Patterns of maternal and neonatal vascular adaptations in placental disease

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PATTERNS OF MATERNAL AND NEONATAL VASCULAR ADAPTATIONS IN PLACENTALDISEASE

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We declare that **Iosifina Stergiotou** has performed under our supervision the studies presented in the thesis **"Patterns of maternal and neonatal vascular adaptations in placental disease"**.

This dissertation has been structured following the normative for PhD thesis as a compendium of publications, to obtain the degree of **International Doctor in Medicine** and the mentioned studies are ready to be presented to a Tribunal.

Eduard Gratacós Solsona
Thesis Director

Fátima Crispi Brillas
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To my family

LIST OF ORIGINAL PUBLICATIONS

The present dissertation has been structured following the normative for PhD thesis, as a compendium of publications, to obtain the degree of International Doctor in Medicine. It was approved by the *Comisión de Doctorado de la Facultad de Medicina* on the 18th May, 2012. Projects included in this thesis belong to the same research line, leading to three articles published or submitted for publication in international journals:

1. **Stergiotou I**, Crispi F, Valenzuela-Alcaraz B, Bijnens B, Gratacos E. Patterns of maternal vascular remodeling and responsiveness in early- versus late-onset preeclampsia. Am J Obstet Gynecol 2013;209:558.e1-558.e14.
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2. **Stergiotou** I,Bijnens B, Cruz-Lemini M, Figueras F, Gratacos E, Crispi F. Maternal subclinical vascular changes in fetal growth restriction with and without preeclampsia.
   Submitted to Am J Obstet Gynecol, *Impact factor:* 3.877, *Quartile:* 1st

3. **Stergiotou** I, Crispi F, Valenzuela-Alcaraz B, Cruz-Lemini M, Bijnens B, Gratacos E. Aortic and carotid Wall thickness in term small-for-gestational age newborns and relationship with signs of severity, Ultrasound Obstet Gynecol 2013. doi: 10.1002/uog.13245.
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CONTENTS

1. INTRODUCTION

The impact of placental disease in maternal and neonatal vasculature was the main scope of this thesis. Subclinical vascular changes, assessed at placental disease diagnosis, could reflect increased risk of future cardiovascular disease (CVD). Thus, identification of mothers and neonates at risk of adverse long term cardiovascular outcome could provide an opportunity for lifestyle and risk factors' modification with the aim of reducing morbidity and mortality. We therefore planned to assess various structural and functional vascular indices in mothers and neonates affected by placental disease. Confirmation of our hypothesis could also improve our understanding of common and distinct features of the disease components.

1.1 CARDIOVASCULAR DISEASE

1.1.1 Prevalence and risk factors of cardiovascular disease

Cardiovascular disease is the main cause of death worldwide, representing 30% of all deaths. The World Health Organization (WHO) estimated a total of 17.3 million deaths from CVD in 2008, and this number is steadily increasing. Coronary heart disease and stroke account for the 3/4 of deaths and affect people in their mid-adult life. The remaining 1/4 of deaths can be attributed to other causes, such as rheumatic heart disease, peripheral arterial disease, pulmonary embolism and congenital heart disease.[1] Behavioral risk factors, such as tobacco use, unhealthy diet and obesity, physical inactivity, high blood pressure, diabetes and raised lipids account for 80% of coronary disease and stroke and are generally modifiable.[1,2] Consequently, the burden of CVD can be reduced by prioritizing and addressing them first. On the other side, determinants of CVD such as globalization,

population ageing, poverty, stress and genetic require implementation of social, economic and cultural measures.[1]

1.1.2 Cardiovascular disease in women

The risk of dying due to CVD is largely underestimated in women.[3]This is in part due to gender-related differences in risk factors, symptoms, and treatment.[4] In 2004, the American Heart Association expanded its focus on female-specific clinical recommendations. Updates have been issued on 2007 and 2011 focusing on more gender-specific analyses and publishing more definitive recommendations. In 2007 the panel emphasized that pregnancy represents a unique opportunity to identify women's lifelong cardiovascular risk.[5] But, it was not until the 2011 review when the list of recommendations implicated pregnancy complications at the evaluation of cardiovascular risk.[6] The expert panel was based on recent reports of literature elucidating that pregnancy complications such as gestational hypertension, preeclampsia (PE) and fetal growth restriction (FGR) are associated with maternal CVD morbidity and mortality later in life.[7-12] It suggested that a woman's careful history should always include information on pregnancy complications and that these women should be appropriately referred by obstetricians to a primary care physician or cardiologist in the postpartum period.[6]

1.1.3 Cardiovascular disease in offspring and fetal programming

Apart from lifestyle and pregnancy risk factors, considerable data link low birth weight, due to intrauterine growth restriction, to increased offspring risk of vascular disease in later adult life. This is considered to be the result of programming through processes associated with low rates of fetal and infant growth such as adult blood pressure, glucose tolerance, plasma concentrations of fibrinogen, factor VII, and apolipoprotein B.[13] The developmental origins of adultl disease model, proposed by Barker has been confirmed in recent studies[14-16] but

certainly needs further clarification in the context of defining effective diagnostic and preventive interventions to improve CVD outcomes.

1.2 PLACENTAL DISEASE AND LONG-TERM CARDIOVASCULAR OUTCOME

1.2.1 Placental disease definition

Although PE, FGR, and preterm labour may differ in their clinical manifestations, recent studies suggest that they may be considered as a single disease process.[17,18] Restricted endovascular invasion is the chief mechanism involved together with chronic hypoxia, and placental ischemia.[17] Placental histologic findings support indeed the classification of PE, FGR, and preterm labour under the scheme of ischemic placental disease. Lesions characteristic of placental ischemia such as hemosiderin deposition, necrosis, and atherosis, are commonly found in placentas of pregnancies complicated by PE[19,20] and FGR.[20–22] The common pathophysiology of these entities is further supported by epidemiological studies demonstrating their link to increased risk for CVD later in life. Specifically, Smith et al. in a well-organized retrospective observational study reported that PE, preterm birth and birthweight in the lowest quintile for gestational age, were all significantly associated with an increased risk of CVD or death during the subsequent 19 years.[23] Their associations remained significant after adjusting by maternal diet and lifestyle.[23] Similarly, Pell and al. demonstrated that FGR, preterm birth, and a history of spontaneous abortion are all associated with an increased risk of subsequent cerebrovascular disease in the mother.[24] Follow up data spread between 14-19 years and there were significant independent associations with all three conditions after adjustment for the potential confounding effects of maternal age, height, socioeconomic status, and preeclampsia.[24]

1.2.2 Long-term cardiovascular outcome

The positive association between preeclampsia and later life cardiovascular disease has been attributed to shared cardiovascular risk factors.[12,25-27]Thus, the same conditions that have been implicated in the pathogenesis of preeclampsia are also strong risk factors for future development of CVD, including insulin resistance, diabetes mellitus, obesity, chronic hypertension and renal disease.[8]It is also possible that the hypertensive pregnancy disorder itself could induce irreversible vascular and metabolic changes that are associated with increased risk of CVD.[28] Yet, a recent prospective population study from Norway suggested that cardiovascular risk factors that are present before a hypertensive pregnancy are more important determinants of subsequent cardiovascular risk factors than the hypertensive pregnancy itself.[12]

The existing evidence for the common pathophysiology and outcomes of PE and FGR led us to investigate whether they also share vascular structural and functional changes.

1.3 PREECLAMPSIA

1.3.1 Definition of preeclampsia

Preeclampsia (PE) is a multisystem disorder complicating 5-10% of pregnancies and is unique to human pregnancies.[29] PE is featuring as a leading cause of maternal mortality (15-20% in developed countries), morbidity (short and long term), preterm birth, perinatal mortality and fetal growth restriction (FGR).[30] Namely, the clinical manifestations of PE are characterized by a maternal component (hypertension and proteinuria with and without multisystem involvement) and a fetal component (FGR).[30] Diagnostic criteria of PE have not been standardized until 2000-1.[31,32] PE is defined according to the International Society Study of the Hypertension in Pregnancy as a resting blood pressure of ≥ 140/90 mm Hg on 2 occasions at least 4 hours apart and proteinuria of ≥ 300 mg/L or a 2+ urine dipstick beyond 20 weeks of gestation in a previously normotensive woman.[31,32]

1.3.2 Pathophysiology and classification of preeclampsia

The precise pathophysiology of preeclampsia remains a subject of extensive research, but it is likely to be multifactorial. Superficial placentation plays a crucial role in the development of preeclampsia, driven possibly by immune maladaptation with subsequently hypoxia and reduced concentration of angiogenic factors in the maternal circulation.[30] The final preeclamptic phenotype is further modulated by preexisting maternal cardiovascular and metabolic risk factors and results in pronounced inflammatory responses.[30]The onset, severity, and progression is significantly affected by the maternal response.[30]

Previous studies have supported that classifying PE into early and late-onset disease differentiates two distinct clinical forms with pathophysiological specific features. Thus, early PE is commonly associated with placental insufficiency, intrauterine growth restriction and adverse maternal and perinatal outcomes.[33,34,35] Conversely, late-onset PE is associated with minor placental involvement and milder clinical disease.[33,34,35]Intrinsic placental factors are more frequently altered in early PE,[33,34]while late PE is usually associated with predisposing maternal factors.[36]

1.3.3 Maternal cardiac adaptations in preeclampsia

Recently, interesting data indicate that early PE is associated with stage B heart failure (asymptomatic left ventricular dysfunction/hypertrophy), a high prevalence of essential hypertension and an increased cardiovascular risk status within few years postpartum.[37] Conversely, global diastolic dysfunction was detected more frequently in late PE, while the observed left ventricular remodeling was considered an adaptive response to maintain myocardial contractility.[38] These findings suggest that women with a history of hypertensive disorders in pregnancy could benefit from earlier intervention to prevent premature CDV.[25]The first specific aim of this thesis was to investigate if a cohort of preeclamptic women stratified according to gestational age at diagnosis presents distinct types of vascular structural and functional changes already at the time of diagnosis.

1.4 FETAL GROWTH RESTRICTION

1.4.1 Definition and classification of fetal growth restriction

Appropriate fetal growth depends on the fetal genetic growth potential and is further regulated by fetal, maternal health and placental function. If any of these prerequisites is not fulfilled, fetal growth is challenged.[39]Yet, the precise mechanism of how various conditions affect fetal growth potential still remains unclear. FGR is one of the most common conditions affecting ongoing pregnancies.[40]The actual diagnostic assessment of growth is based on the fetal biometry. Concurrent measurement of the head circumference, abdominal circumference, and femur length allows quantification of the estimated fetal weight (EFW). An EFW below the 10th percentile for gestational age is considered FGR.[39]

There is a current tendency to distinguish between early and late FGR if it occurs before or after 32 weeks gestation.[41]The importance of gestational age at diagnosis is reflected on the severity of placental dysfunction and the prognosis. Early FGR is mainly characterized by severe placental insufficiency expressed by abnormal umbilical artery.[39,41] On the other hand, late FGR is associated with a milder placental dysfunction, illustrated merely by brain redistribution and abnormal uterine arteries.[42,43]

1.4.2 Maternal cardiac and metabolic outcomes of fetal growth restriction

Recent findings suggest that early PE and preterm severe FGR could share similar maternal cardiovascular responses such as impaired myocardial relaxation and left ventricular diastolic dysfunction.[44] Both conditions present also lipid profile alterations and insulin resistance.[45] In addition, late FGR present minor maternal cardiac dysfunction,[46] changes in lipid and glucose metabolism,[47] as well as signs of minor endothelial dysfunction.[33,48] Maternal long-term cardiovascular outcome is more severe in PE than in FGR, [44,45,49,50] as mediated by metabolic, inflammatory, angiogenic and cardiac parameters.[44,45,51-54] The second aim of this thesis was to investigate if pregnant women with normotensive FGR, stratified according to

gestational age at diagnosis, present distinct types of vascular structural and functional changes.

1.4.3 Intrauterine adaptations to fetal growth restriction

FGR fetuses showed echocardiographic and biochemical signs of cardiac dysfunction compared with appropriate for gestational age (AGA) fetuses from early stages.[55,56] The fetal cardiovascular adaptation to hypoxia and undernutrition is thought to represent a central adaptive mechanism and induces both cardiac[57,58] and vascular remodeling [15,16] that persists until childhood.[58] Furthermore early FGR children present neurocognitive difficulties and neurobehavioral impairment.[59] Recent evidence has demonstrated that also late FGR fetuses, often referred as term small for gestational age (SGA) due to normal umbilical artery, are characterized by subtle cardiovascular remodeling.[55,60] These cases have worse cardiovascular[61] and also neurodevelopmental[62] outcomes in postnatal life. Over recent years several prognostic factors, including abnormal cerebroplacental ratio[63] and uterine artery Doppler,[42]and birth weight below the 3rd centile,[64] have been identified as predictors of poor neonatal outcome, and consequently as surrogate markers of true growth restriction among near term SGA fetuses. Although these factors are associated with poorer neonatal outcome, they don't characterize well fetal cardiac changes[61] and it is unknown whether they also classify well the risk of abnormal vascular outcome. The third aim of this thesis was to investigate if vascular assessment is feasible in FGR neonates. The forth aim was to evaluate vascular structural and functional changes in late FGR neonates stratified according to stages of severity.

1.5 VASCULAR ASSESSMENT

1.5.1 Vascular remodeling

Vascular remodeling is a physiologic vascular process occurring as a result of hemodynamic changes and plays a major role in the clinical manifestation of cardiovascular disease. This

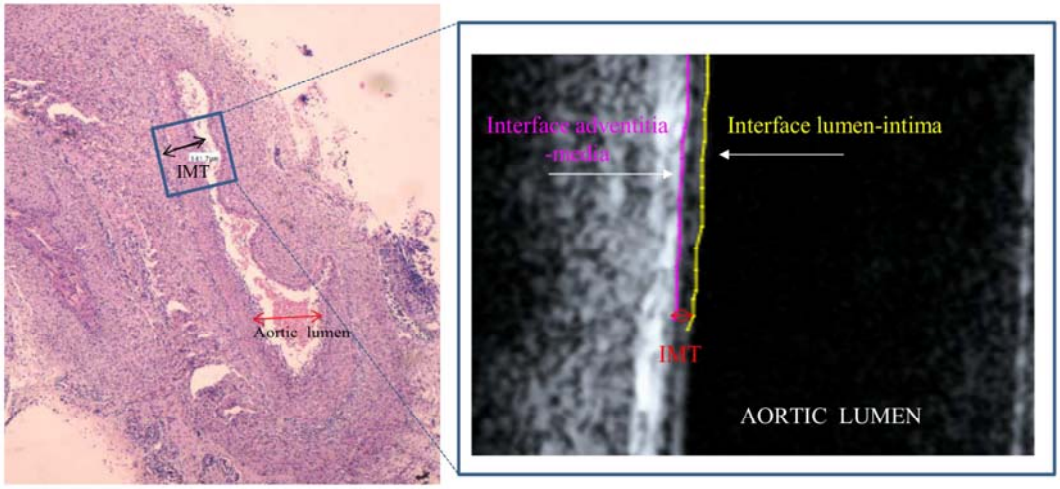
phenomenon was first described by Glagov in 1987 in a postmortem study of human coronary arteries.[65] Glagov and colleagues observed that the lumen area of atherosclerotic human coronary arteries was effectively maintained constant until stenosis exceeded 40%. Since then, coronary arteries are viewed as structures modifying shape and size to adapt plaque accumulation. Although the mechanism was initially described in atherosclerosis, a wide range of pathophysiological conditions, such as angioplasty, hypertension, and flow diversion are associated with vascular remodeling.[66] Remodeling in our studies is defined as the modification of arterial shape and size (intima media thickness and lumen diameters) to adapt to hemodynamic changes.[67]

1.5.2 Vascular intima media thickness

Intima Media Thickness (IMT) measurement is an important non-invasive imaging technique for the prediction for human cardiovascular disease.[68] Carotid IMT evaluation is considered the most useful tool for refining CVD risk in patients at intermediate CVD risk and identifying asymptomatic patients at high risk who might benefit from timely intervention.[69] IMT is measured by means of B-mode ultrasound scanning preferably in the far wall of the investigated vessel and stretches between the two echogenic lines that correspond to the lumen-intima and the media-adventitia interfaces (Figure 1).[69] There is a strong association between coronary risk factors and increased IMT, including associations with smoking, diabetes, age, sex, total cholesterol, low density lipoprotein, hypertension and peripheral vascular disease.[70]

Measurement of carotid IMT during pregnancy may provide useful information about the cardiovascular and metabolic profile of women. Cardiovascular risk factors are present for years before pregnancy and suggest that unfavorable metabolic profiles predisposing to PE are in place early in life.[71]

Figure1. The left image depicts a pathology picture of a cross section of an aorta of a growth restricted neonate. The tunicas media, and intima layers are denoted in black, while aortic lumen is denoted in red (hematoxylin-eosin stain; 10xmagnification) (Courtesy of Dr. Alfons Nadal). The right image shows the corresponding ultrasound B-mode depiction of intima media thickness



Since the first description of developmental origins of adult disease model in 1989 by Barker,[72] clinical data has confirmed associations between FGR and aortic IMT adaptations in newborns.[15,16] Furthermore, young children of 5 years of age, born FGR were found to present different cardiac shape , subclinical systolic and diastolic dysfunction and also increased carotid IMT. Studies in neonates have targeted mostly aortic IMT. However, no information was available on carotid IMT during neonatal age, although carotid arteries are technically more accessible than aorta in newborns as they lie superficially in the nuchal area. Moreover, no information exists on aorta and carotid IMT reproducibility in neonatal period, and if vascular changes in FGR neonates are consistent with severity criteria.

1.5.3 Arterial stiffness indices

The concept of aortic stiffness entails an understanding of the heterogeneity along the arterial tree. The elastic properties of conduit arteries vary with more elastic proximal arteries and stiffer distal arteries.[73] Arterial stiffness is known to be an independent risk factor for

cardiovascular events irrespective of previous cardiovascular diseases, age, and diabetes. Its assessment is becoming increasingly used in clinical examinations.[73] Increased arterial stiffness is one of the earliest detectable manifestations of adverse functional changes within the vessel wall.[74] Arterial stiffness can either be estimated locally at specific arterial sites (carotid, radial, brachial) or regionally over a given arterial segment length (carotid-aorta).[75]Local estimates of arterial stiffness can be described in terms of distensibility and wall stress coefficients, and are usually obtained through measurement of arterial changes in diameter and local distending pressure by means of ultrasound imaging.[74,76]They are indicated for mechanistic analyses in pathophysiology.[73]Carotid stiffness may be of particular interest, since in that artery atherosclerosis is frequent.[73]

Information on arterial stiffness is essential in order to complete vascular assessment and improve stratification of cardiovascular risk. Cardiovascular morbidity has been assessed by several arterial stiffness indices such as pulse wave velocity, augmentation index, distensibility and circumferential wall stress.[76,77,78] PE has been associated with increased augmentation index and pulse wave velocity, especially the early form.[54,79] In neonates arterial stiffness has been less frequently evaluated. In a recent study, a decrease of aortic distensibility in the compromised preterm FGR infants suggests that the structure of the aortic wall is altered.[80]In our studies, the term arterial stiffness is used interchangeably with the terms vascular function and responsiveness, which is considered a more generic term applied to both arterial structural and functional changes.

1.5.4 Inferior vena cava evaluation

Venous compliance, the capacity to accommodate plasma volume, is one of the most prominent features of normal venous system. Inferior vena cava is a compliant vein reflecting venous responsiveness to haemodynamic changes.[81,82,83]The concept of venous dysfunction in PE consists on a blunted venous adaptation that may persist postpartum.[84]The growing

evidence that venous maladaptation could be part of the altered maternal haemodynamics in hypertensive pregnancies[85] is a new research subject that could enhance our understanding of the pathophysiology of placental disease.

1.6 CLINICAL RELEVANCE OF VASCULAR STUDIES IN PLACENTAL DISEASE

Cardiovascular disease is the main cause of death worldwide with coronary heart disease and stroke accounting for the vast majority of deaths. Although it affects people in their mid-adult life, scientists are identifying risk factors earlier in life. Normal pregnancy constitutes a metabolic and cardiovascular stress test as it entails increase in arterial stiffness,[86] altered lipid and lipoprotein profiles,[87] and impaired glucose tolerance.[88] Furthermore, pregnancy complications attributed to placental disease such as PE[7,8,11,89] and FGR[14,15,72,90,91] are now considered cardiovascular risk factors. Although PE and FGR seem to be heterogeneous entities, they could actually represent a single disease process. One reason is that they share defective placentation as a common etiology. Moreover, both entities are similarly characterized by maternal and fetal sequelae. PE is a major cause of short and long term maternal mortality and morbidity, preterm birth, perinatal mortality and FGR. FGR affects not only fetal wellbeing but also increases maternal risk for cardiovascular disease.[23,24] The vascular structural index of IMT and arterial stiffness functional indices are standard diagnostic procedures in assessing cardiovascular risk in asymptomatic adults and can be easily applied in clinical practice.[69,73,76,77] However, their clinical application in neonatal period has been limited until now.[15,16]

The main aim of this thesis was to identify patterns of vascular changes, in mothers and neonates affected by placental disease that could possibly enable the stratification of vascular risk as partr of public health screening programs. To achieve this aim, four projects were designed. Two projects encompassed maternal vascular assessment in PE (**project 1**) and in FGR (**project 2**). The further two projects involved vascular assessment in neonatal

period with the aim to describe first its clinical feasibility and reproducibility (**project 3**) and subsequently its ability to classify FGR cases in stages of severity (**project 4**).

2. HYPOTHESIS

2.1 MAIN HYPOTHESIS

The main hypothesis of the thesis was that placental disease affects vascular structure and function in mothers and offspring.

2.2 SPECIFIC HYPOTHESES

2.2.1 Early and late PE present distinct maternal vascular structural and functional changes already at the time of diagnosis

2.2.2 Maternal vascular adaptations are present in FGR, mainly in early FGR, already at the time of diagnosis

2.2.3 Stages of placental insufficiency in SGA neonates classify the risk of abnormal vascular outcome

3. OBJECTIVES

3.1 MAIN OBJECTIVE

The general aim of this thesis was to evaluate vascular structure and function in mothers and offspring affected by placental disease

3.2 SPECIFIC OBJECTIVES

3.2.1 To evaluate maternal carotid IMT, distensibility and circumferential wall stress together with inferior vena cava collapsibility in early- and late-onset preeclamptic women

3.2.2 To evaluate maternal blood pressure, carotid IMT, distensibility, circumferential wall stress and inferior vena cava collapsibility in FGR pregnancies with and without PE, also 2subclassified into early and late disease

3.2.3 To assess carotd and aortic IMT reproducibility in neonates

3.2.4 To assess blood pressure and carotid/aortic IMT in late-onset growth restricted newborns classified by surrogate markers in different stages of severity

4. METHODS

4.1 POPULATIONS' DESCRIPTION AND DEFINITIONS

The prospective cohort studies of antenatal maternal vascular assessment, carried out over a 3 year period from April 2010 to October 2012 at the Department of Maternal-Fetal Medicine in Hospital Clinic (Barcelona), included 100 preeclamptic pregnancies and 64 normotensive pregnancies affected by FGR and a total of 110 normally grown and normotensive control pregnancies. The prospective cohort study of neonatal vascular assessment, carried out simultaneously as the previous studies, included 67 term newborns prenatally diagnosed as SGA and 134 appropriate for gestational age control neonates. The local Ethics Committee of the Hospital Clinic of Barcelona approved all studies, and informed written consent was obtained from each pregnant woman included in the studies and from the parents of each participating neonate.

PE was defined according to the International Society Study of the Hypertension in Pregnancy as a resting blood pressure of ≥ 140/90 mm Hg on 2 occasions at least 4 hours apart and proteinuria of ≥ 300 mg/L or a 2+ urine dipstick beyond 20 weeks of gestation in a previously normotensive woman.[31] FGR or SGA were defined as EFW and confirmed birthweight below 10th centile.[39]

4.1.1 Populations included in study 1

The pregnant women with PE were recruited and evaluated at or after 24h of diagnosis while admitted to the hospital. Preeclamptic pregnancies were subdivided into: A) 50 cases of early-onset PE defined as PE occurring before the 34th week of gestation and B) 50 cases of late-onset PE occurring after the 34th week of gestation. Additionally, 100 normotensive controls, attending antenatal clinic with uncomplicated pregnancies, were matched 1 to 1 by maternal age and gestational age at exploration to preeclamptic women. Only healthy non-

smoking women, not affected by current or anterior pregnancy complications or intrauterine growth restriction, were included in the control group.

4.1.2 Populations included in study 2

This prospective cohort study included 110 normally grown and normotensive control pregnancies, 64 normotensive pregnancies affected by FGR and 60 FGR pregnancies complicated with PE. Normotensive FGR cases were further subclassified into early-onset (<32 weeks of gestation at clinical onset) or late onset (>32 weeks of gestation).[41]FGR pregnancies were recruited and evaluated at the time of diagnosis. Women with diabetes mellitus, renal and connective tissue disease were excluded from the study.

4.1.3 Populations included in study 3

This study included 23 neonates to assess the interobserver reproducibility of carotid and aortic IMT in neonates. Furthermore, the intraobserver reproducibility was evaluated in the whole sample of 201 neonates.

