haemodynamic changes are required for optimal utero-placental circulation and fetal growth. There is evidence supporting a role of maternal arterial dysfunction in both preeclamptic pregnancies (PE) and fetal growth restriction (FGR), but the pathway leading up to that remains to be elucidated[1]. PE has long been considered a placental disorder, but very recently, this concept has been questioned. Instead, it has been suggested that most cases of PE originate from a maternal cardiovascular inability to cope with the great demands of the feto-placental unit[2].

Regardless of exactly what triggers PE and FGR, the maternal cardiovascular system in pregnancy is of uttermost interest. In order to correctly interpret deviant cardiovascular responses in pregnancies, normal vascular adaptation to pregnancy must first be described. Some aspects of the vascular tree have been thoroughly studied during pregnancy, but other parts remain insufficiently explored. In particular, only a few studies have examined different aspects of vascular function in the same women longitudinally during normal pregnancy.

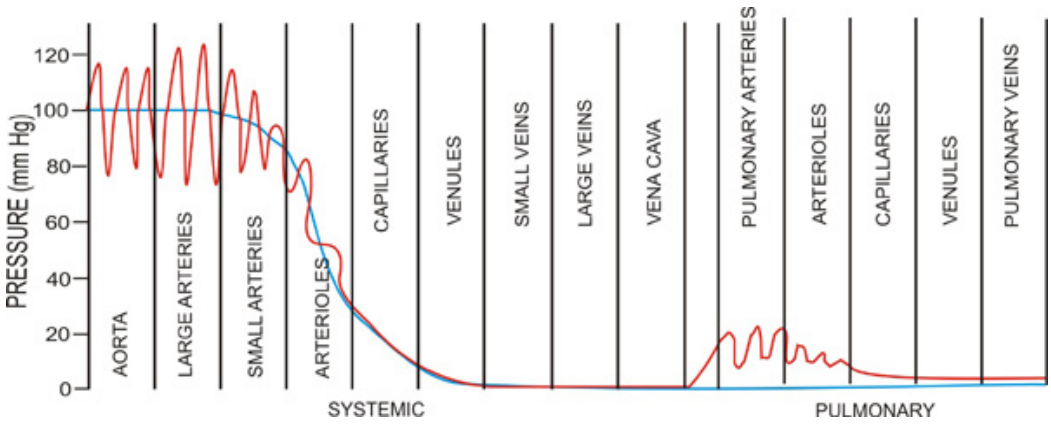
The rationale for this thesis was firstly that a better knowledge of vascular physiology during normal pregnancy is of great importance, secondly that exploring the association between maternal vascular function, fetal growth and placental function could lead to a better understanding of the relation between maternal cardiovascular function, fetal growth and placental function. This could provide a better opportunity to identify women and/or their offspring at risk of complications during pregnancy or in later in life.

2 BACKGROUND

2.1 THE CARDIOVASCULAR SYSTEM

The cardiovascular tree consists of the heart, large elastic arteries, muscular arteries, arterioles, capillaries, venules and the veins. The heart pumps the blood into the pulmonary and systemic circulation in a pulsative fashion; systole includes the contraction of the ventricles, and diastole the contraction of the atria and the filling of the ventricles. Thus, the blood flow oscillates from the heart, generating a pulse wave that moves along the circulatory system. The muscular arteries distribute the blood into the periphery and can modify this pulse wave propagation through changes in vascular tone. Next, the blood flow reaches the microcirculation that consists of arterioles, capillaries and venules. The arterioles are the primary site of vascular resistance as they stand for the greatest change in blood flow and pressure; once the blood flow reaches the capillaries it is continuous as shown in Figure 1. The capillaries are the site for tissue oxygenation, gas exchange and nutrition. Pre-capillary sphincters with smooth muscular cells regulate the capillary blood flow through relaxation and constriction as a response to local and nervous factors. The venous circulation carries the blood back to the heart.

Figure 1. Blood pressure along the circulatory system



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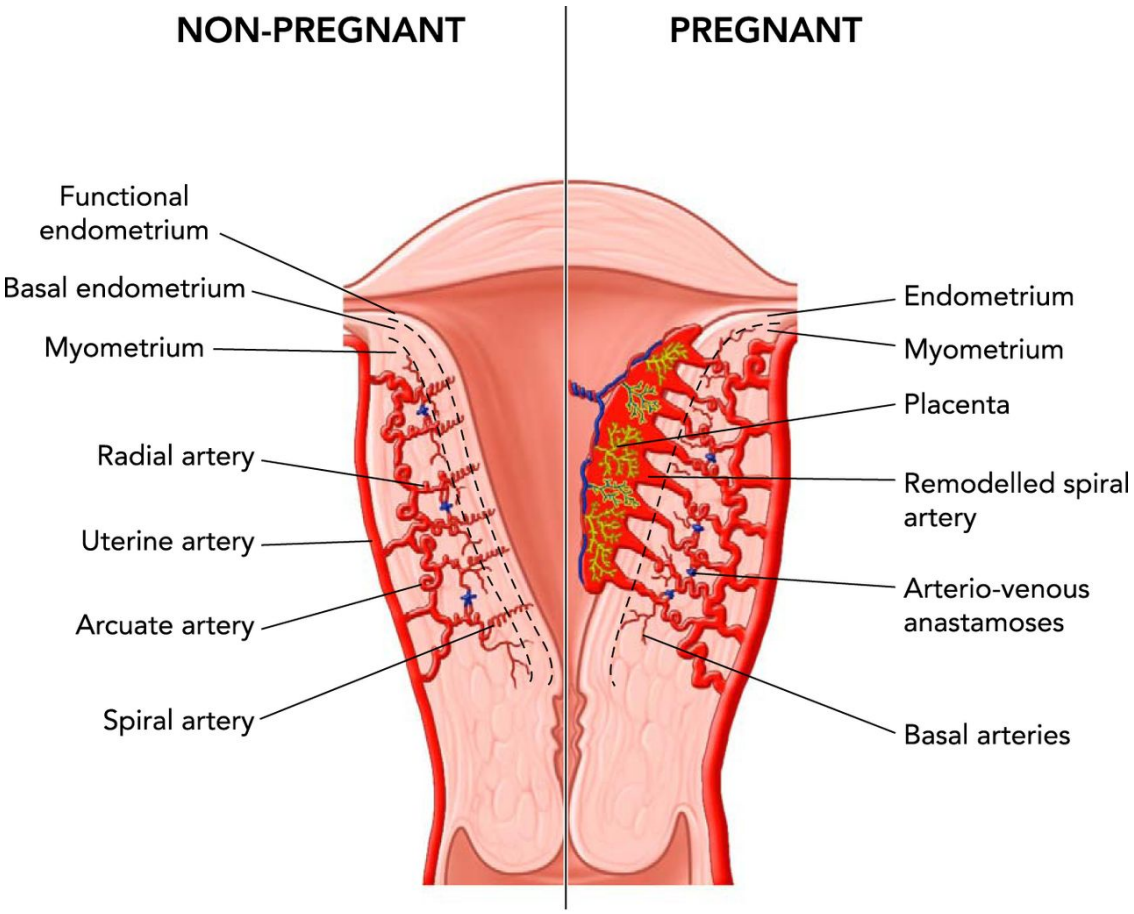
Utero-placental circulation

The uterine arteries arise from the internal iliac arteries and provide the major blood supply to the uterus. Between the uterine arteries, the vaginal artery, and the ovarian arteries, anastomoses are formed and arcuate arteries give rise to large radial arteries that will enter the endometrium to form the spiral arteries. During gestation, trophoblasts invade the spiral

arteries and replace the vascular smooth muscle and endothelial layer, forming low resistance vessels that are almost independent of maternal vasoconstriction[3]. Through these tubes, maternal blood will enter the intervillous space in spurts forming large lacunae where villi will bathe, and nutritional exchanges occur. This type of placenta is called haemochorial placenta and is unique to humans and higher primates[4]. At term, approximately 60-100 spiral arteries open up into the intravilleous space[5]. The diameter of the uterine artery is doubled by 21 week's gestation, but there is no, or only little, change in the diameter of the internal iliac arteries[6]. During pregnancy, different parts of the systemic and utero-placental circulation differ in responsiveness to vasoactive factors with decreasing sensitiveness to most, but not all, vasoconstrictors closer to the intervilleous space[7]. The anatomy of the blood vessels during the menstrual cycle and during pregnancy is shown in Figure 2.

Figure 2. The anatomy of the blood vessels in the uterus during the menstrual cycle (non-pregnant), and during pregnancy (pregnant).



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The widely dilated placental circulation is dependent on a rapid and extensive increase in systemic blood flow, maintained through increased cardiac output and intravascular volume.

2.2 Preeclampsia

Preeclampsia occurs in 3-5% of pregnancies and is characterized by the new onset of hypertension (brachial BP ≥140/90 mmHg) and *one* of the following criteria: proteinuria, end-organ dysfunction, or fetal growth restriction. Thus, proteinuria is no longer considered to be mandatory for the diagnosis of PE[8]. PE is characterized by an imbalance in pro-angiogenetic and anti-angiogenetic factors and a generalized maternal endothelial dysfunction. The origins of the disorder remain unclear, as discussed in the Introduction. Women that develop PE are more likely to show a shallow implantation of the placenta and decreased spiral artery remodelling[9], but the majority of placentas from preeclamptic pregnancies are normal[10]. PE is often divided into early- and late-onset PE (diagnosed before or after 34+0 weeks gestation, respectively). Ninety percent of PE occurs after 34 weeks gestation. Pregnancies that develop early-onset PE are more likely to have an abnormal placental morphology, and there is more effective screening for early-onset PE, but further research is needed in order to determine whether early- and late onset PE are separate disorders, or merely different severity levels of the same disease[11]. PE is still one of the leading causes of maternal mortality and morbidity worldwide, and the only cure for PE is delivery of the baby and the placenta.

2.2 Cardiovascular investigations and a window of opportunity

Women with a history of PE or fetal growth restriction have an increased risk of cardiovascular disease later in life[12 13]. Vascular ageing starts early, and subclinical atherosclerosis predicts cardiovascular events many years before they appear[14]. Hypertensive disorders during pregnancy and cardiovascular disease share many risk factors[15], and might arise from the same vascular vulnerability existing already before the pregnancy[16]. Pregnancy can therefore be considered a window on future health.

There are several methods available for discovering early signs of subclinical atherosclerosis; those used in **Study I-IV,** are shown in Table 1.

Table 1. Vascular examinations

Method	Measures	Association with cardiovascular events	References
FMD (Flow mediated dilatation in the brachial artery)	Endothelial function in conduit arteries	Early event in atherogenesis Predicts cardiovascular events and mortality.	Celermajer et al 1992[17], Matsuzawa et al 2015 [18]
PWV (Pulse wave velocity)	Aortic and arterial stiffness	Predicts cardiovascular events and mortality	Ben-Shlomo et al 2014[19]
AIx (Augmentation index)	Wave reflection; Arterial stiffness and endothelial function	Sensitive in younger individuals. Predicts cardiovascular events and mortality.	Vlachopoulus et al 2010[20] McEniery et al 2005[21]
Brachial Blood pressure (BP)	BP in the upper limb	Predicts cardiovascular events and mortality	Rapsomaniki et al 2014[22]
Central BP	BP at the aortic root	Relates closer to cardiovascular events and death than does brachial BP	Roman et al 2007[23] Vlachopoulus et al 2010[20]
Common carotid artery intima media thickness (CCA-IMT)	Arterial structure and atherosclerosis	Predicts cardiovascular events	Eigenbrodt et al 2007[24]
Laser Doppler Fluxmetri with Iontophoreses	Skin microvascular reactivity and endothelial function	Associated with cardiovascular risk. Currently no evidence of predictive value.	I. Jzerman et al 2003[25] Hellman et al 2015[26]

2.3 CARDIOVASCULAR FUNCTION IN PREGNANCY

Many of the methods available for discovering early signs of subclinical atherosclerosis have been used on pregnant women. A summary of the main findings on vascular function in normal and hypertensive pregnancies is shown in Table 2.

