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Vascular Dysfunction in Women With a History of Preeclampsia and Intrauterine Growth Restriction

Insights Into Future Vascular Risk

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Background—Women with a history of placental disease are at increased risk for the future development of vascular disease. It is unknown whether preexisting endothelial dysfunction underlies both the predisposition to placental disease and the later development of vascular disease. The aim of this study was to assess vascular function in postpartum women and to determine whether differences emerged depending on the presentation of placental disease.

Methods and Results—Women with a history of early-onset preeclampsia (n=15), late-onset preeclampsia (n=9), intrauterine growth restriction without preeclampsia (n=9), and prior normal pregnancy (n=16) were studied 6 to 24 months postpartum. Flow-mediated vasodilatation and flow-independent (glyceryl trinitrate–induced) vasodilatation were studied through the use of high-resolution vascular ultrasound examination of the brachial artery. Arterial stiffness was assessed by pulse-wave analysis (augmentation index). Laboratory assessment included circulating angiogenic factors (vascular endothelial growth factor, soluble fms-like tyrosine kinase 1, placental growth factor, and soluble endoglin). Flow-mediated vasodilatation was significantly reduced in women with previous early-onset preeclampsia and intrauterine growth restriction compared with women with previous late-onset preeclampsia and control subjects (3.2±2.7% and 2.1±1.2% versus 7.9±3.8% and 9.1±3.5%, respectively; $P<0.0001$). Flow-independent vasodilatation was similar among all groups. Similarly, the radial augmentation index was significantly increased among women with previous early-onset preeclampsia and intrauterine growth restriction, but not among late preeclamptic women and control subjects ($P=0.0105$). Circulating angiogenic factors were similar in all groups.

Conclusion—Only women with a history of early-onset preeclampsia or intrauterine growth restriction without preeclampsia exhibit impaired vascular function, which might explain their predisposition to placental disease and their higher risk of future vascular disease. **(*Circulation*. 2010;122:1846-1853.)**

Key Words: endothelium ■ preeclampsia ■ vasodilation ■ women ■ vascular diseases ■ placenta diseases ■ postpartum women

Abnormal placentation resulting in preeclampsia and intrauterine growth restriction (IUGR) is a major cause of both maternal and perinatal morbidity and mortality.[1,2] Early-onset preeclampsia is commonly associated with IUGR, abnormal uterine, and umbilical artery blood flow, as well as adverse maternal and neonatal outcomes. In contrast, late-onset preeclampsia is often associated with milder maternal disease, lower rates of fetal involvement, and perinatal outcomes that are typically favorable.[2–4] Occasionally, severe placental disease can result in IUGR without evidence of preeclamptic manifestations or maternal endothelial dysfunction. It has been suggested that these variable presentations of abnormal placentation should be regarded as distinct disease entities.[2–4]

Clinical Perspective on p 1853

Although the symptoms of preeclampsia resolve over a number of weeks after delivery,[5] a growing body of literature has suggested that maternal vascular dysfunction may persist for years, manifest by an increased risk for the development of hypertension, stroke, coronary artery disease, and end-stage renal disease.[6–9] Although most of these epidemiological studies included both early- and late-onset preeclampsia, evidence suggests that the increased risk for future vascular disease is more pronounced in women with early-onset disease. In fact, women with a history of severe preeclampsia necessitating delivery before 37 weeks of gestation have an

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8-fold higher risk for death caused by cardiovascular disease than women without such a history.[8] Similarly, infant birth weight has been noted to have an inverse relationship to maternal risk for cardiovascular disease.[10,11]

Recent studies critical to the understanding of the pathogenesis of preeclampsia have described the release of soluble antiangiogenic factors from an abnormal placenta that are injurious to the vascular endothelium. Excess soluble fms-like tyrosine kinase 1 (sFlt-1) binds placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), preventing their interaction with receptors located on the vascular endothelial cells.[12] Soluble endoglin (sEng), also released from an ischemic placenta, deleteriously affects vascular tone by blocking the activation of endothelial nitric oxide synthase.[13] Whether damage to the vascular endothelium from circulating angiogenic factors results in an increased risk of future vascular disease or whether preexisting endothelial dysfunction underlies both the predisposition to placental disease and the later development of vascular disease is presently unknown.

