Changes in Endothelial Function Precede the Clinical Disease in Women in Whom Preeclampsia Develops

Faisel Khan, Jill J.F. Belch, Maureen MacLeod, Gary Mires

Abstract—Endothelial dysfunction is important in the pathophysiology of preeclampsia. No study has examined endothelial function sequentially at different gestations before development of the clinical syndrome and after delivery (to compare maternal from placental influences). We sought to determine whether endothelial function changes before the clinical development of preeclampsia. We measured skin microvascular function using iontophoresis of acetylcholine and sodium nitroprusside, and laser Doppler imaging at 22, 26, 34 weeks’ gestation and 6 weeks postpartum in women identified as being at increased risk of preeclampsia, based on uterine artery Doppler waveforms at 18 to 20 weeks, and controls with normal Doppler waveforms. Fifty-four women remained normotensive and preeclampsia developed in 15. In normotensive and preeclamptic women, acetylcholine responses were augmented during pregnancy compared with postpartum responses (P<0.001). Sodium nitroprusside responses were augmented during pregnancy compared with those postpartum (P<0.005) in preeclamptic women only. Microvascular responses were augmented in women in whom preeclampsia developed, compared with those in normotensive women, at 26 and 34 weeks for acetylcholine (P<0.05 and P<0.001, respectively) and at 22 and 26 weeks for sodium nitroprusside (P<0.05 and P<0.02, respectively). Postpartum acetylcholine and sodium nitroprusside responses were not significantly different between preeclamptic and normal women. Microvascular responses are enhanced during pregnancy in women in whom preeclampsia develops to a level above that seen in normotensive women. These changes precede the onset of clinical disease and might be related to a compensatory increased sensitivity of the microcirculation to nitric oxide. (Hypertension. 2005;46:1123-1128.)

Key Words: endothelium-derived factors ■ hypertension ■ microcirculation ■ nitric oxide ■ preeclampsia ■ pregnancy

Preeclampsia (PE) continues to be a leading cause of maternal and perinatal morbidity and mortality. Characteristic hemodynamic changes occur during normal human pregnancy, which include peripheral vasodilatation, reductions in total vascular resistance, and decreased arterial blood pressure. The precise reasons for such changes are not clear, but recent evidence points to changes in endothelial function as contributory factors.1,2

In PE there is accumulating evidence for a pathogenic model whereby deficient trophoblast invasion of the maternal spiral arteries leads to a poorly perfused feto-placental unit.3 This results in secretion of a factor(s) by the placenta into the maternal circulation, which activates the vascular endothelium.4,5 However, although endothelial dysfunction appears to be a key player in the pathophysiology of PE, there appears to be a difference in the way in which endothelial damage is expressed depending on the particular vascular bed studied. For example, arterial endothelial function, assessed using ultrasound measurements of flow-mediated dilatation, is diminished in women who have PE compared with normal pregnant women.6–8 Conversely in the microcirculation, endothelium-dependent vasodilatation is increased in women who have PE to levels over and above those seen in normal pregnancy.9 After pregnancy, however, both arterial and microvascular endothelial functions are diminished in women who had PE.10–12 This suggests that either endothelial dysfunction is present before pregnancy and predisposes women to PE, or that PE induces long-term changes in endothelial function, which could have implications for development of cardiovascular disease in later life.

There is no study however, that has examined vascular responses sequentially at different gestations before the clinical syndrome of PE develops and after delivery (to compare maternal from placental influences), and compared these changes to those in women who remain normotensive. The purpose of this study, therefore, was to measure endothelial function at 22, 26, and 34 weeks’ gestation and 6 weeks postpartum in women identified as being at increased risk for PE, based on uterine artery Doppler waveforms at 18 to 20 weeks, and control subjects with normal Doppler waveforms.
We chose uterine arterial notching as a means of identifying a cohort of women at increased risk of PE based on the findings from a previous study that demonstrated a relative risk of early onset PE of 26.2 (95% CI, 11.0 to 62.4) in women with bilateral uterine arterial notching at 18 to 20 weeks’ gestation. We sought to determine whether changes in endothelial function in PE occur before the clinical development of the disease and whether such changes persist after delivery.

