Altered Retinal Flicker Response Indicates Microvascular Dysfunction in Women With Preeclampsia

Andreas Brückmann, Christin Seeliger, Thomas Lehmann, Ekkehard Schleußner, Dietmar Schlembach

Abstract—Flicker-induced dilatation is reduced in patients with cardiovascular risk, and the following arteriolar constriction is reduced with aging, leading to a reduced arteriolar amplitude and, thereby, indicating microvascular endothelial dysfunction. As endothelial dysfunction is associated with preeclampsia, we assessed retinal flicker response during pregnancy and postpartum. Between 2006 and 2013, women were recruited from University Hospital Jena and Prenatal Diagnostic Center Erfurt, Germany, of which 34 women with preeclampsia, 45 women with normal pregnancy, and 22 nonpregnant controls were included in the study. Women with normal pregnancy were matched for age, nulliparity, smoking, previous gestational hypertensive disorders, and family history of cardiovascular disease. Nonpregnant women were age-matched, nulliparous, nonsmoking, without family history of cardiovascular disease. Retinal vessel measurement using Dynamic Vessel Analyzer consisted of 50-seconds baseline acquisition, followed by three 20-second flicker and 80-second relaxation periods. Arteriolar constriction and arteriolar amplitude were reduced during pregnancy (P=0.001 and P=0.008) and postpartum (P=0.018 and P=0.034) in women with preeclampsia, adjusted for age, body mass index, mean arterial pressure, baseline diameter, and family history of cardiovascular disease. Flicker-induced dilatation was unchanged within the groups and throughout the study period. The unchanged flicker-induced dilatation may support a preserved autoregulatory competence of the microvasculature, and the diminished arteriolar amplitude, mainly because of the absence of the arteriolar constriction, indicates a commenced retinal microvascular dysfunction in women with preeclampsia during pregnancy and postpartum. Mechanisms responsible for altered retinal flicker response in preeclampsia need to be clarified in further studies. (Hypertension. 2015;66:900-905. DOI: 10.1161/HYPERTENSIONAHA.115.05734.) • Online Data Supplement

Key Words: endothelium ■ flicker ■ microvascular ■ preeclampsia ■ pregnancy ■ retinal

Preeclampsia occurs in 2% to 3% of all pregnancies and is one of the major causes of maternal and perinatal morbidity and mortality. It is considered as a disease of the maternal endothelium, and the proper function of any endothelium includes maintenance of a barrier and regulation of vascular tone. In preeclampsia, vasoactive substances, such as sFlt-1, are released into the maternal circulation, resulting in reduced nitric oxide (NO) bioavailability, vasoconstriction of the microvasculature, increased peripheral resistance, and hypertension.

Several attempts have been described to quantify the degree of peripheral vascular resistance, endothelial dysfunction (EDF), and microvascular constriction indirectly; however, a direct method of visualizing and measuring microvascular changes during pregnancy may advance understanding of the development of preeclampsia and support clinical risk stratification in pregnancy.

Hypertensive retinopathy has been found in preeclampsia, including generalized or focal arteriolar narrowing. The retinal vasculature is known to be responsive to vascular endothelial growth factor, which is decreased in preeclampsia. Similarly, vascular endothelial growth factor inhibitors induce arteriolar constriction in ophthalmic diseases.

The dynamic behavior of the microvasculature can be assessed measuring retinal vessels in response to flickering light, and the reduction in neurovascular coupling may be a consequence of EDF. This light-evoked reaction induces both flow-mediated dilatation of larger retinal vessels nearby and vasoconstriction in more distant vessels to redistribute the blood flow to certain regions. The evoked vasodilatation of retinal vessels is a measure of microvascular endothelial function because it is impaired in known diseases with EDF, such as diabetes mellitus or hypertension, and it is altered after NO synthase inhibition and endothelial removal. Minor alterations in retinal flicker response (RFR) were associated with aging, suggesting microvascular changes in biomechanical properties of the endothelium. However, the determination of dynamic retinal vascular behavior involves the endothelium and underlying autoregulatory mechanisms.
We therefore postulated that retinal vessels might display microvascular dysfunction in preeclampsia and, consequently, evaluated RFR in women with preeclampsia during pregnancy and postpartum.