Table 2 Main findings on vascular function in normal and hypertensive pregnancy

Method	Findings in normal pregnancies	References	Findings in hypertensive pregnancies	References
FMD (Flow mediated dilatation in the brachial artery)	Increases in normal pregnancy, decreases near term	Van Balen et al 2017[27]	Lower FMD among women who subsequently develop PE	Noori 2010[28] Savvidou 2003[29]
PWV (Pulse wave velocity)	Either no change or decrease. Increases slightly in the third trimester.	Mahendru et al 2014[30] Khalil et al 2009[31]	Increased PWV proceeds preeclampsia	Hausvater et al 2012[32] Foo et al 2017[33]
AIx (Augmentation index)	Decreases during pregnancy with a nadir in the second trimester.	Khalil et al 2009[31]	Increased augmentation index in, and before, preeclampsia	Hausvater et al 2012[32] Foo et al 2017[33]
Brachial Blood pressure (BP)	Initial reduction, followed by an increase in the third trimester.	Macdonald-Wallis et al 2015[34] Grindheim et al 2012[35]	Increased BP at 8 weeks' gestation and no or small decrease in mid-gestation	Macdonald-Wallis et al 2012[36]
Central BP	Decreases more than brachial BP in the first part of gestation.	Fujime et al 2012[37] Mahendru et al 2014[30]	Higher in women subsequently developing preterm PE	Khalil et al 2014[38] Macdonald-Wallis et al[36]
Common carotid artery dimensions (CCA-IMT)	Either no change or slightly increase in IMT near term	Akhter et al 2013[39]	Thicker intima, a thinner media of the CCA were found in women with PE	Akhter et al 2017[40]
Laser Doppler Fluxmetri with Iontophoreses)	Limited knowledge. Increased microvascular reactivity.	Stewart et al 2007[41]	Conflicting results on increased or decreased microvascular reactivity	Agra et al 2017[42] Davis et al 2001[43] Khan et al 2005[44]

2.3.1 Maternal haemodynamics and hormonal changes

Normal pregnancy is associated with systemic vasodilatation that occurs as early as 3-4 weeks after conception[45]. When conception occur, levels of estrogen and progesterone increase due to the extended function of the corpus luteum and the increased levels of human chorionic gonadotropin (hCG) produced by the trophoblasts. There is a positive correlation between estrogen, progesterone and vasodilatation[46]. Also, relaxine, a powerful dilator of systemic resistance arteries, rises during pregnancy [47]. Peripheral vascular resistance decreases to a plateau of 35-40% below pre-pregnant values in the second trimester and then increases slightly until term. This decrease in peripheral vascular resistance likely triggers the renin–angiotensin–aldosterone system so as to retain sodium and increase plasma volume. The increase in plasma volume results in a lower haemoglobin level than in the non pregnant state. Plasma volume increases to approximately 50% over pre-pregnant values by 32 weeks gestation[48]. Heart rate increases throughout the entire pregnancy and rises to 20-25% above pre-pregnant levels[30].

In preeclamptic pregnancies, the decrease in vascular resistance is absent or insufficient, plasma volume expansion is inadequate and haemoglobin levels are higher than in normal pregnancies. Reduced plasma volume expansion is also associated with fetal growth restriction[48 49].

2.3.2 Blood pressure

In most studies of normal pregnancy, a decrease in both central and brachial blood pressure with a mid-trimester drop has been found, followed by a progressive increase until term. Diastolic BP (and thus mean arterial pressure) decrease more than systolic BP[35] Central blood pressure reflects the pressure in the aorta and has been shown to more strongly relate to vascular disease and outcome than does brachial blood pressure, especially in young individuals [23]. Pulse pressure is the difference between systolic and diastolic blood pressure and is largely determined by the elasticity of blood vessels. Elevated pulse pressure has been reported early in preeclamptic pregnancies[50].

2.3.3 Endothelial function

Other than being a semipermeable barrier between blood and tissue, the endothelium is also involved in regulating haemostasis, inflammation, angiogenesis and blood pressure. Increased blood flow and shear stress (the mechanical tension on the endothelial cells) stimulate the endothelial cells to produce nitric oxide (NO), which dilates the smooth vascular cells behind

the endothelial layer. As the vessels dilate, blood flow increases and blood pressure decreases. This dilatation is called *endothelium dependent vasodilatation* and can be assessed clinically by deliberate changes in blood flow, for example after occlusion of a vessel that is causing ischemia (FMD), or by the pharmacological administration of ACh into the blood stream. Vasodilatation can also occur without the endothelium being involved through the production of NO by other actors such as smooth muscular cells, neurons and macrophages. This dilatation is called *endothelium independent vasodilatation* and can be assessed clinically after the administration of NO-donors like glyceryl trinitrate (GTN) or sodium nitroprusside (SNP). Traditionally, endothelium dependent vasodilatation is considered to be a predictor of cardiovascular events[51]; however, recently it has been demonstrated that also endothelium independent vasodilatation is associated with cardiovascular risk factors[52]. Thus, when investigating vasodilatation it is important to measure both endothelium-dependent as well as -independent vasodilatation in order to correctly distinguish between endothelial function and vasodilatation derived through other mechanisms. Endothelial function can be assessed both in the larger transport vessels, and in the microcirculation[17 53]. It is well established that endothelial function in both conduit arteries and in the microcirculation, are independently associated with cardiovascular risk factors[17 25 54]. However, they represent very different aspects of vascular function; where FMD and FMD/GTN reflect endothelial function in larger transport vessels[55], while skin microvascular reactivity merely reflects capillary circulation important for tissue oxygenation and nutrition. There are different regulatory mechanisms involved in different vascular beds, and endothelial function in large and small arteries can therefore not be considered to reflect the same thing. It has been suggested that microvascular dysfunction precedes endothelial dysfunction in larger arteries[26].

During normal pregnancy, a general vasodilatation is necessary in order to hold the rapidly increasing plasma volume and maintain a stable blood pressure. The mechanism behind this is unclear; however, decreased endothelial responsiveness to vasoconstrictors as well as a hormone - mediated increased availability of nitric oxide (NO) is probably partly accountable[56 57]. A vascular inability to dilate and endothelial dysfunction during pregnancy is associated with hypertensive disorders during pregnancy as shown in Table 2 [48 58 59].

Endothelial function in the large artery bed

Flow mediated dilatation of the brachial artery (FMD) is widely used to assess endothelium dependent conduit vessel dilatation mediated by shear stress stimulus during reactive hyperaemia[60]. Several studies report that FMD and brachial diameter is increased in the

second half of normal gestation, and decreased in hypertensive pregnancies [27 28], Table 2. Only one small previous study investigated endothelium independent vasodilatation during pregnancy and found no differences compared to non-pregnant individuals[61].

Endothelial function in the skin microcirculation

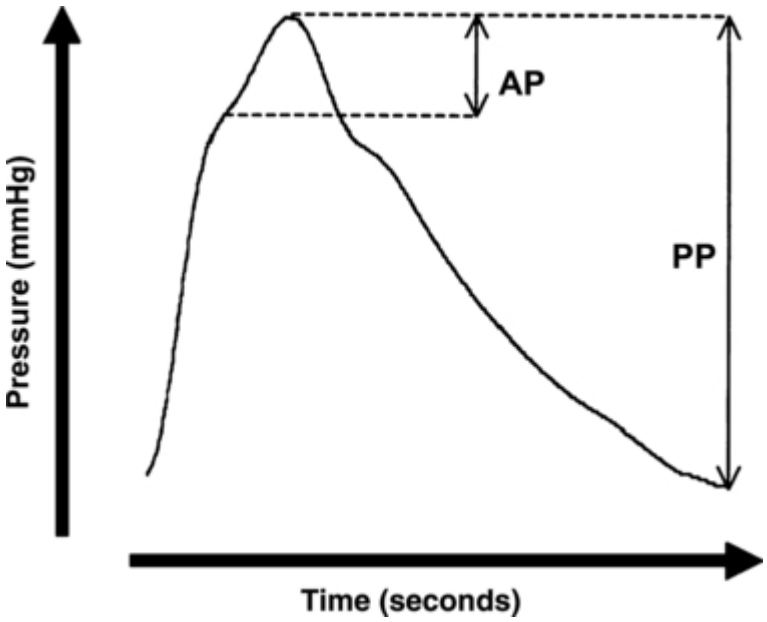
The microcirculation consists of arterioles, capillaries and venules and is important for tissue oxygenation and nutrition. In hypertensive patients, increased resistance due to microvascular dysfunction is an important component of high blood pressure[26]. Iontophoreses and Laser Doppler Fluxmetri (LDF) can assess endothelium dependent and independent microvascular reactivity[53], and is associated with cardiovascular risk[25]. There is limited knowledge concerning microvascular reactivity during normal and complicated pregnancy, Table 2. Previous studies have reported increased microvascular reactivity in normal pregnancy[41]. A very recent study reported microvascular dysfunction in women 12 months after severe PE[62].

2.3.4 Arterial stiffness and structure

Arterial stiffness and augmentation index

Pulse wave velocity (PWV) is the propagation speed of the pulse wave that moves along the aorta and the other large elastic arteries. When these vessels become stiffer, the pulse wave moves faster through the system and PWV increases. PWV is also determined by blood pressure and is closely related to cardiovascular risk[63], Table 1. Also, the reflected pulse wave can be quantified through the augmentation index (AIx), and is an independent predictor of cardiovascular events since it contains information about the reflective site (arterial resistance and endothelial function) [21], Figure 3. There is a consensus that AIx decreases during normal pregnancy, reaching a nadir in the second trimester, whereas studies on PWV show inconsistent results with either no change or a decrease during normal pregnancy[33], Table 2.

Figure 3. Augmentation index



AP = augmented pressure, i.e the increase in systolic blood pressure due to the reflected pulse wave. PP = pulse pressure. Augmentation index (AIx) is calculated as AP/PP x100 (%)

Arterial structure

Structural elastic arterial changes during pregnancy can be measured by ultrasonography of the carotid artery. Common carotid artery intima-media thickness (CCA-IMT) is a marker of subclinical vascular disease and is closely related to CVD risk[64]. A few previous studies investigated IMT and CCA diameter during pregnancy and found indices of increased CCA diameter and unaltered or slightly increased IMT during normal pregnancy, and increased CCA stiffening toward term has also been observed in healthy pregnancies [39 65]. In women with PE, a significantly thicker mean common carotid artery intima, thinner media, and higher I/M ratio than in normal pregnancy have been reported [66], Table 2.

2.3.5 Uterine artery Doppler measurements

Blood flow of the uterine artery increases from 50-60 mL/minute in the late first trimester, to 500-750 mL/minute at term[67]. During the first half of pregnancy, uterine artery remodels and under normal conditions, at 24 weeks gestation, uterine artery Doppler measurement should demonstrate a continuous blood flow during diastole. If a high resistance to blood flow is present, the pulsatility index (PI), in which the difference between peak systolic velocity and end diastolic velocity divided by time-averaged velocity, becomes high. A high PI in the uterine artery in the second trimester means a higher risk of PE and /or FGR [68]. Uterine artery

resistance has been presumed to reflect placental conditions and is associated with spiral remodelling[69]. However, ophthalmic artery Doppler assessment is as effective as uterine artery assessment in predicting FGR and/or PE [70]. Furthermore, uterine artery pulsatility index is highly dependent on heart rate[71], which raises the question of what uterine artery PI really reflects: placental circulation or merely general maternal vascular adaptation to pregnancy[2 69]?

2.3.6 Cardiac structure and function

As a result of the rapidly increasing volume, and a fall in total peripheral resistance, normal pregnancy is a state of increased preload and a reduced afterload[72]. Preload is defined by the amount of blood that returns to the right atrium, and afterload is the amount of resistance the left ventricle has to overcome in order to eject blood. Cardiac output increases up to 40%, which is mainly achieved by increased heart rate and by structural changes. Left atrial size, left ventricular relative wall thickness and left ventricular mass increases throughout pregnancy, and a recent meta-analysis stated that normal pregnancy is associated with a predominantly concentric LV remodelling [73].

Findings on systolic and diastolic function in normal pregnancy are somewhat divergent[74 75], and there is evidence of chamber diastolic dysfunction near term in approximately one fifth of all normal pregnancies [76].

In women subsequently developing PE, abnormal LV remodelling and impaired myocardial relaxation can be observed. In cases of severe PE, both diastolic and systolic chamber dysfunction can be present[77-79].