4.1.4 Populations included in study 4

Cases and controls were identified in fetal life and evaluated in the neonatal period during their first week of life. This prospective cohort study included: A) 35 SGA with signs of severity defined by EFW and confirmed birth weight below 3rd percentile or abnormal uterine artery mean pulsatility index (PI) (above 95th percentile) or cerebro-placental ratio (below 5th percentile); B) 32 SGA without signs of severity defined by EFW and birth weight between 3rd and 10th percentile together with normal uterine artery mean PI and cerebro-placental ratio; and C) 134 controls defined by EFW and confirmed birth weight above 10th percentile with no pregnancy complications. Controls were matched 2 to 1 with cases by gender and gestational age at delivery (±1 week). Exclusion criteria were chromosomal or genetic disorders, monochorionic twin pregnancy and evidence of infection.

4.2. BASELINE DATA COLLECTION

Maternal information was obtained on age, reproductive history, smoking habits, medical history and medications in the interview at the day of the scan and through medical history notes. Height and weight were measured and body mass index (BMI) was calculated in kg/m^2.

Gestational age was defined as completed weeks of gestation based on the first trimester routine ultrasound dating. Feto-placental Doppler examination included uterine arteries, umbilical artery and middle cerebral artery. Cerebro-placental ratio was calculated as a simple ratio of the middle cerebral artery pulsatility index divided by the umbilical artery pulsatility index.[92]

Pregnancy outcome such as gestational age at delivery, mode of delivery, birth weight, birth weight centile, Apgar score and umbilical pH were also recorded. Fetal and neonatal weight centile were calculated according to local reference curves.[93]

4.3 MATERNAL VASCULAR ASSESSMENT

The maternal vascular exploration was performed in a quiet environment with the pregnant women in a supine position with slight hyperextension and rotation of the neck in an opposite direction to the probe. The assessment protocol included initially blood pressure measurement by a validated ambulatory automated Omron 5 Series device. Carotid ultrasound assessment was subsequently performed by a single experienced investigator using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA). Longitudinal clips of the far wall of both carotid arteries were obtained approximately 1 cm proximal to the bifurcation using a 13-MHz linear-array transducer. Carotid IMT measurements were performed offline according to a standardized protocol based on a trace method with the assistance of a computerized program (Siemens Syngo Arterial Health Package). To obtain IMT, three end- diastolic frames were selected across a length of 10 mm and analyzed for mean and maximum IMT in end-diastole, and the average reading from

these three frames was calculated. Simultaneous electrocardiogram (ECG) recording ensured an accurate R-wave still frame selection. Systolic and diastolic arterial diameters were acquired at the sites corresponding at the IMT measurements. They were evaluated offline with the same computerized program at end systole and end diastole.

Table 1. Parameters included in the maternal arterial assessment

	Definition	Technique
Structural parameters		
Intima Media Thickness (mm)	Distance between leading edge of the adventitia media interface to the leading edge of intima lumen interface	2-D ultrasound measurement
Systolic lumen diameter (mm)	measurement of arterial lumen during systole	2-D ultrasound measurement
Diastolic lumen diameter (mm)	measurement of arterial lumen during diastole	2-D ultrasound measurement
Arterial functional parameters		
Distensibility ($kPa^{-1}10^{-3}$)	arterial stiffness index	2*(systolic artery diameter-diastolic artery diameter)/ (diastolic artery diameter)*(brachial pulse pressure)
Circumferencial Wall Stress (kPa)	arterial stiffness index	Mean blood pressure * diastolic lumen diameter/2*IMT
Venous functional parameters		
Collapsibility	Vascular distensibility	IVCe-IVCi
Collapsibility Index	Vascular distensibility	IVCe-IVCi/ IVCe

IMT: intima media thickness; brachial pulse pressure: systolic blood pressure- diastolic blood pressure

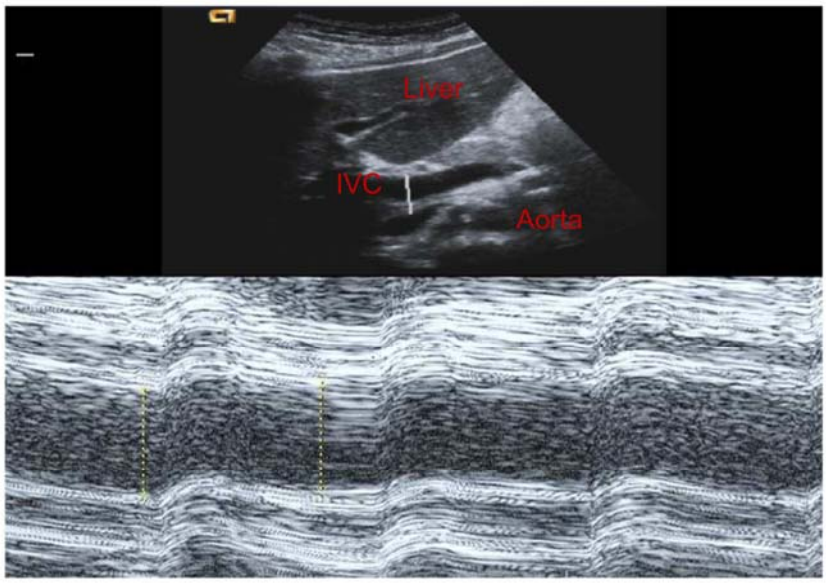
*Mean blood pressure: (2 * diastolic blood pressure+ systolic blood pressure) / 3*

IVC: inferior vena cava; IVCe: IVC during expiration; IVCi :IVC during inspiration

Arterial distensibility was calculated as 2*1000*(systolic carotid artery diameter-diastolic carotid artery diameter)/ (diastolic carotid artery diameter)*(brachial pulse pressure).[94] Pulse pressure denotes the difference between systolic and diastolic blood pressure and was converted from millimeters of mercury to kilopascals (kPa; 1 mm Hg=0.133 kPa). Circumferential Wall Stress was calculated as: CWS = MBP x dD/2xcIMT where MBP denotes mean blood pressure and was also converted from millimeters of mercury to kilopascals, dD diastolic diameter and cIMT carotid intima-media thickness.[76]

IVC diameter changes were evaluated during the respiratory cycle by ultrasound M-Mode as depicted in Figure 2. All examinations were performed in the supine position with 6-MHz curvilinear transducer placed in a subxyphoid plane. Sagittal sections of the upper part of IVC behind the liver were obtained. Respiratory variations of the vessel diameter were evaluated offline during inspiration (IVCi) and expiration (IVCe) with Siemens Syngo Arterial Health Package. The difference between IVCe and IVCi is defined as collapsibility and collapsibility index is the ratio IVCe-IVCi/ IVCe.[81,82,83]

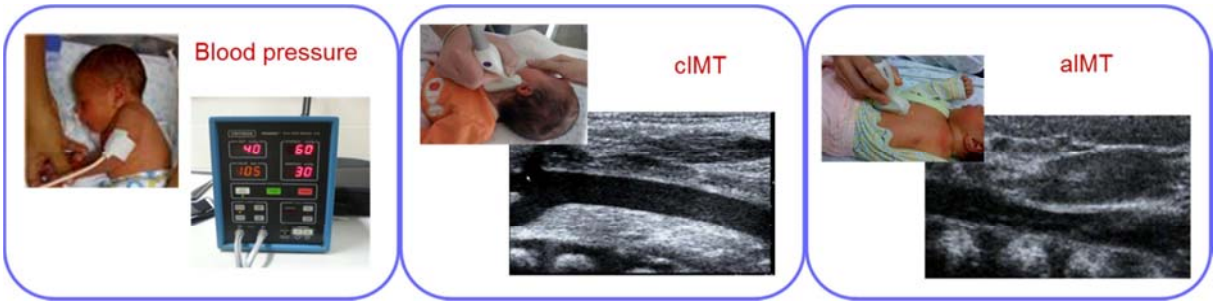
Figure 2.Anatomical relations of inferior vena cava as depicted in 2-D ultrasound. Illustration of the inferior vena cava respiratory variations by ultrasound M-mode. IVC denotes inferior vena cava



4.4 NEONATAL VASCULAR ASSESSMENT

Neonatal vascular assessment included blood pressure measurement and ultrasound assessment of aorta and carotid artery. A controlled environment contributed to the achievement of all neonatal measurements.

Figure 3.Neonatal vascular assessment



Blood pressure was obtained from brachial artery using a validated ambulatory automated Omron 5 Series device. An appropriate cuff size covering the 40 percent of the arm circumference was used to ensure accurate measurement.[95] Each newborn's blood pressure was evaluated twice during quiescence and the average was determined.

Carotid and aorta ultrasound assessment was performed by skilled sonographers using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA). Longitudinal clips of the far wall of both carotid arteries were obtained approximately 1 cm proximal to the bifurcation using a 13-MHz linear-array transducer (Figure 4). Longitudinal clips of the far wall of the proximal abdominal aorta were obtained in the upper abdomen by a 10-MHz linear probe (Figure 5). Carotid and aorta IMT measurements were performed offline according to a standardized protocol based on a trace method with the assistance of a computerized program (Siemens Syngo Arterial Health Package). To obtain IMT, three end-diastolic frames were selected across a length of 10 mm and analyzed for mean and maximum IMT, and the average reading from these three frames was calculated. Systolic and diastolic arterial diameters were acquired at the sites corresponding at the IMT measurements in end-diastole.

Figure 4.Identification of common carotid artery and delineation of intima media thickness in the far arterial wall. B denotes carotid bifurcation, IC internal carotid artery, EC external carotid artery

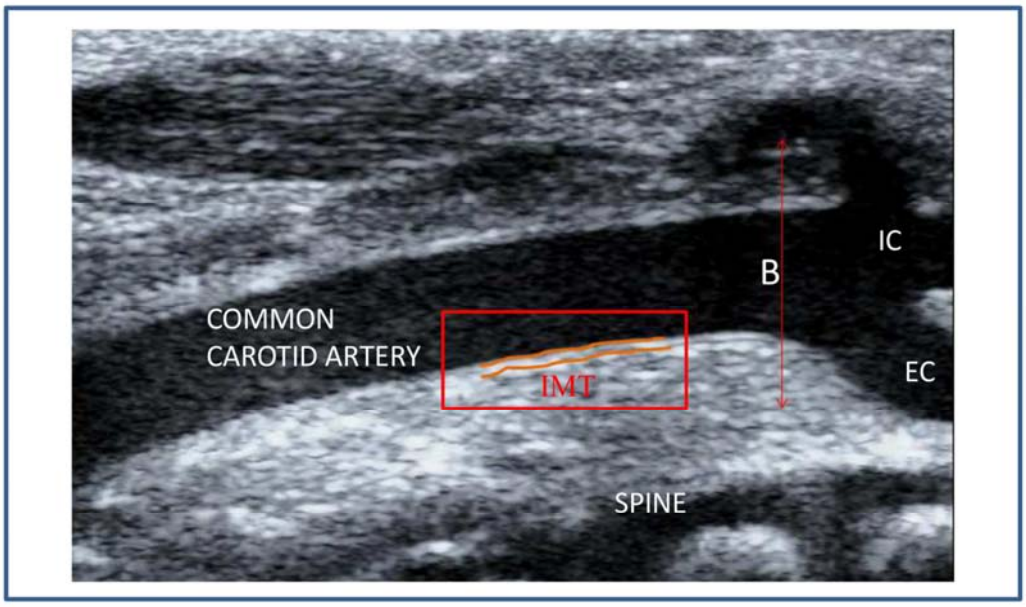


Figure 5.Identification of abdominal aorta and delineation of intima media thickness in the far arterial wall beyond diaphragm. TA denotes thoracic aorta, AA denotes abdominal aorta and D diaphragm

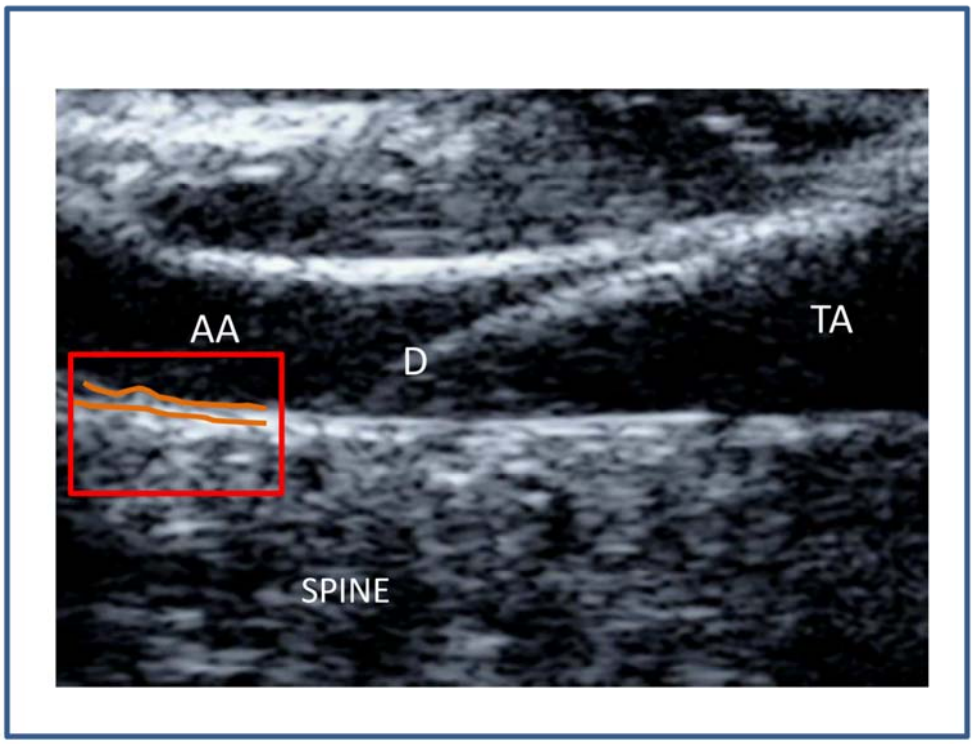


Table 2. Parameters for neonatal arterial assessment

	Definition	Technique
Carotid		
Intima Media Thickness (mm)	Distance between leading edge of the adventitia media interface to the leading edge of intima lumen interface	2-D ultrasound measurement
Intima Media Thickness/ neonatal weight (mm/kg)	Intima media thickness adjusted by neonatal weight	IMT/neonatal weight
Intima Media Thickness / diameter	Intima media thickness adjusted by diameter	IMT/carotid diastolic lumen
Aorta		
Intima Media Thickness (mm)	Distance between leading edge of the adventitia media interface to the leading edge of intima lumen interface	2-D ultrasound measurement
Intima Media Thickness/ neonatal weight (mm/kg)	Intima media thickness adjusted by neonatal weight	IMT/neonatal weight
Intima Media Thickness / diameter	Intima media thickness adjusted by diameter	IMT/aortic diastolic lumen
IMT: intima media thickness		

4.5 VASCULAR WALL THICKNESS REPRODUCIBILITY

Intra-observer variability was determined for neonatal mean and maximum IMT by comparing data from three measurements of the same sonographer. Inter-observer variability for the same parameters was similarly evaluated by comparing data from two sonographers at one occasion.

4.6 STATISTICAL ANALYSIS

The analyses were carried out using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA). All statistical tests were performed at the 2-sided 5% level of significance. Normality was evaluated by the Shapiro-Wilk test. Results are presented as median (interquartile range) or as percentage after checking parametric assumptions by Kolmogorov-Smirnov test. One way analysis of variance based on log transformed data adjusted with

Bonferroni post-hoc test and Pearson chi square were used to compare quantitative and qualitative data respectively. Feto-placental Doppler ultrasound measurements were converted into Z-scores.[92,96,97]

4.6.1 Statistical analysis in study 1

Sample size was calculated for detecting a 10% difference in maternal cIMT. For a 90% power and 5% type I error level, the estimated sample size was 41 women per study group and therefore we finally decided to include 50 pregnant women per group. Results are presented as median (interquartile range) or as percentage. Comparisons of vascular parameters among study groups were performed by regression analysis adjusting by maternal age, maternal body mass index and gestational age at evaluation. Additionally, polynomial orthogonal contrasts were constructed in order to test for linear trends of parameters across severity groups. Finally, in order to assess potential confounders, data was analyzed after excluding smokers and chronic hypertensive cases

4.6.2 Statistical analysis in study 2

Sample size was calculated for detecting a 15% difference in carotid IMT.30 For an 80% power and 5% type I error level, the estimated sample size was 20 women per study group and therefore we finally included at least 20 pregnant women per group. Comparisons of vascular parameters among study groups were performed by multiple linear regression analysis adjusting by maternal age, maternal body mass index maternal smoking and gestational age at evaluation.

4.6.3 Statistical analysis in study 3

The intra-observer reproducibility was assessed by calculating the intra class correlation coefficients and coefficients of variation (CV). The inter-observer reproducibility was assessed by calculating the CV for each parameter.[98]

4.6.4 Statistical analysis in study 4

Sample size was calculated taking into account comparison between control and SGA neonates both with and without signs of severity. We used balanced ANOVA type of analysis for 2 factors and 3 groups, considering standard deviation (SD) of 0.08 mm[15] and detectable contrast of 0.07 for both factors (cIMT and aIMT). An estimated sample size of 32 women per study group was achieved for a power of more than 90% and 5% type I error level.[99]

5. STUDIES

5.1 STUDY 1

Patterns of maternal vascular remodeling and responsiveness in early versus late onset preeclampsia

Stergiotou I, Crispi F, Valenzuela-Alcaraz B, Bijnens B, Gratacos E.

Am J Obstet Gynecol 2013;209:558.e1-558.e14.

Impact factor: 3.877

OBSTETRICS

Patterns of maternal vascular remodeling and responsiveness in early- versus late-onset preeclampsia

Iosifina Stergiotou, MD; Fatima Crispi, MD, PhD; Brenda Valenzuela-Alcaraz, MD; Bart Bijnens, MD, PhD; Eduard Gratacos, MD, PhD

OBJECTIVE: We sought to assess vascular structure and function in early- and late-onset preeclampsia (PE) at the time of diagnosis.

STUDY DESIGN: We evaluated 100 PE cases subdivided into 50 early- and 50 late-onset cases according to gestational age at onset (</>34 weeks), and 100 controls paired by maternal age and gestational age at scan with cases. Carotid intima-media thickness (IMT), distensibility, and circumferential wall stress together with inferior vena cava (IVC) collapsibility were assessed by ultrasound.

RESULTS: Early PE was characterized by increased carotid IMT diameters, and arterial stiffness with no significant changes in IVC parameters as compared to normotensive pregnancies. Late PE was characterized by significantly increased carotid IMT and lumen diameters as compared to controls while arterial stiffness, as expressed by distensibility, did not provide pronounced changes. A significant decrease of IVC collapsibility index was also observed in late PE as compared to controls.

CONCLUSION: The current data suggest that distinct vascular adaptations in early and late PE could reflect different pathophysiologic mechanisms. Future studies are warranted to further assess the complex etiologies and clinical expressions of the 2 entities.

Key words: carotid intima-media thickness, circumferential wall stress, distensibility, inferior vena cava collapsibility, preeclampsia

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Preeclampsia (PE) is a multisystem disorder complicating 5-10% of pregnancies and a leading cause of maternal mortality and morbidity.[1] A substantial body of literature has elucidated in recent years that PE is a cardiovascular risk factor, predictive of subsequent cardiovascular disease and death.[2-5] Two recent metaanalyses established a 3-fold risk for hypertension and a 2-fold risk of ischemic heart disease and stroke in women with a history of PE.[2,3] The positive association between PE and later-life cardiovascular disease has been attributed to shared cardiovascular risk factors.[6-9] Cardiovascular morbidity is currently assessed by carotid intima-media thickness[10] (IMT) and arterial stiffness indices such as pulse wave velocity, augmentation index, distensibility, and circumferential wall stress (CWS).[11-13] Additionally, inferior vena cava (IVC) is a compliant vein reflecting venous responsiveness to hemodynamic changes.[14-16] Evaluation of these parameters in preeclamptic women has provided conflicting results, which could possibly be explained by the heterogeneity of PE syndrome.[17-24]

Recent data have supported that classifying PE into early- and late-onset disease differentiates 2 distinct clinical forms with pathophysiological specific features. Thus, early PE is commonly associated with placental insufficiency, intrauterine growth restriction, and adverse maternal and perinatal outcomes.[25-27] Conversely, late-onset PE is associated with minor placental involvement and milder clinical

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disease.[25–27] Intrinsic placental factors are more frequently altered in early PE[25,26] while late PE is usually associated with predisposing maternal factors.[28]

In this study, we hypothesized that early and late PE present distinct types of vascular structural and functional changes already at the time of diagnosis. Thus, we sought to comprehensively describe carotid IMT, carotid distensibility, CWS, and IVC collapsibility in normotensive, early-onset preeclamptic, and late-onset preeclamptic women to explore the existence of these differences.

Materials and Methods

Study population

This prospective cohort study included 100 normotensive and 100 preeclamptic pregnancies. PE was defined according to the International Society for the Study of the Hypertension in Pregnancy as a resting blood pressure of ≥140/90 mm Hg on 2 occasions at least 4 hours apart and proteinuria of ≥300 mg/L or a 2+ urine dipstick >20 weeks of gestation in a previously normotensive woman.[29] Further, the affected pregnancies were subdivided into: (1) 50 cases of early-onset PE defined as PE occurring <34th week of gestation; and (2) 50 cases of late-onset PE occurring >34th week of gestation.

The pregnant women with PE were recruited and evaluated within ≥24 hours of diagnosis while admitted to the Department of Obstetrics at the Hospital Clinic, University of Barcelona. All were receiving intravenous fluids and anticonvulsive and/or antihypertensive medication. Women without confirmed history of PE, diabetes mellitus, and renal and connective tissue disease were included in the study. Smokers and women with history of chronic hypertension were considered initially eligible. Women were considered as having chronic hypertension if hypertension (blood pressure of ≥140/90 mm Hg) predated pregnancy or developed <20th week of pregnancy.[29]

The normotensive group comprised women attending antenatal clinic with uncomplicated pregnancies matched 1 to 1 by maternal age and gestational age at exploration to preeclamptic women. Only healthy nonsmoking women, not affected by current or anterior pregnancy complications or intrauterine growth restriction, were included in the control group.

Information on maternal demographic characteristics, reproductive history, and current pregnancy clinical data was obtained in the interview at the day of the scan and through medical history notes. Fetoplacental Doppler examination included uterine arteries, umbilical artery, and middle cerebral artery. Cerebroplacental ratio was calculated as a simple ratio of the middle cerebral artery pulsatility index divided by the umbilical artery pulsatility index.[30] Pregnancy outcome such as gestational age at delivery, mode of delivery, birthweight, birthweight centile, Apgar score, and umbilical pH were recorded a posteriori. In all pregnancies gestational age was calculated based on the crown-rump length at first-trimester ultrasound.[31] Fetal and neonatal weight centile were calculated according to local reference curves.[32] Ethics approval was received by the local research ethics committee and written informed consent form was obtained from patients.

Vascular assessment

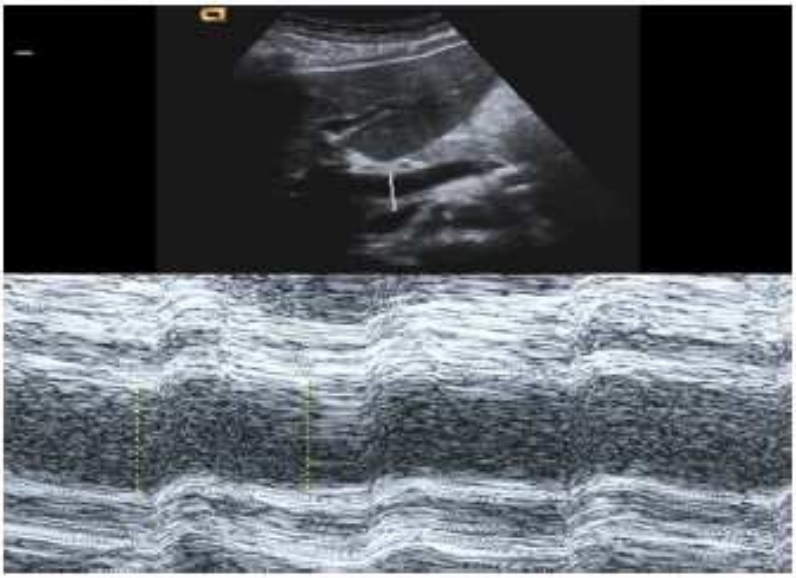
The study was designed to evaluate several components of vascular structure and function. Remodeling is defined as the modification of arterial shape and size (IMT and lumen diameters) to adapt to hemodynamic changes.[33] Arterial distensibility and CWS are measures of arterial stiffness and consequently of arterial function. IVC collapsibility is defined as venous distensibility in response to hemodynamic changes. And finally, "responsiveness" is considered a generic term applied to both arterial and venous structural and functional changes.