Studies have demonstrated endothelial dysfunction in the forearm vasculature of women with a history of preeclampsia 6 to 27 months after delivery.[14–16] These studies included both early- and late-onset preeclampsia. In addition, data are currently limited and inconsistent with respect to maternal vascular function in IUGR pregnancies, and no data exist on endothelial function after delivery in women with a history of a normotensive IUGR pregnancy, a unique group who do not display maternal endothelial dysfunction during pregnancy.

Accordingly, the aim of this study was to examine whether vascular function is impaired in postpartum women with placental syndromes by measuring endothelial function and arterial stiffness. We hypothesized that vascular function would be influenced by the disease presentation and would vary among women with a history of early-onset preeclampsia, late-onset preeclampsia, or normotensive IUGR. Because altered angiogenesis may represent a bridging pathophysiological mechanism between placental syndromes and future vascular disease, we evaluated whether endothelial dysfunction is associated with altered levels of angiogenic factors, including VEGF, sFlt-1, PlGF, and sEng.

Methods

Subjects

Women with a history of preeclampsia and IUGR, and healthy control subjects were recruited 6 to 24 months after their delivery from the high-risk maternal-fetal medicine services at Mount Sinai Hospital and Sunnybrook Health Sciences Centre in Toronto, Ontario, Canada. We identified 82 women with either preeclampsia or IUGR; 21 were excluded after a careful review of their medical histories. An additional 28 women qualified, but declined participation. Therefore, the study group included 24 women with a history of preeclampsia in their last pregnancy, 9 women with a history of IUGR without preeclampsia, and 16 control subjects with a prior normal pregnancy. The previously preeclamptic women were further subdivided into early-onset preeclampsia, diagnosed before 34 weeks of gestation (15 women), and late-onset preeclampsia, diagnosed after 34 weeks of gestation (9 women).

Preeclampsia was defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy. These criteria require the appearance of a diastolic blood pressure of ≥90 mm Hg measured on 2 occasions at least 4 hours apart, in combination with proteinuria (≥300 mg/24 h or 2+ dipstick) developing after 20 weeks of gestation, in a previously normotensive woman.[17] IUGR was defined as birth weight below the 5th percentile accompanied by abnormal umbilical artery Doppler examination, defined as absence or reverse of end-diastolic velocity. All patients in the IUGR group had early-onset IUGR and were delivered before 34 weeks of gestation because severe growth restriction. The combination of low birth weight and abnormal flow of indices indicates placental insufficiency as the cause of growth restriction, and other causes of IUGR such as infection, anomalies, and abnormal chromosomes were excluded in all cases. Women who had completed a normal singleton pregnancy 6 to 24 months before the study served as control subjects and were recruited from the low-risk obstetrics clinic at Mount Sinai Hospital and via public advertisements.

Exclusion criteria for all groups included current or past hypertension, diabetes mellitus, pregestational renal disease, body mass index >30 kg/m^2, and multiple gestations in the index pregnancy. Given that smoking has profound effects on endothelial function,[18] past and current smokers, and women living with smokers were excluded. Women using oral contraceptive agents were also excluded because estrogen has a favorable effect on endothelial dysfunction.[19,20] All studies were conducted during the nonmenstrual phase of the cycle.

The study was approved by the Research Ethics boards of all participating hospitals. All women gave informed written consent before entering the study.

Study Protocol

All studies were carried out at the Renal Physiology Laboratory at Toronto General Hospital in a quiet, warm (25°C), temperature-controlled room the morning after an overnight fast. A detailed clinical history, including family history of hypertension, diabetes mellitus, or cardiovascular disease, and obstetric history were obtained with a structured interview questionnaire. Outcome data from previous pregnancies were confirmed by review of medical records. When available, results of the screen for thromboembolic disease (lupus anticoagulant, anti-cardiolipin antibody, gene for factor V Leiden, prothrombin gene, antithrombin III level, and protein C and S levels) were noted, along with placental Doppler reports. Height and weight were recorded, and blood pressure measurements were performed in the lying position after 10 minutes of rest. For each woman, the average of 2 readings was used. Blood samples were collected and assayed for insulin, glucose, C-reactive protein, triglycerides, total cholesterol, low-density lipoprotein, and high-density lipoprotein, and the second morning urine was tested for microalbumin and creatinine. Blood samples were also collected for serum measurements of VEGF, sFlt-1, sEng, and PlGF. These samples were processed immediately and stored at −70°C.