Myocardial mechano-energetic efficiency

A way of examining whether the rapid LV remodelling remains beneficial throughout the course of pregnancy is to examine myocardial mechano-energetic efficiency (MEE). MEE can be measured as the ratio between systolic work and energy consumption, and expresses the amount of oxygen consumed by each contraction per second. Thus, a low MEE indicates inefficient LV performance with high energy waste, which is associated with increased cardiovascular risk in patients with hypertensive LV hypertrophy[80]. Despite extensive research on cardiac function during pregnancy, MEE during normal and complicated pregnancy remains to be explored.

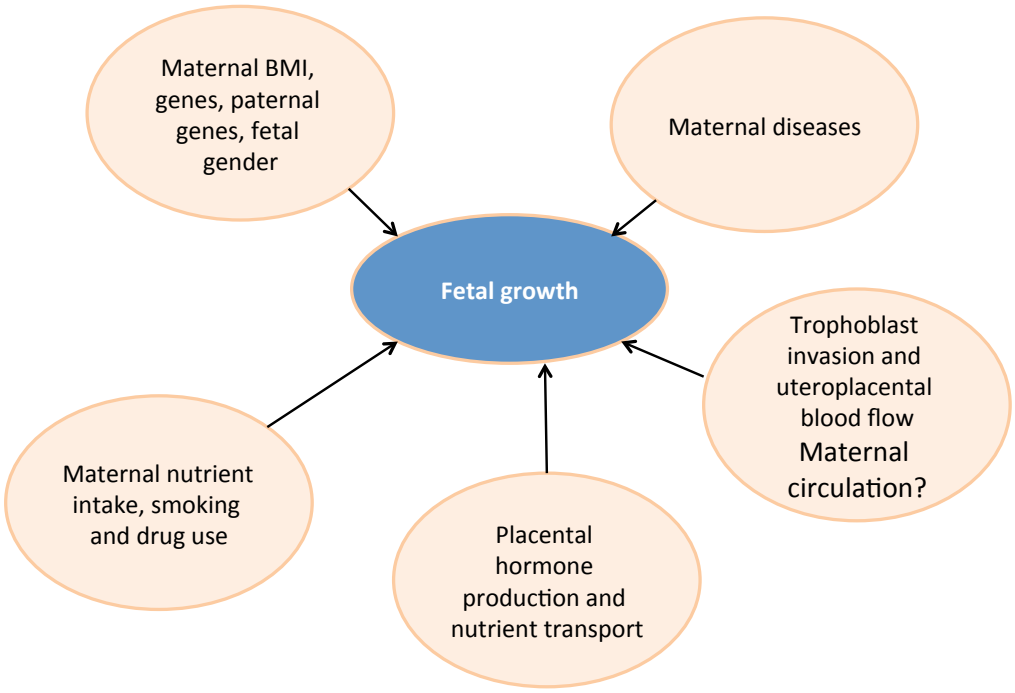
Ventricular-arterial coupling

Elastance refers to the change in blood pressure for a given change in blood volume. A high elastance is beneficial as it means a greater sensitivity to volume change. The interaction between LV function and the arterial system can be assessed as the ratio between arterial elastance and LV end-systolic elastance, i.e. ventricular-arterial coupling (VAC)[81]. The greatest efficiency is achieved when elastances are matched, and even in hypertensive disorders changes in LV elastance and arterial elastance often occur parallel with a maintained VAC [82]. There is insufficient knowledge regarding the importance and predictive value of VAC during pregnancy. A few studies investigated VAC in normal and complicated pregnancy and found either no change or decreased VAC[83 84].

2.4 FETAL GROWTH

Fetal growth is determined by a variety of genetic, hormonal and environmental factors [85], as shown in Figure 4.

Figure 4 Determinants of fetal growth



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When some of these factors prevent the fetus from reaching its growth potential, fetal growth restriction, FGR (also called intrauterine growth restriction IUGR), is present. FGR affects 3-7% of all pregnancies (depending on the definition), and is a well-known complication of

preeclampsia (PE), but does also appear in normotensive pregnancies[86]. Sometimes FGR develops as a result of a deficient placentation, where a shallow trophoblast invasion and altered remodelling of the maternal spiral arteries will subsequently cause diminished perfusion and hypoxia in the placenta [87]. However, in the majority of pregnancies complicated by FGR, placental histology is normal[10].

Fetal growth restriction defined

FGR can be divided into early-onset (diagnosed ≤ 32 weeks gestation, or late-onset (diagnosed >32 weeks) FGR, and is associated with hypertensive disorders during pregnancy in 70% and 10% of all cases, respectively[88]. There is no consensus on the definition of FGR, but the most used definition is an estimated fetal weight (EFW) < 10th or < the 5th centile[89]. The terms FGR and small for gestational age (SGA) are not interchangeably, and SGA only refer to the fact that the estimated fetal weight or the actual birth weight is smaller than the reference population, and might or might not be associated with growth impairment. In Sweden, FGR during gestation is classified as EFW < 22% than estimated mean for gestational age. A birth weight or EFW < 22% is equal to an EFW or birth weight below the 2.3th percentile and to -2 SD [90]. Customized birth weight centiles have been developed so as to adjust for factors known to have a major impact on fetal growth; sex of the neonate, maternal height, weight, ethnicity and gestational age and parity[91 92].

Identification of FGR and neonates that are small for gestational age (SGA) is crucial for appropriate antenatal and postnatal care. SGA is associated with preterm labour, placental abruption, neonatal morbidity and mortality, and long-term adverse effects in adulthood[93 94]. Delivery of an SGA infant is also associated with maternal cardiovascular morbidity or mortality in later life[95-97]. Therefore, prediction is important as it probably improves the outcome[98]. The combination of second trimester uterine artery Doppler and serum markers, such as first trimester levels of PAPP-A, can predict FGR to some extent, but does not fulfil the criteria for an effective screening tool[99].

2.5 CARDIOVASCULAR FUNCTION AND FETAL GROWTH

Growing evidence supports the idea of a relation between maternal cardiovascular function, and fetal growth. There is a fairly strong evidence that second and third trimester maternal blood pressure is inversely correlated with fetal growth[100 101]. The same relationship is less clear, but probably present, already in the first trimester[102 103].

Lower plasma volumes have been reported in pregnancies complicated by fetal growth restriction[48]. Impaired FMD has been reported in the third trimester, and 6-24 months after, pregnancies complicated by fetal growth restriction [104-106]. Whether a dysfunctional endothelium is present before the pregnancy, or occurs as a response *to* pregnancy, is uncertain. Fetal growth was associated with mid-gestation endothelium dependent microvascular reactivity in one previous study[107].

An attenuated AIx in early pregnancy has been reported to be associated with fetal growth restriction in women with chronic hypertension[108], and another study showed that first trimester maternal arterial elastance was associated with birth weight[109].

Pre-pregnancy to second trimester changes in CO, PVR and cardiac index correlated to birth weight Z-score in a previous study[110], and another study reported that women with FGR had altered left ventricular geometry, impaired myocardial relaxation, and higher prevalence of diastolic chamber dysfunction, as compared to women with normal gestation[111]. Both CO and heart rate correlated to birth weight in several previous studies[71 110 112].

2.6 PAPP-A

Pregnancy-associated plasma protein A (PAPP-A) was first discovered in the serum of pregnant women in 1974 [113], but it was not until the early 1990s when Brambati et al described lower levels of PAPP-A in the serum of pregnant women with aneuploid fetuses, that the research accelerated[114]. Knowledge of the biological effect of PAPP- A was lacking until 1999, when it was discovered that PAPP-A is a proteinase that enhances the tissue availability of insulin-like growth factor 1 (IGF-1) by cleavage of IGF-binding proteins[115]. PAPP-A is also detectable in low levels in non-pregnant individuals [116], and is synthesized by several cell types, including vascular smooth muscle cells and endothelial cells. PAPP-A is increased in patients with acute injury or unstable atherosclerotic disease[117-119], and although PAPP-A seems to be important for normal tissue repair, inhibition of PAPP-A has proved to be a potential treatment for a variety of diseases such as atherosclerosis, cancer, fragility and nephropathy[120].

PAPP-A during pregnancy

PAPP-A is produced by the placenta during pregnancy and occurs in increasing amounts in maternal serum. The exact mechanisms behind the effects of PAPP-A during pregnancy remain unclear; several different functions have been suggested including matrix mineralization, microvascular endothelial cell proliferation and angiogenesis[119 121 122]. Low first trimester PAPP-A levels are associated with both chromosomal anomalies and adverse

pregnancy outcomes, such as small size for gestational age, preterm labour, preeclampsia, and stillbirth [123 124]. PAPP-A is therefore generally considered to reflect placental invasion and circulation, a theory that is further strengthened by the fact that low levels of first trimester PAPP-A in maternal circulation is associated with reduced number of capillaries and smaller capillary diameters in the placenta[125 126]. Interestingly, the majority of the amount of PAPP-A during pregnancy is distributed in the maternal circulation; it has been demonstrated that the concentration of PAPP-A seen in the umbilical cord at term is 1.000-fold less than in the maternal circulation[127]. Nevertheless, PAPP-A seems to be crucial for a normal fetal growth[128].

PAPP-A is increased in the third trimester in preeclamptic pregnancies, as compared to normal pregnancies[129 130], and elevated levels of PAPP-A and E-selectin were reported in women 10 years after preeclamptic pregnancies [118]. Despite this, one previous study could not find any association between high first trimester PAPP-A levels and adverse pregnancy outcome[131]. More research is needed in order to understand the complex role of both the friend and foe that PAPP-A represents.

3 AIMS OF THE THESIS

- To investigate alterations in vascular structure and function, ranging from the aorta to the microcirculation, longitudinally during normal pregnancy.

- To assess cardiac function, myocardial mechano-energetic efficiency and ventricular-arterial coupling longitudinally during normal pregnancy.

- To explore whether there is an association between cardiac structure and function during pregnancy, and fetal growth.

- To examine whether there is an association between maternal endothelial function in the first trimester, and fetal growth.

- To investigate whether there is an association between levels of circulating maternal pregnancy-associated plasma protein A (PAPP-A), supposedly reflecting placental function, and first trimester maternal vascular function.

4 METHODS

4.1 STUDY DESIGNS

Table 3. Overview of study designs and analyses methods

	Study Design	Number of Participants	Analyses Methods
Study I	Prospective longitudinal observational study	52	Linear mixed model
Study II	Prospective longitudinal observational study	52	Linear mixed model, Pearson correlation coefficient
Study III	Cross-sectional observational study	56	Linear regression, ANOVA, Kruskal-Wallis test
Study IV	Cross-sectional observational study	53	Linear regression, Mann-Whitney U test, Student`s *t*-test

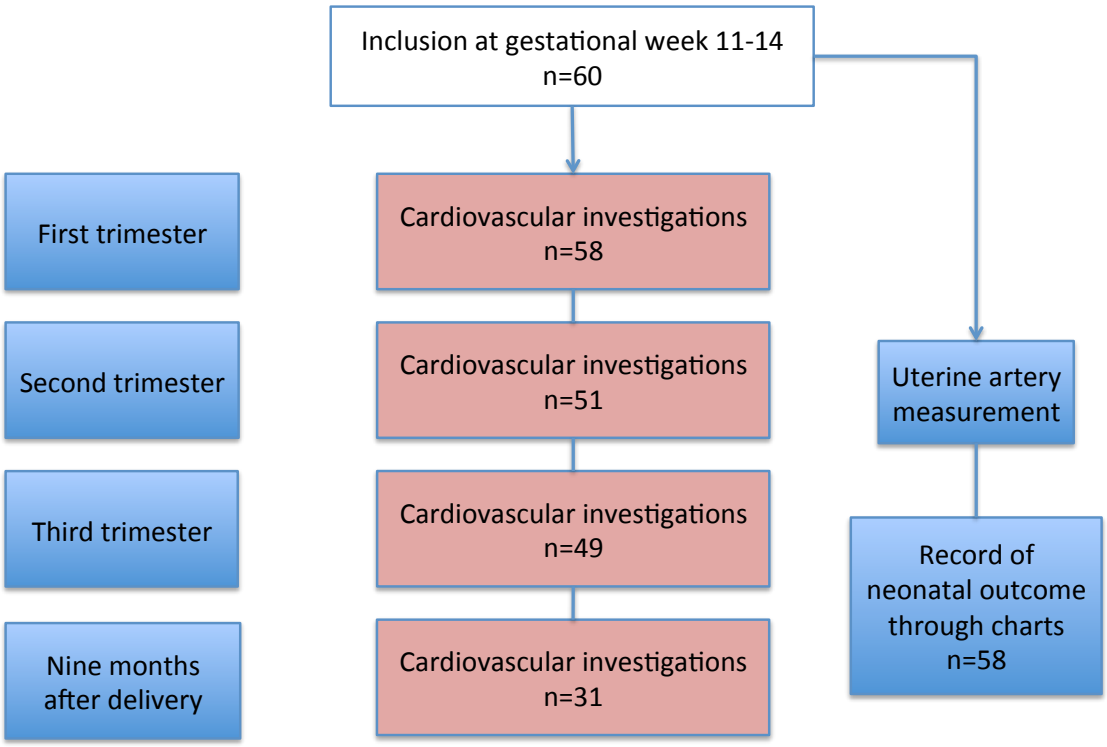
4.2 STUDY POPULATION

All four studies (**Study I-IV**) are based on one single cohort of women examined longitudinally during and after their first pregnancy. Previously healthy non-smoking women were enrolled in our institution during the routinely performed first trimester combined ultrasound and biochemical screening (CUB-test) for Down´s syndrome. Since one of our primary aims with this project was to investigate whether maternal vascular function was associated with fetal growth, we aimed to include both women with normal fetal size and smaller fetal size than expected in the first trimester. Further inclusion criteria were singleton pregnancy, regular menstrual cycles, reliable data for last menstrual period, and no observed malformations of the fetus at inclusion.