The exploration was performed in a quiet environment with the pregnant women in a supine position with slight hyperextension and rotation of the neck in an opposite direction to the probe. The assessment protocol included initially blood pressure measurement by a validated ambulatory automated Omron 5 Series device (Omron Healthcare, Kyoto, Japan). Carotid ultrasound assessment was subsequently performed by a single experienced investigator using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA). Longitudinal clips of the far wall of both carotid arteries were obtained approximately 1 cm proximal to the bifurcation using a 13-MHz linear-array transducer. Carotid IMT measurements were performed offline according to a standardized protocol based on a trace method with the assistance of a computerized program (Siemens Syngo Arterial Health Package; Siemens Medical Systems). To obtain IMT, 3 end-diastolic frames were selected across a length of 10 mm and analyzed for mean and maximum IMT in end-diastole, and the average reading from these 3 frames was calculated. Simultaneous electrocardiogram recording ensured an accurate R-wave still-frame selection. Systolic and diastolic arterial diameters were acquired at the sites corresponding at the IMT measurements. They were evaluated offline with the same computerized program at end-systole and end-diastole.

Arterial distensibility was calculated as: 2 × 1000 × (systolic carotid artery diameter − diastolic carotid artery diameter)/ (diastolic carotid artery diameter) × (brachial pulse pressure).[34] Pulse pressure denotes the difference between systolic and diastolic blood pressure and was converted from millimeters of mercury to kilopascals (kPa; 1 mm Hg = 0.133 kPa). CWS was calculated as: CWS = MBP × dD/2 × carotid IMT, where MBP denotes mean blood pressure and was also converted from millimeters of mercury to kilopascals, and dD denotes diastolic diameter.[12]

IVC diameter changes were evaluated during the respiratory cycle by ultrasound M-mode as depicted in Figure 1. All examinations were performed in the supine position with 6-MHz curvilinear transducer placed in a subxyphoid plane. Sagittal sections of the upper part of IVC behind the liver were obtained. Respiratory variations of the vessel diameter were evaluated offline during inspiration (IVCi) and expiration (IVCe) with Siemens Syngo Arterial Health Package. The difference between IVCe and IVCi is defined as collapsibility and collapsibility index is the ratio IVCe − IVCi/IVCe.[14–16]

FIGURE 1
Inferior vena cava respiratory variations by ultrasound M-mode



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Statistical analysis

The analyses were carried out using a statistical package (SPSS, version 17; IBM Corp, Armonk, NY). All statistical tests were performed at the 2-sided 5% level of significance. Sample size was calculated for detecting a 10% difference in carotid IMT. For a 90% power and 5% type I error level, the estimated sample size was 41 women per study group and therefore we finally decided to include 50 pregnant women per group. Results are presented as median (interquartile range) or as percentage and fetoplacental Doppler ultrasound measurements were converted into z-scores.[30,35,36] One-way analysis of variance based on log-transformed data adjusted with Bonferroni post hoc test and Pearson χ^2 were used to compare quantitative and qualitative data, respectively. Comparisons of vascular parameters among study groups were performed by regression analysis adjusting by maternal age, maternal body mass index, and gestational age at evaluation. Additionally, polynomial orthogonal contrasts were constructed to test for linear trends of parameters across severity groups. Finally, to assess potential confounders, data were analyzed after excluding smokers and chronic hypertensive cases (Appendix; Supplementary data).

RESULTS

Study populations

Clinical and perinatal data are presented in Table 1. Maternal characteristics were similar among the study groups regarding maternal age, ethnicity, and hematocrit, which is major factor of blood viscosity. However, there was a cluster of traditional risk factors that were found to be increased in the preeclamptic study groups. Body mass index was significantly increased in the late PE group and both systolic and diastolic first-trimester blood pressures were higher in preeclamptic groups. Furthermore, there was an increased prevalence of nulliparity, smoking, chronic hypertension, previous PE, and proteinuria in both preeclamptic groups as compared to controls. As expected, cases, mainly women with early PE, had worse fetoplacental Doppler and perinatal outcomes as compared to controls.

Vascular assessment

Results on vascular parameters are shown in Table 2 and Figure 2. By definition, blood pressure values were significantly increased in preeclamptic groups as compared to normotensive controls.

Mean carotid IMT did not differ significantly between early preeclamptic and normotensive women but it was significantly increased in late PE as compared to controls (mean carotid IMT: 0.015 mm [SE 0.010] per early PE, $P = .142$ and mean carotid IMT: 0.037 mm [SE 0.012] per late PE, $P = .002$, respectively). There was a significant linear tendency for higher mean carotid IMT values across groups ($P < .001$). Maximum carotid IMT was significantly increased in both early and late preeclamptic cases as compared to controls ($P = .01$ and $P < .01$, respectively). Similarly to mean carotid IMT, a linear trend for higher values across groups was observed ($P < .001$). Regarding lumen systolic and diastolic diameters, we noted significantly increased diameters in both early and late PE as compared to normotensive subjects. Furthermore, both systolic and diastolic diameters were significantly increased in early PE as compared to late PE even after adjusting by maternal age, maternal body mass index, and gestational age at evaluation ($P = .035$ and $P < .001$, respectively). Regarding arterial stiffness, carotid distensibility was reduced significantly in early PE when compared to controls ($P = .013$), while this reduction did not reach statistical significance in late PE ($P = .262$). However, a significant linear trend demonstrated differences among groups ($P = .008$). CWS was significantly increased in both preeclamptic groups as compared to controls (both early and late PE $P < .001$). Similarly to arterial distensibility, it showed a linear trend for differences across study groups ($P < .001$).

Results on IVC respiratory variation are also presented in Table 2. Although the vessel diameters were similar among study groups during IVCi and IVCe, there was a significant decrease in collapsibility and collapsibility index in late as compared to early PE ($P = .044$

TABLE 1
Baseline characteristics and perinatal outcomes of study groups

Characteristic	Control	Early PE	Late PE	P value[b]
n	100	50	50	
Maternal characteristics				
Maternal age, y	32.0 (6)	34.5 (9)	34.0 (9)	.708
Body mass index, kg/m^2	23.1 (4.1)	23.7 (6.4)	25.1 (7.4)[a]	.014
Nulliparity, n (%)	39 (39)	38 (76)[a]	35 (70.0)[a]	.028
White ethnicity, n (%)	62 (62)	31 (62)	20 (40.0)	.163
Chronic hypertension, n (%)	0 (0)	9 (18)[a]	8 (16)[a]	.001
Previous PE, n (%)	0 (0)	7 (14)[a]	4 (8)	.059
Smoking, n (%)	0 (0)	9 (18)[a]	4 (8)[a]	< .001
First-trimester systolic BP, mm Hg	110 (18)	120 (26)[a]	120 (18)[a]	< .001
First-trimester diastolic BP, mm Hg	65 (13)	80 (20)[a]	80 (15)[a]	< .001
First-trimester mean BP, mm Hg	80 (13)	93 (23)[a]	93 (16)[a]	< .001
Proteinuria, mg/24 h	—	2093 (3376)	906 (1362)	< .001
Hematocrit, n (%)	35 (4)	33 (5)	34 (5)	.035
Fetal ultrasound				
Gestational age at fetal scan, wk	33.4 (1.5)	31.4 (3.6)[a]	35.2 (2.4)	< .001
Uterine artery mean PI, z-scores	−0.56 (0.30)	3.18 (2.05)[a]	2.63 (2.50)[a]	.002
Umbilical artery PI, z-scores	−0.33 (0.72)	0.52 (2.19)[a]	0.50 (0.57)	.023
Middle cerebral artery PI, z-scores	0.12 (1.31)	−0.96 (1.97)[a]	−0.20 (2.22)	.026
Cerebroplacental ratio, z-scores	0.37 (0.04)	−1.72 (3.08)[a]	−1.09 (2.31)[a]	.001
Delivery data				
Gestational age at diagnosis, wk	—	30.0 (3)	36.2 (3)	< .001
Gestational age at delivery, wk	39.3 (2.5)	32 (2.4)[a]	37.1 (2.7)[a]	< .001
Cesarean section, n (%)	16 (16)	47 (94)[a]	32 (64)[a]	< .001
Male sex, n (%)	28 (28)	24 (48)	17 (34)	.404
Birthweight, g	3580 (1250)	1405 (697)[a]	2095 (682)[a]	< .001
Birthweight percentile	50 (7)	1 (7)[a]	17 (47)[a]	< .001
5-min Apgar score	10 (0)	8 (2)[a]	10 (0)	< .001
Umbilical artery pH	7.25 (0.08)	7.23 (0.11)	7.26 (0.10)	.130
Days in NICU, n (%)	2 (2)	47 (94)[a]	14 (28)[a]	< .001

Data are presented as median (interquartile range) or percentage (n). All Doppler parameters are presented as z-scores.

BP, blood pressure; *NICU*, neonatal intensive care unit; *PE*, preeclampsia; *PI*, pulsatility index.

[a] $P < .05$ as compared to controls; [b] *P* value calculated by 1-way analysis of variance or Pearson χ^2 test.

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and $P = .043$, respectively). Furthermore, late PE was characterized by a decrease in collapsibility index as compared to controls ($P = .029$). A significant linear decrease was observed across study groups ($P = .026$).

Finally, to assess potential confounders, data were analyzed after excluding smokers and chronic hypertensive cases, showing similar results for early and late PE in most parameters (Supplementary data).

COMMENT

This study provides evidence that maternal vascular characteristics of preeclamptic women differ between early- and late-onset PE, possibly due to the distinct pathogenesis of the 2 entities.

TABLE 2
Results on maternal vascular assessment among study groups

Characteristic	Control	Early PE	Late PE	*P* value[c]
n	100	50	50	
Gestational age at maternal evaluation, wk	34.1 (4)	31.0 (4)[a]	36.5 (3)[a]	< .001
BP				
Systolic BP at examination, mm Hg	110 (13)	145 (15)[a]	143 (15)[a]	< .001
Diastolic BP at examination, mm Hg	70 (11)	90 (11)[a]	90 (11)[a]	< .001
Mean BP at examination, mm Hg	83 (11)	109 (13)[a]	108 (11)[a]	< .001
Carotid arteries				
Mean cIMT, mm	0.408 (0.06)	0.425 (0.09)	0.439 (0.08)[a]	< .001
Maximum cIMT, mm	0.505 (0.07)	0.530 (0.08)[a]	0.558 (0.08)[a]	< .001
Carotid systolic diameter, mm	5.950 (0.600)	7.100 (0.750)[a]	6.525 (0.720)[a,b]	< .001
Carotid diastolic diameter, mm	5.450 (0.630)	6.500 (0.850)[a]	5.975 (0.740)[a,b]	< .001
Carotid distensibility, $kPa^{-1}10^{-3}$	32.14 (15.06)	23.85 (14.75)[a]	27.80 (21.53)	.008
Circumferential wall stress, kPa	12.470 (2.99)	20.183 (4.85)[a]	18.625 (5.81)[a]	< .001
Inferior vena cava				
Diameter at inspiration, mm	8.750 (5.78)	9.550 (2.55)	10.000 (1.80)	.207
Diameter at expiration, mm	9.950 (5.38)	10.600 (2.68)	10.750 (1.55)	.315
Collapsibility, mm	0.950 (0.50)	0.900 (0.83)	0.700 (0.53)[b]	.082
Collapsibility index, n (%)	11.0 (7)	9.4 (6)	6.6 (4)[a,b]	.026

Data are presented as median (interquartile range).

BP, blood pressure; *cIMT*, carotid intima media thickness; *PE*, preeclampsia.

[a] $P < .05$ as compared to controls calculated by regression adjusted by maternal age, maternal body mass index, gestational age at evaluation, and group; [b] $P < .05$ as compared to early PE calculated by regression adjusted by maternal age, maternal body mass index, gestational age at evaluation, and group; [c] *P* calculated by linear trends across severity groups.

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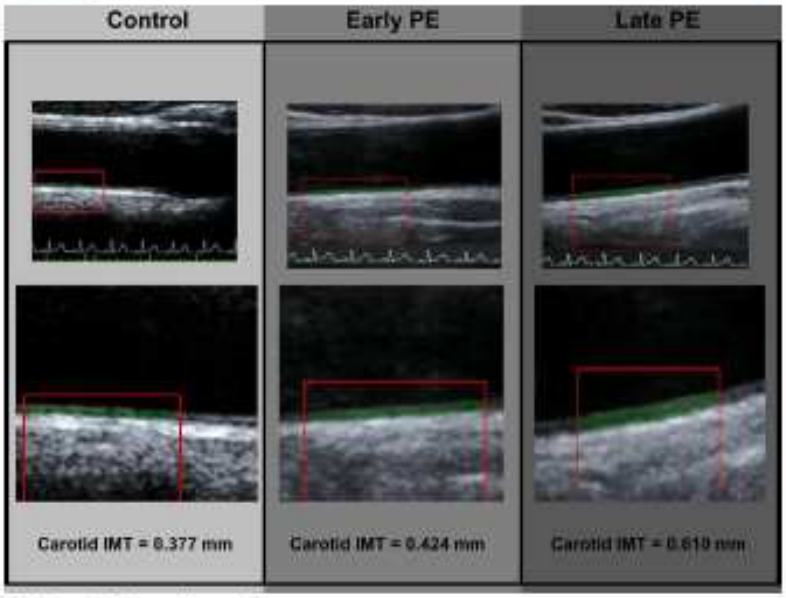
Early PE was characterized by increased carotid IMT, lumen diameters and arterial stiffness, but with no significant changes in IVC collapsibility as compared to normotensive pregnancies. The increase in carotid IMT and arterial stiffness is consistent with previous studies.[17-23] The release of factors from an underperfused placenta in early PE[25-28] may cause vascular dysfunction and high blood pressure. Enlarged IMT could possibly represent an adaptive response to preserve the arterial wall stress.[17] Furthermore, disturbances in endothelial function may affect arterial elasticity and consequently enhance stiffness.[22] Alternatively, high blood pressure is considered a predisposing factor for increased arterial stiffness.[21,22] In accordance to recently published data,[20] lumen diameters in early PE are significantly increased as compared to controls. The interpretation of this finding is challenging as lumen diameter depends on the complex interaction of parameters such as cardiac output, blood volume, heart rate, and vessel diameter. Interestingly, cardiac output was found to be low at midgestation in untreated women destined to develop early PE[38,39] but normalized after treatment.[39] Thus, the significant increase of cardiac output in early PE after initiation of treatment could possibly explain our observation. To evaluate venous functioning in PE, we assessed IVC collapsibility. Despite previous evidence of reduced venous distensibility in the forearm in early PE,[40] IVC collapsibility was similar as compared with control pregnancies. Venous distensibility differs in distinct vascular beds[41] and our finding may be explained by the lack of history of maternal vascular maladaptation. In any case, the 9-fold increase in cardiovascular mortality[4] in early preeclamptic women could be possibly attributed to the cardiovascular changes observed in these patients.[23]

On the other hand, late PE was characterized by more prominent carotid IMT but by less pronounced changes in lumen diameters and arterial stiffness as compared to early PE. A significant decrease of IVC collapsibility was also observed in late PE. Carotid IMT was expected to present comparable values in early and late PE due to observed similar blood pressure. However, mean carotid IMT was found to be increased in late PE. This finding suggests that an earlier vascular impairment, possibly due to maternal predisposition, has occurred. In accordance to previous data,[21,42] and despite similar blood viscosity characteristics such as hematocrit,[43] arterial

FIGURE 2
Carotid IMT measurement by ultrasound in control and in early and late PE subjects



IMT, intima-media thickness; *PE*, preeclamptic.
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stiffness was increased in late PE but to a lesser degree as compared to early PE, since a significant linear tendency for both parameters was observed. Interestingly, it has been previously described that arterial stiffness could be similar or even decreased in hypertensive as compared to normotensive patients.[12] The hypothesis that timely arterial hypertrophy could favor a "structural" increase in compliance by decreasing the relative amount of connective tissue has been postulated.[12] Decreased collapsibility index in late PE, suggesting reduced venous reserve capacity is in support of the concept of maternal preexisting vascular maladaptation. Regarding arterial diameters, less prominent increase in carotid diameters in late PE is concordant with milder changes in cardiac output as compared to early PE.[44] In this context, the development of less prominent vascular disorders in late PE as compared with early PE could provide support to the concept of a milder (2-fold) increased risk for cardiovascular disease.

Our data are in agreement with current notions suggesting differences in the etiology and pathogenesis of early and late PE. Early PE is considered essentially a placental-mediated disease, where the role of maternal predisposition is much lower in comparison with late PE. In early PE, the release of factors from the hypoxic placenta may initiate a sequence of endothelial dysfunction and damage. A lower prevalence of subclinical previous disease in this subset of patients with PE is supported by our findings of a lower carotid IMT. Furthermore, the lower arterial susceptibility in early onset PE as found in this study also supports that the vascular insult is of a more acute nature in these patients. Increased arterial stiffness may exacerbate the systolic portion of pressure wave leading to severe systolic and diastolic dysfunction and it could be one of the mechanisms to explain increased severity of hypertensive disease in early PE.[21,45] Conversely, late PE is most probably associated with maternal constitutional factors and a lower placental involvement.[25–28] Our finding of a linear tendency for different stiffness might reflect differences in the severity of disease. However, it could as well reflect changes due to a higher prevalence of chronic clinical or subclinical vascular disease in women with late PE. If this was so, preexisting susceptibility for cardiovascular disease might explain a better maternal adaptation and therefore milder vascular disease resulting in lower distensibility index. Thus, our data support that maternal hemodynamic responsiveness might be better preserved in late PE, which is in line with the relatively better cardiovascular outcome as compared with early PE.

The strength of the present study was mainly to evaluate for the first time vascular structure and function in preeclamptic women at diagnosis differentiating between early and late PE. We acknowledge that our study has limitations due to the limited approach of cardiovascular physiology. It is evident that understanding the mechanism of circulatory adaptations in preeclamptic pregnancies implies considering all aspects of cardiac, arterial, and venous structure and function. Furthermore, we acknowledge that our sample size could not have been sufficient enough to demonstrate statistical significant results for all parameters, despite performing a power calculation. The inclusion of cases of preeclamptic women with history of smoking and chronic hypertension could raise concerns due to their potential confounding influence. However, we do not believe that is the case, as most parameters show similar results in early- and late-onset PE even after excluding smokers and chronic hypertensive patients (data shown in Appendix). It would have been also interesting to include preconceptional and postpartum evaluation of our sample. As PE is now considered a cardiovascular risk factor, further investigation on its implications is mandatory.

In summary, the current data suggest that distinct vascular adaptations in early and late PE reflect different etiologies and clinical expressions. Future studies are warranted to assess the complex pathophysiologic mechanisms of PE and the potential utility of our measurements

to select mothers at higher risk who may benefit from timely diagnosis and treatment. ■

REFERENCES

1. World Health Organization Collaboration. WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia 2011. Department of Reproductive Health and Research. Available at: http://www.who.int/maternal_child_adolescent/documents/pre-eclampsia_eclampsia/en/. Accessed June 18, 2013.
2. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ 2007;335:974-7.
3. McDonald SD, Malinowski A, Zhou Q, Yusuf S, Devereaux PJ. Cardiovascular sequelae of preeclampsia/eclampsia: a systematic review and meta-analyses. Am Heart J 2008;156:918-30.
4. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort. Hypertension 2010;56:166-71.
5. Valdivieso C, Garovic VD, Ouyang P. Preeclampsia and hypertensive disease in pregnancy: their contributions to cardiovascular risk. Clin Cardiol 2012;35:60-5.
6. Roberts JM, Hubel CA. Pregnancy: a screening test for later life cardiovascular disease. Womens Health Issues 2010;20:304-7.
7. Magnussen EB, Vatten LJ, Lund-Nilsen TI, Salvesen KA, Davey Smith G, Romundstad PR. Prepregnancy cardiovascular risk factors as predictors of pre-eclampsia: population-based cohort study. BMJ 2007;335:978-81.
8. Romundstad PR, Magnussen EB, Smith GD, Vatten LJ. Hypertension in pregnancy and later cardiovascular risk: common antecedents? Circulation 2010;122:579-84.
9. Haville EW, Viikari JS, Raitakari OT. Preconception cardiovascular risk factors and pregnancy outcome. Epidemiology 2011;22:724-30.
10. Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography carotid intima-media thickness task force, endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008;21:93-111.
11. Boutouyrie P, Tropeano AI, Asmar R, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;39:10-5.
12. Laurent S, Girerd X, Mourad JJ, et al. Elastic modulus of the radial artery wall material is not increased in patients with essential hypertension. Arterioscler Thromb 1994;14:1223-31.
13. Laurent S, Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. Hypertension 2007;49:1202-6.
14. Feissel M, Michard F, Faller JP, Teboul JL. The respiratory variation in inferior vena cava diameter as a guide to fluid therapy. Intensive Care Med 2004;30:1834-7.
15. Sefidbakht S, Assadsangabi R, Abbasi HR, Nabavizadeh A. Sonographic measurement of the inferior vena cava as a predictor of shock in trauma patients. Emerg Radiol 2007;14:181-5.
16. Dipti A, Soucy Z, Surana A, Chandra S. Role of inferior vena cava diameter in assessment of volume status: a meta-analysis. Am J Emerg Med 2012;30:1414-9.
17. Blaauw J, Van Pampus MG, Van Doormaal JJ, et al. Increased intima-media thickness after early onset preeclampsia. Obstet Gynecol 2006;107:1345.
18. Haukkamaa L, Moilanen L, Kattainen A, et al. Pre-eclampsia is a risk factor of carotid artery atherosclerosis. Cerebrovasc Dis 2009;27:599-607.
19. Goynumer G, Yucel N, Adali E, Tan T, Baskent E, Keskag C. Vascular risk in women with a history of severe preeclampsia. J Clin Ultrasound 2013;41:145-50.
20. Yuan LJ, Xue D, Duan YY, Cao TS, Yang HG, Zhou N. Carotid intima-media thickness and arterial stiffness in preeclampsia by analysis with a radio-frequency ultrasound technique. Ultrasound Obstet Gynecol 2013 [Epub ahead of print] doi: 10.1002/uog.12409. http://onlinelibrary.wiley.com/doi/10.1002/uog.12409/pdf.
21. Khalil A, Jauniaux E, Harrington K. Antihypertensive therapy and central hemodynamics in women with hypertensive disorders in pregnancy. Obstet Gynecol 2009;113:846-54.
22. Tihtonen KM, Kööbi T, Uotila JT. Arterial stiffness in preeclamptic and chronic hypertensive pregnancies. Eur J Obstet Gynecol Reprod Biol 2008;139:180-6.
23. Yinon Y, Kingdom JC, Odutayo A, et al. Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: insights into future vascular risk. Circulation 2010;122:1846-53.
24. McDonald SD, Ray J, Tao K, et al. Measures of cardiovascular risk and subclinical atherosclerosis in a cohort of women with a remote history of preeclampsia. Atherosclerosis 2013;229:234-9.
25. Crispi F, Dominguez C, Llurba E, Martin-Gallán P, Cabero L, Gratacós E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in preeclampsia and in isolated intrauterine growth restriction. Am J Obstet Gynecol 2006;195:201-7.
26. Crispi F, Llurba E, Dominguez C, Martin-Gallán P, Cabero L, Gratacós E. Predictive value of angiogenic factors and uterine artery Doppler for early- versus late-onset pre-eclampsia and intrauterine growth restriction. Ultrasound Obstet Gynecol 2008;31:303-9.
27. Valensise H, Vasapollo B, Gagliardi G, Novelli GP. Early and late preeclampsia: two different maternal hemodynamic states in the latent phase of the disease. Hypertension 2008;52:873-80.
28. Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. Hypertension 2008;51:970-5.
29. National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. Am J Obstet Gynecol 2000;183:S1-22.
30. Baschat AA, Gembruch U. The cerebroplacental Doppler ratio revisited. Ultrasound Obstet Gynecol 2003;21:124-7.
31. Robinson HP, Sweet EM, Adam AH. The accuracy of radiological estimates of gestational age using early fetal crown-rump length measurements by ultrasound as a basis for comparison. Br J Obstet Gynaecol 1979;86:525-8.
32. Figueras F, Meler E, Iraola A, et al. Customized birthweight standards for a Spanish population. Eur J Obstet Gynecol Reprod Biol 2008;136:20-4.
33. Berk BC, Korshunov VA. Genetic determinants of vascular remodeling. Can J Cardiol 2006;22(Suppl):6-11B.
34. Stefanadis C, Stratos C, Boudoulas H, Kourouklis C, Toutouzas P. Distensibility of the ascending aorta: comparison of invasive and non-invasive techniques in healthy men and in men with coronary artery disease. Eur Heart J 1990;11:990-6.
35. Arduini D, Rizzo G. Normal values of pulsatility index from fetal vessels: a cross-sectional study on 1556 healthy fetuses. J Perinat Med 1990;18:165-72.
36. Gómez O, Figueras F, Fernández S, et al. Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation. Ultrasound Obstet Gynecol 2008;32:128-32.
37. Bots ML, Hofman A, Grobbee DE. Increased common carotid intima-media thickness: adaptive response or a reflection of atherosclerosis? Findings from the Rotterdam study. Stroke 1997;28:2442-7.
38. Melchiorre K, Sutherland G, Sharma R, Nanni M, Thilaganathan B. Mid-gestational maternal cardiovascular profile in preterm and term pre-eclampsia: a prospective study. BJOG 2013;120:496-504.
39. Visser W, Wallenburg HC. Central hemodynamic observations in untreated preeclamptic patients. Hypertension 1991;17:1072-7.
40. Sakai K, Imaizumi T, Maeda H, et al. Venous distensibility during pregnancy: comparisons between normal pregnancy and preeclampsia. Hypertension 1994;24:461-6.
41. Gyselaers W. Hemodynamics of the maternal venous compartment: a new area to explore in obstetric ultrasound imaging. Ultrasound Obstet Gynecol 2008;32:716-7.
42. Kalhura C, Savvidou MD, Anderson JM, McEniery CM, Nicolaides KH. Maternal arterial stiffness in pregnancies affected by preeclampsia. Am J Physiol Heart Circ Physiol 2009;297:H759-64.