Experimental Procedures

Brachial artery reactivity was determined by recording diameter changes in the brachial artery in response to increased blood flow generated during reactive hyperemia (flow-mediated dilatation [FMD]) and glyceryl trinitrate (GTN)–induced dilatation. The right brachial artery was scanned 2 to 5 cm above the antecubital fossa with high-resolution B-mode vascular ultrasound (Vividi, 7- to 15-MHz linear-array transducer, GE/Vingmed, Madison, Wis). Longitudinal, ECG-gated, end-diastolic images were acquired over 6 cardiac cycles, and the brachial arterial diameter was determined for each image with integrated software, the results of which were averaged. The end-diastolic diameter was measured by the distance between the junctions of media and adventitia. After baseline images were recorded, the blood pressure cuff was inflated at the level of the midforearm to >200 mm Hg for 5 minutes and then suddenly deflated. After cuff deflation, the increase in blood flow was measured (reactive hyperemia), along with the change in vessel diameter (FMD), which was measured for 5 minutes after deflation. Thereafter, we allowed 10 minutes for recovery of the vessel, after which an additional resting scan was performed. A sublingual GTN spray (400 μg) was then administered, and the changes in diameter were measured over 5 minutes. FMD and GTN-induced dilatation were expressed as the maximal percentage change in the brachial

Table 1. Baseline Clinical Characteristics and Biochemical Parameters

	Control (n=16)	Late Preeclampsia (n=9)	Early Preeclampsia (n=15)	IUGR (n=9)	P
Age, y	34±1	34±1	35±1	33±2	0.86
White, n (%)	11 (69)	7 (78)	8 (53)	5 (56)	0.60
Family history, n (%)	8 (50)	2 (22)	10 (67)	3 (33)	0.15
BMI, kg/m^2	22.1±0.6	23.2±1.0	24.6±1.0	24.0±1.2	0.19
Primiparous, n (%)	9 (56)	7 (78)	7 (47)	5 (56)	0.52
Time after delivery, mo	14±1	15±2	14±2	8±1	0.02
MAP, mm Hg	78±1	86±3	85±2	82±3	0.04
Heart rate, bpm	63±2	69±3	67±1	65±3	0.24
Biochemical parameters					
Fasting glucose, mmol/L	4.6±0.1	4.8±0.2	4.8±0.1	4.6±0.1	0.67
Fasting insulin, pmol/L	23.3±2.4	40.4±10.8	29.7±5.9	45.3±8.5	0.08
HOMA index	0.50±0.04	0.78±0.18	0.61±0.12	0.84±0.15	0.14
Total cholesterol, mmol/L	4.1±0.1	4.4±0.3	4.8±0.3	4.4±0.3	0.26
HDL cholesterol, mmol/L	1.5±0.1	1.3±0.1	1.3±0.1	1.3±0.1	0.24
LDL cholesterol, mmol/L	2.3±0.1	2.6±0.3	3.0±0.2	2.6±0.3	0.10
Triglycerides, mmol/L	0.8±0.1	1.0±0.2	1.0±0.1	1.2±0.4	0.34
CRP, mg/L	4.4±1.0	4.3±0.9	3.9±0.6	5.0±1.8	0.93
Microalbumin/creatinine ratio >2.8 mg/mmol, n (%)	0 (0)	1 (11)	2 (13)	1 (11)	0.54

BMI indicates body mass index; MAP, mean arterial pressure; HOMA, homeostatic model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and CRP, C-reactive protein.

arterial diameter after reactive hyperemia and administration of GTN, respectively. FMD is also reported as FMD/flow, in which flow (reactive hyperemia) is defined as the percentage change between maximal flow after cuff deflation and the baseline flow before cuff inflation. Endothelial dysfunction was defined as an endothelium-mediated dilatation <4.5% because this cutoff point has been shown to predict coronary events.[21] A single observer (Y.Y.) obtained all measurements and was blinded to each participant's clinical details. The technique for measurement of brachial artery FMD is reproducible in our laboratory with intraobserver variability of 0.26±0.01%, which compares favorably with previous reports.[22]