During 2008 to 2012, 60 women were recruited, two were excluded due to malformations of the fetus and a few further participants chose to decline some of the examinations due to discomfort. Several women chose not to participate in the last examination post partum.

Given this, 58 women participated in the first examination, 51 in the second, 49 in the third and 31 in the fourth examination.

Flow-chart of study protocol



Cardiovascular investigations included brachial blood pressure, heart rate, central blood pressure, carotid-femoral and carotid-radial pulse wave velocity, augmentation index, flow-mediated vasodilatation in the brachial artery, Laser Doppler fluxmetri in the skin microcirculation, ultrasound of the carotid arteries, and echocardiography.

Blood samples were also collected for current and later analyses. Non pregnant values were obtained nine moths post partum. Fetal growth was sonographically estimated in gestational week 11-14, 24 and 32. Uterine artery Doppler indices were measured in the second trimester (gestational week 22-24). Data on maternal background characteristics and pregnancy outcome (complications, birth weight etc.) were obtained through questionnaires and from medical charts.

4.3 CARDIOVASCULAR EXAMINATIONS

Blood pressure and heart rate

Study I-IV

Brachial BP and heart rate was calculated as the mean of three readings obtained 1 minute apart with the participant in the supine position, using an oscillometric device (Omron 705IT, Omron Healthcare, Kyoto, Japan) on the right arm with an appropriately sized cuff.

Arterial stiffness, central blood pressure and augmentation index

Study I and **II**

Pulse wave analysis was performed using a SphygmoCor device (AtCor Pty, West Ryde, NSW, Australia) according to current recommendations[132]. Radial artery waveforms were obtained by applanation tonometry and calibrated using brachial systolic and diastolic BP; the central aortic waveform was calculated by device software using the generalized transfer function, and central blood pressure values were derived. The augmentation index was calculated through the software and corrected to a heart rate of 75 bpm. Recordings were repeated at the level of the common carotid artery (CCA) and the femoral artery, and pulse wave velocity (PWV) was calculated from the direct (carotid-to-radial and carotid-to-femoral) path length.

Arterial structure

Study I

Ultrasonographic examinations of the left and right CCA were performed using a Vivid 7 Dimension ultrasound device with a 5 or 7 MHz linear transducer (GE Medical System, Horten, Norway) and the subjects in the supine position, as described elsewhere[133]. An ECG signal synchronized the image analysis to the end of diastole. The vessels were scanned at the level of the bifurcation, and three B-mode images from the longitudinal view, as well as a short sequence of real-time images, were recorded on videotape. CCA intima-media thickness was measured along a 10 mm-long segment just proximal to the carotid bulb. The intima-media thickness was assessed as the mean value of the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of far and near walls in the right and left carotid arteries.

Endothelial function

Study I, III and **IV**

FMD

Post-ischemic hyperemia induced flow mediated dilatation (FMD) of the forearm vascular bed, which is considered to reflect endothelium dependent vasodilatation, was assessed in the non-dominant arm, according to current recommendations[134]. Vasodilatation was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 5 minutes, followed by release. Brachial artery blood flow and diameter was measured proximal to the cuff by a Vivid 7 Dimension ultrasound device with a 9-MHz linear transducer. The mean values of three measurements of arterial diameter performed at end diastole were calculated at rest and at 30, 60, and 90 seconds after cuff release. After a washout period of at least 10 minutes to regain stable resting conditions, 0.4 mg glyceryl trinitrate given as sublingual spray was used to assess endothelium independent vasodilatation. Relative changes in brachial artery diameter after administration of GTN were calculated from rest to 4 minutes following drug administration. In order to properly assess endothelial functiion we calculated the endothelial function index by the ratio of the maximum relative increase in FMD/GTN, as previously proposed[135] . In our laboratory, the coefficient of variation for FMD is 15%. Local shear rate was calculated as blood velocity/brachial artery internal diameter.

Skin microcirculation

Endothelium dependent and independent forearm skin microvascular dilatation was assessed using laser Doppler perfusion imaging and iontophoretic administration of acetylcholine (ACh) and sodium nitroprusside (SNP)[136], as shown in Figure 5. Electrode chambers (LI611 Drug Delivery Electrode Imaging; Perimed, Järfälla, Sweden) were attached to the volar side of the forearm and filled with 0.5 ml of either ACh (2%) or SNP (2%). A battery-powered iontophoresis controller (Perilont 382b; Perimed) provided a direct current (0.1 mA for 60 s) for drug iontophoresis. Skin microvascular flux (expressed in arbitrary units, AU) before, during, and after iontophoresis was recorded (PeriScan PIM II; Perimed) continuously up to 10 and 14 minutes after iontophoresis of Ach and SNP, respectively, and peak microvascular flux was determined. To assess endothelial dependant vasodilatation and also account for non-specific vascular reactivity in the skin microcirculation, the ratio ACh/SNP was calculated. In our laboratory, coefficients of variation for peak microvascular flux after iontophoreses of ACh and SNP are 11% and 20%, respectively.

Figure 5. Laser Doppler Fluxmetry with Iontophoresis for investigation of skin microcirculation. Figure shown with permission from Perimed AB, Järfälla, Sweden.

Uterine artery pulsatility index

Study III

Uterine artery pulsatility index (PI) was measured abdominally in gestational week 22-24. The ultrasound probe was held in the sagittal axis and moved laterally. B-mode ultrasound examination with colour Doppler was used to visualize the vessels lateral to the uterus and identify the uterine artery at the apparent crossover with the external iliac artery. A pulsed-wave Doppler gate was then placed over the uterine artery with an angle of insonation of less than 60 °. The PI, calculated as the difference between peak systolic velocity and end diastolic velocity divided by time-averaged velocity, was measured in both vessels, and the mean value was calculated.

Cardiac structure and function

Study II

Transthoracic echocardiography was performed in the left antecubital position with a Vivid 7 system (General Electric, Horten, Norway) equipped with a phased array 3.5 MHz transducer (Doppler frequency 5.0–3.5 MHz). Measurements were performed on a minimum of three cardiac beats, from which mean values were calculated. Measurements of LV dimension in end-systole (LVDes) and end-diastole (LVDed), and interventricular septum (IVS) and

posterior wall thickness (PWT) in diastole were made from M-mode recordings[137]. LV mass was calculated as ($0.8 \times [1.04 \times ([LVDed + IVS + PWT]^3 - LVDed^3) + 0.6$ g]), and divided by body height$^{2.7}$ to obtain LV mass index[138]. LV volumes were calculated from area tracings in four- and two-chamber views using the modified Simpson's rule, and the ejection fraction was calculated accordingly[139]. Longitudinal LV systolic function was also evaluated by the mitral annular plane systolic excursion measured by M-mode at four sites (septal, lateral, inferior, and anterior part of the mitral annulus) from an apical view[140]. Left and right atrial areas were measured in four-chamber view. The right ventricular (RV) outflow end-diastolic dimension was measured in parasternal long axis view. The RV inflow end-diastolic basal diameter was measured from the apical four-chamber view[141].

Conventional pulsed wave Doppler echocardiography was used for recordings of mitral inflow to evaluate LV diastolic function[140]. The peaks of early (E) and late (A) mitral flow velocities were measured, and the E/A ratio was calculated. The deceleration time from mitral E wave and isovolumic relaxation time were measured.

Doppler tissue imaging echocardiography was performed with pulsed wave Doppler in the apical four-chamber view. We measured mitral annular velocities in the septal, lateral, inferior, and anterior insertion sites of mitral leaflets. Systolic mitral annular velocity (s) and early (e'), and late (a') diastolic velocities were measured off-line. The E/e' mean is expressed as the mean of e' sept and e' lat; and e'/a' mean ratio was calculated as a mean of the velocities in the septal, lateral, inferior, and anterior measurements. The E/e' was used as an estimate of LV filling pressure. Doppler tissue imaging was also used in the evaluation of RV systolic function by measuring the maximal systolic myocardial velocity of the free wall of RV with the sample volume placed at the tricuspid annulus.

MEE was calculated as the ratio between external work and total energy consumption[80]. To estimate external myocardial work, stroke work (SW) was calculated as systolic BP x stroke volume x 0.014. The conversion factor 0.014 transforms mmHg x cm^3 into gram-meters[142]. Total energy consumption or myocardial oxygen consumption (MVO2) measured non-invasively can be approximated by using the formula MVO2 ≈ heart rate x systolic BP[143]. Thus, the ratio between external work and total energy consumption can be simplified as MEE = stroke volume/heart rate, where heart rate is divided by 60. MEE is strongly related to LV mass[80], and thus, to obtain an estimate of MEE per g, MEE was divided by LV mass.

Indices of ventricular-arterial coupling were calculated as effective arterial elastance (LV end-systolic pressure/stroke volume), LV end-systolic elastance (LV end-systolic pressure/LV end-systolic volume), ventricular-arterial coupling ratio (effective arterial elastance/LV end-systolic elastance), total vascular compliance (stroke volume/[systolic BP – diastolic BP]), and total peripheral resistance (mean arterial pressure/cardiac output)[144].

4.3 FETAL SIZE AND NEONATAL OUTCOME

Study II and **III**

Biparietal diameter (BPD) and crown rump length (CRL) was measured at inclusion in gestational week 11-14. Δ GA was calculated as gestational age discrepancy, i.e difference in observed and expected size of fetus, expressed in days. Gestational age was assessed from femur length and biparietal diameter in weeks 16-18 according to the Hadlock formula[18]. All sonographers were certified by, and in accordance with, the guidelines of the Fetal Medicine Foundation in London.

Information on gestational week at delivery, birth weight, gender, and any pregnancy complications was obtained from medical charts on the delivery wards. Birth weight centile was calculated using a customized centile calculator taking into account the gestational age of delivery, maternal booking weight, height, parity, ethnic group and sex of the neonate[92].

4.4 BLOOD SAMPLES

Study I - IV

Blood samples were collected at the time of the vascular examinations at gestational week 11-14 and analysed for fasting glucose, cholesterol, and creatinine by standard procedures. Venous blood for PAPP-A measurements was collected in serum separator tubes, allowed to clot at room temperature for at least 30 min, centrifuged at 1700-2000 G for 10 min. Serum was separated and stored at 2-8°C until analysis on the same or the following day by immunoassay (AutoDelfia analyser, PerkinElmer, Waltham, MA, USA). Since PAPP-A levels are highly related to gestational age, PAPP-A multiples of median (MoM) were calculated as a measure of how individual results deviates from the median according to current gestational age.

4.5 STATISTICS

The sample size was originally calculated from the initial study design to detect a 15% difference in FMD (with 2α 0.05 and β 0.80), between two groups; one group (cases) with

fetuses that had a gestational age discrepancy of 7 or more days less as would have been expected according to menstrual data, and another group (controls) with no or up to 7 days gestational age discrepancy. We estimated that 25 women were needed in each group.