Methods

Study Group and Outcome Definitions

An outline of the experimental design is shown in Figure 1, and Table 1 shows the sample distribution of subjects into subsequent groupings. Uterine arterial waveform analysis is routinely performed at 18 to 20 weeks’ gestation at Ninewells Hospital, Dundee, and was used as a screening test to identify a cohort of women at high risk for an adverse pregnancy outcome. Women in whom bilateral uterine arterial notching was identified at this gestation and control women (with normal Doppler waveforms) were recruited into the study. Women with any underlying condition likely to compromise renal function, such as diabetes or renal disease, were excluded from the study. PE was defined as the presence of a systolic pressure ≥140 mm Hg or a diastolic pressure of ≥90 mm Hg on ≥2 separate occasions after week 20 of pregnancy in a woman who was normotensive at booking (first trimester) and 6 weeks postnatally, in the presence of significant proteinuria (either >300 mg/L in a 24-hour collection or ≥2+ on a voided random urine sample in the absence of urinary tract infection). Gestational hypertension was defined as hypertension arising de novo in pregnancy without proteinuria.

The individualized birth weight ratio was used to define intrauterine growth restriction; individualized birth weight ratio corrects birth weight for gestational age, taking into account confounding variables of maternal height and weight, ethnic origin, parity, and infant sex and was calculated using the UK Perinatal Institutes online calculator. Pregnancies with an individualized birth weight ratio <10 were defined as growth-restricted.

The study was approved by the Tayside Committee on Medical Research Ethics and conducted according to the principles outlined in the Declaration of Helsinki. All subjects gave written informed consent to their participation.

Microvascular Function

Measurements of microvascular function were performed at 22, 26, and 34 weeks’ gestation, and at 6 weeks after delivery in a laboratory set at 22±1°C. Participants were seated with their arms supported at heart level. We assessed forearm microvascular function, as described previously, by measuring skin vascular responses to iontophoresis of acetylcholine (ACh; Sigma-Aldrich Co. Ltd) and sodium nitroprusside (SNP; David Bull Laboratories). Iontophoresis is a drug-delivery method that stimulates the migration of charged ions across the skin noninvasively and without inducing systemic effects. We used a delivery current of 100 µA and administered each drug as a successive accumulation of 10-, 20-, 40-, and 80-second doses, ie, effectively, 1, 2, 4, and 8 milli coulomb (mC). Using a current of 100 µA and large-diameter electrodes does not elicit...
Table 2. Characteristics of Subject Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal (n=54)</th>
<th>Preeclampsia (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Booking systolic BP (mm Hg)</td>
<td>112±12</td>
<td>117±8</td>
</tr>
<tr>
<td>Booking diastolic BP (mm Hg)</td>
<td>67±8</td>
<td>69±8</td>
</tr>
<tr>
<td>Maximum systolic BP (mm Hg)</td>
<td>122±11</td>
<td>150±15*</td>
</tr>
<tr>
<td>Maximum diastolic BP (mm Hg)</td>
<td>78±7</td>
<td>99±9*</td>
</tr>
<tr>
<td>Gestation at delivery (wk)</td>
<td>36.4±4.1</td>
<td>35.3±3.4*</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3417±541</td>
<td>2509±1108*</td>
</tr>
</tbody>
</table>

*P<0.001.
Values are means±SEM.

vasodilatation via nonspecific galvanic effects, because a low charge density (maximum of 2.5×10^−2 mC/mm²) is generated.17

Microvascular skin blood flow was assessed using laser Doppler imaging (moorLDI; Moor Instruments Ltd). This technique works by scanning a 2-mW helium–neon laser across the surface of the skin. Light that is backscattered from moving erythrocytes undergoes a shift in frequency proportional to their velocity, according to the Doppler principle. The resulting color-coded image represents skin blood flow over the scan area; a relative measure called the laser Doppler flux. Baseline scans of skin perfusion (without drug delivery) were taken before the iontophoresis protocol was administered. The median laser Doppler flux over the baseline and drug-delivery site was calculated for each image using dedicated image-processing software (Moor Instruments Ltd). For each dose response, the mean of the 2 highest stable flux values was calculated, and this was divided by the baseline measurement to give a ratio representing the change in flow.

Table 1 shows the pregnancy outcome for the notched and normal Doppler waveform groups. There were no significant differences in vascular responses to ACh and SNP between women with normal and abnormal Doppler waveforms at any time point (Table 3).

Baseline skin perfusion (mean±SE in arbitrary perfusion units [PU]) was not significantly different between normal and preeclamptic women at 22 weeks (55.5±1.9 versus 52.5±3.0 PU, respectively), 26 weeks (56.4±2.1 versus 51.6±2.6 PU), 34 weeks (53.1±1.8 versus 50.9±4.3 PU), and postpartum (51.6±1.9 versus 58.8±4.0 PU).

