Association of Maternal Endothelial Dysfunction With Preeclampsia

John C. Chambers, MD, MRCP
Luca Fusi, MD, FRCOG
Iqbal S. Malik, MD, MRCP
Dorian O. Haskard, DM, FRCP
Michael De Swiet, MD, FRCP
Jaspal S. Kooner, MD, FRCP

Context Preeclampsia is believed to result from release of placental factors that damage maternal vascular endothelium. However, because most studies have been conducted during pregnancy, it has not been possible to separate maternal from placental mechanisms underlying endothelial dysfunction in preeclampsia.

Objective To determine whether endothelial function is impaired in nonpregnant women with previous preeclampsia and whether endothelial dysfunction is mediated by oxidative stress.

Design and Setting Case-control study conducted at 3 hospital maternity units in London, England, between July 1997 and June 2000.

Participants A total of 113 women with previous preeclampsia (n=35 with recurrent episodes; n=78 with a single episode) and 48 women with previous uncomplicated pregnancies, all of whom were at least 3 months (median, 3 years) postpartum.

Main Outcome Measures Brachial artery flow-mediated (endothelium-dependent) and glyceryl trinitrate–induced (endothelium-independent) dilatation were compared between previously preeclamptic women and controls. To investigate oxidative stress, these measurements were repeated after administration of ascorbic acid, 1 g intravenously, in 15 cases and 15 controls.

Results Mean (SD) flow-mediated dilatation was lower in women with previous preeclampsia compared with controls (recurrent group, 0.9% [4.1%]; single-episode group, 2.7% [3.5%]; and control group, 4.7% [4.3%]; \( P \leq .001 \)). In contrast, glyceryl trinitrate–induced dilatation was similar in the 3 groups (recurrent, 19.5% [5.9%]; single-episode, 21.0% [8.0%]; and control, 21.0% [8.3%]; \( P = .65 \)). Impaired flow-mediated dilatation in previously preeclamptic women was not accounted for by recognized vascular risk factors. Ascorbic acid administration increased flow-mediated dilatation in previously preeclamptic women (baseline, 2.6% [3.3%]; after administration, 5.6% [3.0%]; \( P = .001 \)) but not in controls (baseline, 6.2% [3.3%]; after administration, 6.7% [5.0%]; \( P = .72 \)).

Conclusions Our results indicate that endothelial function is impaired in women with previous preeclampsia and is not explained by established maternal risk factors but is reversed by antioxidant ascorbic acid administration.

JAMA. 2001;285:1607-1612 www.jama.com

Author Affiliations: National Heart and Lung Institute (Drs Chambers, Malik, Haskard, and Kooner), Institute of Obstetrics and Gynecology (Dr Fusi), Institute of Reproductive and Developmental Biology (Dr De Swiet), Imperial College School of Medicine, Hammersmith Hospital, London, England.

Corresponding Author and Reprints: J. S. Kooner, MD, FRCP, National Heart and Lung Institute, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, England (e-mail: j.kooner@ic.ac.uk).

See also Patient Page.
ENDOTHELIAL DYSFUNCTION AND PREECLAMPSIA

cies (n=48) between July 1997 and June 2000. Cases and controls were at least 3 months (median, 3 years) postpartum. Women with previous preeclampsia were identified from the maternity units of Ealing, Hammersmith, and Queen Charlotte’s Hospitals, London. Criteria for preeclampsia were a blood pressure greater than 140/90 mm Hg after the 20th week of gestation, accompanied by a proteinuria of 2+ on urinalysis or proteinuria greater than 300 mg in a 24-hour collection.9

Of a total of 485 women with preeclampsia who were sent invitations to participate, at least 75 had moved, leaving 410 who received the invitation. One hundred twenty-eight replied to the invitation, and 113 agreed to participate (28% acceptance rate). There was no evidence for selection bias in recruitment: in particular, responders and nonresponders were of similar age and parity, and they had similar blood pressure at their initial prenatal visit. Controls were women with uncomplicated deliveries at the same hospitals. Exclusion criteria for both patients and controls included established atherosclerosis, malignancy, major organ failure (including hepatic or renal failure), vasculitis, systemic infection, recent major surgery or trauma, and known diabetes. The study was approved by the local ethics committee, and all participants gave written informed consent.