In all studies mean or median values were analysed depending on whether the data was normally distributed (as assessed by Shapiro-Wilks test). In **Study I** and **II**, data were analysed over time using a linear mixed model with appropriate post-hoc tests for pairwise comparisons. Correlations were assessed using Pearson´s correlation coefficient. Multivariate analyses with adjustment for covariates were performed using linear regression (**Study III** and **IV**). In **Study III**, participants were divided into tertiles according to FMD, GTN and ACh values and analysed with ANOVA or Kruskal-Wallis test in order to detect potential confounding factors. For the same reason, in **Study IV**, participants were divided into two groups according to PAPP-A levels and analysed with Students *t*-test or Mann-Whitney U-test respectively. The null hypothesis was rejected when $p < 0.05$.

4.6 ETHICAL CONSIDERATIONS

Ethical permits have been obtained for this project from the Regional Ethics Committee in Stockholm, Sweden. All subjects provided written informed consent to participate in the study. We have ensured that the participation in this study has been voluntarily. The confidentiality of the participants has been respected. Some of the investigations could cause discomfort, and all participants were informed regarding this. We did put neither the mother nor the fetus in any danger and used only safe methods previously evaluated in pregnant women. If needed, we made sure that proper medical care was provided.

The research was independent, of high quality and performed in well-established research-labs by trained staff. Since the topic is of great importance and to a great extent still unexplored, the time and effort put in by the participants was, by our account, reasonable.

5 RESULTS

5.1 SUBJECT CHARACTERISTICS

Subject characteristics for the entire cohort at inclusion are shown in Table 4. Neonatal outcome is shown in Table 5. 60 women were enrolled; two participants were excluded due to lethal malformation of the fetus and several participants choose not to participate in all cardiovascular examinations.

Table 4. Subject characteristics of the entire cohort at inclusion (n=58)

Characteristic	Mean±SD or %	Range
Age (years)	31.8±3.6	24–43
Height (cm)	167.3±5.5	154–182
Weight (kg)	65.2+11.7	50–106
Body mass index (kg/m2)	23.2±3.6	18–35
Serum creatinine (μmol/L)	51.2±7.3	31–71
Serum total cholesterol (mmol/L)	4.4±0.8	3.0–7.0
Serum hs-CRP (mg/L)	4.1±3.3	5–19.6
Plasma glucose (mmol/L)	4.5±0.2	3.9–5.1
Systolic blood pressure (mmHg)	107 ± 7	92–129
Diastolic blood pressure (mmHg)	61 ± 6	48–77
Δ GA discrepancy at inclusion, days	-4.5±3.8	-13–+3
Weight gain during pregnancy, kg	12.7±3.8	6–21
Ethnicity = Caucasian	56/58 = 97%	

GA, gestational age

Table 5. Neonatal outcome for the entire cohort (n=58)

Characteristic	Mean±SD or %	Range
Birth weight (g)	3448±368	2630–4120
Birth-weight centile	42±26	2–98
Gestational age at delivery (days)	280±11	237–296
Gender = girl	33/58 = 57%	
SGA ≤ 10th percentile	8/58 = 14%	
SGA ≤ 5th percentile	2/58 = 3.4%	
Mild preeclampsia	2/58 = 3.4%	
Severe preeclampsia	0/58 = 0%	
Mode of delivery		
Caesarean section	9/58 = 16%	
Vacuum-assisted vaginal delivery	4/58 = 7%	
Vaginal delivery	45/58 = 77%	

SGA, small for gestational age

Information on pregnancy outcome was extracted from medical charts and is therefore complete (n=58). In **Study I** and **II**, we wanted to investigate cardiovascular function in normal pregnancy and thus, the four participants who developed pregnancy complications, i.e. PE or SGA ≤ 5th percentile, were excluded.

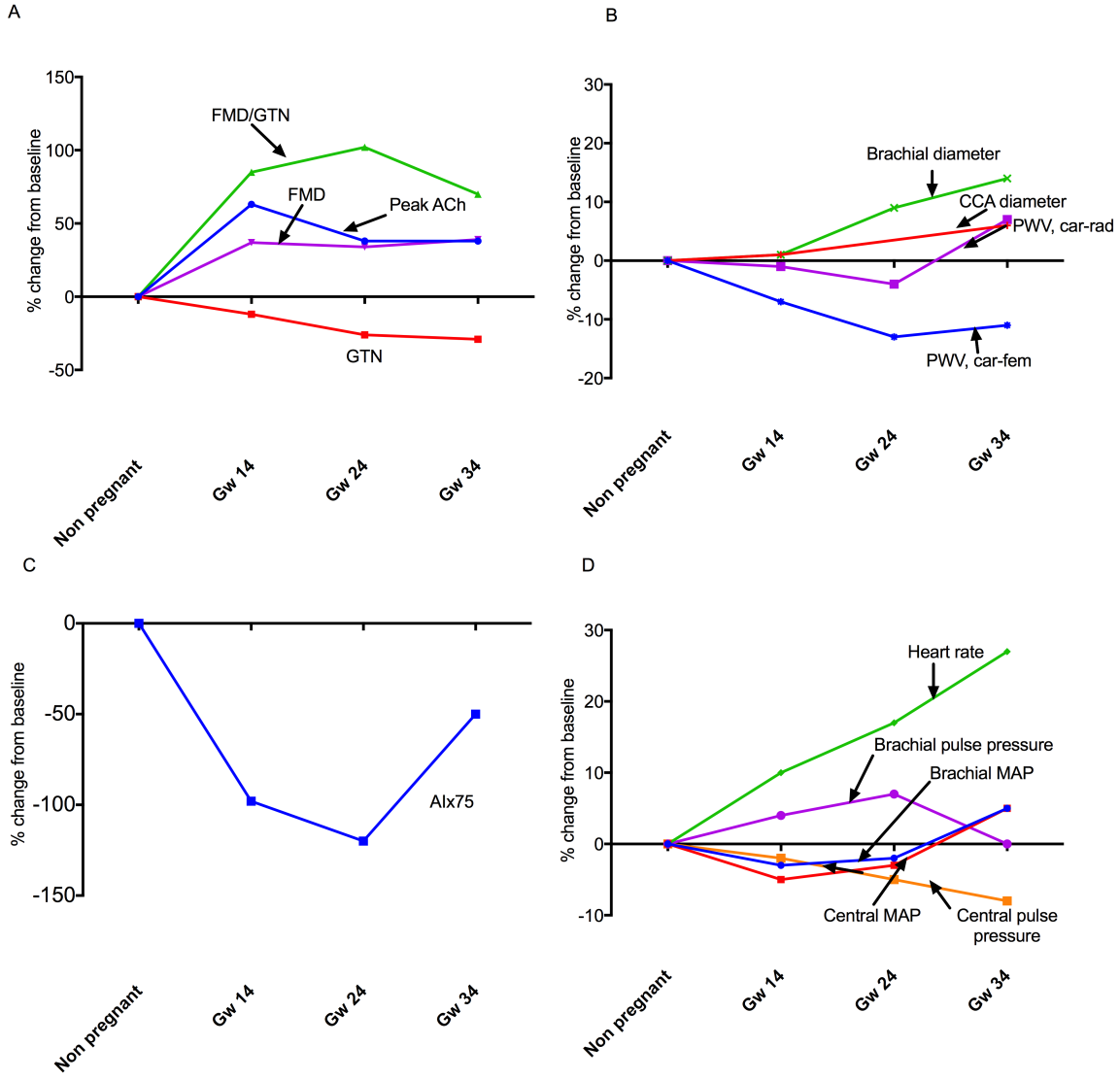
5.2 STUDY 1: VASCULAR STRUCTURE AND FUNCTION IN NORMAL PREGNANCY

5.2.1 Summary of main findings

Brachial blood pressure (BP) decreased in early pregnancy followed by an increase in the third trimester. Central BP decreased more than brachial BP in the first trimester. Heart rate increased linearly with each trimester. Carotid-femoral pulse wave velocity (PWV) and

augmentation index decreased during pregnancy with a nadir in the second trimester. Examinations of endothelial function showed an early and persistent improvement in endothelial function of the brachial artery. Also, endothelium dependent microvascular reactivity increased during pregnancy. Conduit artery diameters (brachial and common carotid artery) increased progressively during pregnancy. Microvascular reactivity increased. Changes in vascular structure and function during pregnancy, presented as percentage change from a non-pregnant state at 9 months postpartum is presented in Figure 6

Figure 6 Changes in vascular structure and function during pregnancy, presented as percentage change from a non-pregnant state at 9 months postpartum



A endothelial function; B arterial structure; C arterial stiffness; D blood pressure and heart rate. FMD, flow-mediated vasodilatation; GTN, vasodilatation after glyceryl trinitrate; ACh, acetylcholine; CCA, common carotid artery; PWV, pulse wave velocity: AIx, augmentation index; MAP, mean arterial pressure

5.3 STUDY II: CARDIAC STRUCTURE AND FUNCTION IN NORMAL PREGNANCY

5.3.1 Summary of main findings

Left atrial diameter increased during normal pregnancy. Left ventricular (LV) wall thickness increased during, while LV and right ventricular (RV) dimensions remained essentially unchanged. Thus, relative wall thickness, LV mass, and LV mass indexed by height$^{2.7}$ increased during pregnancy. The changes in LV wall thickness were largely confined to the posterior wall.

Left ventricular stroke volume and cardiac output increased. Late mitral flow velocity (A peak velocity) increased and the E/A ratio decreased with advancing gestation, while deceleration time and isovolumic relaxation time remained unchanged. Diastolic myocardial fibre tissue Doppler velocity (e'/a´ ratio) decreased during pregnancy and reached a nadir in the third trimester.

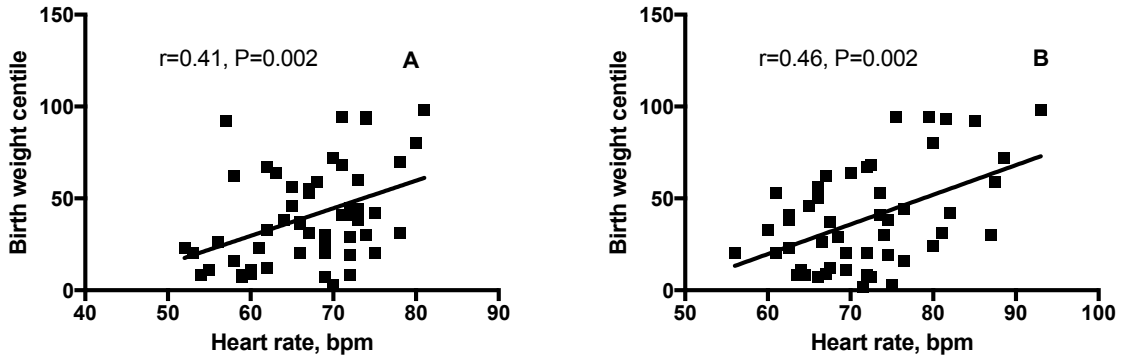
Both stroke work and energy consumption increased throughout the course of pregnancy and thus, MEE remained unchanged.

Total peripheral resistance and effective arterial elastance decreased in parallel during the course of pregnancy. Thus, ventricular–arterial coupling ratio remained unchanged.

5.3.2 Cardiac function and offspring birth weight

Heart rate was associated with birth weight centile in the first (r = 0.41, P = 0.002 and second (r = 0.46, *P* = 0.002), trimesters, Figure 7. Other indices of cardiac function were not related to birth weight centile or birth weight.

Figure 7, Relationship between birth weight centile and (A) first trimester heart rate, and (B) second trimester heart rate



5.4 STUDY III: FETAL GROWTH AND MATERNAL VASCULAR FUNCTION

5.4.1 Summary of main findings

Maternal first trimester vascular reactivity in the brachial artery following ischemia (FMD), and sublingual glyceryl trinitrate (GTN), related positively to birth weight centile. Also maternal endothelium dependent and independent microvascular reactivity were associated with birth weight centile in a similar way.

5.4.2 Related findings

The study subjects were divided into tertiles according to the exposure (FMD) in order to detect potential confounding factors. There were no differences in age, BMI or blood pressure between the groups. Uterine artery pulsatility index was higher in women with the lowest FMD, as compared to the other two groups with higher FMD. However, when performing a univariate analyses using linear regression, first trimester FMD did not predict uterine artery pulsatility index ($r = 0.20$, $P = 0.18$),

A sub-group analysis of women with low (≤ 5%) FMD (n=8) showed that first trimester fetal size, as well as birth weight centile, was decreased among women with low FMD as compared to women with normal (> 5%) FMD.