43. Shen TW, Wang CH, Lai YH, Hsu BG, Liou HH, Fang TC. Use of cardio-ankle vascular index in chronic dialysis patients. Eur J Clin Invest 2011;41:45-51.
44. Melchiorre K, Sutherland GR, Baltabaeva A, Liberati M, Thilaganathan B. Maternal cardiac dysfunction and remodeling in women with pre-eclampsia at term. Hypertension 2011;57:85-93.
45. Melchiorre K, Sutherland GR, Watt-Coote I, Liberati M, Thilaganathan B. Severe myocardial impairment and chamber dysfunction in preterm pre-eclampsia. Hypertens Pregnancy 2012;31:454-71.

5.2 STUDY 2

Maternal subclinical vascular changes in fetal growth restriction with and without preeclampsia.

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MATERNAL SUBCLINICAL VASCULAR CHANGES IN FETAL GROWTH RESTRICTION WITH AND WITHOUT PREECLAMPSIA

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CONDENSATION

Normotensive FGR presents similar changes in maternal vascular thickness as compared to PE possibly due to a common placental etiology

SHORT VERSION OF TITLE

Maternal vascular remodeling in fetal growth restriction and preeclampsia.

ABSTRACT

Objective: To assess maternal vascular structure and function in fetal growth restriction (FGR) pregnancies with and without preeclampsia (PE) at the time of diagnosis.

Study design: We evaluated 124 pregnant women with FGR subdivided in 60 FGR with and 64 FGR without PE, together with 110 controls. The group of FGR without PE was further subdivided according to the gestational age at diagnosis into early (< 32 weeks) or late-onset (≥32 weeks). Maternal carotid intima-media thickness (IMT), blood pressure (BP), carotid artery distensibility (CAD) and circumferential wall stress (CWS), and inferior vena cava (IVC) collapsibility were assessed by ultrasound at the time of FGR diagnosis.

Results:

FGR cases with PE showed increased carotid IMT, blood pressure and CWS, together with reduced CAD and IVC collapsibility. FGR without PE had as a whole increased IMT and blood pressure, but similar CAD and ICV collapsibility to controls. When subclassified according to gestational age, only early-onset FGR had significantly increased IMT and CWS, but both groups had increased BP.

Conclusion: FGR without PE shares some subclinical vascular features with PE, which further reinforces the notion that, at least in a proportion of cases, there is a common placental disease that influences maternal cardiovascular features.

KEYWORDS: Carotidintima media thickness; fetal growth restriction; preeclampsia; vascular remodeling.

INTRODUCTION

Fetal growth restriction (FGR) refers to the failure of the fetus to achieve its growth potential. It remains one of the main causes of poor perinatal outcome, affecting 5-8 % of ongoing pregnancies.[1,2] FGR is commonly associated to preeclampsia (PE) as both entities are attributed to poor placental implantation and may be considered a similar disease process, mainly the early-onset form.[3-6] Early-onset FGR is associated with PE in about 50-70% of cases[7] and is characterized by severe placental insufficiency.[8,9] Late-onset FGR presents milder placental involvement and maternal disease,[8-11] while less than 10% of cases are complicated by PE.[7]

The common pathophysiology of PE and FGR is further supported by long-term maternal cardiovascular complications in both entities.[12-15] Recent findings suggest that early PE and FGR could share similar maternal cardiac responses, such as impaired myocardial relaxation and left ventricular diastolic dysfunction.[12] Both conditions present also lipid profile alterations, such as increased cholesterol concentrations and a trend for insulin resistance.[17] Late-onset PE and FGR are also characterized by changes in maternal metabolism,[18,19] cardiac and endothelial function.[8,10,11]

Vascular structural and functional changes are considered independent risk factors for long-term cardiovascular events.[20-24] Vascular structural remodeling can be evaluated by carotid intima-media thickness (IMT)[25] and lumen diameters, while vascular function can be assessed by arterial stiffness indices such as pulse wave velocity, augmentation index, distensibility (CAD) and circumferential wall stress (CWS).[26-28] Inferior vena cava compliance is also reflecting venous responsiveness to haemodynamic changes.[29-31] These parameters have been previously applied to assess susceptibility to cardiovascular morbidity in women with prior or index

preeclamptic pregnancies.[20-24] However, vascular responsiveness in normotensive FGR pregnancies has not been yet evaluated.

In this study, we hypothesized that normotensive women with FGR might present maternal vascular structural and functional changes similar to those with PE. Thus, we sought to comprehensively describe maternal carotid IMT and CAD, CWS and IVC collapsibility in a prospective cohort study including FGR pregnancies with and without PE, subclassified into early and late disease.

MATERIALS AND METHODS

Study population

This was a prospective cohort study of antenatal maternal vascular assessment, carried out over a 3 year period from July 2010 to October 2012, including 110 normally grown and normotensive control pregnancies, 64 normotensive pregnancies affected by FGR and 60 FGR pregnancies complicated with PE. Pregnant women were recruited while attending the antenatal clinic of Hospital Clinic in Barcelona. The control group comprised women attending routine antenatal clinic with uncomplicated pregnancies and with EFW and birthweight above 10th centile. Only healthy non-smoking women, not affected by current or anterior pregnancy complications were included in the control group. FGR was defined as estimated fetal weight and confirmed birthweight below 10th centile. In order to assess potential differences according to different subsets of FGR, normotensive FGR cases were further subclassified into early-onset ($\leq$32 weeks of gestation at clinical onset) or late onset (>32 weeks of gestation).[24,32]PE was defined according to the International Society Study of the Hypertension in Pregnancy as a resting blood pressure of ≥ 140/90 mm Hg on 2 occasions at least 4 hours apart and proteinuria of ≥ 300 mg/L or a 2+ urine

dipstick beyond 20 weeks of gestation in a previously normotensive woman.[33] Eighty percent of preeclamptic women included in this study were also recruited for a previous study from our group.[24] Cases were evaluated at the time of diagnosis. Women with diabetes mellitus, renal and connective tissue disease were excluded from the study.

Information on maternal demographic characteristics, reproductive history and current pregnancy clinical data including first trimester maternal blood pressure was obtained in the interview at the day of the maternal vascular evaluation and through medical history notes. In all pregnancies gestational age was calculated based on the crown-rump length at first trimester ultrasound.[34]All pregnancies underwent a feto-placental Doppler examination including uterine arteries, umbilical artery and middle cerebral artery. Cerebro-placental ratio was calculated as a simple ratio of the middle cerebral artery pulsatility index divided by the umbilical artery pulsatility index.[35]Pregnancy outcome such as gestational age at delivery, mode of delivery, birth weight, birth weight centile, Apgar score and umbilical pH were also recorded. Fetal and neonatal weight centile were calculated according to local reference curves.[36] Ethics approval was received by the local Research Ethics Committee and written informed consent form was obtained from patients.

Maternal vascular assessment

Maternal vascular assessment was performed during third trimester for normotensive controls and at diagnosis for FGR cases with or without PE. The exploration was performed in a controlled environment with the pregnant women in a decubitus position. The assessment protocol included initially blood pressure measurement by a validated ambulatory automated Omron 5 Series device (Omron Healthcare, Kyoto, Japan). Carotid ultrasound assessment was subsequently performed with slight hyperextension and rotation of the neck in an opposite direction to the probe by a

single experienced investigator using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA). Longitudinal clips of the far wall of both carotid arteries were obtained approximately 1 cm proximal to the bifurcation using a 13-MHz linear-array transducer. Carotid IMT measurements were performed offline according to a standardized protocol based on a tracemethod with the assistance of a computerized program (SiemensSyngo Arterial Health Package, Siemens Medical Systems). To obtain IMT, three end-diastolicframes were selected across a length of 10 mm and analyzed for mean and maximum IMT in end-diastole, and the average reading from these three frames wascalculated. Simultaneous electrocardiogram (ECG) recording ensured reliable detection of R-wave and therefore reliable clinically evaluation. Systolic and diastolic arterial diameters were acquired at the sites corresponding at the IMT measurements. They were evaluated offline with the same computerized program at end systole and end diastole.

Arterial distensibility was calculated as CAD = 2 x 1000 x (systolic carotid artery diameter-diastolic carotid artery diameter) / (diastolic carotid artery diameter) x (brachial pulse pressure).[37] Pulse pressure denotes the difference between systolic and diastolic blood pressure and was converted from millimeters of mercury to kilopascals (kPa; 1 mm Hg=0.133 kPa). Circumferential Wall Stress was calculated as: CWS = MBP x dD/2 x cIMT where MBP denotes mean blood pressure and was also converted from millimeters of mercury to kilopascals, dD diastolic diameter and cIMT carotid intima-media thickness.[27]

IVC diameter changes were evaluated during the respiratory cycle by ultrasound M-Mode as depicted in Figure 1. All examinations were performed in the supine position with 6-MHz curvilinear transducer placed in a subxiphoid plane. Sagittal sections of the upper part of IVC behind the liver were obtained. Respiratory variations of the vessel diameter were evaluated offline during inspiration (IVCi) and expiration (IVCe)

with Siemens Syngo Arterial Health Package. The difference between IVCe and IVCi is defined as collapsibility and collapsibility index is the ratio IVCe-IVCi/ IVCe.[29-31]

Statistical analysis

The analyses were carried out using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA). All statistical tests were performed at the 2-sided 5% level of significance. Sample size was calculated for detecting a 15% difference in carotid IMT. For an 80% power and 5% type I error level, the estimated sample size was 20 women per study group. Results are presented as median (interquartile range) or as percentage and feto-placental Doppler ultrasound measurements were converted into *Z*-scores.[35,38,39] One way analysis of variance based on log transformed data adjusted with Bonferroni post-hoc test and Pearson chi square were used to compare quantitative and qualitative data respectively. Comparisons of vascular parameters among study groups were performed by multiple linear regression analysis adjusting by maternal age, maternal body mass index, maternal smoking and gestational age at evaluation. Additionally, polynomial orthogonal contrasts were constructed in order to test for linear trends of parameters across severity groups.

RESULTS

Study populations

Clinical and perinatal data of the study populations are presented in Table 1. As expected, FGR cases with or without PE demonstrated a higher prevalence of maternal smoking and assisted reproductive technologies. In addition, FGR cases with PE had higher maternal BMI, paternal age and prevalence of nulliparity. First trimester maternal blood pressure was higher in both FGR groups with significantly higher values in the cases that later developed PE.As expected, FGR cases had a lower birthweight centile together with worse feto-placental Doppler and perinatal outcome, especially in those cases complicated by PE.

Maternal vascular assessment in FGR and PE

Results on maternal vascular parameters in FGR and PE are shown in Table 2. Both FGR cases with or without PE had a significant increase of maternal blood pressure, carotid IMT and CWS at third trimester as compared to controls. In addition, normotensive FGR had a non-significant tendency to higher CAD and IVC collapsibility index together with similar arterial diameters as compared to controls. On the contrary, FGR cases with PE showed significantly lower CAD and non-significant lower IVC collapsibility index with increased diameters as compared to controls and normotensive FGR.

Results in early- and late-onset FGR

Results of fetal ultrasound and maternal vascular characteristics in normotensive FGR cases, subdivided into early and late-onset, are presented in Table 3. As expected, both FGR groups had worse feto-placental Doppler parameter results. Both early and late-onset normotensive FGR had significantly increased first and third trimester blood pressure values as compared to controls. Early-onset normotensive FGR was associated with significantly increased carotid IMT and CWS with no significant changes in CAD and collapsibility index as compared with controls. Late-onset normotensive FGR showed similar results as compared with controls for both structural and functional maternal vascular parameters.

COMMENT

This study provides evidence that normotensive FGR pregnant women present signs of vascular remodeling similar to those observed in PE. These adaptations were more prominent in early-onset FGR cases.

The most relevant result of this study is the significant increase of carotid IMT and BP in normotensive FGR as compared to controls. Despite similar structural changes however, vascular function, reflected by CAD and IVC collapsibility index, differed considerably between FGR and PE. Our data, along with previous reports, contributes to a better understanding of the shared and distinct features of FGR and PE. Both entities present signs of placental and endothelial dysfunction,[8] as well as similar structural cardiac adaptations.[8,10,40] In this context, increased carotid IMT and BP could possibly represent a subclinical manifestation of the maternal disease in FGR. The preservation or even the increase of vascular distensibility in FGR could be attributed to less pronounced cardiovascular impairment and a timely maternal adaptation in FGR.[8,10] Conversely, severe maternal systemic disease in PE enhances arterial and venous stiffness that could lead to cardiovascular decompensation and injury.[41] Thus, the minor vascular dysfunction in normotensive FGR could support the concept of a milder (2-fold) increased risk for cardiovascular disease as compared with the markedly higher 8-fold risk for early PE.[13,16]

Maternal responses in early and late normotensive FGR were further assessed in our study. Early FGR presented increased BP, carotid IMT and CWS, while in late FGR less prominent adaptations occurred with only BP showing significant differences. Interestingly, first and third trimester maternal BP was significantly increased in both early and late FGR as compared with controls. The disparity in maternal BP values in FGR among our and previous results[7,10,12] could be explained by differences in FGR definition and study populations. Overall, these results indicate that early and late

FGR share common pathophysiologic traits, but the observed differences imply that they might be distinct clinical diseases. Actually, the very severe early-onset FGR with abnormal umbilical artery demonstrated similar vascular behavior as early PE (supplemental data). Our findings are in keeping with previous studies reporting different cardiovascular responses for early and late FGR[10,12] that might possibly encompass distinct future cardiovascular outcomes. Epidemiologic studies have indeed provided evidence of worst cardiovascular outcome in women with history of early rather than late FGR.[10,11,18,42]

The main strength of the present study was to evaluate for the first time maternal vascular structure and function in FGR pregnant women at diagnosis, distinguishing among different subsets of normotensive FGR. Overall, our data suggest that vascular features in the normotensive FGR could be attributed to placental involvement, which further induces subclinical maternal vascular adaptations. Whether vascular IMT could be used as a prognostic imaging biomarker to improve stratification of long term maternal cardiovascular outcome in FGR needs to be further investigated in large prospective studies.

This study has some limitations. Studying mainly the circulatory and not cardiac adaptations could account as a limitation of our study. Furthermore, we acknowledge that our sample size couldnot have been sufficient enough mainly women comparing different subsets of FGR, as demonstrated by several trends not reaching statistical significance. We also acknowledge that several potential confounders such as smoking could have affected our maternal vascular results. However, all analyses were adjusted by maternal age, smoking and body mass index in order to minimize this effect. Finally, as FGR is emerging as a cardiovascular risk factor, further postpartum follow up is warranted to be confirmed in long term studies.

In summary, pregnancies with FGR presented subclinical signs of maternal vascular remodeling, which is relevant with long term increased cardiovascular risk. Distinct vascular adaptations in early and late FGR could reflect different etiologies and clinical expressions. Future studies are warranted to assess the complex pathophysiologic mechanisms of FGR and the potential utility of our measurements to select to mothers at higher risk that may benefit from timely diagnosis and treatment.

REFERENCES

1. Gardosi J. Clinical strategies for improving the detection of fetal growth restriction. Clin Perinatol 2011;38:21-31.

2. Figueras F, Gardosi J. Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. Am J Obstet Gynecol 2011;204:288-300.

3. Ananth CV, Peltier MR, Chavez MR, Kirby RS, Getahun D, Vintzileos AM. Recurrence of ischemic placental disease. Obstet Gynecol 2007;110:128-33.

4. Norwitz ER. Defective implantation and placentation: laying the blueprint for pregnancy complications. Reprod Biomed Online 2006;13:591–9.

5. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. Am J Obstet Gynecol 2006;195: 40-9.

6. Yinon Y, Kingdom JC, Odutayo A, et al. Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: insights into future vascular risk. Circulation 2010;122:1846-53.

7. Crovetto F, Crispi F, Scazzocchio E, et al. Performance of first trimester integrated screening for early and late small for gestational age newborns. Ultrasound Obstet Gynecol 2014; 43:34-40

8. Crispi F, Domínguez C, Llurba E, Martín-Gallán P, Cabero L, Gratacós E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in preeclampsia and in isolated intrauterine growth restriction. Am J Obstet Gynecol 2006;195:201-7.

9. Crispi F, Hernandez-Andrade E, Pelsers M et al. Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses. Am J Obstet Gynecol 2008; 199:254.

10. Bamfo JE, Kametas NA, Chambers JB, Nicolaides KH. Maternal cardiac function in fetal growth-restricted and non-growth-restricted small-for- gestational age pregnancies. Ultrasound Obstet Gynecol 2007;29:51–57.

11. Kanagalingam MG, Nelson SM, Freeman DJ, et al.Vascular dysfunction and alteration of novel and classic cardiovascular risk factors in mothers of growth restricted offspring. Atherosclerosis 2009;205:244-50.

12. Melchiorre K, Sutherland GR, Liberati M, Thilaganathan B.Maternal cardiovascular impairment in pregnancies complicated by severe fetal growth restriction.Hypertension 2012;60:437-43.

13. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardio- vascular disease death: prospective evidence from the child health and –

development studies cohort. Hypertension 2010;56:166–171.

14. Davey Smith G, Harding S, Rosato M. Relation between infants' birth weight and mothers' mortality: prospective observational study. BMJ 2000;320:839–40.

15. Smith GC, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. Lancet 2001;357:2002-6

16. Irgens HU, Reisæter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. BMJ 2001;323:1213–1217.

17. Manten GT, Sikkema MJ, Voorbij HA, Visser GH, Bruinse HW, Franx A. Risk factors for cardiovascular disease in women with a history of pregnancy complicated by preeclampsia or intrauterine growth restriction. Hypertens Pregnancy 2007;26:39-50.

18. Caruso A, Paradisi G, Ferrazzani S, Lucchese A, Moretti S, Fulghesu AM. Effect of maternal carbohydrate metabolism on fetal growth. Obstet Gynecol 1998;92:8-12.

19. Hermes W, Tamsma JT, Grootendorst DC, et al. Cardiovascular risk estimation in women with a history of hypertensive pregnancy disorders at term: a longitudinal follow-up study. BMC Pregnancy Childbirth 2013;4:13:126.

20. Blaauw J, Van Pampus MG, Van Doormaal JJ, Fokkema MR, Fidler V, Smit AJ, Aarnoudse JG. Increased intima-media thickness after early onset preeclampsia. Obstet Gynecol 2006;107:1345.

21. Haukkamaa L, Moilanen L, Kattainen A, et al. Pre-eclampsia is a risk factor of carotid artery atherosclerosis. Cerebrovasc Dis 2009;27:599-607.

22. Goynumer G, Yucel N, Adali E, Tan T, Baskent E, Karadag C. Vascular risk in women with a history of severe preeclampsia. J Clin Ultrasound 2013;41:145-50.

23. Yuan LJ, Xue D, Duan YY, Cao TS, Yang HG, Zhou N. Carotid intima-media thickness and arterial stiffness in preeclampsia by analysis with a radio-frequency ultrasound technique. Ultrasound Obstet Gynecol 2013;42:644-52

24. Stergiotou I, Crispi F, Valenzuela-Alcaraz B, Bijnens B, Gratacos E. Patterns of maternal vascular remodeling and responsiveness in early- versus late-onset preeclampsia. Am J Obstet Gynecol 2013;209:558.e1-558.e14.

25. Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008;21:93–111

26. Boutouyrie P, Tropeano AI, Asmar R, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;39:10–5.

27. Laurent S, Girerd X, Mourad JJ, et al. Elastic modulus of the radial artery wall material is not increased in patients with essential hypertension. Arterioscler Thromb 1994;14:1223-31

28. Laurent S, Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. Hypertension 2007;49:1202-6.

29. Feissel M, Michard F, Faller JP, Teboul JL. The respiratory variation in inferior vena cava diameter as a guide to fluid therapy. Intensive Care Med 2004;30:1834-7.

30. Sefidbakht S, Assadsangabi R, Abbasi HR, Nabavizadeh A. Sonographic measurement of the inferior vena cava as a predictor of shock in trauma patients.Emerg Radiol 2007;14:181-5.

31. Dipti A, Soucy Z, Surana A, Chandra S. Role of inferior vena cava diameter in assessment of volume status: a meta-analysis. AmJEmergMed 2012;30:1414-1419

32. Savchev S, Figueras F, Sanz-Cortes M, et al. Evaluation of an optimal gestational age cut-off for the definition of early and late-onset fetal growth restriction. Fetal Diagn Ther 2013. DOI: 10.1159/000355525. Accessed February 12, 2014

33. National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. Am J Obstet Gynecol 2000;183:S1-S22.

34. Robinson HP, Sweet EM, Adam AH. The accuracy of radiological estimates of gestational age using early fetal crown-rump length measurements by ultrasound as a basis for comparison. Br J Obstet Gynaecol 1979;86:525-8

35. Baschat AA, Gembruch U. The cerebroplacental Doppler ratio revisited. Ultrasound Obstet Gynecol 2003;21:124-7.

36. Figueras F, Meler E, Iraola A, et al. Customized birthweight standards for a Spanish population. Eur J Obstet Gynecol Reprod Biol 2008;136:20-4

37. Stefanadis C, Stratos C, Boudoulas H, Kourouklis C, Toutouzas P. Distensibility of the ascending aorta: comparison of invasive and non-invasive techniques in healthy men and in men with coronary artery disease. Eur Heart J 1990;11:990–6.

38. Arduini D, Rizzo G. Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses. J Perinat Med 1990;18:165-72.

39. Gómez O, Figueras F, Fernández S, et al. Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation. Ultrasound Obstet Gynecol 2008;32:128-32.

40. Vasapollo B, Valensise H, Novelli GP, et al. Abnormal maternal cardiac function and morphology in pregnancies complicated by intrauterine fetal growth restriction. Ultrasound Obstet Gynecol 2002;20:452-7.

41. Tihtonen KM, Kööbi T, Uotila JT. Arterial stiffness in preeclamptic and chronic hypertensive pregnancies. Eur J Obstet Gynecol Reprod Biol 2006;128:180-6.

42. Paradisi G, Mattoli MV, Tomei C, et al. Cardiovascular risk factors in healthy women with previous small for gestational age infants. J Obstet Gynaecol Res 2011;37:1397–1404.

Table1. Baseline characteristics and perinatal outcomes of the study groups.

Characteristic	**Controls**	**Normotensive FGR**	**FGR with PE**	***P-value*[c]**
N	***110***	***64***	***60***	
Parental baseline characteristics				
Maternal age (years)	32.0 (6)	33.0 (7)	34.0 (8)	0.081
Paternal age (years)	34.0 (7)	34.0 (7)	37.0 (9)[a]	***0.004***
Maternal body mass index (kg/m²)	23 (4.1)	21.7 (4.0)	24 (8.5)[ab]	***0.001***
Paternal body mass index (kg/m²)	25.5 (4)	24.9 (4)	25.9 (5)	***0.027***
Nulliparity (%)	55 (50)	40 (62)	41 (68)[a]	0.093
White ethnicity (%)	66 (60)	50 (78)[a]	34 (57)[b]	0.069
Smoking (%)	0 (0)	15 (23)[a]	8 (12.5)[a]	***<0.001***
ART (%)	0 (0)	5 (8)[a]	11 (18)[a]	***<0.001***
Maternal first trimester BP				
First trimester systolic BP(mmHg)	110 (15)	112 (17)	121 (25)[ab]	***<0.001***
First trimester diastolic BP(mmHg)	65 (13)	71 (14)[a]	80 (21)[ab]	***<0.001***

First trimester mean BP (mmHg)	80 (13)	85 (13)[a]	95 (21)[ab]	***<0.001***
Third trimester fetal ultrasound				
Gestational age at fetal scan (weeks)	33.5 (2.1)	35.0 (7.7)[a]	32.0 (4.7)	***0.025***
Uterine artery mean PI (z-scores)	-0.68 (0.30)	0.18 (2.23)	2.98 (2.47)[ab]	***<0.001***
Umbilical artery PI (z-scores)	-0.89 (0.64)	0.56 (1.66)	0.55 (2.12)	0.051
Middle cerebral artery PI (z-scores)	0.19 (2.08)	-0.39 (1.78)[a]	-1.16 (1.66)[ab]	***<0.001***
Cerebro-placental ratio (z-scores)	0.37 (0.04)	-0.29 (2.97)[a]	-1.38 (2.36)[a]	***<0.001***
Delivery data				
Gestational age at delivery (weeks)	40.0 (1.6)	36.0 (2.6)[a]	33.1 (4.8)[ab]	***<0.001***
Caesarian section (%)	24(22)	8(36)[a]	50(83)[ab]	***<0.001***
Birth weight (g)	3450 (520)	2500 (645)[a]	1670 (850)[ab]	***<0.001***
Birth weight percentile	53 (30)	1 (5)[a]	2 (3)[a]	***<0.001***
5-min Apgar score	10(0)	10(0)	10(1)[a]	***<0.001***
Umbilical artery pH	7.25 (0.08)	7.23 (0.09)	7.24 (0.11)	0.089
Days in NICU (%)	0 (0)	6 (15)	18 (22)[a]	***<0.001***

Data are presented as median (interquartile range) or percentage (N).