Radial artery waveforms were recorded using a micromanometer (SPC-301, Millar Instruments, Houston, Tex), and a corresponding aortic pulse pressure waveform was generated via a mathematical transfer function (SphygmoCor, AtCor Medical Systems Inc, Sydney, Australia).[23,24] Augmentation index, an estimate of systemic arterial stiffness, was calculated as the difference between the second and first systolic peaks and is expressed as a percentage of the aortic pulse pressure. Because there is an inverse linear relationship between augmentation index and heart rate, augmentation index was standardized to a heart rate of 75 bpm.[25] At least 3 measurements were taken for each patient, and an average value was obtained. The interoperator variability and reproducibility of the augmentation index have been validated to be 0.4±6.4%. Our group has previously published and validated the measurements of brachial artery activity and radial arterial waveforms.[26–29]

Laboratory Determinations

Standard laboratory assays were used to determine insulin, glucose, C-reactive protein, and cholesterol profiles. The homeostatic model assessment index was calculated to determine insulin resistance.[30] Urine microalbumin was measured by an immunoturbidimetric assay, and urine creatinine was measured with the standard Jaffe reaction. Microalbuminuria was defined as a ratio of albumin to creatinine >2.8 mg/mmol. Serum measurements of free VEGF, sFlt-1, sEng, and PlGF were performed by ELISA kits according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn). All samples were run in duplicate, and the protein levels were calculated from a standard curve derived from known concentrations of the respective recombinant proteins.

Statistical Analysis

Data are presented as mean±SEM. On the basis of previous work in our laboratory that revealed a variance of 10% in measurements of FMD, 9 subjects in each group were required to detect a difference between groups of at least 6% (80% power and 5% type 1 error).[26,31] To assess for baseline differences between the 4 groups, ANOVA or a χ^2 test was used when appropriate, and significance was defined as a value of $P<0.05$. We then used an ANCOVA or logistic regression when appropriate to assess differences among the outcomes of interest, adjusting for baseline covariates that proved significantly different between the 4 groups. Finally, a Bonferroni adjustment was used for posthoc comparisons of the measurements of endothelial function between the 4 groups; therefore, significance was decreased to a value of $P<0.008$ to adjust for the multiple comparisons. Statistical analyses were performed with SAS version 9.2 (SAS Institute Inc, Cary, NC).

Results

Baseline Characteristics

The baseline clinical and biochemical characteristics of the study groups are summarized in Table 1. There were no significant differences in age, ethnicity, body mass index, or prevalence of family history of hypertension, diabetes mellitus, or cardiovascular disease. Approximately 50% of the women in each group were primiparous, with the exception of a higher rate (78%) among women with late-onset preeclampsia that did not reach statistical significance. As expected, birth weight and gestational age at delivery were significantly lower in women with a history of early preeclampsia and

Table 2. Vascular Function

	Control (n=16)	Late Preeclampsia (n=9)	Early Preeclampsia (n=15)	IUGR (n=9)	*P*
FMD, %	9.14±0.90	7.93±1.33	3.25±0.70*†	2.14±0.44*†	<0.0001
FMD/flow, units	0.245±0.039	0.290±0.080	0.075±0.016*†	0.070±0.019*†	0.0015
GTN-mediated dilatation, %	18.00±1.14	20.04±1.46	16.04±0.93	15.96±1.33	0.1985
Endothelial dysfunction, n (%)	2 (13)	2 (22)*	14 (93)*†	8 (89)*†	0.0024

Data are presented as mean±SEM. Endothelial dysfunction is defined as a FMD of <4.5%. *P* values are adjusted for baseline covariates.

*$P<0.008$ versus control; †$P<0.008$ versus late preeclampsia.

IUGR compared with women with previous late preeclampsia and control subjects. The birth weight was 3417±88, 2828±251, 971±89, and 841±133 g in the control, late preeclampsia, early preeclampsia, and IUGR groups, respectively ($P<0.001$). The gestational age at delivery was 39.6±0.3, 37.6±0.7, 29.1±0.8, and 29.2±0.9 weeks in the control, late preeclampsia, early preeclampsia, and IUGR groups, respectively ($P<0.001$). Of the 15 women with previous early-onset preeclampsia, 13 (87%) had associated fetal growth restriction during their pregnancy compared with only 2 of the women (22%) with late-onset preeclampsia. The interval from delivery to day of study was significantly shorter in the IUGR group compared with the other 3 groups ($P=0.02$).