Methods
Clinical history, including past history of hypertension, diabetes, habitual smoking, alcohol intake, and drug therapy, was recorded for all subjects. The mean of 3 blood pressure readings was calculated for each participant who sat for 10 minutes while the readings were measured by a mercury sphygmomanometer. Height, weight, and waist-hip girth ratio were recorded according to standardized protocols. Blood samples were collected in the fasting state (overnight) and assayed for total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride and glucose levels (AU800 multi-channel analyzer; Olympus Optical Ltd [UK]; Middlesex, England), and total plasma homocysteine,10 recognized determinants of vascular endothelial dysfunction.11,12 Measurements were also made of plasma soluble E-selectin and intercellular adhesion molecule-1 (ICAM-1) by radioimmunoassay (R & D Systems, Abingdon, England), as biochemical markers of endothelial activation.11 Subjects were not investigated at any specific time in relation to their menstrual cycle.

For each participant, brachial artery flow-mediated dilatation (endothelium dependent), and glyceryl trinitrate-induced dilatation (endothelium independent), were measured as described below. To investigate the role of oxidant stress mechanisms in the observed vascular responses, brachial artery measurements were repeated before and 1 hour after administration of ascorbic acid (1 g in 100 mL of normal saline intravenously infused over 30 minutes) in 15 patients and 15 controls selected as nonsmoking, nonobese (body mass index [BMI] <25 kg/m²), normotensive (blood pressure <140/90 mm Hg), with normal total cholesterol (<250 mg/dL [<6.5 mmol/L]) and normal fasting plasma glucose (108 mg/dL [<6.0 mmol/L]) levels.

Vascular Endothelial Function
Brachial artery flow-mediated dilatation was measured using a 7.0-MHz linear array transducer (Acuson 128XP/10 system; Mountain View, Calif), and high resolution ultrasonic vessel wall tracking system (Vadirec; Ingenious Systems; Arnhem, the Netherlands) as previously described.13 In brief, the brachial artery was scanned longitudinally, and the brachial artery diameter was measured at end diastole. After the baseline resting scan, a pneumatic cuff placed at the level of the mid-forearm was inflated to 300 mm Hg for 4.5 minutes. The second scan was performed 55 to 65 seconds after cuff deflation. Fifteen minutes was allowed for vessel recovery, after which the second baseline scan was performed. Glyceryl trinitrate (400 µg) was then administered and the fourth scan of the brachial artery undertaken. Brachial artery velocity and blood flow, determinants of flow-mediated dilatation, were measured using pulsed wave Doppler analysis 1 minute prior to inflation and 10 seconds after deflation of the pneumatic cuff. Velocity data were recorded with online angle correction on superVHS tape and analyzed offline. Peak systolic velocity and velocity time integral were calculated as an average over 5 beats. Resting and hyperemic brachial artery blood flow were derived from the velocity time integral, by standard methods.11 Vessel diameter and blood flow were measured by 2 independent observers unaware of each participant’s clinical details or the type and stage of the study. The technique for measurement of brachial artery flow–mediated dilatation is reproducible in our laboratory. The intraindividual, between-day coefficient of variation for flow-mediated dilatation is 3%, which compares favorably with other centers.12

Data Processing and Statistical Analysis
Based on previous studies in our laboratory,14 we expected an SD for flow-mediated dilatation of 4% in healthy controls. We planned to study 50 individuals in each of the 3 groups (recent preeclampsia, single episode of preeclampsia, and controls), offering a 90% power to detect, at a significance level of 5%, a true difference of 3% in flow-mediated dilatation between groups, a difference comparable to that identified in other pathological states.11,16-18

Data were analyzed using SPSS, Version 10.0 (SPSS Inc, Chicago, Ill) statistical package. Continuous data are expressed as mean (SD) or as mean (95% confidence interval [CI]). The differences between the 3 groups were investigated using analysis of variance for continuous data, and the χ² test for categorical data. Post hoc t tests were used to localize differences, with Bonferroni adjustment for multiple comparisons. Thereafter, the contribution of possible confounding effects to the
In contrast, there were no significant differences in glyceryl trinitrate–induced, endothelium-independent dilatation between the 3 groups (recurrent episode, 19.5% [5.9%]; single episode, 21.0% [8%]; control, 21.0% [8.3%]; P = .65). Baseline brachial artery diameter was higher in women with previous preeclampsia compared with controls but not when corrected for body surface area (Table 2). There were no significant differences between the groups in brachial artery velocity or blood flow either at rest or during the reactive hyperemia after deflation of the pneumatic cuff (Table 2).