Methods

Recruitment of Subjects

In this prospective observational study, 35 women with preeclampsia (PE) and 49 women at a corresponding gestational age with hitherto uncomplicated pregnancies (NP), matched for age, nulliparity, current smoking, previous gestational hypertensive disorders, and family history of cardiovascular disease (CVD), were enrolled after written informed consent between 2006 and 2013 from University Hospital Jena and Prenatal Diagnostic Center Erfurt, Germany. The Ethics Committee of the University of Jena approved the study (1884-10/06). Women with multiple pregnancy, chronic hypertension, renal disease, pregestational or gestational diabetes mellitus, and overt CVD were not included. Twenty-two nonpregnant age-matched (to PE), nulliparous, nonsmoking controls (NC) without family history of CVD and without hormonal contraceptive use were measured on the 5th day of menstrual cycle to state a normal RFR, without possible confounders.

Five pregnant women had to be excluded from final analysis because of later development of gestational diabetes mellitus (PE=1, NP=2) and HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome (NP=2). For postpartum analysis (at least >10 weeks postpartum), 27 PE women and 36 NP women with uncomplicated pregnancies remained (PE: 2 relocations and 5 withdrawals; NP: 3 relocations and 6 withdrawals; Figure S1 in the online-only Data Supplement).

According to International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines, preeclampsia was defined as new onset of hypertension (≥140/90 mm Hg) with proteinuria ≥300 mg/d or ≥2+ on dipstick or as severe when systolic blood pressure (BP) was ≥160 mm Hg and diastolic BP ≥110 mm Hg and when massive proteinuria occurred (≥5000 mg/d). Onset of hypertension was defined as early-onset preeclampsia and ≥3 weeks as late-onset preeclampsia.

BP was measured on both upper arms with an ambulatory BP monitor (Boso Medicus Control), and hypertension was confirmed in a second measurement. Systolic BP and diastolic BP were averaged, and mean arterial pressure (MAP) was calculated (MAP=2×diastolic BP+systolic BP)/3. Body mass index (BMI) was calculated from weight and height measurements. Patient records at 11 to 13 weeks were screened for previous BP readings (to rule out potential preexisting hypertension) and BMI.

Uterine Artery Doppler

Transabdominal sonography with color Doppler was used to measure pulsatility index (PI) of the right and left uterine artery, and the average PI of the 2 arteries was recorded (UtA-PI).

Retinal Flicker Response

Participants were seen by an ophthalmologist before retinal analysis to screen for visual abnormalities or ophthalmologic diseases. Afterward, RFR was measured with Dynamic Vessel Analyzer, Imedos, Jena, Germany, as previously described.

After marking an arteriole and venule segment of ≥1 mm in the superior temporal region (0.5–2 disk diameters from optic disc margin), the vessel diameters were continuously (25x/second) measured (Figure 1). The 350-second measurement cycle starts with a 50-second baseline diameter acquisition, followed by three 20-second flicker and 80-second relaxation periods (Figure S2). Mean relative diameter change from baseline is calculated by the Dynamic Vessel Analyzer software. The resulting arteriolar sum curve of the 3 flicker cycles consists of a shortened baseline, flicker-induced dilation (FID), maximum arteriolar constriction (MAC), and relaxation period. The arteriolar amplitude (AA) is the percentage change from peak FID to maximum constriction (Figure 2). Additional information on this method can be found in the online-only Data Supplement.

Statistical Analyses

IBM SPSS Statistics Version 19 for Windows Release 19.0.0 was used for data analysis. After assessment of normal distribution with Kolmogorov–Smirnov Test, comparisons were made with univariate and multivariate ANOVA and with unpaired and paired t test for continuous data and with χ² test for categorical data between PE, NP, and NC and between pregnancy and postpartum. After bivariate correlation analysis (Spearman) to evaluate confounding factors for MAC and AA, adjustments in univariate and multivariate ANOVA were made for age, arteriolar baseline diameter, MAP, BMI, and family history of CVD (which includes family history of hypertension, stroke, and myocardial infarction). Bonferroni post hoc analysis was used for multiple comparisons. Data are expressed as mean (SD).

Results

Clinical Characteristics of Cohort

There were no differences in age, parity, smoking, previous gestational hypertensive disorders, and family history of CVD between PE and NP. PE group had higher BMI, systolic BP, diastolic BP, and MAP at 11 to 13 weeks and UtA-PI at inclusion and were delivered earlier of smaller infants (Table 1). Nine (26.5%) women had early onset preeclampsia, 5 (14.7%) had severe preeclampsia, 23 (67.6%) were on antihypertensive therapy (methyldopa), and 8 (23.5%) showed intrauterine growth restriction (birth weight <10th percentile). At inclusion and postpartum, PE group had increased BMI and BP values compared with the NP and NC group (Table 2). None of the participants had any visual symptoms or ophthalmologic diseases.

Retinal Flicker Response

The results of RFR are summarized in Table 3, all of which were normally distributed. Baseline diameter and FID of the arteriole and venule were not significantly different between groups throughout the study period.