5.5 STUDY IV: PAPP-A AND MATERNAL VASCULAR FUNCTION

5.5.1 Summary of main findings

PAPP-A correlated positively to first trimester maternal skin microvascular endothelial function index (peak ACh/peak SNP), but not to peak ACh nor to peak SNP. PAPP-A was also inversely related to maternal first trimester vascular reactivity in the brachial artery following ischemia (FMD).

5.5.2 Related findings

A comparison of women with low (< median) and high (≥ median) PAPP-A MoM levels revealed that women with high PAPP-A levels had higher hs-CRP levels, and a tendency to higher cholesterol levels than women with low PAPP-A. There were no differences in BMI, fasting p-glucose or blood pressure between the groups.

6 METHODOLOGICAL CONSIDERATIONS

6.1 SYSTEMATIC ERROR

Systematic error occurs when the selection of the study group is incorrect, if the variables are incorrectly classified, or if the results lack adjustment for factors that interact with these variables.

6.1.1 Selection bias

If the study group recruited differs in their exposure-to-outcome distribution from the target population, selection bias occurs. Selection bias can also occur in the process of getting study participants to remain in the study.

All four studies (**Study I-IV**) are based on a cohort of previously healthy, non-smoking women examined longitudinally during and after their first pregnancy. Since one of our primary aims with this project was to investigate if maternal vascular function was associated with fetal growth, we intended to include both women with normal fetal size and smaller fetal size than expected in the first trimester. Thus, at the start of this research project, we included women in two groups; one group (cases) with fetuses that had a gestational age discrepancy of 7 or more days less as would have been expected according to menstrual data, and another group (controls) with no or up to 7 days gestational age discrepancy. However, as we analysed our data, it became apparent that the data on last menstrual period, and thus gestational age discrepancy, was very uncertain and difficult to dichotomize. We recruited 38 controls and 22 participants with small fetuses (gestational age discrepancy of 7 or more days), of which two were excluded due to lethal malformations of the fetus. In the control group, gestational age discrepancy varied from -6 to + 3 days, as compared to -7 to -13 days in the group with small foetuses. We performed analyses according to our initial hypothesis and found no differences in either vascular measurements or neonatal outcome between the groups. Two participants developed mild PE, both in the group with normal first trimester fetal size. Of the eight participants with offspring growth below the 10^{th} percentile, 3 were present in the group with small first trimester fetal size, and 5 in the other. Both offspring with a birth weight below the 5^{th} percentile had normal first trimester fetal size. Nevertheless, an important issue that comes with this approach must be discussed is how our initial inclusion criteria based on cases and controls, could bias the results. Though most cases of postponed due dates are attributable to uncertainty in LMP, or to fetal gender[145], it may also reflect early growth restriction. Small first trimester fetal growth is associated with

aneuploidy, higher diastolic BP, higher maternal age, and to preterm delivery, low birth weight and SGA, but not with PE[102 146]. Hence, this inclusion criterion has left us with higher maternal age, an overrepresentation of girls among the offspring, and a mean birth weight centile that is below the expected. The fact that two participants were excluded due to lethal malformations of the fetus is not representative of the general population. On the other hand, the other background characteristics presented in Tables 4 and 5 are in accordance with a larger population. In **Study I**, fetal size (i.e gestational age discrepancy) in the first trimester was analysed as a continuous variable and found to be different in the group with a supposedly dysfunctional endothelium dependent vasodilatation in the brachial artery (FMD) as compared to women with normal FMD. Although the results should be interpreted with great caution since one of the groups only consisted of 8 women, one cannot ignore the fact that small first trimester fetal size nevertheless may be of importance, and that we might have found differences between the groups with larger sample sizes. Thus, our initial inclusion criteria probably lead us to limitations in external validity, but with a wider range of fetal growth pattern, and a better opportunity to explore some of our aims.

On the other hand, in **Study I and II**, we wanted to investigate normal cardiovascular adaptation to pregnancy. In order to do so we decided to move our focus to pregnancy outcomes, and disregard first trimester fetal size. Thus, we only included women with normal pregnancy outcomes and excluded those who that came to develop any pregnancy complications such as hypertensive disorders and/or fetal growth restriction ($\leq 5^{th}$ percentile). Concerns can be raised as to weather this procedure left us with a group of pregnant women with non-representative vascular function and a limited external validity. However, the fact that the occurrence of hypertensive disorders and/or fetal growth restriction in our study sample was at, or rather below, the expected level, indicates that our study group was still, to some extent, representative of the target population. Furthermore, since we only included women with normal pregnancy outcomes, it should be justified to consider the vascular adaptation to pregnancy normal and beneficial in all included participants.

Loss to follow-up

Many women chose not to participate in the last examination post partum; 58 women participated in the first examination, 51 in the second, 49 in the third and 31 in the forth. However, data on maternal background characteristics and pregnancy outcome (complications, birth weight etc.) were obtained through questionnaires, and from medical charts, and were complete for all participants.

If the participants that do not remain in the study differ in their exposure-to-outcome distribution from those who completes all study visits, selection bias could occur. To investigate whether that was the case in **Study I and II**, we performed subgroup analyses of women who completed all study visits. Women who completed all study visits showed similar baseline characteristics and neonatal outcomes as those who did not complete all visits, and participants who completed all study visits revealed no differences in cardiovascular structure and/or function, as compared to the results from the entire study population.

Misclassification

In **Study I and II,** data representing the non-pregnant state were obtained nine months after delivery, and there is substantial epidemiological and research evidence to suggest that pregnancy has a significant and protracted postpartum effect on the maternal cardiovascular system including blood pressure and PWV, but not on CO or FMD [147]. To obtain the most accurate results possible, the reference values should be obtained before conception[30]. Furthermore, at the last examination, we did not record menstrual phase, known to impact vascular function[148]. It is possible that this has left us with differential misclassification that has affected our results.

6.1.2 Confounding

A confounding factor is a variable that influences both the exposure and the outcome, and affects the association.

In **Study I and II**, variables were measured repeatedly in the same women, and we generally present crude data and did not adjust for the characteristics of the participants. In **Study I,** we choose to adjust pulse wave velocity for blood pressure, and augmentation index for blood pressure and heart rate, according to previous recommendations[33].

In **Study II**, we performed a secondary analysis on the impact of body size on cardiac structure and function during pregnancy since the range of body weight among our participants was large, although representative of a Swedish population. Our results suggest that body mass index can affect most indices of cardiac structure and function. However, the longitudinal changes appeared to be parallel in women with a body mass index below 25 and those with a higher body mass index. Thus, since the primary aim of this study was to investigate longitudinal changes, this suggests that the large range of body weight did not bias our results. Furthermore, correcting cardiac measurements to weight or body suface area

(BSA) during pregnancy is probably not accurate since BSA cannot be considered a good proxy for the change in body size during pregnancy[149].

In **Study III,** outcome (birth weight centile) and exposures (indices of vascular function) were defined. A number of confounding factors could hypothetically affect the associations studied. To deal with this we used birth weight centiles in order to take into account the major determinants of birth weight; gestational age at delivery, maternal booking weight, height, parity, ethnicity and sex of the neonate. Furthermore, to explore potential confounding factors for the vascular parameters, we grouped participants into tertiles according to different exposures, and performed multivariate regression analysis with known potential confounders for FMD[150]. However, it is still possible that the results are affected by residual confounding.

Also in **Study IV**, residual confounding could be a major issue since knowledge is limited regarding what affects, and what is affected by, PAPP-A[151]. Our sample size was too small to investigate that satisfactorily. Thus, the results of Study IV should be interpreted with great caution and be taken primarily as hypothesis generating.

6.2 RANDOM ERROR

Random error refers to the unpredictable fluctuations in measurements due to limitations of the measurement device. This variability is due to chance and can usually be reduced by increasing the sample size.

6.2.1 Precision

Cardiovascular examinations and analysis are resource intense, which limited our study group size and increases the risk of type II error. All cardiovascular examinations were performed in well-established research-labs by a limited number of trained staff. However, all four studies could have benefited from a larger study group.

FMD

FMD is highly user-dependent, but has nevertheless been proven to have good reproducibility and low observer variability. In our laboratory, the coefficient of variation for FMD is 15%. However, FMD is sensitive to a variety of factors including probe position, stress, sleeping disorders, caffeine consumption, and menstrual phase. It is therefore important to standardize as many variables as possible in the study protocol. Our study protocol included 10 minutes of rest before the examination, lateral position to avoid caval compression, and no intake of caffeinated drinks at least 4 hours prior to the examination.

Examination of skin microcirculation

Assessing skin microvascular reactivity with laser Doppler perfusion imaging following iontophoretic administration of vasoactive factors is influenced by a wide range of factors; room temperature, stress, intake of caffeine, and body position. Our study protocol included the same parameters as for FMD and, importantly, a recorded room temperature of 22-24°C. In our laboratory, coefficients of variation for peak microvascular flux after iontophoreses of ACh and SNP are 11% and 20%, respectively.

Examination of carotid arteries

Examination of the carotid arteries with ultrasound is also highly user-dependent. In our laboratory, the coefficient of intra-observer and inter-observer variability were 1% and 8%, respectively. CCA-IMT measurements are extremely small and thus, image depth and resolution is of great importance. Patient position and translation artefact from pulsatile jugular vein can affect the results. In **Study I**, a Vivid 7 Dimension ultrasound device with a 5- or 7- MHz linear transducer was used. Previous studies indicate that the precision of the results could have improved by using high-frequency ultrasound, which allows identification of the intima and media separately[152].

Pulse wave velocity, central blood pressure and augmentation index

Pulse wave velocity was analysed in **Study I-III** and is gold standard for measuring arterial stiffness. There is no consensus on which device is most valid during pregnancy[33]. It is important to measure the path length from the carotid to the femoral artery without taking into account the curvature of the pregnant uterus.

We used the SphygmoCor device to assess PWV, and to derive central blood pressures and augmentation index. The SphygmoCor uses radial artery waveforms, brachial blood pressure and the generalized transfer function to derive central blood pressure. One problem with this technique is that any presence of brachial-to-radial amplification of the pulse wave might lead to an underestimation of central blood pressure[153].

Cardiac examinations

Transthoracic echocardiography was performed as described in the Methods section. In our laboratory, the intra-assay coefficient of variation, as calculated on LV mass is 1%. This method is user-dependent, and the results are highly influenced by loading conditions. Since loading conditions change a great deal during pregnancy, the examination should

preferably be completed with measurements of myocardial tissue movement, also called strain. In order to do this, we used Doppler tissue imaging echocardiography. This method is well validated and provides valuable information in hypertensive pregnancies[78]. There are other methods for evaluating myocardial tissue indices, such as 2D and 3D speckle-tracking echocardiography, but whether those methods provide any additional information that could be important during pregnancy, remains to be studied.

Examinations of placental function

Placental function is difficult to measure. Up until today, no method for evaluating placental function in vivo has proved to fulfill the needs for a noninvasive, simple and accurate method. The most widely spread method for assessing placental function in vivo is uterine artery Doppler measurements, as discussed above[2 69]. Other methods available are 3D ultrasound, MRI and power Doppler[154-156], but whether those methods provide any additional information that could be useful during pregnancy, remain to be studied[157]. In **Study III,** the uterine artery pulsatility index was examined in gestational week 24 by trained sonographers. The examination should be performed at rest and in a lateral position. Exercise, heart rate and uterine contractions could affect the result.

In **Study IV**, PAPP-A was considered a proxy for early placental function, and the accuracy of that can be questioned. However, apart from the well-established relationship between low levels of PAPP-A and adverse pregnancy outcome, previous studies also show that first trimester PAPP-A levels are significantly associated with placental thickness, morphology, placental vascularization and 3D placental Doppler indices[125 158 159].