Table 1. Clinical and Biochemical Characteristics of Controls and Women With Previous Preeclampsia

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>Single-Episode Preeclampsia</th>
<th>Recurrent Preeclampsia</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>48</td>
<td>78</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>35 (6)</td>
<td>34 (5)</td>
<td>37 (5)</td>
<td>.11</td>
</tr>
<tr>
<td>Current cigarette smoking, No. (%)</td>
<td>9 (19)</td>
<td>7 (9)</td>
<td>3 (9)</td>
<td>.21</td>
</tr>
<tr>
<td>Family history of hypertension, No. (%)</td>
<td>12 (25)</td>
<td>43 (55)</td>
<td>16 (46)</td>
<td>.004</td>
</tr>
<tr>
<td>Hypertension†</td>
<td>0 (0)</td>
<td>12 (15)</td>
<td>10 (29)</td>
<td>.001</td>
</tr>
<tr>
<td>Diabetes‡</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.9 (3.9)</td>
<td>25.7 (5.3)</td>
<td>27.0 (4.6)</td>
<td>.01</td>
</tr>
<tr>
<td>Waist-hip girth ratio</td>
<td>0.77 (0.05)</td>
<td>0.79 (0.05)</td>
<td>0.81 (0.06)</td>
<td>.004</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>103 (10)</td>
<td>116 (16)</td>
<td>118 (17)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL§</td>
<td>182 (35)</td>
<td>186 (35)</td>
<td>197 (42)</td>
<td>.15</td>
</tr>
<tr>
<td>HDL-C, mg/dL§</td>
<td>60 (12)</td>
<td>60 (17)</td>
<td>56 (17)</td>
<td>.42</td>
</tr>
<tr>
<td>Total to HDL cholesterol ratio</td>
<td>3.1 (0.8)</td>
<td>3.3 (1.0)</td>
<td>3.7 (1.2)</td>
<td>.02</td>
</tr>
<tr>
<td>Fasting triglycerides, mg/dL</td>
<td>80 (35)</td>
<td>80 (62)</td>
<td>89 (53)</td>
<td>.60</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL¶</td>
<td>81 (9.0)</td>
<td>83 (7.2)</td>
<td>85 (10.6)</td>
<td>.12</td>
</tr>
<tr>
<td>Total homocysteine, μmol/L</td>
<td>8 (2)</td>
<td>9 (3)</td>
<td>9 (3)</td>
<td>.30</td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td>162 (71)</td>
<td>209 (116)</td>
<td>166 (89)</td>
<td>.02</td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>286 (93)</td>
<td>262 (83)</td>
<td>270 (75)</td>
<td>.51</td>
</tr>
</tbody>
</table>

Continuous data are expressed as mean (SD) unless otherwise indicated. ICAM-1 indicates intercellular adhesion molecule 1. Hypertension was considered if a physician diagnosed chronic hypertension or subject’s blood pressure was greater than 140/90 mm Hg at the time of investigation. Diabetes was considered with physician diagnosis of diabetes or a fasting plasma glucose level of greater than 126 mg/dL (7.0 mmol/L). To convert total cholesterol and high-density lipoprotein cholesterol (HDL-C) from mg/dL to mmol/L, multiply by 0.02586. To convert triglycerides from mg/dL to mmol/L, multiply by 0.01129. To convert fasting plasma glucose from mg/dL to mmol/L, multiply by 0.05551.

Table 2. Brachial Artery Diameter and Blood Flow Characteristics in Controls and Women With Previous Preeclampsia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>Single-Episode Preeclampsia</th>
<th>Recurrent Preeclampsia</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline diameter, mm</td>
<td>3.28 (0.46)</td>
<td>3.42 (0.45)</td>
<td>3.64 (0.46)</td>
<td>.002</td>
</tr>
<tr>
<td>Diameter/body surface area, mm²</td>
<td>1.9 (0.2)</td>
<td>2.0 (0.3)</td>
<td>2.0 (0.2)</td>
<td>.24</td>
</tr>
<tr>
<td>Maximum velocity, cm/s</td>
<td>42 (11)</td>
<td>38 (9)</td>
<td>40 (10)</td>
<td>.11</td>
</tr>
<tr>
<td>Velocity time integral, cm</td>
<td>8 (1)</td>
<td>7 (1)</td>
<td>7 (1)</td>
<td>.36</td>
</tr>
<tr>
<td>Blood flow, mL/min</td>
<td>46 (33)</td>
<td>43 (24)</td>
<td>58 (33)</td>
<td>.06</td>
</tr>
</tbody>
</table>