Although numerically higher in previously preeclamptic subjects with an overall statistically significant value of $P=0.04$, between-group differences in mean arterial pressure did not reach statistical significance. All study groups exhibited similar fasting glucose and insulin levels and therefore homeostatic model assessment indexes. Furthermore, no significant differences were observed between the groups in concentrations of total cholesterol, high-density lipoprotein, low-density lipoprotein, serum triglycerides, or C-reactive protein. The prevalence of microalbuminuria was low in all groups.

Vascular Function

Even after adjustment for potential baseline covariates (Table 1), FMD was significantly reduced in women with previous early preeclampsia and in women with previous IUGR compared with women with previous late preeclampsia and control subjects (3.25±0.70% and 2.14±0.44% versus 7.93±1.33% and 9.14±0.90%, respectively; $P<0.0001$; Table 2 and Figure 1). Similarly, FMD corrected for the flow stimulus (FMD/flow) was significantly decreased in the early preeclampsia group and the IUGR group compared with the late preeclampsia and control groups ($P=0.0015$; Table 2). Both parameters were similar between women with previous late preeclampsia and control subjects. Interestingly, in the early preeclampsia group, impaired FMD was driven by the 13 of 15 women who also had fetal growth restriction (8.0±5.7% versus 2.4±1.3%). In addition, 93% and 89% of the early preeclampsia and IUGR women, respectively, exhibited endothelial dysfunction defined as FMD <4.5%, whereas only 22% of late preeclamptic women and 12.5% of the control subjects met the criteria for endothelial dysfunction ($P=0.0024$; Table 2). In contrast, GTN responsiveness was similar among the 4 groups ($P=0.1985$; Table 2).

Augmentation index of the radial artery adjusted for baseline covariates was significantly increased among women with previous early-onset preeclampsia and in women with previous normotensive IUGR pregnancies compared with control subjects (27.8±5.3 and 28.7±5.7 versus 15.1±8.9, respectively; $P=0.0105$; Figure 2). Women with late preeclampsia had an intermediate value of 20.6±7.1, which did not reach statistical significance for between-group differences ($P=0.08$). Placental Doppler reports were available for 11 of 15 patients with previous early preeclampsia and 8 of 9 patients with IUGR. Of interest, there was a positive correlation between the mean placental pulsatile index and the arterial augmentation index ($R^2=0.24$, $P=0.03$). However, it is difficult to draw any strong conclusions between any potential relationship because data were not available for all study subjects. Screening for thrombophilias, as mentioned, occurred at the discretion of the managing clinician and therefore was available in 28 of 33 subjects with placental disease. None of healthy control subjects were assessed for a thrombophilia. Only 1 patient with early preeclampsia (decreased antithrombin III level) and 1 patient with IUGR (protein S deficiency) were noted to have abnormalities, suggesting that thrombophilias did not factor significantly into our vascular findings.

Postpartum markers of angiogenesis are shown in Table 3. No significant differences were found between the 4 groups

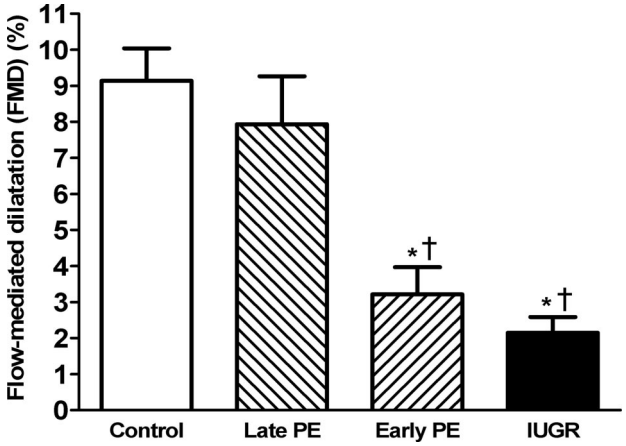

Figure 1. FMD expressed as the maximal percentage change in the brachial arterial diameter after reactive hyperemia. Error bars represent the SEM. *$P<0.008$ vs control; †$P<0.008$ vs late preeclampsia (PE).