All Doppler parameters are presented as Z-scores.

[a]P<0.05 as compared to controls.

[b]P<0.05 as compared to normotensive FGR

[c]P value calculated by one-way ANOVA or Pearson's chi-square test

PE, preeclampsia; *IVF*, in vitro fertilization; PI, pulsatility index; *NICU*, neonatal intensive care unit.

Table 2. Results on maternal vascular assessment among the study groups

Characteristic	Controls	Normotensive FGR	FGR with PE	*P-value*[c]
N	***110***	***64***	***60***	
Gestational age at maternal evaluation (weeks)	34.4(3)	35.4(7)	32.4(5)	0.485
Blood pressure				
Systolic BP at examination (mmHg)	110 (14)	114 (20)[a]	145 (17)[ab]	***<0.001***
Diastolic BP at examination (mmHg)	69 (12)	73 (14)[a]	91 (15)[ab]	***0.004***
Mean BP at examination (mmHg)	82 (12)	88 (14)[a]	109 (15)[ab]	***<0.001***
Maternal carotid arteries ultrasound				
Mean cIMT(mm)	0.409 (0.04)	0.436 (0.08)[a]	0.425 (0.10)[a]	***0.008***
Maximum cIMT(mm)	0.504 (0.06)	0.535 (0.07)[a]	0.542 (0.11)[a]	***<0.001***
Carotid systolic diameter (mm)	6.000 (0.450)	6.000 (0.680)	6.750 (0.740)[ab]	0.701
Carotid diastolic diameter (mm)	5.550 (0.550)	5.470 (0.550)	6.150 (0.840)[ab]	0.773
Carotid Distensibility ($kPa^{-1}10^{-3}$)	31.97 (15.73)	34.94 (17.62)	25.30 (18.94)[ab]	0.656
Circumferencial Wall Stress (kPa)	12.236 (3.07)	14.006 (3.36)[a]	19.785 (5.72)[ab]	***0.001***
Maternal inferior Vena Cava ultrasound evaluation				
Diameter at inspiration	8.700 (5.10)	6.600 (3.10)	9.750 (2.60)	0.135
Diameter at expiration	9.900 (4.60)	7.600 (4.10)	10.600 (2.75)[ab]	0.169
Collapsibility	0.950 (0.50)	0.900 (0.85)	0.800 (0.57)	0.646
Collapsibility Index	0.105 (0.06)	0.120 (0.08)	0.073 (0.04)[b]	0.146

Data are presented as median (interquartile range).

[a]*P<0.05* as compared to controls calculated by regression adjusted by maternal age, body mass index, smoking and gestational age at evaluation.

[b]*P<0.05* as compared to FGR without PE calculated by regression adjusted by maternal age, body mass index, smoking and gestational age at evaluation.

[c]*P* calculated by linear trends across severity groups

FGR, fetal growth restriction; *PE*, preeclampsia; *BP*, blood pressure; *cIMT*, carotid intima-media thickness

Table 3. Results on maternal vascular assessment among the normotensive FGR study cases subclassified by gestational age to early- and late- onset FGR

Characteristic	**Controls**	**Normotensive late FGR**	**Normotensive early FGR**	***P-value*[c]**
N	**110**	**44**	**20**	
Fetal ultrasound evaluation				
Gestational age at fetal scan (weeks)	33.5 (2.1)	38.1 (2.5)	28.5 (4.1)[ab]	***<0.001***
Uterine artery mean PI (z-scores)	-0.68 (0.30)	0.10 (1.93)	1.21 (1.95)	0.353
Umbilical artery PI (z-scores)	-0.89 (0.64)	0.32 (1.49)[a]	1.95 (3.13)[a]	***<0.001***
Middle cerebral artery PI (z-scores)	0.19 (2.08)	-0.01 (1.68)	-1.25 (1.51)[a]	***0.001***
Cerebro-placental ratio (z-scores)	0.37 (0.04)	-0.84 (1.60)[a]	-2.38 (1.96)[ab]	***<0.001***
Maternal BP				
First trimester Systolic BP (mmHg)	110 (15)	115 (20)	123 (14)[ab]	***0.009***
First trimester Diastolic BP (mmHg)	65 (13)	66 (10)[a]	71 (17)[a]	***0.021***
First trimester mean BP (mmHg)	80 (13)	84 (11)[a]	90 (14)[a]	***0.006***

Systolic BP at examination (mmHg)	110 (14)	115 (19)[a]	114 (19)[a]	***0.005***
Diastolic BP at examination (mmHg)	69 (12)	73 (13)	73 (14)[a]	0.081
Mean BP at examination (mmHg)	82 (12)	88 (14)[a]	89 (14)[a]	***0.015***
Maternal carotid arteries				
Mean cIMT (mm)	0.409 (0.04)	0.417 (0.08)	0.476 (0.10)[ab]	***<0.001***
Maximum cIMT (mm)	0.504 (0.06)	0.532 (0.06)	0.560 (0.16)[ab]	***<0.001***
Carotid systolic diameter (mm)	6.000 (0.450)	6.000 (0.590)	5.975 (0.660)	0.789
Carotid diastolic diameter (mm)	5.550 (0.550)	5.475 (0.580)	5.475 (0.590)	0.423
Carotid Distensibility ($kPa^{-1}10^{-3}$)	31.97 (15.73)	32.79(17.35)	37.29 (16.29)	0.121
Circumferencial Wall Stress (kPa)	12.236 (3.07)	13.590(3.41)	14.549(5.26)[ab]	***<0.001***
Inferior Vena Cava				
Diameter at inspiration	8.700 (5.10)	7.250 (2.50)	5.700 (1.40)[a]	0.055
Diameter at expiration	9.900 (4.60)	8.350 (3.35)	6.500 (1.80)[a]	***0.036***
Collapsibility	0.950 (0.50)	1.000 (1.15)	0.800 (0.20)	0.363
Collapsibility Index	0.105 (0.06)	0.124 (0.10)	0.120 (0.08)	0.385

Data are presented as median (interquartile range).

[a]*P<0.05* as compared to controls calculated by regression adjusted by maternal age, body mass index, smoking and gestational age at evaluation

[b]*P<0.05* as compared to normotensive late FGR calculated by regression adjusted by maternal age, body mass index, smoking and gestational age at evaluation

[c]*P* calculated by linear trends across severity groups

FGR, fetal growth restriction; *BP*, blood pressure; *cIMT*, carotid intima-media thickness

5.3 STUDY 3

Aortic and carotid wall thickness in term small-for-gestational age newborns and relationship with signs of severity

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AORTIC AND CAROTID WALL THICKNESS IN TERM SMALL-FOR-GESTATIONAL AGE NEWBORNS AND RELATIONSHIP WITH PRENATAL SIGNS OF SEVERITY

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KEY WORDS

aortic intima-media thickness; blood pressure; carotid intima-media thickness; late onset intrauterine growth restriction; small-for-gestational age

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ABSTRACT

Objective: To assess carotid and aortic intima-media thickness (IMT) in term growth restricted newborns with and without prenatal signs of severity.

Methods: This was a prospective study comprising 67 cases diagnosed prenatally and 134 normally grown newborns. Cases were sub-classified into small-for-gestational age (SGA) with no signs of severity and those with signs of severity, defined as a birth weight below 3rd percentile, or abnormal uterine artery Doppler or cerebro-placental ratio. Blood pressure and vascular IMT were evaluated.

Results: SGA newborns showed a trend for higher values of blood pressure. IMT values were significantly increased in SGA, with and without signs of severity, as compared with controls. The magnitude of the increase was higher in SGA with signs of severity.

Conclusions: Vascular IMT was increased in SGA irrespective of the presence or absence of prenatal signs of severity. The results challenge the notion of "constitutionally small" SGA, and support that the majority of SGA represent forms of true growth restriction and suffer fetal cardiovascular programming.

INTRODUCTION

Fetal growth restriction (FGR) is one of the most common conditions affecting ongoing pregnancies[1]. A substantial body of evidence has reported a wide spectrum of unfavorable perinatal and lifelong effects associated with FGR[2-6]. The pathophysiology linking growth restriction to the adverse short and long term outcomes could be possibly attributed to the profound changes in fetuses' metabolism[7], cardiovascular system[8,9] and brain structure[10]. The fetal cardiovascular adaptation to hypoxia and undernutrition is thought to represent a central adaptive mechanism and induces both cardiac [9,11] and vascular remodeling[12,13]. Vascular intima-media thickness (IMT) is a standard diagnostic procedure in assessing cardiovascular risk in asymptomatic adults[14]. Recently, an inverse relation between aortic IMT[12,13], arterial stiffness[15,16] and low birth weight is reported.

There is a current tendency to distinguish between early and late FGR if it occurs before or after 32 weeks gestation[17]. The importance of gestational age at diagnosis is reflected on the severity of placental dysfunction and the prognosis. Early FGR is mainly characterized by severe placental insufficiency expressed by abnormal umbilical artery[17,18]. On the other hand, late FGR is associated with a milder placental dysfunction, illustrated merely by brain redistribution and abnormal uterine arteries[19,20]. Recent evidence has demonstrated that late FGR fetuses, often referred as term small for gestational age (SGA) due to normal umbilical artery, are characterized by subtle cardiovascular remodeling[8,21]. These cases have worse cardiovascular[22] and neurodevelopmental[23] outcomes in postnatal life than initially anticipated. Over recent years several prognostic factors, including abnormal cerebroplacental ratio[24] and uterine artery Doppler[19], and birth weight below the 3rd centile[25], have been identified as predictors of poor neonatal outcome, and

consequently as surrogate markers of true growth restriction among near term SGA fetuses. Although these factors are associated with poorer neonatal outcome, it is unknown whether they also classify well the risk of abnormal vascular outcome.

We hypothesized that vascular structure and function in SGA neonates could be similarly anticipated by the aforementioned prognostic factors of placental insufficiency. We explored this hypothesis by conducting a prospective cohort study to assess blood pressure and vascular IMT in late-onset growth restricted newborns classified by surrogate markers in different stages of severity.

METHODS

Study population

This was a prospective cohort study including 201 term newborns prenatally diagnosed as small or appropriate for gestational age and delivered after 37 weeks, subdivided into: A) SGA with signs of severity defined by estimated fetal weight (EFW) and confirmed birth weight below 3rd percentile or abnormal uterine artery mean pulsatility index (PI) (above 95th percentile) or cerebro-placental ratio (below 5th percentile); B) SGA without signs of severity defined by EFW and birth weight between 3rd and 10th percentile together with normal uterine artery mean PI and cerebro-placental ratio; and C) controls defined by EFW and confirmed birth weight above 10th percentile with no pregnancy complications. Prenatal ultrasonographic examination was performed using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA) with a 6-MHz curvilinear transducer. Feto-placental Doppler examination before delivery was considered and included uterine arteries, umbilical artery and middle cerebral artery. Cerebro-placental ratio was calculated as a simple ratio of the middle cerebral artery PI divided by the umbilical artery PI[26].

Information on maternal demographic characteristics, reproductive history and pregnancy outcome was obtained in the interview at the day of the scan and a posteriori through medical history notes. Gestational age at delivery, mode of delivery, birth weight, birth weight centile, Apgar score and umbilical pH were also recorded. Cases and controls were identified in fetal life among pregnancies attended in the Department of Maternal-Fetal Medicine in Hospital Clinic (Barcelona) and evaluated in the neonatal period during their first week of life. Controls were matched 2 to 1 with cases by gender and gestational age at delivery (±1 week). Exclusion criteria were chromosomal or

genetic disorders, monochorionic twin pregnancy and evidence of infection. In all pregnancies gestational age was calculated based on the crown-rump length at first trimester ultrasound[27]. Fetal and neonatal weight centile were calculated according to local reference curves[28]. The study received ethical approval by the local Research Ethics Committee and written informed consent form was obtained from parents.

Neonatal vascular assessment

A controlled environment contributed to the achievement of all neonatal measurements. Blood pressure was obtained from brachial artery using a validated ambulatory automated Omron 5 Series device. An appropriate cuff size covering the 40 percent of the arm circumference was used to ensure accurate measurement[29]. Each newborn's blood pressure was evaluated twice during quiescence and the average was determined. Due to the lack of recently published blood pressure normal values for our population of healthy newborns, systolic and diastolic blood pressure percentiles were calculated using local standards (supplemental data).

Carotid and aorta ultrasound assessment was performed by skilled sonographers using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA). Longitudinal clips of the far wall of both carotid arteries were obtained approximately 1 cm proximal to the bifurcation using a 13-MHz linear-array transducer. Longitudinal clips of the far wall of the proximal abdominal aorta were obtained in the upper abdomen by a 10-MHz linear probe. Carotid and aorta IMT measurements were performed offline according to a standardized protocol based on a trace method with the assistance of a computerized program (Siemens Syngo Arterial Health Package), as depicted in Figure 1. To obtain IMT, three end-diastolic frames were selected across a length of 10 mm and analyzed for mean and maximum IMT, and the average reading from these three frames was calculated. Systolic and diastolic arterial diameters were acquired at the sites corresponding at the IMT measurements in end-diastole.

Vascular wall thickness reproducibility

Intra-observer variability was determined for each parameter by comparing data from three measurements of the same sonographer and inter-observer variability by comparing data from two sonographers at one occasion.

Statistical analysis

The intra-observer reproducibility was assessed by calculating the intra class correlation coefficients and coefficients of variation (CV). The inter-observer reproducibility was assessed by calculating the CV for each parameter[30].

Sample size was calculated taking into account comparison between control and SGA neonates both with and without signs of severity. We used balanced ANOVA type of analysis for 2 factors and 3 groups, considering standard deviation (SD) of 0.08 mm[12] and detectable contrast of 0.07 for both factors (cIMT and aIMT). An

estimated sample size of 32 women per study group was achieved for a power of more than 90% and 5% type I error level[31]. Results are presented as median (interquartile range) or as percentage after checking parametric assumptions by Kolmogorov-Smirnov test. One way analysis of variance based on log transformed data adjusted with Bonferroni post-hoc test and Pearson chi square were used to compare quantitative and qualitative data respectively. Doppler ultrasound measurements were converted into *Z*-scores[26,32,33]. Models for vascular results were adjusted by multiple linear regression by gender, gestational age at birth, age at evaluation. Additionally, polynomial orthogonal contrasts were constructed in order to test for linear trends of parameters across severity groups. All statistical tests were performed at the 2-sided 5% level of significance. The analyses were carried out using the statistical package SPSS version 17 (SPSS Inc., Chicago, IL, USA).

RESULTS

Study populations

Clinical and perinatal data are presented in Table 1. Maternal characteristics were similar among the study groups with the exception of a higher prevalence of nulliparity and smokers in the SGA group with signs of severity as compared to controls. Median birthweight of control neonates was 3350g (minimum 2530g and maximum 4400g). By definition, both SGA groups had a lower birth weight; median birthweight for SGA without signs of severity was 2603g (minimum 2190g and maximum 2920g), while median birthweight for SGA with signs of severity was 2200g (minimum 1630g and maximum 2650g). SGA cases with signs of severity had worse prenatal Doppler as compared to controls. Most of SGA with signs of severity were characterized by EFW <p3 and only 30% of them presented abnormal uterine

artery Doppler or cerebroplacental ratio. As expected, SGA cases with signs of severity had a higher prevalence of cesarean section and admission to the neonatal intensive care unit.

Neonatal vascular assessment

Results on intra- and inter-observer reproducibility are presented in tables 2 and 3. Results on vascular parameters are shown in table 4 and figure 2.

Blood pressure values were similar among groups with a non-significant tendency for higher values in SGA without and with signs of severity.

Mean and maximum carotid artery were significantly increased in both SGA cases as compared to controls (mean cIMT: 0.017 mm [SE 0.008], P=0.041 and maximum cIMT: 0.035 mm [SE 0.011], P=0.002 per SGA without signs of severity; mean cIMT: 0.023 mm [SE 0.010], P=0.028 and maximum cIMT: 0.026 mm [SE 0.013], P=0.050 per SGA with signs of severity). These differences remained significant after normalization of IMT values by neonatal weight and vessel diameter. There was a significant linear tendency for higher values across groups (P<0.001, P=0.001 for mean and maximum IMT, respectively). Mean aortic IMT showed a non significant trend for higher values between controls and SGA cases (mean aIMT: 0.023 mm [SE 0.017], P=0.194 per SGA without signs of severity; mean aIMT: 0.036 mm [SE 0.018], P=0.055 per SGA with signs of severity), that became significantly different after adjusting by neonatal weight and vessel diameter. Furthermore, maximum aorta IMT was significantly increased in both SGA cases as compared to controls (maximum aIMT: 0.055 mm [SE 0.018], P=0.003 per SGA without signs of severity and maximum aIMT: 0.057 mm [SE 0.022], P=0.010 per SGA with signs of severity). Similarly to previous parameters, the association remained significant after normalization of IMT values by neonatal weight and vessel diameter.

DISCUSSION

This study demonstrates that term SGA cases with and without signs of severity show increased vascular IMT, irrespective of the absence of signs of severity.

No significant differences for both systolic and diastolic blood pressure within groups were demonstrated in our study. However, a tendency for higher values in SGA newborns with signs of severity was shown as compared to SGA without signs of severity. No prior studies aimed at analyzing blood pressure of neonates with late-onset growth restriction. Yet, it is well documented that cases with growth restriction have higher blood pressure values than controls in childhood[11,34,35]. Nevertheless, our results are concordant with previous data showing no differences between preterm controls and early-onset growth restricted neonates[15]. The non significant tendency to higher blood pressure values in our neonates could be explained by our limited sample size or selection of a different postnatal age at examination. The lack of differences in the neonatal period could also be explained by an intrinsic limitation of measuring blood pressure in the neonatal period.

Vascular IMT was increased in growth restricted neonates irrespective whether they presented signs of severity or not. The differences in both SGA cases were statistically significant after adjusting by neonatal weight and vessel diameter. These results are in agreement with previous data demonstrating associations between low birth weight and aortic IMT adaptations in newborns and young children[11-13]. We showed a significant linear trend for higher values of IMT in relation to growth restriction severity. Although there was a tendency to worse values in SGA cases with signs of severity, SGA neonates without signs of severity also presented clear changes suggesting vascular remodeling, without though significant differences among the two groups. This association challenges the notion of "constitutionally

small" SGA, and suggests that SGA contain a substantial proportion of cases representing forms of true fetal growth restriction. The observed changes in SGA neonates without signs of severity suggest that severity markers, which are useful to predict adverse perinatal outcome, such as uterine artery pulsatility index, cerebroplacental ratio, estimated fetal weight less than the 3rd percentile[19,24,25], fail to predict a normal postnatal cardiovascular outcome. These results are in line with previous studies evaluating postnatal long term cardiac function in SGA[22]. Thus, the need to develop biomarkers allowing the detection of long term cardiovascular outcome in term low birth weight subjects could not be overemphasized. Whether vascular IMT could be used as a prognostic imaging biomarker, to improve stratification of risk among term growth restricted newborns, needs to be further investigated in large prospective studies.

In order to complete vascular assessment with further information, additional vascular parameters were included in our study (provided in the supplemental data). Arterial tensile stress, a major determinant of arterial hemodynamic forces[36], and arterial distensibility showed comparable values between cases and controls. The interpretation of these results is challenging. Theoretically, these findings were not anticipated when considering the previously reported increased afterload and arterial stiffness in fetuses and preterm growth restricted neonates when evaluated shortly after delivery[11,15,37]. However, as previously mentioned the neonatal period is a transitional pathophysiological state for cardiovascular regulation. The vascular behavior in our cohort would support the hypothesis that high placental resistance and subsequently increased afterload and raised blood pressure occur merely during fetal life[11,15,37]. In the immediate postnatal period however, the multiple stimuli that are responsible for the aforementioned changes would cease to exert their influence.

Consequently, tensile stress would show a behavior similar to blood pressure during this period. This assumption could be in line with our previously published results in 3-6 years old children with late-onset growth restriction without signs of severity. These children showed a non significant trend for higher values of normalized mean cIMT values and significant linear tendency for this parameter across stages of severity[22]. It seems therefore that despite normalization of hemodynamic conditions in the early neonatal period, mild changes still persist in childhood.

This study has strengths and limitations. To our knowledge, it was the first time to focus on structural and functional parameters of carotid and aorta as distinct components of the vascular tree[38-40]. Furthermore, the thorough classification of neonates according to their prenatal data in stages of severity has allowed observing vascular remodeling in all growth restricted neonates. The adjustment of results by both neonatal weight and lumen diameter further confirmed this finding. On the other side, our relatively limited sample size could account for the non significant trend for higher values in blood pressure. We also acknowledge that the different prevalence of maternal smoking among groups could have partly affected our results. Finally, long term pediatric follow up of our cohort is warranted in order to better understand the pathophysiology of cardiovascular remodeling in growth restriction and subsequently implement preventive strategies.

In conclusion, our study suggests that SGA neonates, both with and without signs of severity, demonstrate similar changes in vascular structure and function. These results challenge the notion of "constitutionally small" SGA, and support that the majority of SGA represent forms of true growth restriction and suffer fetal cardiovascular programming. Identifying these neonates at risk of vascular remodeling could make a significant contribution to screening public health

programs, given that growth restriction occurs in 10% of general maternity population.

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REFERENCES

1. Gardosi J. Clinical strategies for improving the detection of fetal growth restriction. Clin Perinatol 2011; 38: 21-31.

2. M Kady S., Gardosi J. Perinatal mortality and fetal growth restriction. Best Pract Res Clin Obstet Gynaecol 2004; 18: 397–410.

3. Barker DJP, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 1989; 2: 577-580.

4. Skilton MR, Viikari JS, Juonala M, Laitinen T, Lehtimäki T, Taittonen L, Kähönen M, Celermajer DS, Raitakari OT. Fetal growth and preterm birth influence cardiovascular risk factors and arterial health in young adults: the Cardiovascular Risk in Young Finns Study. Arterioscler Thromb Vasc Biol 2011; 31: 2975-81.

5. Kaijser M, Bonamy AK, Akre O, Cnattingius S, Granath F, Norman M, Ekbom A.. Perinatal risk factors for diabetes in later life. Diabetes 2009; 58: 523-6.

6. Martyn CN, Gale CR, Jespersen S, Sherriff SB. Impaired fetal growth and atherosclerosis of carotid and peripheral arteries. Lancet 1998; 352: 173-8.

7. Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 1999; 353: 1789-92.

8. Crispi F, Hernandez-Andrade E, Pelsers MM, Plasencia W, Benavides-Serralde JA, Eixarch E, Le Noble F, Ahmed A, Glatz JF, Nicolaides KH, Gratacos E.. Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses. Am J Obstet Gynecol 2008; 199: 254.

9. Girsen A, Ala-Kopsala M, Makikallio K, Vuolteenaho O, Rasanen J. Cardiovascular hemodynamics and umbilical artery N-terminal peptide of proB-type

natriuretic peptide in human fetuses with growth restriction. Ultrasound Obstet Gynecol 2007; **29**: 296-303.

10. Batalle D, Eixarch E, Figueras F, Muñoz-Moreno E, Bargallo N, Illa M, Acosta-Rojas R, Amat-Roldan I, Gratacos E. Altered small-world topology of structural brain networks in infants with intrauterine growth restriction and its association with later neurodevelopmental outcome. Neuroimage 2012; 60: 1352-66.

11. Crispi F, Bijnens B, Figueras F, Bartrons J, Eixarch E, Le Noble F, Ahmed A, Gratacós E. Fetal growth restriction results in remodeled and less efficient hearts in children. Circulation 2010; **121**: 2427-36.

12. Skilton MR, Evans N, Griffiths KA, Harmer JA, Celermajer DS. Aortic wall thickness in newborns with intrauterine growth restriction. Lancet 2005; 365: 1484–6.

13. Koklu E, Kurtoglu S, Akcakus M, Koklu S, Buyukkayhan D, Gumus H, Yikilmaz A. Increased aortic intima-media thickness is related to lipid profile in newborns with intrauterine growth restriction. Horm Res 2006; 65: 269-75.

14. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS; American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008; **21**: 93–111

15. Mori A, Uchida N, Inomo A, Izumi S. Stiffness of systemic arteries in appropriate- and small-for-gestational-age newborn infants. Pediatrics 2006;118:1035–41.

16. Tauzin L, Rossi P, Giusano B, Gaudart J, Boussuges A, Fraisse A, Simeoni U. Characteristics of arterial stiffness in very low birth weight premature infants. Pediatr Res 2006; 60: 592–6.

17. Savchev S, Figueras F, Sanz-Cortes M, Cruz-Lemini M, Triunfo S, Botet F, Gratacos E. Evaluation of an optimal gestational age cut-off for the definition of early and late-onset fetal growth restriction. Fetal Diagn Ther 2013. In press

18. Baschat AA. Pathophysiology of fetal growth restriction: implications for diagnosis and surveillance. Obstet Gynecol Surv 2004; 59: 617-27.

19. Severi FM, Bocchi C, Visentin A, Falco P, Cobellis L, Florio P, Zagonari S, Pilu G. Uterine and fetal cerebral Doppler predict the outcome of third-trimester small-for-gestational age fetuses with normal umbilical artery Doppler. Ultrasound Obstet Gynecol 2002; **19**: 225-8.