In women with normal pregnancies, vascular responses to ACh were not significantly different at 22, 26, and 34 weeks but were significantly enhanced compared with postpartum responses (ANOVA P<0.001, P<0.001, and P<0.05, respectively) (Table 4). Post-hoc analysis comparing postpartum responses with those at 22 and 26 weeks showed significant differences at doses of 2, 4, and 8 mC (P<0.01). For comparisons between postpartum responses and those at 34 weeks, significant differences were found at 2 mC (P<0.05) and at 4 and 8 mC (P<0.01). Peak ACh responses were 13% higher at 34 weeks compared with postpartum responses. In contrast, SNP responses were similar at all time points (Table 4).

In preeclamptic women, there was a progressive increase in ACh responses from 22 to 26 and 34 weeks (ANOVA, P<0.01 for both), followed by a decrease postpartum. Post-
The findings of the present study in the microcirculation show that normal pregnancy is associated with a steady increase in endothelium-dependent vasodilatation, which returns to normal levels postpartum. In contrast, women in whom PE develops show a progressive increase in endothelium-dependent vasodilatation during pregnancy to levels over and above that seen in normal pregnancy. Endothelium-independent, nitric oxide-mediated vascular responses are also significantly increased during pregnancy in women in whom PE develops. Importantly, these changes precede the onset of clinical disease by weeks or months. We propose the hypothesis that women in whom PE develops have an increased sensitivity of the microcirculation to nitric oxide during pregnancy, perhaps as a compensatory mechanism to offset impaired placental perfusion.

During normal pregnancy, the augmented response to acetylcholine, but not to sodium nitroprusside, suggests enhanced production of endothelium-derived substances rather than a generalized increased responsiveness of the microvessels. Several mediators might be involved, such as nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF). Our findings in the microcirculation are consistent with those seen in large vessels of normal pregnant women.1

In women with PE, however, our findings in the microcirculation are not consistent with those in large vessels. Flow-mediated dilatation is significantly reduced in women with established PE compared with responses in normal pregnant women.6-8 Brachial artery infusions of acetylcholine also produce diminished endothelium-dependent vasodilatation in established PE. Whether these changes are transient during PE or more permanent could not be determined from these studies as all were performed at varying single time points and no comparison was made with postpartum responses. Savvidou et al did find that changes in endothelial function predated the development of PE by 10 weeks.7 In this regard, our results support this finding but the way in which endothelial dysfunction is expressed during pregnancy differs between large and small vessels.

**TABLE 4. Comparison of Dose-Responses (Expressed as a Ratio of Response Over Baseline) by ANOVA to ACh and SNP in Normotensive (n=54) and PE Women (n=15)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Acetylcholine</th>
<th>Sodium Nitroprusside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mC</td>
<td>2 mC</td>
</tr>
<tr>
<td>22 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>2.6±0.2</td>
<td>4.6±0.3</td>
</tr>
<tr>
<td>PE</td>
<td>2.4±0.2</td>
<td>4.5±0.4</td>
</tr>
<tr>
<td>26 weeks</td>
<td>2.7±0.2</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Normal</td>
<td>3.2±0.3</td>
<td>5.4±0.6</td>
</tr>
<tr>
<td>PE</td>
<td>2.4±0.2</td>
<td>4.2±0.3</td>
</tr>
<tr>
<td>34 weeks</td>
<td>3.2±0.4</td>
<td>6.2±0.9</td>
</tr>
<tr>
<td>Normal</td>
<td>2.0±0.1</td>
<td>3.6±0.2</td>
</tr>
<tr>
<td>Post partum</td>
<td>2.3±0.2</td>
<td>4.0±0.4</td>
</tr>
</tbody>
</table>

Footnotes refer to comparisons of dose-responses. See Results text for post-hoc analyses at individual doses. Values are means±SE.
*P<0.05, †P<0.005 comparing post partum ACh responses with those at 22, 26, and 34 weeks (P<0.001, P<0.001, and P<0.05, respectively) in normal women. P<0.001 comparing normal vs PE responses at 22 and 34 weeks for ACh and at 22 weeks for SNP.
§P<0.01 comparing ACh responses at 22 weeks with those at 26 and 34 weeks, and SNP responses at 22 and 26 weeks in PE women.
¶P<0.05 comparing normal vs PE responses at 26 weeks for ACh and at 22 weeks for SNP.
Enhanced microvascular responses to acetylcholine have been shown previously in women who have PE by Davis et al., but the time of onset of these changes could not be determined from their study. In contrast to our study, they did not find any differences in responses to sodium nitroprusside. The reason for this could be that their measurements were made using single point laser Doppler flowmetry as opposed to imaging over a larger area as we did, which gives a better representation of skin perfusion. They did, however, show a trend toward an increased response but conducted their study at a single time point only (between 34 and 36 weeks’ gestation) when the effects might not be as apparent. In keeping with this, we saw a smaller difference in SNP responses at 34 weeks’ compared with those at 22 and 26 weeks’ gestation in preeclamptic women.