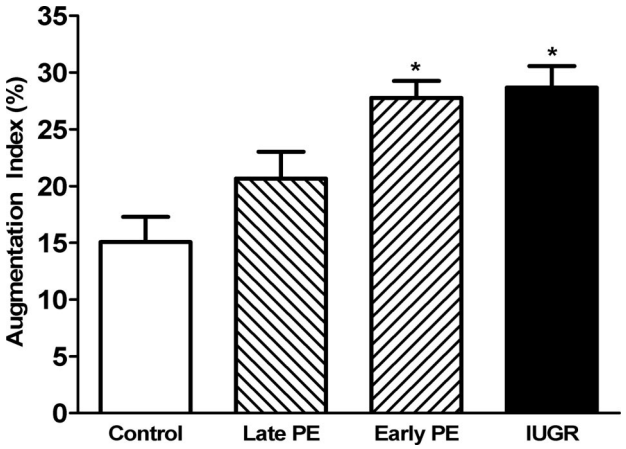

Figure 2. Augmentation index at a heart rate of 75 measured at the radial artery. Error bars represent the SEM. *$P<0.008$ vs control. PE indicates preeclampsia.

in serum concentrations of sFlt-1, sEng, VEGF, and PlGF. Furthermore, none of the measured biochemical or angiogenic factors correlated with any of the measures of vascular function.

Discussion

In recent years, epidemiological studies have clearly delineated a link between placental disease and future vascular disease. An increased future risk of hypertension, cardiovascular disease, stroke, and end-stage renal disease has been noted in women with a history of preeclampsia.[6,8,9,32] The risk is further increased in women with early-onset disease, multiple complicated pregnancies, and concomitant poor fetal growth or death.[6,8,32] Of interest, preterm birth, even in the absence of maternal signs and symptoms of preeclampsia, is associated with a significantly increased risk for the development of cardiovascular disease and stroke, with hazard ratios of 2.95 (95% confidence interval, 2.12 to 2.70) and 1.91 (95% confidence interval, 1.26 to 2.91), respectively.[8] Although this study using administrative health data was unable to identify the precise cause for preterm birth, isolated IUGR was likely to have contributed to the increased risk noted in this and other similar studies.[10,11]

It has been suggested that vascular dysfunction may be the predisposing factor for abnormal placentation and may represent the link between placentation defects and the development of vascular disease later in life.[33,34] Alternatively, the resultant endothelial damage from circulating angiogenic factors after a pregnancy complicated by preeclampsia may be the predisposing factor for future vascular disease. Although we cannot be sure without prospectively designed studies that assess women before pregnancy, both the aforementioned epidemiological literature and our results suggest that the former hypothesis is more likely. Our first significant observation was that FMD is reduced in women with previous early-onset preeclampsia and in women with previous IUGR without preeclampsia 6 to 24 months postpartum. In contrast, women with a history of late-onset preeclampsia exhibited normal FMD similar to control subjects. Using pulse-wave analysis, we then demonstrated that arterial stiffness is increased in both women with a history of early preeclampsia and women with previous normotensive IUGR relative to women with previous late-onset preeclampsia and healthy control subjects.

Table 3. Angiogenic Factors

	Control (n=16)	Late Preeclampsia (n=9)	Early Preeclampsia (n=15)	IUGR (n=9)
sFlt, pg/mL	176.4±9.8	196.4±13.1	169.3±10.5	183.1±13.1
sEng, pg/mL	4.1±0.2	4.1±0.2	3.9±0.2	3.5±0.2
VEGF, pg/mL	275±45	365±60	225±48	350±60
PlGF, pg/mL	6±1	8±1	8±1	7±1

Data are presented as mean±SEM.

Our data confirm the results of previous studies showing that endothelial function is impaired in women with previous preeclampsia.[15,16,35,36] However, previous studies did not differentiate between early- and late-onset preeclampsia or include patients with isolated IUGR. Because early-onset preeclampsia and late-onset preeclampsia are considered by some to be different disease entities,[3,4] we divided the preeclamptic women into early onset (<34 weeks) and late onset (≥34 weeks). In early-onset disease, underperfusion of the placenta is the predominant precipitating factor, whereas in late-onset preeclampsia, there is often minimal or no placental involvement.[37,38] In support of the concept that these conditions represent different disease entities, we found that FMD in the women with previous late-onset preeclampsia was comparable to that in the control group and significantly higher than in women with early-onset preeclampsia.