20. Cruz-Martínez R, Figueras F, Hernandez-Andrade E, Oros D, Gratacos E. Fetal brain Doppler to predict cesarean delivery for nonreassuring fetal status in term small-for-gestational-age fetuses.Obstet Gynecol 2011; **117**: 618-26.

21. Comas M, Crispi F, Cruz-Martinez R, Figueras F, Gratacos E. Tissue Doppler echocardiographic markers of cardiac dysfunction in small-for-gestational age fetuses. Am J Obstet Gynecol 2011; **205**: 57.e1-6.

22. Crispi F, Figueras F, Cruz-Lemini M, Bartrons J, Bijnens B, Gratacos E.. Cardiovascular programming in children born small for gestational age and relationship with prenatal signs of severity. Am J Obstet Gynecol 2012; **207**: 121.e1-9.

23. Figueras F, Oros D, Cruz-Martinez R, Padilla N, Hernandez-Andrade E, Botet F, Costas-Moragas C, Gratacos E. Neurobehavior in term, small-for-gestational age infants with normal placental function. Pediatrics 2009; **124**: e934–941.

24. Bahado-Singh RO, Kovanci E, Jeffres A, Oz U, Deren O, Copel J, Mari G. The Doppler cerebroplacental ratio and perinatal outcome in intrauterine growth restriction. Am J Obstet Gynecol 1999; **180**: 750–756.

25. Savchev S, Figueras F, Cruz-Martinez R, Illa M, Botet F, Gratacos E. Estimated weight centile as a predictor of perinatal outcome in small-for-gestational-age pregnancies with normal fetal and maternal Doppler indices. Ultrasound Obstet Gynecol 2012; **39**: 299-303.

26. Baschat AA, Gembruch U. The cerebroplacental Doppler ratio revisited. Ultrasound Obstet Gynecol 2003; **21**: 124-7.

27. Robinson HP, Sweet EM, Adam AH. The accuracy of radiological estimates of gestational age using early fetal crown-rump length measurements by ultrasound as a basis for comparison. Br J Obstet Gynaecol 1979; **86**: 525-8

28. Figueras F, Meler E, Iraola A, Eixarch E, Coll O, Figueras J, Francis A, Gratacos E, Gardosi J. Customized birthweight standards for a Spanish population. Eur J Obstet Gynecol Reprod Biol 2008; **136**: 20-4

29. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004; **114**(2 Suppl 4th Report): 555-76.

30. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; **1**: 307–310.

31. Lenth, R. V. (2006-9). Java Applets for Power and Sample Size. http://www.stat.uiowa.edu/~rlenth/Power [11 September 2013]

32. Arduini D, Rizzo G. Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses. J Perinat Med 1990; **18**: 165-72.

33. Gómez O, Figueras F, Fernández S, Bennasar M, Martínez JM, Puerto B, Gratacós E. Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation. Ultrasound Obstet Gynecol 2008; **32**: 128-32.

34. Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens 2000; **18**: 815–31.

35. Whincup PH, Bredow M, Payne F, Sadler S, Golding J. Size at birth and blood pressure at 3 years of age. The Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC). Am J Epidemiol 1999; **149**: 730–39.

36. Bots ML, Hofman A, Grobbee DE. Increased common carotid intima-media thickness. Adaptive response or a reflection of atherosclerosis? Findings from the Rotterdam Study. Stroke 1997; **28**: 2442-7.

37. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC, Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study.Circulation 2008; **117**: 649-59.

38. Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. J Am Coll Cardiol 2011; **57**: 1511-22.

39. McGill HC Jr, McMahan CA, Herderick EE, Tracy RE, Malcom GT, Zieske AW, Strong JP. Effects of coronary heart disease risk factors on atherosclerosis of selected regions of the aorta and right coronary artery. PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. Arterioscler Thromb Vasc Biol 2000; **20**: 836-45.

40. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke, G L, Wolfson, S K Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction

and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999; 340: 14-22.

Table1. Baseline characteristics and perinatal outcomes of the study groups.

Characteristics	Controls	SGA without signs of severity	SGA with signs of severity	*P value*[†]
N	***134***	***32***	***35***	
Maternal characteristics				
Maternal age (years)	32.0 (8.3)	32.5 (11)	31.0 (9)	0.820
Body mass index (kg/m²)	22.8 (3.8)	23.0 (5.1)	21.4 (4.5)	0.190
Nulliparity (%)	78 (58.0)	20 (62.5)	27 (77.1)*	0.132
White ethnicity (%)	74 (55.2)	18 (56.6)	22 (62.8)	0.906
Smoking (%)	22 (16.4)	8 (25.0)	13 (37.1)*	**0.028**
Prenatal ultrasound				
Gestational age at prenatal scan (weeks)	34.1 (4.0)	37.7 (2.0)*	37.6 (1.3)*	***<0.001***
Uterine artery mean PI (z-scores)	-0.93 (2.56)	0.36 (1.29)	-0.72 (1.22)	**0.050**
Umbilical artery PI (z-scores)	-0.04 (0.42)	0.06 (0.67)	0.19 (1.29)*	**0.040**
Middle cerebral artery PI (z-scores)	1.06 (1.10)	0.48 (1.18)	-0.39 (1.94)	0.345
Cerebro-placental ratio (z-	-0.29 (1.48)	-0.31	-0.71	0.353

scores)		(1.10)	(2.18)	
Delivery data				
Gestational age at delivery (weeks)	39.5 (1.8)	38.7 (1.5)	38.5 (1.2)*	**0.012**
Caesarian section (%)	22 (16.0)	6 (18.7)	15 (42.5)*	**0.006**
Caesarian section for non reassuring fetal status (%)	4 (2.9)	2 (6.2)	5 (14.2)*	**0.033**
Male gender (%)	78 (58)	19 (59)	20 (57)	0.983
Birth weight (g)	3350 (545)	2603 (273)*	2200 (290)*	*<0.001*
Birth weight percentile	55 (50)	4 (3)*	1 (1)*	*<0.001*
5-min Apgar score	10 (0)	10 (0)	10 (0)	0.464
Umbilical artery pH	7.24 (0.10)	7.26 (0.09)	7.25 (0.08)	0.346
Days in NICU	0 (0)	0 (0)	4 (3)*	*<0.001*

Data are presented as median (interquartile range) or percentage (N).

**P*<0.05 as compared to controls.

†P value calculated by one-way ANOVA or Pearson's chi-square test.

All Doppler parameters are presented as Z-scores.

SGA, small-for-gestational age; *PI*, pulsatility index;

NICU, neonatal intensive care unit.

Table 2. Intra-observer reproducibility of neonatal carotid and aortic arteries intima-media thickness evaluation.

	Correlation coefficient	Coefficient of variation
	ρ	CV, %
Mean right cIMT	0.827 (0.790-0.859)	2.7%
Mean left cIMT	0.870 (0.842-0.895)	2.6%
Maximum right cIMT	0.908 (0.887-0.926)	2.8%
Maximum left cIMT	0.921 (0. 903-0.937)	2.6%
Mean aIMT	0.861 (0.830-0.887)	2.6%
Maximum aIMT	0.934 (0.919-0.947)	2.1%

ρ indicates intraclass correlation coefficient (Lower bound-higher bound); CV, coefficient of variation; cIMT, carotid artery intima-media thickness; aIMT, aortic intima-media thickness.

Table 3. Inter-observer reproducibility of neonatal aortic and carotid arteries intima-media thickness evaluation.

	Coefficient of variation
	CV, %
Mean right cIMT	1.3%
Mean left cIMT	1.5%
Maximum right cIMT	1.2%
Maximum left cIMT	1.0%
Mean aIMT	7.6%
Maximum aIMT	4.5%

CV, coefficient of variation; cIMT, carotid artery intima-media thickness; aIMT, aortic intima-media thickness.

Table 4. Results on neonatal vascular assessment in the study groups.

Characteristic	Controls	SGA without signs of severity	SGA with signs of severity	*Linear tendency P-value‡*
N	***134***	***32***	***35***	
Blood pressure				
Systolic BP (mmHg)	82 (17)	85 (20)	86 (13)	0.143
Systolic BP percentile	57 (50)	57 (48)	62 (48)	0.420
Diastolic BP (mmHg)	46 (15)	48 (15)	52 (16)	0.107
Diastolic BP percentile	44 (53)	53 (50)	65 (51)	0.324
Carotid arteries				
Mean cIMT(mm)	0.226 (0.06)	0.247 (0.05)*	0.268 (0.08)*	*<0.001*
Mean cIMT / neonatal weight (mm/kg)	0.067 (0.02)	0.090 (0.02)*	0.090 (0.03)*	*<0.001*
Mean cIMT / diameter	0.094 (0.04)	0.117 (0.04)*	0.112 (0.03)*	*<0.001*
Maximum cIMT(mm)	0.303 (0.08)	0.336 (0.08)*	0.341 (0.10)*	*0.001*
Maximum cIMT / neonatal weight (mm/kg)	0.090 (0.02)	0.122 (0.02)*	0.120 (0.04)*	*<0.001*
Max cIMT / diameter	0.126 (0.04)	0.148 (0.05)*	0.157 (0.04)*	*<0.001*
Aorta				

Mean aIMT(mm)	0.419 (0.10)	0.493 (0.20)	0.446 (0.12)	*<0.001*
Mean aIMT / neonatal weight (mm/kg)	0.127 (0.03)	0.183 (0.08)*	0.210 (0.06)*†	*<0.001*
Mean aIMT / diameter	0.085 (0.03)	0.104 (0.05)*	0.113 (0.04)*	*<0.001*
Maximum aIMT(mm)	0.547 (0.11)	0.622 (0.20)*	0.604 (0.15)*	*0.002*
Maximum aIMT / neonatal weight (mm/kg)	0.165 (0.05)	0.222 (0.08)*	0.284 (0.09)*†	*<0.001*
Maximum aIMT /aortic diameter	0.111 (0.02)	0.128 (0.07)*	0.141 (0.05)*	*<0.001*

Data are presented as median (interquartile range).

**P<0.05* as compared to controls calculated by regression adjusted by gender, gestational age at birth, age at evaluation and group

†P<0.05 as compared to SGA without signs of severity calculated by regression adjusted by gender, gestational age at birth, age at evaluation and group

‡P<0.05 calculated by linear trends across severity groups

SGA, small-for-gestational age; *BP*, blood pressure;

cIMT, carotid intima-media thickness; *aIMT*, aortic intima-media thickness.

Figure 1. Illustration of the mean carotid and aortic intima-media thickness (IMT) assessment by ultrasound in control, small-for-gestational age neonates without signs of severity and small-for-gestational age neonates with signs of severity.

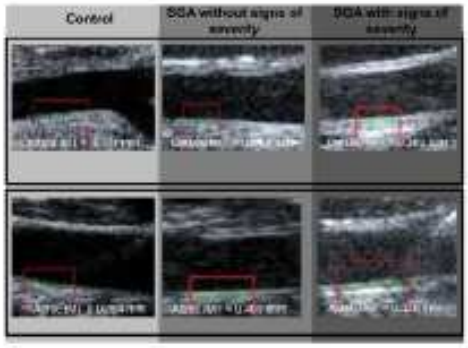
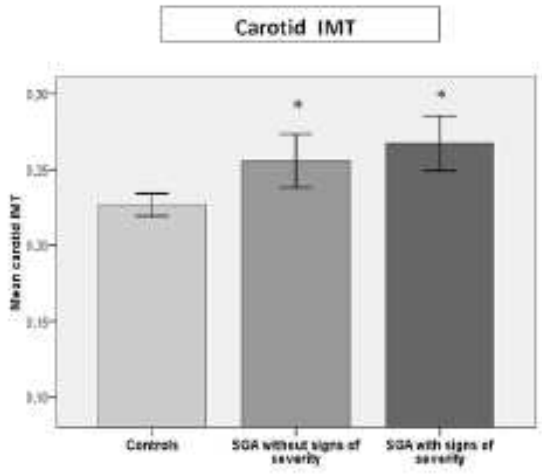
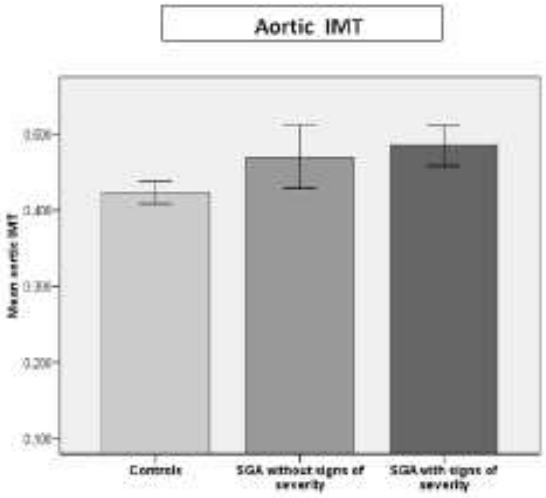


Figure 2. Vascular intima-media thickness (IMT) in controls, late-onset growth restricted neonates without signs of severity and late-onset growth restricted neonates with signs of severity. Data are presented as mean (95% CI). *$P<0.05$ as compared to controls adjusted by gender, gestational age at birth, age at evaluation and group.





6. RESULTS

Overall, our results demonstrated that vascular parameters presented structural and functional adaptations depending on the degree and early or late-onset of placental disease. Regarding maternal vascular changes, both PE and FGR presented significant alterations, with distinct patterns among early and late-onset disease. Regarding neonatal assessment, SGA newborns presented vascular remodeling irrespective of the severity of placental affectation.

6.1. Maternal vascular characteristics in PE (study 1)

Baseline characteristics were similar among the study populations (controls, early and late PE) included in study 1, with similar maternal age, ethnicity and hematocrit, which is major factor of blood viscosity. However, maternal body mass index was significantly increased in the late PE group and both systolic and diastolic booking blood pressures were higher in both preeclamptic groups. Furthermore, there was an increased prevalence of nulliparity, smoking, chronic hypertension, previous PE, proteinuria in both preeclamptic groups as compared to controls. As expected, cases, mainly women with early PE, had worse feto-placental Doppler and perinatal outcomes as compared to controls.

Results on vascular parameters are shown in table 3 and figure 6. By definition, blood pressure values were significantly increased in preeclamptic groups as compared to normotensive controls. Mean carotid artery IMT did not differ significantly between early preeclamptic and normotensive women but it was significantly increased in late PE as compared to controls (mean cIMT: 0.015 mm [SE 0.010] per early PE, P=0.142 and mean cIMT: 0.037 mm [SE 0.012] per late PE, P=0.002, respectively). There was a significant linear tendency for higher mean carotid IMT values across groups (P<0.001).

Table 3. Results on maternal vascular assessment among the study groups.

Characteristic	Controls	Early PE	Late PE	P-valuec
N	*100*	*50*	*50*	
Gestational age at maternal evaluation (weeks)	34.1 (4)	31.0 (4)a	36.5(3) [a]	<0.001
Maternal BP				
Systolic BP at examination (mmHg)	110 (13)	145 (15) [a]	143 (15)[a]	<0.001
Diastolic BP at examination (mmHg)	70 (11)	90 (11)[a]	90 (11)[a]	<0.001
Mean BP at examination (mmHg)	83 (11)	109 (13)[a]	108 (11)[a]	<0.001
Carotid arteries				
Mean cIMT(mm)	0.408 (0.06)	0.425 (0.09)	0.439 (0.08)[a]	***<0.001***
Maximum cIMT(mm)	0.505 (0.07)	0.530 (0.08)[a]	0.558 (0.08)[a]	***<0.001***
Carotid systolic diameter (mm)	5.950 (0.600)	7.100 (0.750)[a]	6.525 (0.720)[ab]	***<0.001***
Carotid diastolic diameter (mm)	5.450 (0.630)	6.500 (0.850)[a]	5.975 (0.740)[ab]	***<0.001***
Carotid Distensibility ($kPa^{-1}10^{-3}$)	32.14 (15.06)	23.85 (14.75)[a]	27.80(21.53)	***0.008***
Circumferencial Wall Stress (kPa)	12.470(2.99)	20.183(4.85)[a]	18.625(5.81)[a]	***<0.001***
Inferior Vena Cava				
Diameter at inspiration	8.750 (5.78)	9.550 (2.55)	10.000 (1.80)	0. 207
Diameter at expiration	9.950 (5.38)	10.600 (2.68)	10.750 (1.55)	0.315
Collapsibility	0.950 (0.50)	0.900 (0.83)	0.700 (0.53)[b]	0.082
Collapsibility Index	11.0 (7)	9.4 (6)	6.6 (4)[ab]	***0.026***

Data are presented as median (interquartile range).

[a]*P<0.05* as compared to controls calculated by regression adjusted by maternal age, body mass index and gestational age at evaluation

[b]*P<0.05* as compared to early PE calculated by regression adjusted by maternal age, body mass index and gestational age at evaluation

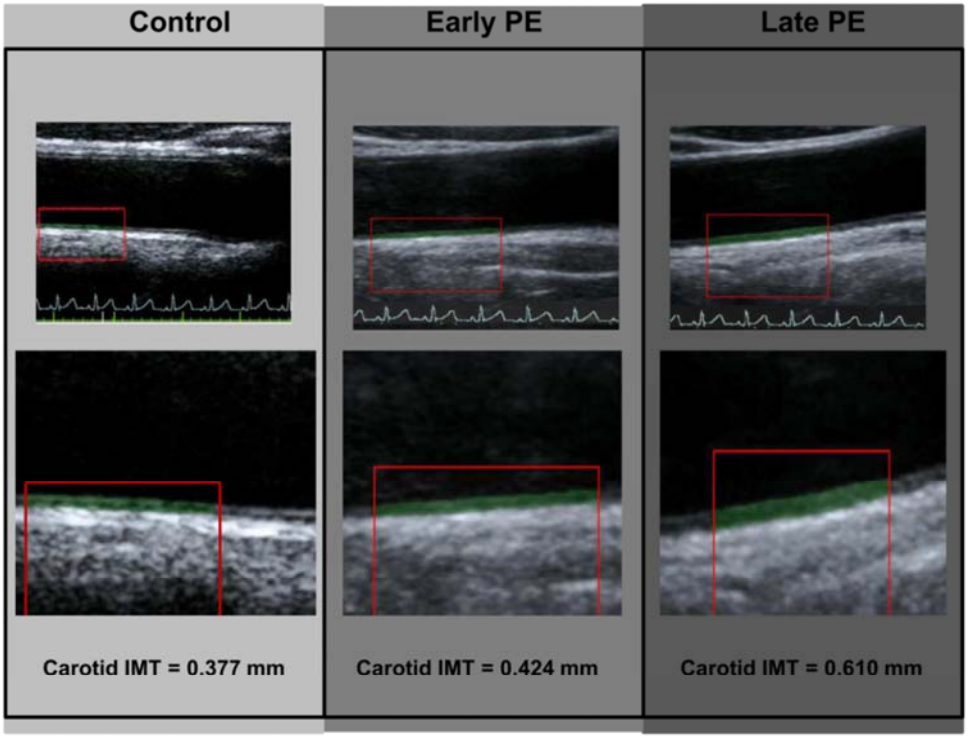
[c]*P* calculated by linear trends across severity groups

PE, preeclampsia; *BP*, blood pressure; *cIMT*, carotid intima-media thickness

Maximum carotid artery IMT was significantly increased in both early and late preeclamptic cases as compared to controls (P=0.01 and P<0.01, respectively). Similarly to mean carotid artery IMT, a linear trend for higher values across groups was observed (P<0.001). Regarding lumen systolic and diastolic diameters, we noted significantly increased diameters in both early and late PE as compared to normotensive subjects. Furthermore, both systolic and diastolic diameters were significantly increased in early PE as compared to late PE even after adjusting by maternal age, maternal body mass index and gestational age at evaluation (P=0.035 and P<0.001, respectively). Regarding arterial stiffness, carotid distensibility was reduced significantly in early PE when compared to controls (P=0.013), while this reduction did not reach statistical significance in late PE (P=0.262). However, a significant linear trend demonstrated differences among groups (P=0.008). Circumferential wall stress was significantly increased in both preeclamptic groups as compared to controls (both early and late PE P<0.001).Similarly to arterial distensibility, it showed a linear trend for differences across study groups (P<0.001).

Results on inferior vena cava respiratory variation are also presented in Table 3. Although the vessel diameters were similar among study groups during inspiration and expiration, there was a significant decrease in collapsibility and collapsibility index in late as compared to early PE (P=0.044 and P=0.043, respectively). Furthermore, late PE was characterized by a decrease in collapsibility index as compared to controls (P=0.029). A significant linear decrease was observed across study groups (P=0.026).

Figure 6. Illustration of the carotid intima-media thickness (IMT) measurement by ultrasound in control, early and late preeclamptic (PE) subjects.



6.2 Maternal vascular characteristics in FGR (study 2)

FGR cases with or without PE demonstrated a higher prevalence of maternal smoking and assisted reproductive technologies. In addition, FGR cases with PE had higher maternal BMI, paternal age and prevalence of nulliparity. First trimester maternal blood pressure was higher in both FGR groups with significantly higher values in the cases that later developed PE. As expected, FGR cases had a lower birthweight centile together with worse feto-placental Doppler and perinatal outcome, especially in those cases complicated by PE.

Results on maternal vascular parameters in FGR and PE are shown in Figure 7 and Table 4.

Both FGR cases with or without PE had a significant increase of maternal blood pressure, carotid IMT and CWS at third trimester as compared to controls. In addition, normotensive FGR had a non-significant tendency to higher CAD and IVC collapsibility index together with similar arterial diameters as compared to controls. On the contrary, FGR cases with PE showed significantly lower CAD and non-significant lower IVC collapsibility index with increased diameters as compared to controls and normotensive FGR.

Results of fetal ultrasound and maternal vascular characteristics in normotensive FGR cases, subdivided into early and late-onset, are presented in Table 5. As expected, both FGR groups had worse feto-placental Doppler parameter results. Both early and late-onset normotensive FGR had significantly increased first and third trimester blood pressure values as compared to controls. Early-onset normotensive FGR was associated with significantly increased carotid IMT and CWS with no significant changes in CAD and collapsibility index as compared with controls. Late-onset normotensive FGR showed similar results as compared with controls for both structural and functional maternal vascular parameters.

Table 4. Results on maternal vascular assessment among the study groups

Characteristic	Controls	Normotensive FGR	FGR with PE	*P-value*[c]
N	***110***	***64***	***60***	
Gestational age at maternal evaluation (weeks)	34.4(3)	35.4(7)	32.4(5)	0.485
Maternal BP				
Systolic BP at examination (mmHg)	110 (14)	114 (20)[a]	145 (17)[ab]	***<0.001***
Diastolic BP at examination (mmHg)	69 (12)	73 (14)[a]	91 (15)[ab]	***0.004***
Mean BP at examination (mmHg)	82 (12)	88 (14)[a]	109 (15)[ab]	***<0.001***
Maternal carotid arteries				
Mean cIMT(mm)	0.409 (0.04)	0.436 (0.08)[a]	0.425 (0.10)[a]	***0.008***
Maximum cIMT(mm)	0.504 (0.06)	0.535 (0.07)[a]	0.542 (0.11)[a]	***<0.001***
Carotid systolic diameter (mm)	6.000 (0.450)	6.000 (0.680)	6.750 (0.740)[ab]	0.701
Carotid diastolic diameter (mm)	5.550 (0.550)	5.470 (0.550)	6.150 (0.840)[ab]	0.773
Carotid Distensibility ($kPa^{-1}10^{-3}$)	31.97 (15.73)	34.94 (17.62)	25.30 (18.94)[ab]	0.656
Circumferencial Wall Stress (kPa)	12.236 (3.07)	14.006 (3.36)[a]	19.785 (5.72)[ab]	***0.001***
Inferior Vena Cava				
Diameter at inspiration	8.700 (5.10)	6.600 (3.10)	9.750 (2.60)	0.135
Diameter at expiration	9.900 (4.60)	7.600 (4.10)	10.600 (2.75)[ab]	0.169
Collapsibility	0.950 (0.50)	0.900 (0.85)	0.800 (0.57)	0.646
Collapsibility Index	0.105 (0.06)	0.120 (0.08)	0.073 (0.04)[b]	0.146

Data are presented as median (interquartile range).