Fetal measurements and customized birth weight centiles

Gestational age at inclusion was assessed on measurements of the biparietal diameter (BPD) if it was < 21mm, and otherwise on crown rump length (CRL)[160]. Expected date of delivery was assessed in gestational week 16-18, according to the current Swedish recommendation at that time[160]. According to the American Congress of Obstetricians and Gynecologists (ACOG) gestational age should be assessed before 14 weeks gestation, however, although the accuracy of the measurement in the first trimester is only marginally better than in the second trimester[161]. Nevertheless, the error in the estimation of gestational age could have had a severe impact on the results in **Study III**, and additional data based on gestational age and birth weight centile calculated from the last menstrual period (LMP) would have been of interest. The use of customized birth weight centiles can be questioned, since there is no evidence of improved outcome when using them[162]. However, we

considered customized birth weight centiles to be the best available tool for estimating each infant's growth in relation to optimal growth potential.

Statistics

One of the major strengths of Study II and IV is the longitudinal design in which the entire vascular tree was investigated repeatedly in the same women during and after pregnancy. This provides us with a unique opportunity to compare and evaluate the interaction between different parts of the cardiovascular system. However, one issue of concern that is associated with this design is the multiple testing problem and the risk of type 1 error. One way of dealing with that problem is to use a higher threshold for significance levels, but that commonly requires larger study groups. One can argue that if several examinations and results points in the same direction, these concerns could be set aside, although, our results should nevertheless be interpreted with some caution due to this.

6.3 EXTERNAL VALIDITY

External validity reflects whether the results of a study can be extrapolated to a larger population.

All cardiovascular exams were performed by a limited number of trained and well-experienced stuff, and thus, the precision in **Study I** and **II** was supposedly satisfactory. However, as discussed above, the initial inclusion criteria might have left us with a group of pregnant women with non-representative vascular function and limited external validity. Nevertheless, the fact that the delivery-related variables reflected a Swedish obstetric context could indicate that our results are replicable at least to healthy, pregnant Swedish women.

The extent to which the findings in **Study III** and **IV** can be transferred to a larger group of pregnant women is limited. This is because of the small sample size and the very small proportion of complicated pregnancies. Hence, the results could and should not be extrapolated to complicated pregnancies.

7 DISCUSSION

7.1 STRUCTURAL CHANGES

The heart and the aorta

Study II found an increase in LV wall thickness and a subsequent increase in LV mass during pregnancy. This is in agreement with other studies showing a 15-25% increase in LV wall thickness and an increase up to 50% in LV mass during normal pregnancy[73] The increase in LV wall thickness during pregnancy in our study occurred in the third trimester and was largely confined to the LV posterior wall, while changes in the interventricular septum were small. This is in agreement with findings in a recent meta-analysis[73]. We observed LVOT and aortic root diameter to remain unaltered, and there were no changes in LV diameters in systole or diastole, or in RV diastolic diameter. Previous studies have reported either no change or else an increase in LVOT and aortic root diameter, and a slight increase in RVEDD in the third trimester[163 164]. Thus, our findings extend other observations[73] to suggest that normal pregnancy is associated with a predominantly concentric LV remodelling in response to the increased circulatory demand during pregnancy.

Elastic (conduit) arteries

In **Study I**, we observed a progressive increase in the diameter of common carotid arteries and brachial artery during pregnancy, more prominent in the brachial artery than in the common carotid artery (CCA). CCA- intima media thickness decreased, possibly due to distension. A few previous studies investigated IMT and CCA diameter during pregnancy and found indices of increased CCA diameter and unaltered IMT during normal pregnancy, although, increased CCA stiffening toward term have also been observed in healthy pregnancies [39 65].

Microcirculation

Baseline microvascular reactivity was markedly increased during pregnancy as compared to baseline conditions; possible indicating increased vessel size also in the arterioles.

7.1.1 Structural changes in context

We observed profound structural changes throughout the vascular tree during normal pregnancy, reaching from the heart to the microcirculation. Interestingly, although the heart

remodels extensively during pregnancy, the LV and RV blood volumes remain unaltered, and thus, the extra blood volume necessary for sufficient fetal growth must instead be held more peripheral and in the venous system. Our results might imply that the more peripheral a vessel, the greater change in vessel diameter. Further studies are needed to evaluate this.

7.2 FUNCTIONAL CHANGES

The heart and the aorta

In the **Study II**, stroke volume increased progressively throughout the trimesters, whereas CO reached a plateau in the second trimester. There is agreement that stroke volume and CO increases during normal pregnancy but the time course, magnitude, and maintenance, in particular during the last trimester, of these alterations remain unclear[74 165] The results from our longitudinal study extend to findings from a recently published meta-analysis based on mostly small studies, both longitudinal and cross sectional, suggesting a non linear increase in CO until the second or early third trimester [166]. Taken together, our findings and those by others[72 167], suggest that CO increases in response to the large reduction in total peripheral vascular resistance during early pregnancy, and is probably driven by an increase in heart rate and stroke volume, where heart rate may be more important during later pregnancy.

Cardiac diastolic function and/or dysfunction during normal pregnancy has been a topic of great interest during the past few years, since evidence of chamber diastolic dysfunction near term has been observed in approximately one fifth of all normal pregnancies [76]. Additionally, chamber diastolic dysfunction is evident in women subsequently developing preterm PE [153].

We found a progressive reduction in the E/A ratio during normal pregnancy, indicating decreased diastolic function, in agreement with others[168 169]. The E/A ratio is affected by blood pressure and heart rate[170], which may be potential confounding factors. However, there were directionally similar progressive changes in the e´/a ´ratio, a more sensitive marker of diastolic function with less potential confounding by increased preload [171 172]. Furthermore, the progressive increase in E/e´ during pregnancy and left atrial enlargement, findings of increased cardiac filling pressures usually associated with increased LV stiffness and decreased diastolic function, indicate a progressive impairment of diastolic function during normal pregnancy. These findings, in agreement with others[75 169], are probably an effect of the pregnancy-induced increase in LV wall thickness. Thus, our results suggest that the increase in preload in addition to the concentric LV remodelling leads to higher filling pressure and slower relaxation in diastole, not due to fibrosis or stiffness, but merely due to a

thicker myocardium. Thus, the impaired diastolic function should not be considered to be dysfunctional.

A typical finding in hypertension-induced cardiac remodelling is disproportionate myocardial fibrosis and impaired early diastolic relaxation, which is present before LV hypertrophy develops[173]. In contrast, animal studies suggest that temporary cardiac remodelling associated with volume overload and LV hypertrophy during pregnancy is accompanied by upregulation of vascular endothelial growth factor and increased myocardial angiogenesis, with no increase in cardiac fibrosis[174]. E-deceleration time and isovolumic relaxation time are sensitive echocardiographic indices of early diastolic relaxation, less influenced by heart rate than the E/A ratio[170], and they are associated with myocardial fibrosis[173]. Accordingly, we observed no changes in E deceleration time or isovolumic relaxation time, providing circumstantial evidence to suggest that LV remodelling during normal pregnancy is not associated with myocardial fibrosis.

Another way of examining whether the rapid LV remodelling remains beneficial throughout the course of pregnancy is to examine myocardial mechano-energetic efficiency (MEE). MEE can be measured as the ratio between systolic work and energy consumption, and expresses the amount of oxygen consumed by each contraction per second. Thus, a low MEE indicates inefficient LV performance with high energy wasting, which is associated with increased cardiovascular risk in patients with hypertensive LV hypertrophy[80]. **Study II** is, to the best of our knowledge, the first to examine MEE during normal pregnancy. We demonstrate that myocardial MEE remaines unaltered, despite the rapid increase in LV mass, heart rate and CO. Thus, the myocardium manages to work efficiently in normal pregnancy, also in the third trimester when the demands on cardiac function are accomplished by impairment of diastolic function. To assess the interaction between the heart and the large arteries, indices of ventricular- arterial coupling (VAC) was also measured, and a maintained VAC was observed in all trimesters of normal pregnancy. This can be interpreted as a proof of matched LV and arterial elastances, and an optimal cardiac efficiency. There is a lack of knowledge regarding the importance and predictive value of both MEE and VAC in pregnancy.

Blood pressure, pulse wave velocity and augmentation index

In **Study II,** we found that brachial systolic and diastolic BP decreased somewhat in early pregnancy, followed by an increase in the third trimester, in agreement with most studies during normal pregnancy[34 35]. More important, however, central BP decreased more than

brachial BP during the first part of pregnancy. Our results confirm and extend previous cross sectional studies[35 37] and smaller studies with longitudinal measurements up to 17 weeks postpartum[30], and suggest that central BP is reduced more than brachial BP during early pregnancy.

We found that carotid-femoral PWV, a marker of aortic stiffness[175], decreased during pregnancy. In normal pregnancy, there is probably a significant reduction in PWV to the second trimester, although most studies report that the reduction is BP-dependent[33]. Thus, our findings provide new information in support of reduced aortic stiffness during pregnancy. Both central pulse pressure and augmentation index decreased during pregnancy. The relative changes in augmentation index were larger than those observed for carotid-femoral PWV. This suggests that carotid-femoral PWV and augmentation index, although related, provide different information. Indeed, whereas PWV better reflects aortic stiffness, also endothelial dysfunction and increased arterial resistance are also determinants of augmentation index, which may be a more sensitive early marker of arterial stiffness in younger individuals[21]. Our findings of a reduced augmentation index during pregnancy are in concordance with previous cross-sectional [176]and longitudinal studies[31 37].

In contrast to carotid-femoral PVW, carotid-radial PWV first decreased until mid-pregnancy and then increased until term. This was similar to the observed changes in brachial BP and may reflect the increase in peripheral resistance in late pregnancy.

Endothelial function

In S**tudy** II, we observed an early and persistent improvement in endothelial function in the forearm skeletal muscle vasculature. An increased FMD during pregnancy is in agreement with previous studies [33]. However, it is important to measure indices of both endothelium dependent and independent vasodilatation, as an increased FMD could also be due to increased non-specific vascular smooth muscle responsiveness[52]. Vessel diameter is inversely related to the vascular responses to post-ischemic hyperemia and GTN[17]. Despite the larger resting brachial artery diameter, FMD was markedly enhanced during pregnancy. This inverse relationship to vessel diameter is also a likely reason for the apparent reduced response to GTN. In **Study III**, we demonstrated that impaired maternal endothelial function in the first trimester is associated with insufficient fetal growth[177]. This suggests first trimester endothelial function to be of great importance for maternal and fetal health during pregnancy and beyond.

Microvascular reactivity

We observed increased microvascular reactivity to ACh during pregnancy, suggesting increased endothelium dependent microvascular function. This is consistent with our findings in the forearm skeletal muscle vasculature. However, in contrast to the forearm skeletal muscle vasculature, the response to SNP suggests that also non-specific vascular reactivity in the skin microcirculation is also increased during pregnancy. This is in agreement with findings from a longitudinal study[41], but very few studies have investigated microvascular reactivity during normal pregnancy. Other studies have shown altered endothelium dependent microvascular reactivity in pregnancies complicated by hypertensive disorders or intrauterine growth restriction, but results are diverging[178 179]. In hypertensive patients, increased resistance due to microvascular dysfunction is an important component on the maintenance of high blood pressure[26]

7.2.1 Functional changes in context

We observed early and marked functional changes throughout the vascular tree during normal pregnancy, reaching from the heart to the microcirculation. In the first and second trimester, most indices of vascular function improved: PWV, blood pressures and AIx decreased, endothelial function and microvascular reactivity increased. Cardiac systolic function improved with increasing stroke volumes and cardiac output throughout normal pregnancy. In the third trimester we observed impaired, but not necessary dysfunctional, LV diastolic function as a result of impaired LV relaxation and of increased LV chamber stiffness with increased filling pressures. In addition to this, we also found increased carotid-radial PWV that might indicate, similar to the third trimester increase in blood pressures, an increased peripheral resistance in late pregnancy. MEE and VAC remained unaltered also in the third trimester, suggesting that the structural and functional cardiovascular changes observed during normal pregnancy (including the third trimester), are mainly effective and beneficial. However, the last examination was performed in gestational week 32 and thus, we did not assess cardiovascular function during the last weeks of gestation.

7.3 ASSOCIATION WITH FETAL GROWTH

In **Study III**, we found that maternal vascular vasodilator capacity in the first trimester, assessed both in the brachial artery (largely representing the skeletal muscle vascular bed) and in the skin microcirculation, was positively related to birth weight centile. Both markers of endothelium dependent (i.e. FMD and ACh) and endothelium independent (i.e GTN and

SNP) vasodilatation correlated positively to birth weight centile. Applying a value of FMD ≤ 5% to define endothelial dysfunction, we also observed an association between reduced maternal endothelium dependent vasodilatation and impaired fetal growth already in the first trimester. Furthermore, birth weight centile was very low in this group, which may suggest that a FMD value of 5% or below during the first trimester is a risk marker for impaired fetal growth. However, these results should be confirmed by others, as there were only 8 women in the group with FMD ≤ 5.