Of note, the effect on FMD and not GTN responsiveness suggests endothelial cell (and not vascular smooth muscle cell) dysfunction, which is in agreement with previous literature. Multiple potential putative pathways have been implicated in the pathogenesis of the endothelial cell dysfunction characteristic of preeclampsia. These mechanisms include, but are not limited to, increased cellular fibronectin, von Willebrand factor, cell adhesion molecules, proinflammatory cytokines, the renin-angiotensin system, oxidative stress, and antiangiogenic factors.[39–42] The common end point of these upregulated factors may be to decrease the vascular bioavailability of nitric oxide, along with other vasodilators such as prostacyclin.

Similar to our FMD findings, we demonstrated that radial arterial stiffness is significantly increased only among the early-onset and not the late-onset preeclamptic women, whose augmentation index values were similar to those of the control subjects. In accordance with these results, Khalil et al[43] recently reported a significantly higher augmentation index in early- compared with late-onset preeclampsia when patients were studied during pregnancy. However, other studies were not able to show any differences in the augmentation index between women with preeclampsia and control subjects assessed either during pregnancy or in the postpartum period.[36,44,45] These inconsistent results could be due to the lack of adjustments for possible confounders or the severity of preeclampsia, small sample sizes, or technical issues.[44] A recent study that assessed healthy women through-

out the different phases of the menstrual cycle did not reveal any impact of the menstrual cycle, but noted only moderate reproducibility of results.[46] Our studies were all conducted during the nonmenstrual phase of the cycle, and multiple waveforms were recorded to ensure reproducibility. Our results and the findings reported by Khalil et al[43] suggest that early- but not late-onset preeclampsia is associated with increased arterial stiffness that extends beyond pregnancy and might contribute to adverse vascular outcomes.

We could locate no other study that demonstrated impaired endothelium-dependent vasodilatation and increased arterial stiffness in women with a previous IUGR pregnancy without preeclampsia. This group of patients is unique in that all had pregnancies complicated by severe IUGR with a birth weight below the 5th percentile requiring delivery before 34 weeks of gestation and accompanied by absent or reversed end-diastolic velocity in the umbilical artery, indicating placental insufficiency as the cause of growth restriction. Moreover, none of the patients in this group exhibited clinical signs of preeclampsia and thus represented a pure group of IUGR. Because IUGR has no appreciable clinical impact on the mother, one might expect the endothelial dysfunction in IUGR pregnancies to be confined to the uteroplacental circulation and not to be systemic as in preeclampsia. Supporting this hypothesis, Ramsay et al[47] have reported that endothelial cell activation markers were higher only in preeclamptic, but not in IUGR pregnancies. Savvidou et al[48] showed a greater reduction in brachial artery dilatation during pregnancy in women with preeclampsia than IUGR, suggesting that the degree of endothelial dysfunction may be greater in preeclampsia. However, in our study, the degree of endothelial dysfunction was more pronounced in patients with a previous IUGR pregnancy compared with the early preeclamptic patients. Furthermore, in the early preeclampsia group, women who also had fetal growth restriction had markedly reduced FMD compared with those without fetal growth restriction, suggesting that the degree of endothelial dysfunction is actually more severe in the presence of IUGR. Our results are also supported by a recent study that demonstrated abnormal endothelium-dependent microvascular vasodilatation in normotensive pregnant women with IUGR studied in the third trimester.[49]

It has become clear in recent years that circulating angiogenic factors (sFlt-1 and sEng) released from an ischemic placenta inhibit the actions of VEGF and PlGF, interfere with nitric oxide–mediated vasodilatation, and cause maternal endothelial dysfunction.[12,50,51] It has been hypothesized that damage resulting from maternal exposure to these angiogenic factors during pregnancy may be the cause of future maternal vascular disease, but our study, along with a recent experimental animal data, might suggest otherwise. To assess the long-term effects of preeclampsia on vascular function, that study used a mouse model of sFlt overexpression and injected an adenovirus vector either carrying or not carrying sFlt into the animal at 8 days of gestation. Vascular function was assessed at 6 to 8 months after delivery, and no differences were noted. The authors concluded that in the absence of pregnancy, sFlt does not result in hypertension or altered vascular function that and pregnancy exposure to sFlt has no long-term postpartum effect.[52] In our study, sFlt-1 and sEng were not elevated and VEGF and PlGF were not decreased in women with previous preeclampsia or IUGR despite the presence of endothelial dysfunction. In contrast, Wolf et al[53] found increased levels of sFlt-1 in women with prior preeclampsia. However, the basal levels of sFlt-1 appeared to be too low to influence circulating VEGF, again suggesting that sFlt-1 is not likely to play a clinically significant role in the postpartum state.