[a]*P<0.05* as compared to controls calculated by regression adjusted by maternal age, body mass index, smoking and gestational age at evaluation

[b]*P<0.05* as compared to normotensive FGR calculated by regression adjusted by maternal age, body mass index, smoking and gestational age at evaluation

[c]*P* calculated by linear trends across severity groups

FGR, fetal growth restriction; *PE*, preeclampsia; *BP*, blood pressure; *cIMT*, carotid intima-media thickness

Table 5. Results on maternal vascular assessment among the normotensive FGR study cases subclassified by gestational age to early- and late- onset FGR

Characteristic	Controls	Normotensive late FGR	Normotensive early FGR	*P-value*[c]
N	110	44	20	
Fetal ultrasound evaluation				
Gestational age at fetal scan (weeks)	33.5 (2.1)	38.1 (2.5)	28.5 (4.1)[ab]	***<0.001***
Uterine artery mean PI (z-scores)	-0.68 (0.30)	0.10 (1.93)	1.21 (1.95)	0.353
Umbilical artery PI (z-scores)	-0.89 (0.64)	0.32 (1.49)[a]	1.95 (3.13)[a]	***<0.001***
Middle cerebral artery PI (z-scores)	0.19 (2.08)	-0.01 (1.68)	-1.25 (1.51)[a]	***0.001***
Cerebro-placental ratio (z-scores)	0.37 (0.04)	-0.84 (1.60)[a]	-2.38 (1.96)[ab]	***<0.001***
Maternal BP				
First trimester Systolic BP (mmHg)	110 (15)	115 (20)	123 (14)[ab]	***0.009***
First trimester Diastolic BP (mmHg)	65 (13)	66 (10)[a]	71 (17)[a]	***0.021***
First trimester mean BP (mmHg)	80 (13)	84 (11)[a]	90 (14)[a]	***0.006***
Systolic BP at examination (mmHg)	110 (14)	115 (19)[a]	114 (19)[a]	***0.005***
Diastolic BP at examination (mmHg)	69 (12)	73 (13)	73 (14)[a]	0.081
Mean BP at examination (mmHg)	82 (12)	88 (14)[a]	89 (14)[a]	***0.015***
Maternal carotid arteries				
Mean cIMT (mm)	0.409 (0.04)	0.417 (0.08)	0.476 (0.10)[ab]	***<0.001***
Maximum cIMT (mm)	0.504 (0.06)	0.532 (0.06)	0.560 (0.16)[ab]	***<0.001***
Carotid systolic diameter (mm)	6.000 (0.450)	6.000 (0.590)	5.975 (0.660)	0.789
Carotid diastolic diameter (mm)	5.550 (0.550)	5.475 (0.580)	5.475 (0.590)	0.423
Carotid Distensibility ($kPa^{-1}10^{-3}$)	31.97 (15.73)	32.79(17.35)	37.29 (16.29)	0.121
Circumferencial Wall Stress (kPa)	12.236 (3.07)	13.590(3.41)	14.549(5.26)[ab]	***<0.001***
Inferior Vena Cava				
Diameter at inspiration	8.700 (5.10)	7.250 (2.50)	5.700 (1.40)[a]	0.055
Diameter at expiration	9.900 (4.60)	8.350 (3.35)	6.500 (1.80)[a]	***0.036***
Collapsibility	0.950 (0.50)	1.000 (1.15)	0.800 (0.20)	0.363
Collapsibility Index	0.105 (0.06)	0.124 (0.10)	0.120 (0.08)	0.385

Data are presented as median (interquartile range).

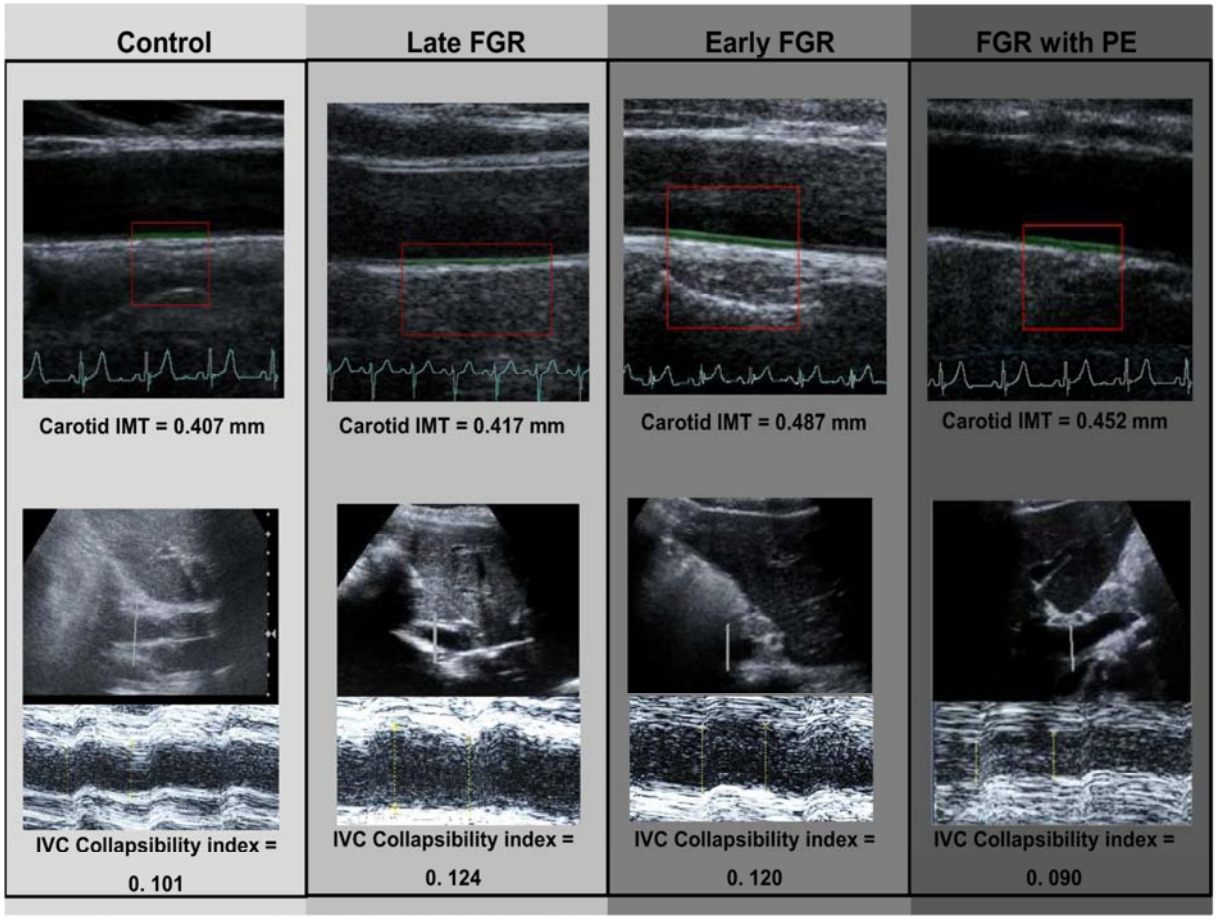
[a]*P<0.05* as compared to controls calculated by regression adjusted by maternal age, body mass index, smoking and gestational age at evaluation

[b]*P<0.05* as compared to normotensive late FGR calculated by regression adjusted by maternal age, body mass index, smoking and gestational age at evaluation

[c]*P* calculated by linear trends across severity groups

FGR, fetal growth restriction; *BP*, blood pressure; *cIMT*, carotid intima-media thickness

Figure7. Illustration of the carotid intima-media thickness (IMT) measurement and inferior vena cava respiratory variations by ultrasound in control, normotensive early FGR, normotensive late FGR and FGR with PE



6.3 Reproducibility of neonatal IMT measurement (study 3)

Results on intra- and inter-observer reproducibility are presented in tables 6 and 7, figures 8 and 9.

Table 6.Intra-observer reproducibility of neonatal carotid and aortic arteries intima- media thickness evaluation.

	Correlation coefficient	Coefficient of variation
	ρ	CV, %
Mean right cIMT	0.827 (0.790-0.859)	2.7%
Mean left cIMT	0.870 (0.842-0.895)	2.6%
Maximum right cIMT	0.908 (0.887-0.926)	2.8%
Maximum left cIMT	0.921 (0. 903-0.937)	2.6%
Mean aIMT	0.861 (0.830-0.887)	2.6%
Maximum aIMT	0.934 (0.919-0.947)	2.1%

ρ indicates intraclass correlation coefficient (Lower bound-higher bound); CV, coefficient of variation; cIMT, carotid artery intima-media thickness; aIMT, aortic intima-media thickness.

Table 7. Inter-observer reproducibility of neonatal aortic and carotid arteries intima- media thickness evaluation.

	Coefficient of variation
	CV, %
Mean right cIMT	1.3%
Mean left cIMT	1.5%
Maximum right cIMT	1.2%
Maximum left cIMT	1.0%
Mean aIMT	7.6%
Maximum aIMT	4.5%

CV, coefficient of variation; cIMT, carotid artery intima-media thickness; aIMT, aortic intima-media thickness.

Figure 8. Bland–Altman plots of the difference versus the mean of the paired measurements between observers of mean carotid IMT (A) and max carotid IMT (B)

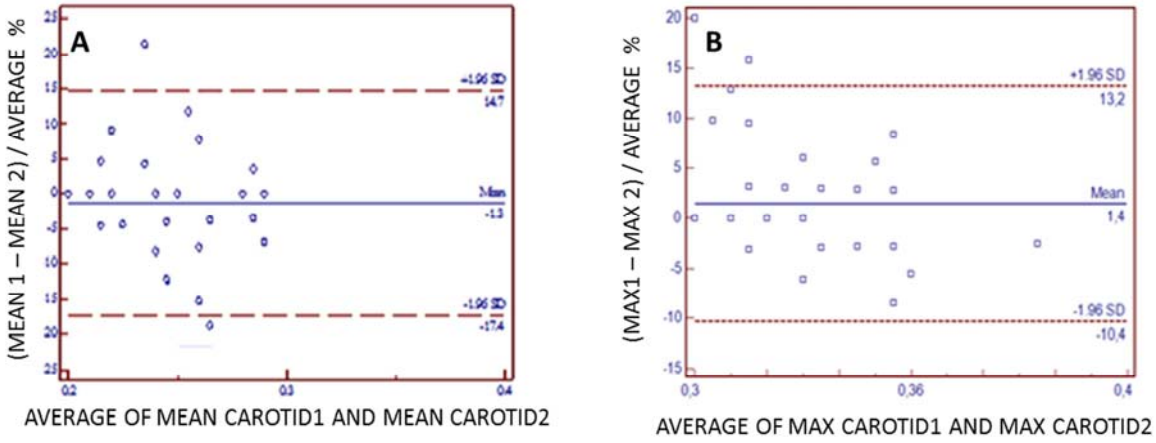
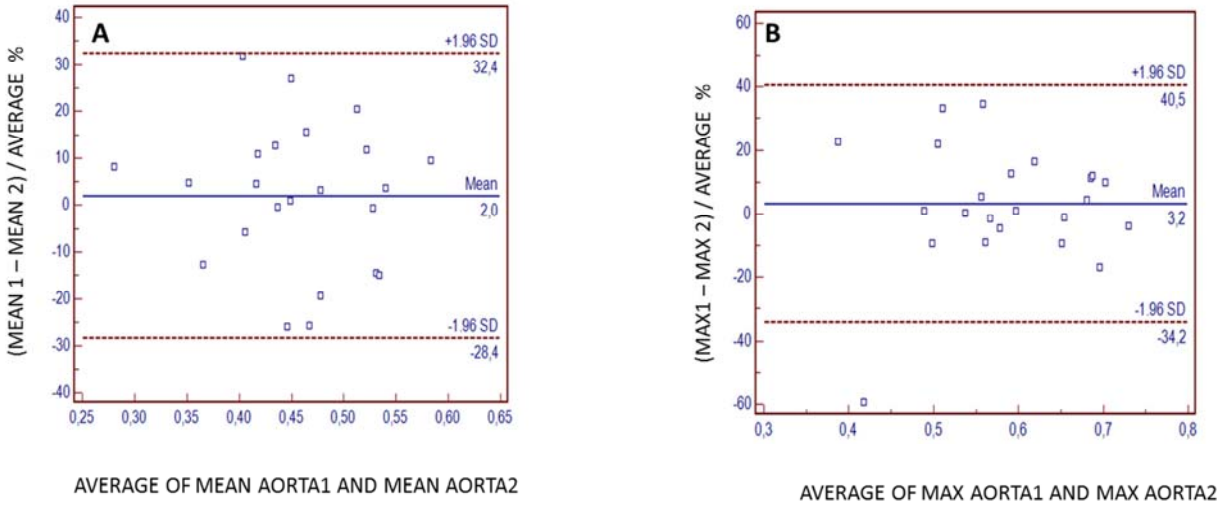


Figure 9. Bland–Altman plots of the difference versus the mean of the paired measurements between observers of mean aortic IMT (A) and max aortic IMT (B)



6.4 Neonatal vascular characteristics in FGR (study 4)

In study 4, the maternal characteristics were similar among the study groups with the exception of a higher prevalence of nulliparity and smokers in the SGA group with signs of severity as compared to controls. By definition, both SGA groups had a lower birth weight. SGA cases with signs of severity had worse prenatal Doppler as compared to controls. Most of SGA with signs of severity were characterized by EFW <p3 and only 30% of them presented abnormal uterine artery Doppler or cerebroplacental ratio. As expected, SGA cases with signs of severity had a higher prevalence of cesarean section and admission to the neonatal intensive care unit.

Results on vascular parameters are shown in table 8 and figure 10.

Blood pressure values were similar among groups with a non-significant tendency for higher values in SGA without and with signs of severity.

Mean and maximum carotid artery were significantly increased in both SGA cases as compared to controls (mean cIMT: 0.017 mm [SE 0.008], P=0.041 and maximum cIMT: 0.035 mm [SE 0.011], P=0.002 per SGA without signs of severity; mean cIMT: 0.023 mm [SE 0.010], P=0.028 and maximum cIMT: 0.026 mm [SE 0.013], P=0.050 per SGA with signs of severity). These differences remained significant after normalization of IMT values by neonatal weight and vessel diameter. There was a significant linear tendency for higher values across groups (P<0.001, P=0.001 for mean and maximum IMT, respectively). Mean aortic IMT showed a non-significant trend for higher values between controls and SGA cases (mean aIMT: 0.023 mm [SE 0.017], P=0.194 per SGA without signs of severity; mean aIMT: 0.036 mm [SE 0.018], P=0.055 per SGA with signs of severity), that became significantly different after adjusting by neonatal weight and vessel diameter. Furthermore, maximum aorta IMT was significantly increased in both SGA cases as compared to controls (maximum aIMT: 0.055 mm [SE 0.018], P=0.003 per SGA without signs of severity

and maximum aIMT: 0.057 mm [SE 0.022], P=0.010 per SGA with signs of severity). Similarly to previous parameters, the association remained significant after normalization of IMT values by neonatal weight and vessel diameter.

Table 8. Results on neonatal vascular assessment in the study groups.

Characteristic	Controls	SGA without signs of severity	SGA with signs of severity	*Linear tendency P-value*[c]
N	*134*	*32*	*35*	
Blood pressure				
Systolic BP (mmHg)	82 (17)	85 (20)	86 (13)	0.143
Systolic BP percentile	57 (50)	57 (48)	62 (48)	0.420
Diastolic BP (mmHg)	46 (15)	48 (15)	52 (16)	0.107
Diastolic BP percentile	44 (53)	53 (50)	65 (51)	0.324
Carotid arteries				
Mean cIMT(mm)	0.226 (0.06)	0.247 (0.05)[a]	0.268 (0.08)[a]	***<0.001***
Mean cIMT / neonatal weight (mm/kg)	0.067 (0.02)	0.090 (0.02)[a]	0.090 (0.03)[a]	***<0.001***
Mean cIMT / diameter	0.094 (0.04)	0.117 (0.04)[a]	0.112 (0.03)[a]	***<0.001***
Maximum cIMT(mm)	0.303 (0.08)	0.336 (0.08)[a]	0.341 (0.10)[a]	***0.001***
Maximum cIMT / neonatal weight (mm/kg)	0.090 (0.02)	0.122 (0.02)[a]	0.120 (0.04)[a]	***<0.001***
Max cIMT / diameter	0.126 (0.04)	0.148 (0.05)[a]	0.157 (0.04)[a]	***<0.001***
Aorta				
Mean aIMT(mm)	0.419 (0.10)	0.493 (0.20)	0.446 (0.12)	***<0.001***
Mean aIMT / neonatal weight (mm/kg)	0.127 (0.03)	0.183 (0.08)[a]	0.210 (0.06)[ab]	***<0.001***
Mean aIMT / diameter	0.085 (0.03)	0.104 (0.05)[a]	0.113 (0.04)[a]	***<0.001***
Maximum aIMT(mm)	0.547 (0.11)	0.622 (0.20)[a]	0.604 (0.15)[a]	***0.002***
Maximum aIMT / neonatal weight (mm/kg)	0.165 (0.05)	0.222 (0.08)[a]	0.284 (0.09)[ab]	***<0.001***
Maximum aIMT /aortic diameter	0.111 (0.02)	0.128 (0.07)[a]	0.141 (0.05)[a]	***<0.001***

Data are presented as median (interquartile range).

[a]*P<0.05* as compared to controls calculated by regression adjusted by gender, gestational age at birth, age at evaluation and group

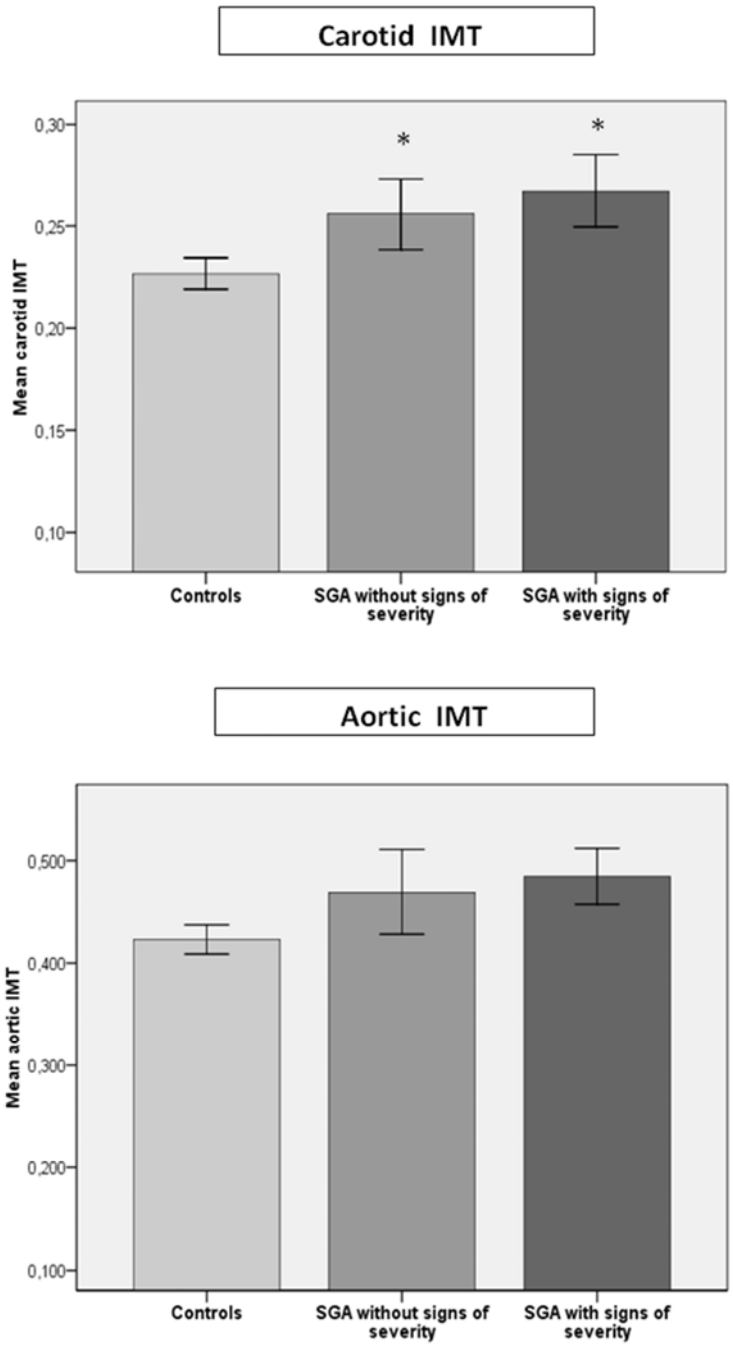
[b]*P<0.05* as compared to SGA without signs of severity calculated by regression adjusted by gender, gestational age at birth, age at evaluation and group

[c]*P <0.05* calculated by linear trends across severity groups

SGA, small-for-gestational age; *BP*, blood pressure;

cIMT, carotid intima-media thickness; *aIMT*, aortic intima-media thickness.

Figure 10. Vascular intima-media thickness (IMT) in controls, late-onset growth restricted neonates without signs of severity and late-onset growth restricted neonates with signs of severity. Data are presented as mean (95% CI). *P<0.05 as compared to controls adjusted by gender, gestational age at birth, age at evaluation and group.



7. DISCUSSION

The main aim of this work was to investigate the vascular adaptations characterizing mothers and offspring affected by placental disease. We considered both vascular structure and vascular function, in order to perceive potential distinct clinical features. Our findings provided evidence that both PE and FGR presented maternal vascular remodeling while vascular function was restricted to the preeclamptic patients. The subclassification into early and late disease allowed us to recognize a common etiology but distinct pathophysiologic paths. Regarding offspring affectation, increased carotid and aortic IMT were present in both SGA with and without signs of severity.

7.1 Maternal vascular changes in early and late PE (study 1)

This study provides evidence that maternal vascular characteristics of preeclamptic women differ between early and late-onset PE.

Early PE was characterized by increased carotid IMT, lumen diameters and arterial stiffness, but with no significant changes in IVC collapsibility as compared to normotensive pregnancies. The release of factors from an underperfused placenta[33,34,35,36]in early PE may cause vascular dysfunction and high blood pressure. Enlarged IMT may represent an adaptive response to preserve arterial wall stress.[100] Disturbances in endothelial function may affect arterial elasticity.[79] Lumen diameters in early PE are significantly increased as compared to controls. The significant increase of cardiac output in early PE after initiation of treatment may explain this observation.[53,101] IVC collapsibility was similar as in control pregnancies. Our finding may be explained by the lack of history of maternal vascular maladaptation.

Carotid IMT was expected to present comparable values in early and late PE due to observed similar BP. However, mean carotid IMT was found to be increased in late

PE. This finding suggests that an earlier vascular impairment, possibly due to maternal predisposition, has occurred.Arterial stiffness was increased in late PE but to a lesser degree than in early PE and timely arterial hypertrophy has been postulated.[76]Preexisting maternal vascular maladaptation could also be supported by the decreased collapsibility index in late PE. The less-prominent increase found in carotid diameters in late PE is concordant with milder changes in cardiac output as compared to early PE.[38]

Maternal predisposition plays a considerably less important role in early PE than in late PE. The release of factors from the hypoxic placenta may initiate endothelial dysfunction. Lower arterial susceptibility in early PE supports that the vascular insult in these patients is of more acute nature. Late PE is probably associated with maternal constitutional factors and less placental involvement. Our finding of different levels of stiffness may reflect differences in the severity of disease. Alternatively, it may reflect changes caused by the greater prevalence of chronic vascular disease in late PE, resulting in milder vascular disease and therefore better cardiovascular outcome.

The strength of the present study was mainly to evaluate for the first time vascular structure and function in preeclamptic women at diagnosis differentiating between early and late PE. We acknowledge that our study has limitations due to the limited approach of cardiovascular physiology. Furthermore, we acknowledge that our sample size could not have been sufficient enough to demonstrate statistical significant results for all parameters, despite performing a power calculation. The inclusion of cases of preeclamptic women with history of smoking and chronic hypertension could raise concerns due to their potential confounding influence. However, we do not believe that is the case, as most parameters show similar results in early and late-onset PE even after excluding smokers and chronic hypertensive patients. It would have been also interesting to include preconceptional and

postpartum evaluation of our sample. As preeclampsia is now considered a cardiovascular risk factor, further investigation on its implications is mandatory.

7.2 Maternal vascular changes in FGR (study 2)

The most relevant result of this study is the significant increase of carotid IMT and BP in normotensive FGR as compared to controls. Despite similar structural changes however, vascular function, reflected by CAD and IVC collapsibility index, differed considerably between FGR and PE. Our data, along with previous reports, contributes to a better understanding of the shared and distinct features of FGR and PE. Both entities present signs of placental and endothelial dysfunction,[33] as well as similar structural cardiac adaptations.[33,46,102]In this context, increased carotid IMT and BP could possibly represent a subclinical manifestation of the maternal disease in FGR. The preservation or even the increase of vascular distensibility in FGR could be attributed to less pronounced cardiovascular impairment and a timely maternal adaptation in FGR.[33,46]Conversely, severe maternal systemic disease in PE enhances arterial and venous stiffness that could lead to cardiovascular decompensation and injury.[79] Thus, the minor vascular dysfunction in normotensive FGR could support the concept of a milder (2-fold) increased risk for cardiovascular disease as compared with the markedly higher 8-fold risk for early PE.[11,103]

Maternal responses in early and late normotensive FGR were further assessed in our study. Early FGR presented increased BP, carotid IMT and CWS, while in late FGR less prominent adaptations occurred with only BP showing significant differences. Interestingly, first and third trimester maternal BP was significantly increased in both early and late FGR as compared with controls. The disparity in maternal BP values in FGR among our and previous results[44,46,104]could be explained by differences in FGR definition and study populations. Overall, these results indicate that early and late FGR share common pathophysiologic traits, but the observed differences imply that they might be distinct clinical diseases. Actually, the very severe early-onset FGR

with abnormal umbilical artery demonstrated similar vascular behavior as early PE (supplemental data). Our findings are in keeping with previous studies reporting different cardiovascular responses for early and late FGR[44,46] that might possibly encompass distinct future cardiovascular outcomes. Epidemiologic studies have indeed provided evidence of worst cardiovascular outcome in women with history of early rather than late FGR.[46,47,48,105]

The main strength of the present study was to evaluate for the first time maternal vascular structure and function in FGR pregnant women at diagnosis, distinguishing among different subsets of normotensive FGR. Overall, our data suggest that vascular features in the normotensive FGR could be attributed to placental involvement, which further induces subclinical maternal vascular adaptations. Whether vascular IMT could be used as a prognostic imaging biomarker to improve stratification of long term maternal cardiovascular outcome in FGR needs to be further investigated in large prospective studies.