Women delivering SGA-offsprings are at higher risk of future cardiovascular disease[97 180]. Our results might imply that impaired vascular vasodilator and/or reduced endothelial function is partly responsible for this association.

In **Study II and III**, we observed an independent association between first and second trimester heart rate and birth weight centile. Our results confirm one previous study on a positive relation between heart rate and birth weight centile [71], and similarly previous studies have demonstrated a positive relation between CO and birth weight[110 112]. This finding is in line with the association between a general vasodilator capacity and birth weight centile, since the increase in heart rate most likely is a result of sympathetic nerve activation in response to vascular dilatation and reduced afterload.

7.4 ASSOCIATION WITH PLACENTAL FUNCTION

Uterine artery Doppler

In **Study III**, we found that the uterine artery pulsatility index was increased in women with the lowest tertile of FMD. However, the relationship was not linear. Nevertheless, our results might suggest that reduced vasodilatation in the brachial artery, at least to some extent, reflects the circulation in the uteroplacental unit. That is, however, given that uterine artery features really reflect placental conditions[2]. Previous studies found no association between FMD and uterine artery pulsatility index in normal pregnancy or before the onset of PE[181 59]. However, in one previous study, women with bilateral uterine artery notch had decreased FMD[182].

PAPP-A

In **Study IV**, we found a positive correlation between forearm skin microvascular endothelium dependent vasodilatation and PAPP-A, whereas FMD (indicative of endothelium dependent vasodilatation in the brachial artery) showed an inverse relation.

During pregnancy, maternal levels of PAPP-A increase rapidly, and low first trimester PAPP-A levels are associated with both chromosomal anomalies and adverse pregnancy outcomes, such as small size for gestational age, preterm labour, preeclampsia, and stillbirth [123 124]. PAPP-A is, therefore, generally considered to reflect placental invasion and circulation, a theory that is further strengthened by the fact that low levels of first trimester PAPP-A in maternal circulation are associated with reduced number of capillaries and smaller capillary diameters in the placenta[125 126]. The reason why some pregnant women have reduced levels of PAPP-A and impaired endothelium dependent skin microvascular reactivity in our study is unclear, but we do know that healthy individuals with a family history of diabetes have functional disturbances in skin microcirculation[183] with an impaired endothelium dependent microvascular dilatation capacity. Accordingly, previous studies reported reduced PAPP-A levels in pregnant women with type-2 diabetes, as compared to healthy controls[184 185]. Furthermore, endothelial function in the skin microcirculation is impaired already in apparently healthy people with a family history of diabetes[183]. Reduced endothelial dependent skin microvascular reactivity and low levels of PAPP-A in the first trimester may therefore be early important markers of metabolic disturbances, and impaired placental function as a result of this. However, whether the observed association between low PAPP-A levels and decreased endothelium dependent microvascular reactivity in **Study IV**, contributes to the increased risk of adverse pregnancy outcome, remains to be explored.

PAPP-A is increased in the third trimester in preeclamptic pregnancies, as compared to normal pregnancies[129 130], and elevated levels of PAPP-A and E-selectin were reported in women 10 years after preeclamptic pregnancies [118]. PAPP-A is synthesized by several cell types, including vascular smooth muscle cells and endothelial cells, and is also detectable in non-pregnant individuals. PAPP-A is increased in patients with acute injury or unstable atherosclerotic disease[117-119]. Despite this, one previous study did not find an association between high first trimester PAPP-A levels and adverse pregnancy outcome[131].

Given that first trimester PAPP-A levels relate to placental function, our results suggest that placental function can be reflected by first trimester endothelium dependent skin microvascular reactivity. We hypothesize that first trimester PAPP-A mainly reflects placental microvascular reactivity and that reduced endothelial dependent skin microvascular reactivity and low levels of PAPP-A in the first trimester may be early important markers of maternal metabolic disturbances and impaired placental function.

More research is needed to confirm our results and to evaluate the significance of the observed (and somewhat conflicting) inverse relation between FMD and PAPP-A.

8 CONCLUSIONS

- There are early and marked structural and functional changes of the maternal vascular system during normal pregnancy. These hemodynamic changes seem to be dependent on normal adaptive endothelial and vascular function.
- Cardiac output increases rapidly during normal pregnancy as a result of an initial increase in heart rate, followed by left atrial enlargement and increased left ventricular wall thickness and mass. In the third trimester, these structural and functional cardiac changes are accomplished by impairment of diastolic function.
- Cardiac efficiency and ventricular arterial coupling remain unaltered throughout pregnancy, suggesting that the structural and functional cardiovascular changes observed during normal pregnancy (including the third trimester), are mainly effective and beneficial.
- There are positive correlations between maternal first and second trimester heart rate, first trimester vascular vasodilator capacity, and fetal growth. These associations are probably partly explained by the fact that the increase in heart rate is a result of sympathetic nerve activation in response to vascular dilatation and reduced afterload.
- Forearm skin microvascular endothelium dependent vasodilatation is positively related to levels of PAPP-A, whereas FMD (indicative of endothelium dependent vasodilatation in the brachial artery) shows an inverse relation. This might suggest that the observed association between maternal cardiovascular fucntion and fetal growth, at least to some extent, can be explained by the fact that maternal skin microvascular reactivity reflects placental capacity.

In summary, normal cardiovascular adaptation to pregnancy includes early and profound structural and functional changes throughout the cardiovascular tree. There are several associations between indices of early maternal cardiovascular adaptation to pregnancy, and fetal growth. Some of these associations might be explained by the fact that maternal vascular function reflects placental circulation.

9 FUTURE PERSPECTIVES

Despite extensive research in the field on hypertensive disorders during pregnancy, the pathogenesis of preeclampsia and fetal growth restriction has not been fully elucidated. Useful predictive markers and preventive strategies are yet to be discovered. In particular, the role and importance of the microcirculation in pregnancy is poorly understood. Although this is believed to be involved in many pregnancy-related complications, further research should evaluate the potential benefit of assessing microvascular reactivity in early pregnancy.

Our findings suggest that endothelial function plays an important role in maternal adaptive capacity to pregnancy. Based on our results presented in the current thesis, further research ought to explore whether there is a predictive value in maternal heart rate, first trimester vascular function in the brachial artery or in the skin microvascular reactivity. If so, studies on the potential benefit of low dose aspirin or other pharmacological interventions to women with impaired first trimester vascular function or lack of increase in heart rate should be carried out.

Currently, the most controversial debate topic in this research field is the role of the placenta in preeclampsia. Over recent decades, the main theory on the cause of preeclampsia has focused on an inadequate implantation of the placenta. Recently, focus has shifted towards the capability of the maternal cardiovascular system to cope with the great demands of pregnancy. Further research should be performed to evaluate shared and disparate factors in placental and systemic circulation. In order to answer the chicken-and-egg question of what comes first, maternal vascular dysfunction or placental hypoperfusion, women must be examined before (and during) pregnancy, and the results should be related to pregnancy events, fetal growth and placental function. Available methods for assessing placental function in vivo are, however, deficient, and thus, improved and more accurate methods are needed.

Cardiac function in normal and hypertensive pregnancies has been extensively explored, although the very recent finding on diastolic dysfunction near term in one fifth of all apparently normal pregnancies needs further evaluation. Our findings suggest that MEE and/or VAC could be useful measurements to distinguish between normal and pathological cardiac alterations in pregnancy. Further studies should be performed to investigate the predictive value in estimating MEE and VAC in complicated pregnancies.

Early signs of endothelial impairment, arterial stiffness, and LV diastolic dysfunction precede preeclampsia and fetal growth restriction, but are also present in normal pregnancies. Further

studies should evaluate whether any of these parameters could have independent predictive values, not only for adverse pregnancy outcome, but also for later cardiovascular events. How can we better use the information on future health that each woman's response to pregnancy contains? Although there is a well-established relation between hypertensive pregnancies and later cardiovascular diseases, preventive strategies are lacking. Future studies should set out to create an effective preventive strategy that reduces this risk. Do pharmacological interventions that have been proved to reduce maternal and fetal risk during pregnancy, also decrease the risk of cardiovascular events later in life?

Low birth weight impairs offspring's endothelial function, and future studies should focus on how, and when, we can interfere with the unfavorable relation between intrauterine exposure to PE or FGR, and offspring cardiovascular impairment. Can pharmacological interventions that reduce maternal and fetal risk during pregnancy, also improve endothelial function and cardiovascular health in the offspring?

10 POPULÄRVETENSKAPLIG SAMMANFATTNING

Bakgrund: När en kvinna blir gravid måste hennes hjärt-kärlsystem öka sin kapacitet rejält för att tillgodose det växande barnets behov. Moderkakan bildas när det befruktade växer fast i livmodern och det är i moderkakan som allt utbyte mellan mamma och foster sker. I slutet av graviditeten flödar en halv liter blod genom moderkakan varje minut. Ca 5% av alla gravida kvinnor drabbas av havandeskapsförgiftning som innebär högt blodtryck, en generell kärlskada och ofta läckage av proteiner i urinen. Ibland är också fostret tillväxthämmat; det växer inte som det ska. Man vet fortfarande inte varför havandeskapsförgiftning uppstår. Tidigare trodde man att havandeskapsförgiftning och tillväxthämning hos barnet oftast berodde på att moderkakan inte bildades på något bra sätt i början av graviditeten, men på senare tid har betydelsen av mammans hjärt-kärlfunktion också lyfts fram. Kan det vara så att havandeskapsförgiftning och tillväxthämning hos barnet beror på att mammans hjärt-kärlsystem inte orkar med att öka sin kapacitet tillräckligt mycket för att tillgodose det växande barnets behov? För att kunna svara på den frågan är det viktigt att känna till hur en normal kapacitetsökning i mammans hjärta och kärl ser ut.
Detta forskningsprojekt genomfördes för att ta reda på det, och för att undersöka hur mammans hjärt- kärlfunktionen under graviditet påverkar barnets tillväxt.

Metod: Vi undersökte 58 kvinnors hjärt- kärlfunktion i graviditetsvecka 11-14, 24 och 34, och sedan nio månader efter förlossningen. Barnets tillväxt mättes med ultraljud och utifrån födelsevikten.

Resultat: I Studie I och II visade vi att samtliga delar av mammans kärlträd ökar sin kapacitet rejält under normal graviditet: hjärtas muskelmassa ökar, större blodmängder pumpas ut med varje slag och hjärtat slår snabbare och snabbare under graviditeten. Aortan blir inte större, men däremot mer elastisk, vilket innebär att större blodvolymer kan passera utan att blodtrycket stiger. De mindre transportkärlen ökar både i diameter och i sin förmåga att anpassa sig till ett ökat blodflöde. De allra minska kärlen i huden uppvisade också ökat blodflöde och bättre förmåga att anpassa sig till det förändrade blodflödet.
I Studie III undersökte vi sambandet mellan mammans kärlfunktion och barnets storlek vid födseln och såg att ju bättre både de små och mellanstora blodkärlen kunde anpassa sig till ett ökat flöde i graviditetsvecka 11-14, desto bättre växte barnen. Vi fann också att barnets tillväxt ökade ju högre vilopuls mamman hade första halvan av graviditeten.

I Studie IV ville vi undersöka om fyndet i studie III berodde på att mammans kärlfunktion speglar moderkakans funktion, och därför undersökte vi sambandet mellan ett protein som bildas av moderkakan, PAPP-A, och mammans kärlfunktion. Vi fann att högre PAPP-A-nivåer korrelerade med ökad reaktivitet i hudens mikrocirkulation, men att det var precis tvärtom i de mellanstora blodkärlen: där var högre PAPP-A-nivåer istället korrelerade med lägre reaktivitet.

Slutsatser: Under normala graviditeter sker stora förändringar i hjärtat och kärlträdet hos gravida kvinnor. Det finns flera samband mellan hjärt- och kärlfunktionen hos mamman under graviditeten, och barnets tillväxt. Det sambandet kan till viss del bero på att moderkakans och kärlträdets funktion hänger ihop.

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