It is possible that other factors contribute to the endothelial dysfunction in the postpartum state, including metabolic variables, which are known to affect vascular reactivity.[54,55] Insulin resistance has been shown to be associated with endothelial dysfunction,[16,56] and there is evidence suggesting that high total cholesterol and low high-density lipoprotein cholesterol levels negatively affect endothelial function.[15,16,57] Previous studies have shown increased cholesterol levels and insulin resistance in women with a history of preeclampsia or IUGR,[42–44] and thus these variables may account for the endothelial dysfunction observed in these women. However, in our study, all 4 groups were comparable in terms of their lipid profiles and measures of insulin resistance; therefore, we assume that these metabolic variables could not be solely responsible for the abnormal endothelium-mediated vasodilatation found in the early preeclamptic and normotensive IUGR women. Similarly, it is unlikely that hypertension alone contributed to the endothelial dysfunction in these women because their blood pressure values, although higher, were not significantly elevated compared with the control group.

We acknowledge that our study has a number of limitations. The number of patients studied was small, which may have limited our ability to detect some between-group differences in vascular parameters. Despite the small sample size and conservative statistical methods, clinically and statistically significant differences were noted in our carefully characterized study cohort. In the future, a larger study that includes perhaps only nulliparous women in whom the clinical diagnosis of preeclampsia is least apt to be erroneous would be of interest. Furthermore, it would be preferable to assess endothelial function preconception to better understand causality, but this would necessitate large population-based studies that might not prove easily feasible with the currently available techniques for measuring endothelial dysfunction. Another limitation of our study is that women with previous IUGR were studied at a shorter interval after delivery than women in the other groups. Many of these women experienced a stillbirth in their previous pregnancy and were keen to get pregnant again as soon as possible and therefore were tested at a relatively short interval after delivery (6 to 12 months). Although we cannot definitively exclude a contribution to our results, our ANCOVA did not suggest that this variable had a significant impact on the outcomes of interest.

Conclusions

This study provides evidence that women with previous early-onset preeclampsia and previous normotensive IUGR pregnancies are characterized by endothelial dysfunction and

increased arterial stiffness. Women with a history of late-onset preeclampsia, however, do not have evidence of vascular dysfunction. These novel findings demonstrating differences in vascular function between these 3 distinct presentations provide a pathophysiological explanation for the differential risk for future vascular disease that has emerged in the epidemiological literature and introduce new measures that may be used to identify and follow potential therapeutic interventions that might prove beneficial in this high-risk group of young women.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Preeclampsia and intrauterine growth restriction can result in devastating pregnancy outcomes. Although initially assumed to be disease entities that resolved quickly postpartum, we are now aware that these pregnancy complications identify women at increased risk for the development of cardiovascular disease. In this study, we examined endothelial function in postpartum women with previous preeclampsia or intrauterine growth restriction, offering a unique approach to in vivo investigation of the early mechanisms of human cardiovascular disease years before the onset of the clinical disease. Our study is the first to show impaired endothelium-dependent vasodilatation and increased arterial stiffness in women with a previous intrauterine growth restriction pregnancy without preeclampsia. Moreover, we divided preeclamptic women into early-onset and late-onset disease and showed that women with previous late-onset preeclampsia do not have evidence of vascular dysfunction. These novel findings demonstrating differences in vascular function between these 3 distinct placental diseases provide a pathophysiological explanation for the differential risk for future vascular disease shown in the epidemiological literature. The abnormal vascular physiological profile identified in women with previous early-onset preeclampsia and normotensive intrauterine growth restriction might explain their increased risk of future vascular disease, whereas late-onset preeclampsia may not fall into this high-vascular-risk category. Our study also provides new measures that can be used to identify those women who are at risk for future cardiovascular disease, allowing the investigation of potential therapeutic interventions that may prove beneficial in this high-risk group of women.