Enhanced production of endothelium-derived substances cannot be solely responsible for the changes seen in women with PE because microvascular responses to both acetylcholine and sodium nitroprusside were significantly altered. It is likely, therefore, that the differences in microvascular responses between women with a normal pregnancy and those with PE result from changes at a level further down from the endothelium and are possibly related to a compensatory increase in the sensitivity to nitric oxide at the smooth muscle level.

A change in the sensitivity of the microvasculature to nitric oxide in PE is of primary importance and is not a consequence of raised blood pressure because enhanced responses to sodium nitroprusside were apparent at 22 weeks’ gestation, before there were any clinical signs of PE. These findings suggest that maternal alterations in the response to nitric oxide might be an important factor in the subsequent development of PE. It has been reported recently that a vasoactive circulating factor(s), capable of altering myometrial endothelial function, is present in plasma samples weeks or months before the diagnosis of preeclampsia. It is possible that one of the actions of this circulating factor(s) is to alter the endothelial production of nitric oxide and subsequently the actions of nitric oxide on smooth muscle.

Maternal abnormalities of endothelial function and nitric oxide responsiveness in women with PE might increase cardiovascular risk in later life. However, we did not find any postpartum differences in microvascular responses between women with normal pregnancies and PE women. One reason for this discrepancy might be that we measured our postpartum subjects after a relatively short period (6 weeks).

The precise role of nitric oxide in PE remains unclear. The vascular changes during normal pregnancy have been attributed, in part, to nitric oxide, although this is not a consistent finding. Forearm vasoconstrictor responses to nitric oxide inhibition have been shown to be reduced in normal pregnancy in 2 studies but not in the study by Bowyer et al. The studies by Anumba et al and Bowyer et al both showed that the effect of nitric oxide inhibition is the same on the vessels of preeclamptic women as in normal pregnancy. Circulating levels of nitrite are reported to be decreased in patients with PE, supporting the concept that diminished nitric oxide synthesis contributes to the pathophysiology of PE. Other studies, however, indicate that in fact higher levels of nitric oxide are produced, perhaps as a compensatory mechanism to offset the impaired placental perfusion in PE. Of interest is the recent study by Wang et al who reported that endothelial nitric oxide synthase expression is decreased in PE. This raises the question as to whether the studies showing increased production of nitric oxide reflect increased endothelial production or whether nitric oxide is produced from other sources. In the present study the increased sensitivity to sodium nitroprusside might result from a compensatory response of the microvessels to decreased expression of nitric oxide synthase.

The incidence of complications in our normal Doppler group was higher than previously reported. However, this study was not designed to assess the sensitivity or specificity of Doppler waveform analysis as a screening test; this has been addressed in previous studies and our experimental design may have contributed to the increased incidence. We used Doppler analyses as a method of identifying a cohort of women at risk for pregnancy complications to facilitate longitudinal plasma sampling. Although the presence of bilateral notching has been shown to adversely affect endothelial function, we did not find any differences in microvascular function between women with and without notches.

The resulting sample size of women with PE was relatively small (n=15), and therefore we must be cautious with generalization of our findings. Nevertheless, we feel that the resulting sample size is sufficient to give us a true reflection of the vascular changes that occur during PE. Ramsay et al used similar methodology to ours in 9 women with diabetes and 16 control subjects and found significant differences within each group when comparing microvascular responses during pregnancy with those in the postnatal period. Additionally, we have previously shown that significant differences in microvascular responses before and after intervention are measurable using a paired comparison in 16 subjects.

Perspectives

We have shown that skin microvascular responses are enhanced in women in whom PE develops. This enhancement in vascular function is over and above that seen in normal pregnancy and possibly results from a compensatory response of the microvasculature to decreased nitric oxide production. Importantly, these changes within the microcirculation precede the onset of clinical disease by weeks or months. Early assessment of vascular function, in women at increased risk for PE, might help in the identification of potentially vulnerable patients, and enable therapeutic strategies to be targeted to this potentially dangerous condition.

Acknowledgments

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References


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