This study has some limitations. Studying mainly the circulatory and not cardiac adaptations could account as a limitation of our study. Furthermore, we acknowledge that our sample size could not have been sufficient enough to compare different subsets of FGR, as demonstrated by several trends not reaching statistical significance. We also acknowledge that several potential confounders such as smoking could have affected our maternal vascular results. However, all analyses were adjusted by maternal age, smoking and body mass index in order to minimize this effect. Finally, as FGR is emerging as a cardiovascular risk factor, further postpartum follow up is warranted to be confirmed in long term studies.

7.3 Reproducibility of neonatal IMT measurements (study 3)

Results on intra- and inter-observer reproducibility of carotid and aortic IMT demonstrated that both parameters are feasible to measure in neonates. As

expected, carotid IMT presents lower coefficients of variation than aorta. Carotid arteries are located superficially in the neck and can be easily visualized by ultrasound. Their ultrasonic assessment does not require any sophisticated technique and is feasible in an outpatients department setting. On the other hand, aorta is located beneath the liver and ultrasound resolution can sometimes be poorer due to intestinal gas. However, high resolution ultrasound technology allowed us to complete the examination in the vast majority of neonates. Thus, good results on reproducibility for both carotid and aortic IMT and good tolerance level on behalf of the neonates allowed to recruit more newborns in order to perform our forth study.

7.4 Carotid and aortic IMT changes in term SGA neonates (study 4)

This study demonstrates that term SGA cases with and without signs of severity show increased vascular IMT, irrespective of the absence of signs of severity.

No significant differences for both systolic and diastolic blood pressure within groups were demonstrated in our study, similarly to previous data on preterm controls and early-onset growth restricted neonates.[80] However, a tendency for higher values in SGA newborns with signs of severity was shown as compared to SGA without signs of severity. Our results could be explained by our limited sample size, the selection of a different postnatal age at examination or the intrinsic limitation of measuring blood pressure in the neonatal period.

Vascular IMT was increased in growth restricted neonates irrespective whether they presented signs of severity and after adjusting by neonatal weight and vessel diameter. We showed a significant linear trend for higher values of IMT in relation to growth restriction severity, without though significant differences among the two SGA groups. This association suggests that SGA contain a substantial proportion of cases representing forms of true fetal growth restriction and that well-established severity markers, such as uterine artery pulsatility index, cerebroplacental ratio, estimated

fetal weight less than the 3rd percentile[42,63,64]fail to predict a normal postnatal cardiovascular outcome.

Arterial tensile stress, a major determinant of arterial hemodynamic forces,[100] and arterial distensibility were also evaluated and showed comparable values between cases and controls. Theoretically, these findings were not anticipated when considering the previously reported increased afterload and arterial stiffness in fetuses and preterm growth restricted neonates when evaluated shortly after delivery.[58,80,106] The vascular behavior in our cohort would support the hypothesis that high placental resistance and raised blood pressure occur merely during fetal life.[58,80,106] In the immediate postnatal period however, the multiple stimuli that are responsible for the aforementioned changes would cease to exert their influence. Consequently, tensile stress would show a behavior similar to blood pressure during this period. This assumption could be in line with our previously published results in 3-6 years old children with late-onset growth restriction without signs of severity. These children showed a non-significant trend for higher values of normalized mean cIMT values and significant linear tendency for this parameter across stages of severity.[61]It seems therefore that despite normalization of hemodynamic conditions in the early neonatal period, mild changes still persist in childhood.

This study has strengths and limitations. To our knowledge, it was the first time to focus on structural and functional parameters of carotid and aorta as distinct components of the vascular tree.[68,74,107] Furthermore, the thorough classification of neonates according to their prenatal data in stages of severity has allowed observing vascular remodeling in all growth restricted neonates. The adjustment of results by both neonatal weight and lumen diameter further confirmed this finding. On the other side, our relatively limited sample size could account for the non-significant trend for higher values in blood pressure. We also acknowledge that the different prevalence of maternal smoking among groups could have partly affected our results. Finally,

long term pediatric follow up of our cohort is warranted in order to better understand the pathophysiology of cardiovascular remodeling in growth restriction and subsequently implement preventive strategies.

8. CONCLUSIONS

1. Distinct maternal vascular structural and functional adaptations in early and late PE reflect different etiologies and clinical expressions

2. Normotensive FGR pregnant women, especially the early-onset FGR cases, present signs of vascular remodeling similar to those observed in PE.

3. Vascular IMT is a feasible and reproducible measurement in the neonatal period

4. FGR neonates, both with and without signs of severity, demonstrate similar changes in vascular structure and function.

9. REFERENCES

1. World Health Organization Collaboration. Cardiovascular diseases.WHO. Http://www.Who.Int/mediacentre/factsheets/fs317/en/. 2. Assessed 24.11.2013

2. World Heart Federation. Cardiovascular disease risk factors.http://www.world-heart federation.org/fileadmin/user_upload/documents/Fact_sheets/2012/PressBackgrounderApril2012RiskFactors.pdf. Assessed 24.11.2013

3. World Heart Federation. Women and cardiovascular disease.http://www.world-heart-federation.org/press/fact-%20sheets/women-and-cardiovascular-disease/

4. Sociedad española de cardiología. La enfermedad cardiovascular mata a casi un 8% más de mujeres que hombres en España. http://www.secardiologia.es/formacion-y-becas/congresos/congreso-sec/sec-2011/3607-enfermedad-cardiovascular-mata-a-casi-un-8-mas-de-mujeres-que-hombres-espana#

5. Mosca L, Banka CL, Benjamin EJ, Berra K, Bushnell C, Dolor RJ, Ganiats TG, Gomes AS, Gornik HL, Gracia C, Gulati M, Haan CK, Judelson DR, Keenan N, Kelepouris E, Michos ED, Newby LK, Oparil S, Ouyang P, Oz MC, Petitti D, Pinn VW, Redberg RF, Scott R, Sherif K, Smith SC Jr, Sopko G, Steinhorn RH, Stone NJ, Taubert KA, Todd BA, Urbina E, Wenger NK; Expert Panel/Writing Group; American Heart Association; American Academy of Family Physicians; American College of Obstetricians and Gynecologists; American College of Cardiology Foundation; Society of Thoracic Surgeons; American Medical Women's Association; Centers for Disease Control and Prevention; Office of Research on Women's Health; Association of Black Cardiologists; American College of Physicians; World Heart Federation; National Heart, Lung, and Blood Institute; American College of Nurse Practitioners. Evidence-based guidelines for cardiovascular disease prevention in women: 2007 update. Circulation 2007;115:1481-501.

6. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Piña IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC Jr, Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V, Wenger NK; American Heart Association. Effectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women—2011 Update : A Guideline From the American Heart Association. J Am Coll Cardiol 2011;57:1404-23.

7. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ 2007;335:974–977.

8. Valdiviezo C, Garovic VD, Ouyang P. Preeclampsia and hypertensive disease in pregnancy: their contributions to cardiovascular risk. Clin Cardiol 2012;35;60-5.

9. Manten GT, Sikkema MJ, Voorbij HA, Visser GH, Bruinse HW, Franx A. Risk factors for cardiovascular disease in women with a history of pregnancy complicated by preeclampsia or intrauterine growth restriction. Hypertens Pregnancy 2007;26:39-50.

10. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. Hypertension 2009;53:944–951. 17.

11. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardio- vascular disease death: prospective evidence from the child health and development studies cohort. Hypertension 2010;56:166–171.

12. Romundstad PR, Magnussen EB, Smith GD, Vatten LJ. Hypertension in pregnancy and later cardiovascular risk: common antecedents? Circulation 2010;122:579-84.

13. Barker DJ. Fetal origins of coronary heart disease. Br Heart J 1993;69:195-6.

14. Kaijser M, Bonamy AK, Akre O, Cnattingius S, Granath F, Norman M, Ekbom A. Perinatal risk factors for ischemic heart disease: disentangling the roles of birthweight and preterm. Circulation 2008;117:405-10.

15. Skilton MR, Evans N, Griffiths KA, Harmer JA, Celermajer DS.Aortic wall thickness in newborns with intrauterine growth restriction. Lancet 2005;365:1484–6.

16. Koklu E, Kurtoglu S, Akcakus M et al. Increased aortic intima-media thickness is related to lipid profile in newborns with intrauterine growth restriction. Horm Res 2006;65:269-75.

17. Ananth CV, Peltier MR, Chavez MR, Kirby RS, Getahun D, Vintzileos AM. Recurrence of ischemic placental disease. Obstet Gynecol 2007;110(1):128-33.

18. Norwitz ER. Defective implantation and placentation: laying the blueprint for pregnancy complications. Reprod Biomed Online 2006;13:591–9.

19. Zhang P, Schmidt M, Cook L. Maternal vasculopathy and histologic diagnosis of preeclampsia: poor correlation of histologic changes and clinical manifestation. Am J Obstet Gynecol 2006;194:1050–6.

20. Salafia CM, Minior VK, Pezzullo JC, Popek EJ, Rosenkrantz TS, Vintzileos AM. Intrauterine growth restriction in infants of less than thirty-two weeks' gestation: associated placental pathologic features. Am J Obstet Gynecol 1995;173:1049–57.

21. Katzman PJ, Genest DR. Maternal floor infarction and massive perivillous fibrin deposition: histological definitions, association with intrauterine fetal growth restriction, and risk of recurrence. Pediatr Dev Pathol 2002;5:159–64.

22. Mardi K, Sharma J. Histopathological evaluation of placentas in IUGR pregnancies. Indian J Pathol Microbiol 2003;46:551–4.

23. Smith GCS, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129 290 births. Lancet 2001;357:2002–6.

24. Pell JP, Smith GC, Walsh D. Pregnancy complications and subsequent maternal cerebrovascular events: a retrospective cohort study of 119,668 births. Am J Epidemiol. 2004;159:336-42.

25. Magnussen EB, Vatten LJ, Smith GD, Romundstad PR.. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. Obstet Gynecol 2009;114:961–970

26. Harville EW, Viikari JS, Raitakari OT. Preconception cardiovascular risk factors and pregnancy outcome. Epidemiology 2011;22:724-30.

27. Roberts JM, Hubel CA. Pregnancy: a screening test for later life cardiovascular disease. Womens Health Issues 2010;20:304-7.

28.Rodie VA, Freeman DJ, Sattar N, Greer IA. Preeclampsia and cardiovascular disease: metabolic syndrome of pregnancy? Atherosclerosis 2004;175:189 –202.

29. World Health Organization Collaboration. WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia 2011.Department of Reproductive Health and Research, WHO.http://www.who.int/maternal_child_adolescent/documents/preeclampsia_eclampsia/en/

30. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365:785-99.

31. National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. Am J Obstet Gynecol 2000;183:S1-S22.

32. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy 2001;20:IX-XIV.

33. Crispi F, Domínguez C, Llurba E, Martín-Gallán P, Cabero L, Gratacós E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in preeclampsia and in isolated intrauterine growth restriction. Am J Obstet Gynecol 2006;195:201-7.

34. Crispi F, Llurba E, Domínguez C, Martín-Gallán P, Cabero L, Gratacós E. Predictive value of angiogenic factors and uterine artery Doppler for early- versus late-onset pre-eclampsia and intrauterine growth restriction. Ultrasound Obstet Gynecol 2008;31:303-9.

35. Valensise H, Vasapollo B, Gagliardi G, Novelli GP. Early and late preeclampsia: two different maternal hemodynamic states in the latent phase of the disease. Hypertension 2008;52:873–80.

36. Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. Hypertension 2008;51:970-5.

37. Melchiorre K, Thilaganathan B.Maternal cardiac function in preeclampsia. Curr Opin Obstet Gynecol 2011;23:440-7.

38. Melchiorre K, Sutherland GR, Baltabaeva A, Liberati M, Thilaganathan B. Maternal cardiac dysfunction and remodeling in women with preeclampsia at term. Hypertension 2011;57:85–93.

39. Baschat AA. Pathophysiology of fetal growth restriction: implications for diagnosis and surveillance. Obstet Gynecol Surv 2004;59:617-27.

40. Gardosi J. Clinical strategies for improving the detection of fetal growth restriction. Clin Perinatol 2011;38:21-31.

41. Savchev S, Figueras F, Sanz-Cortes M, Cruz-Lemini M, Triunfo S, Botet F, Gratacos E. Evaluation of an optimal gestational age cut-off for the definition of early and late-onset fetal growth restriction. Fetal Diagn Ther 2013. DOI: 10.1159/000355525

42. Severi FM, Bocchi C, Visentin A et al. Uterine and fetal cerebral Doppler predict the outcome of third-trimester small-for-gestational age fetuses with normal umbilical artery Doppler. Ultrasound Obstet Gynecol 2002; 19: 225-8.

43. Cruz-Martínez R, Figueras F, Hernandez-Andrade E, Oros D, Gratacos E. Fetal brain Doppler to predict cesarean delivery for nonreassuring fetal status in term small-for-gestational-age fetuses.Obstet Gynecol 2011; 117: 618-26.

44. Melchiorre K, Sutherland GR, Liberati M, Thilaganathan B. Maternal cardiovascular impairment in pregnancies complicated by severe fetal growth restriction. Hypertension 2012;60:437-43.

45. Manten GT, Sikkema MJ, Voorbij HA, Visser GH, Bruinse HW, Franx A. Risk factors for cardiovascular disease in women with a history of pregnancy complicated by preeclampsia or intrauterine growth restriction. Hypertens Pregnancy 2007;26:39-50.

46. Bamfo JE, Kametas NA, Chambers JB, Nicolaides KH. Maternal cardiac function in fetal growth-restricted and non-growth-restricted small-for- gestational age pregnancies. Ultrasound Obstet Gynecol 2007;29:51–57.

47. Caruso A, Paradisi G, Ferrazzani S, Lucchese A, Moretti S, Fulghesu AM. Effect of maternal carbohydrate metabolism on fetal growth. Obstet Gynecol 1998;92:8-12.

48. Kanagalingam MG, Nelson SM, Freeman DJ, Ferrell WR, Cherry L, Lowe GD, Greer IA, Sattar N. Vascular dysfunction and alteration of novel and classic cardiovascular risk factors in mothers of growth restricted offspring. Atherosclerosis 2009;205:244-50.

49. Davey Smith G, Harding S, Rosato M. Relation between infants' birth weight and mothers' mortality: prospective observational study. BMJ 2000;320:839–40.

50. Smith GCS, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129 290 births. Lancet 2001;357:2002–6.

51. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. Am J Obstet Gynecol 2006;195: 40-9.

52. Koopmans CM, Blaauw J, van Pampus MG, Rakhorst G, Aarnoudse JG. Abnormal endothelium-dependent microvascular dilator reactivity in pregnancies complicated by normotensive intrauterine growth restriction. Am J Obstet Gynecol 2009;200:66.e1–66.e6.

53. Melchiorre K, Sutherland GR, Watt-Coote I, Liberati M, Thilaganathan B. Severe myocardial impairment and chamber dysfunction in preterm preeclampsia. Hypertens Pregnancy 2012;31:454–71.

54. Yinon Y, Kingdom JC, Odutayo A, Moineddin R, Drewlo S, Lai V, Cherney DZ, Hladunewich MA. Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: insights into future vascular risk. Circulation 2010;122:1846-53

55. Crispi F, Hernandez-Andrade E, Pelsers M et al. Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses. Am J Obstet Gynecol 2008;199:254.

56. Crispi F, Comas M, Hernández-Andrade E, Eixarch E, Gómez O, Figueras F, Gratacós E. Does pre-eclampsia influence fetal cardiovascular function in early-onset intrauterine growth restriction? Ultrasound Obstet Gynecol 2009;34:660-5.

57. Girsen A, Ala-Kopsala M, Makikallio K, Vuolteenaho O, Rasanen J. Cardiovascular hemodynamics and umbilical artery N-terminal peptide of proB-type natriuretic peptide in human fetuses with growth restriction. Ultrasound Obstet Gynecol 2007;29:296-303.

58. Crispi F, Bijnens B, Figueras F et al. Fetal growth restriction results in remodeled and less efficient hearts in children. Circulation 2010;121:2427-36.

59. Figueras F, Cruz-Martinez R, Sanz-Cortes M, Arranz A, Illa M, Botet F, Costas-Moragas C, Gratacos E.Neurobehavioral outcomes in preterm, growth-restricted infants with and without prenatal advanced signs of brain-sparing. Ultrasound Obstet Gynecol 2011;38:288-94.

60. Comas M, Crispi F, Cruz-Martinez R, Figueras F, Gratacos E. Tissue Doppler echocardiographic markers of cardiac dysfunction in small-for-gestational age fetuses. Am J Obstet Gynecol 2011;205:57.e1-6.

61. Crispi F, Figueras F, Cruz-Lemini M, Bartrons J, Bijnens B, Gratacos E..Cardiovascular programming in children born small for gestational age and relationship with prenatal signs of severity. Am J Obstet Gynecol 2012;207:121.e1-9.

62. Figueras F, Oros D, Cruz-Martinez R, Padilla N, Hernandez-Andrade E, Botet F, Costas-Moragas C, Gratacos E. Neurobehavior in term, small-for-gestational age infants with normal placental function. Pediatrics 2009;124:e934–941.

63. Bahado-Singh RO, Kovanci E, Jeffres A et al. The Doppler cerebroplacental ratio and perinatal outcome in intrauterine growth restriction. Am J Obstet Gynecol 1999;180:750–756.

64. Savchev S, Figueras F, Cruz-Martinez R, Illa M, Botet F, Gratacos E. Estimated weight centile as a predictor of perinatal outcome in small-for-gestational-age pregnancies with normal fetal and maternal Doppler indices. Ultrasound Obstet Gynecol 2012;39:299-303.

65. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. N Engl J Med. 1987;316:1371–1375.

66. Korshunov VA, Schwartz SM, Berk BC. Vascular remodeling: hemodynamic and biochemical mechanisms underlying Glagov's phenomenon. Arterioscler Thromb Vasc Biol 2007;27:1722-8.

67. Berk BC, Korshunov VA. Genetic determinants of vascular remodelling. Can J Cardiol 2006;22 Suppl B:6B-11B

68. O'Leary DH, Polak JF. Intima-media thickness: a tool for atherosclerosis imaging and event prediction. Am J Cardiol 2002;90:18L-21L

69. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008;21:93–111

70. Urbina EM, Srinivasan SR, Tang R, Bond MG, Kieltyka L, Berenson GS; Bogalusa Heart Study. Impact of m ultiple coronary risk factors on the intima-media thickness of different segments of carotid artery in healthy young adults (The Bogalusa Heart Study). Am J Cardiol 2002;90:953-8.

71. Sattar N. Do pregnancy complications and CVD share common antecedents? Atheroscler Suppl 2004;5:3-7

72. Barker DJP, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 1989;2:577-580.

73. Laurent Aortic Stiffness Is an Independent Predictor of All-Cause and Cardiovascular Mortality in Hypertensive Patients Hypertension 2001

74. Cavalcante JL, Lima JA, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. J Am Coll Cardiol 2011; 57: 1511-22.

75. Laurent S. Expert consensus document on arterial stiffness: methodological issues and clinical applications. European Heart Journal 2006;27:2588–2605

76. Laurent S, Girerd X, Mourad JJ, Lacolley P, Beck L, Boutouyrie P, Mignot JP, Safar M. Elastic modulus of the radial artery wall material is not increased in patients with essential hypertension. Arterioscler Thromb 1994;14:1223-31

77. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;39:10–5.

78. Laurent S, Boutouyrie P. Recent advances in arterial stiffness and wave reflection in human hypertension. Hypertension 2007;49:1202-6.

79. Tihtonen KM, Kööbi T, Uotila JT. Arterial stiffness in preeclamptic and chronic hypertensive pregnancies. Eur J Obstet Gynecol Reprod Biol 2006;128:180-6.

80. Mori A, Uchida N, Inomo A, Izumi S. Stiffness of systemic arteries in appropriate- and small-for-gestational-age newborn infants. Pediatrics 2006;118:1035–41.

81. Feissel M, Michard F, Faller JP, Teboul JL. The respiratory variation in inferior vena cava diameter as a guide to fluid therapy. Intensive Care Med 2004;30:1834-7.

82. Sefidbakht S, Assadsangabi R, Abbasi HR, Nabavizadeh A. Sonographic measurement of the inferior vena cava as a predictor of shock in trauma patients.Emerg Radiol 2007;14:181-5.

83. Dipti A, Soucy Z, Surana A, Chandra S. Role of inferior vena cava diameter in assessment of volume status: a meta-analysis. Am J Emerg Med 2012;30:1414-1419

84. Krabbendam I, Spaanderman M. Venous adjustments in healthy and hypertensive pregnancy.Expert Rev Obstet Gynecol 2007;2:671-679

85. Gyselaers W. Hemodynamics of the maternal venous compartment: a new area to explore in obstetric ultrasound imaging. Ultrasound Obstet Gynecol 2008;32:716–717.

86. Robb AO, Mills NL, Din JN, Smith IB, Paterson F, Newby DE, Denison FC. Influence of the menstrual cycle, pregnancy, and preeclampsia on arterial stiffness. Hypertension 2009;53:952-958

87. Lippi G, Albiero A, Montagnana M, Salvagno GL, Scevarolli S, Franchi M, Guidi GC. Lipid and lipoprotein profile in physiological pregnancy. Clin Lab 2007;53:173-177

88. Hodson K, Man CD, Smith FE, Thelwall PE, Cobelli C, Robson SC, Taylor R. Mechanism of insulin resistance in normal pregnancy. Horm Metab Res 2013;45:567-71.

89. McDonald SD, Malinowski A, Zhou Q, Yusuf S, Devereaux PJ. Cardiovascular sequelae of preeclampsia/eclampsia: a systematic review and meta-analyses. Am Heart J 2008;156:918–930.

90. Martyn CN, Gale CR, Jespersen S, Sherriff SB. Impaired fetal growth and atherosclerosis of carotid and peripheral arteries. Lancet 1998;352:173-8.

91. M Kady S., Gardosi J. Perinatal mortality and fetal growth restriction. Best Pract Res Clin Obstet Gynaecol 2004;18:397–410.

92. Baschat AA, Gembruch U. The cerebroplacental Doppler ratio revisited. Ultrasound Obstet Gynecol 2003;21:124-7.

93. Figueras F, Meler E, Iraola A, Eixarch E, Coll O, Figueras J, Francis A, Gratacos E, Gardosi J. Customized birthweight standards for a Spanish population. Eur J Obstet Gynecol Reprod Biol 2008;136:20-4

94. Stefanadis C, Stratos C, Boudoulas H, Kourouklis C, Toutouzas P. Distensibility of the ascending aorta: comparison of invasive and non-invasive techniques in healthy men and in men with coronary artery disease. Eur Heart J 1990;11:990–6.

95. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation,

and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114(2 Suppl 4th Report):555-76.

96. Arduini D, Rizzo G. Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses. J Perinat Med 1990;18:165-72.

97. Gómez O, Figueras F, Fernández S, Bennasar M, Martínez JM, Puerto B, Gratacós E. Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation. Ultrasound Obstet Gynecol 2008;32:128-32.

98. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement.Lancet 1986; 1: 307–310.

99. Lenth, R. V. (2006-9). Java Applets for Power and Sample Size. http://www.stat.uiowa.edu/~rlenth/Power [11 September 2013]

100. Bots ML, Hofman A, Grobbee DE. Increased common carotid intima-media thickness.Adaptive response or a reflection of atherosclerosis?Findings from the Rotterdam Study. Stroke 1997;28:2442-7.

101. Visser W, Wallenburg HC. Central hemodynamic observations in untreated preeclamptic patients. Hypertension 1991;17:1072–7.

102. Vasapollo B, Valensise H, Novelli GP, et al. Abnormal maternal cardiac function and morphology in pregnancies complicated by intrauterine fetal growth restriction. Ultrasound Obstet Gynecol 2002;20:452-7.

103. Irgens HU, Reisæter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. BMJ 2001;323:1213–1217.

104. Crovetto F, Crispi F, Scazzocchio E, Mercade I, Meler E, Figueras F, Gratacos E. Performance of first trimester integrated screening for early and late small for gestational age newborns. Ultrasound Obstet Gynecol 2014;43:34-40

105. Paradisi G, Mattoli MV, Tomei C, Zuppi C, Lulli P, Quagliozzi L, Caruso A. Cardiovascular risk factors in healthy women with previous small for gestational age infants. J Obstet Gynaecol Res 2011;37:1397–1404.

106. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC, Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study.Circulation 2008;117:649-59.

107. McGill HC, Jr., McMahan CA, Herderick E,Tracy RE, Malcom GT, Zieske AW, Strong JP.Effects of coronary heart disease risk factors on atherosclerosis of selected regions of the aorta and right coronary artery. PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. Arterioscler Thromb Vasc Biol 2000;20:836-45.

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