After Cuff Deflation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>Single-Episode Preeclampsia</th>
<th>Recurrent Preeclampsia</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, mm</td>
<td>3.43 (0.48)</td>
<td>3.51 (0.45)</td>
<td>3.67 (0.45)</td>
<td>.06</td>
</tr>
<tr>
<td>Diameter/body surface area, mm²</td>
<td>2.0 (0.3)</td>
<td>2.0 (0.3)</td>
<td>2.1 (0.2)</td>
<td>.92</td>
</tr>
<tr>
<td>Maximum velocity, cm/s</td>
<td>53 (11)</td>
<td>49 (9)</td>
<td>50 (10)</td>
<td>.23</td>
</tr>
<tr>
<td>Velocity time integral, cm</td>
<td>22 (6)</td>
<td>21 (6)</td>
<td>20 (6)</td>
<td>.21</td>
</tr>
<tr>
<td>Blood flow, mL/min</td>
<td>143 (58)</td>
<td>137 (69)</td>
<td>149 (65)</td>
<td>.63</td>
</tr>
</tbody>
</table>

*Data are expressed as mean (SD).

©2001 American Medical Association. All rights reserved.
Regression Analysis of Flow-Mediated Dilatation

In univariate analysis, flow-mediated dilatation was negatively associated with BMI and baseline brachial artery diameter (TABLE 3). In multivariable regression analysis, the relationship between previous preeclampsia and impaired flow-mediated dilatation was independent of age, BMI, waist-hip girth ratio, blood pressure, family history of hypertension, fasting plasma glucose levels, lipid profile, homocysteine concentration, brachial artery diameter, and brachial artery blood flow (P = .008, TABLE 4).

Flow-Mediated Dilatation in Women Without Vascular Risk Factors

In a separate analysis, we compared vascular responses of cases and controls, among women who were nonobese and nonsmoking, who had normal blood pressure and fasting glucose and cholesterol levels (TABLE 5). Flow-mediated, endothelium-dependent dilatation was lower in women with previous preeclampsia compared with those in the control group (previous preeclampsia, 2.5% [3.2%]; control, 4.6% [4.4%]; P = .03), confirming that the relationship between preeclampsia and endothelial dysfunction was independent of risk factors generally associated with vascular disease.

Effects of Ascorbic Acid on Vascular Responses

Administration of ascorbic acid increased flow-mediated dilatation in preeclamptic women but not in controls. In contrast, glyceryl trinitrate–induced dilatation was unchanged after ascorbic acid in both patients and controls (TABLE 6).

COMMENT

Vascular endothelial dysfunction is recognized to be a central disturbance in preeclampsia. Evidence from previous studies suggests that endothelial dysfunction occurs in response to abnormal placentation, which may lead to placental ischemia and release of placental products that damage the maternal vascular endothelium. To identify whether maternal factors, independent of the placenta, contribute to endothelial dysfunction in preeclampsia, we studied the vascular responses of preeclamptic women remote from delivery.

We found that flow-mediated dilatation is reduced in women with previous preeclampsia compared with women with uncomplicated pregnancies, at a median interval of 3 years postpartum. Since flow-mediated dilatation is endothelium dependent, our results demonstrate that vascular endothelial function is impaired in women with previous preeclampsia. Impaired endothelial function was more severe in women with recurrent preeclampsia and was not accounted for by maternal obesity, hypertension, metabolic disturbances associated with insulin resistance, dyslipidemia, elevated homocysteine concentrations, or
brachial artery flow characteristics, which are recognized as potential determinants of vascular function. Endothelial dysfunction 3 years postpartum is also unlikely to be a consequence of the preeclamptic episode since in other situations endothelial dysfunction normalizes once the underlying cause has been removed. Previous studies have shown recovery of endothelial function after lowering risk factors including high cholesterol, triglyceride, and homocysteine concentration; high blood pressure; insulin resistance, physical inactivity, and estrogen deficiency. Endothelial dysfunction is also only transiently impaired by other stimuli such as cigarette smoking and systemic inflammation. Our observations of impaired endothelial function in women with preeclampsia, remote from delivery and independent of known vascular risk factors, suggest that novel factors may contribute to material endothelial dysfunction in preeclampsia.

Recent studies show that endothelium-dependent dilation can be inhibited by N\textsuperscript{G}-monomethyl-L-arginine (L-NAME) infusion, an antagonist of nitric oxide synthase. These observations suggest that endothelium-dependent dilation is largely mediated by the release of nitric oxide although they do not exclude a separate role for prostacyclin and other endothelium-derived relaxing factors. Although nitric oxide activity was not directly measured in our patients, our findings of reduced endothelium-dependent dilation imply that the bioavailability of endothelial nitric oxide may be reduced in preeclamptic women, even in the nonpregnant state. Reduced nitric oxide, the major endothelium-derived vasodilator, promotes vasoconstriction, platelet aggregation, and monocye adhesion, all of which could contribute to the vascular disturbances in preeclampsia.

Increasing evidence suggests that endothelial dysfunction in preeclampsia is mediated by oxidative stress. Recent studies show that antioxidant vitamins improve biochemical markers of endothelial activation and reduce the incidence of preeclampsia in high-risk women. Observations that lipid peroxidation and formation of peroxynitrite are increased in the placentas of women with preeclampsia have led to the hypothesis that the placenta is the principal source of oxidative stress. In our study, impaired endothelium-dependent dilation in women with preeclampsia was normalized by ascorbic acid. Ascorbic acid is a powerful water-soluble antioxidant that scavenges oxygen-derived free radicals, including superoxide anion, which would otherwise interact with nitric oxide and impair its vasoactive functions. Our findings therefore support the presence of oxidative stress, which may contribute to endothelial dysfunction in women with previous preeclampsia.

A further important finding of this study is that blood pressure levels are elevated following an episode of preeclampsia. We found that systolic blood pressure was 14 mm Hg higher and diastolic blood pressure was 8 mm Hg higher in previously preeclamptic women than in controls. These observations indicate that in women with previous preeclampsia, blood pressure distribution is shifted to the right compared with women with normal pregnancies and may precede development of hypertension. This may be of clinical importance since it is increasingly recognized that there is a graded relationship between blood pressure and risk of vascular events, including myocardial infarction and stroke.

In summary, we have found evidence of impaired vascular endothelial function in preeclamptic women, at a median interval of 3 years after delivery. Endothelial dysfunction is not explained by the presence of known vascular risk factors but is reversed by ascorbic acid.

**Table 6. Brachial Artery Diameter and Blood Flow Characteristics at Baseline and After Ascorbic Acid***

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Ascorbic Acid</th>
<th>P Value</th>
<th>Baseline</th>
<th>Ascorbic Acid</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow-mediated dilatation, %</td>
<td>6.2 (3.3)</td>
<td>6.7 (5.0)</td>
<td>.72</td>
<td>2.6 (3.3)</td>
<td>5.6 (3.0)</td>
<td>.001</td>
</tr>
<tr>
<td>Glyceryl trinitrate–induced dilatation, %</td>
<td>21.6 (7.9)</td>
<td>22.5 (4.3)</td>
<td>.71</td>
<td>20.6 (3.2)</td>
<td>21.7 (5.3)</td>
<td>.46</td>
</tr>
</tbody>
</table>

*Values are for the comparison of measurement taken after administration of ascorbic acid (1 g intravenously) with measurements taken at baseline in each study group.

**Author Contributions:** Study concept and design: Chambers, Fusi, De Swiet, and Kooner. Acquisition of data: Chambers, Fusi, Malik, Haskard, De Swiet, and Kooner. Drafting of the manuscript: Chambers, Fusi, De Swiet, and Kooner. Critical revision of the manuscript for important intellectual content: Chambers, Fusi, Malik, Haskard, De Swiet, and Kooner. Statistical expertise: Chambers and Kooner. Obtained funding: Chambers and Kooner. Study supervision: Chambers and Kooner.

**Funding/Support:** This work was supported in part by grant PG00/058 from the British Heart Foundation.

**Acknowledgment:** We thank Mary Rowley and Kim Medlock for their assistance in recruitment and characterization of subjects and Andrew McGregor and Jeff Jean-Marie for performing the vascular studies.

©2001 American Medical Association. All rights reserved.
REFERENCES