Figure 1. Retinal vessel imaging with Dynamic Vessel Analyzer (DVA). The optic disc and its margin are visualized (black double-headed arrow), and in the superior temporal region, an arteriole (red line) and venule segment (blue line) of ≥1 mm are marked in a distance of 0.5–2 disk diameters (white double-headed arrow) from the optic disc margin. Subsequently, the measurement cycle of vessel diameter acquisition starts as shown in Figure S2 in the online-only Supplemental Digital Content.
In bivariate correlation analysis, MAC was positively associated with age, BMI, MAP, and UtA-PI, whereas AA was negatively associated with age, MAP, and UtA-PI and positively associated with family history of CVD (Table S1).

During pregnancy and postpartum, MAC was lower in the PE group compared with the NP and NC group (Table 3; Figure S3). AA was lower in the PE group compared with the NP group during pregnancy and postpartum (Table 3). MAC and arteriolar baseline diameter of PE group were enhanced at postpartum measurement compared with during pregnancy (Table 3). There were no significant differences in RFR parameters between the 2 time points in the NP group. The relationship between preeclampsia and its impairment in MAC and AA was independent of age, arteriolar baseline diameter, MAP, BMI, and family history of CVD (Table 3). There was no significant influence of UtA-PI on MAC and AA (multivariate ANOVA: \(P=0.821\) in the MAC model and \(P=0.108\) in the AA model). Additional adjustment for this parameter did not change the results.

**Discussion**

This is the first study using Dynamic Vessel Analyzer in normal pregnancy and in preeclampsia to evaluate retinal vascular function. We measured RFR during pregnancy and postpartum and in nonpregnant controls. MAC and AA were reduced in PE group compared with the NP and NC group. These changes in RFR persisted several weeks after parturition. Postpartum, MAC and arteriolar baseline diameter were enhanced in the PE group. FID was preserved in PE group at both measurements.

RFR is a function of neurovascular coupling, caused by enhanced retinal ganglion neuronal activity, which primarily dilates capillaries. The secondary increase in blood flow thereby induces an NO-mediated dilatation of larger arterioles and venules, independently of perfusion pressure.\(^{11}\) Endothelium-dependent RFR, which includes FID and MAC, is impaired in several disorders associated with EDF and is paired with stiffened connective tissue.\(^{15-17,19}\) The retinal peripapillary region is a designated low-flow area, and it was found that in an artificial low-flow area, flow-mediated constriction was diminished after endothelial denudation through catheterization, suggesting that this is an endothelium-dependent reaction.\(^{20}\)

In preeclampsia RFR is impaired. Therefore, we suggest that reduced AA and MAC in preeclampsia may indicate pre-aged and stiffened microvessels, which result from EDF and remodeling. We assume that in our results, microangiopathy is evidenced by the diminished MAC, which is further confirmed by the correlation with UtA-PI.

The microvascular endothelium and its basal NO production may be involved in autoregulatory responses to maintain retinal blood flow,\(^{14}\) despite changes of perfusion pressure within a certain range.\(^{12}\) Retinal arteriolar baseline diameter did not change during raised intraocular pressure, which may demonstrate autoregulatory mechanisms that include metabolic and myogenic processes.\(^{21}\) As retinal vessels are free of innervation, central neural actions can be excluded.\(^{22}\) Our findings of steady retinal baseline diameters and preserved FID in PE may support the compensatory and autoregulatory competence of the microcirculation to counteract increased MAP. This is consistent with the results of unaffected FID during raised MAP found in healthy volunteers\(^{23}\) and with unaffected FID after intravenously applied vascular endothelial growth factor inhibitors.\(^{24}\) The flicker response data in our preeclampsia group may describe a condition between normal and chronic hypertension because chronic hypertension results in impaired FID.\(^{13}\)

In preeclampsia, the blood–retinal barrier is disrupted\(^{25}\) and microvascular permeability is increased\(^{26}\) This enables vasoactive factors, such as endothelin-1 and NO, which are increasingly produced in preeclampsia,\(^{27}\) directly to access the retinal vascular smooth muscle cells.\(^{28}\) It is suggested that in

<table>
<thead>
<tr>
<th>Variables</th>
<th>Preeclampsia</th>
<th>Normal Pregnancy</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>34</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Maternal age, y [27 (5)]*</td>
<td>29 (9)</td>
<td>29 (6)</td>
<td>1.000†</td>
</tr>
<tr>
<td>Nulliparous, n (%)</td>
<td>18 (52.9)</td>
<td>28 (62.2)</td>
<td>0.492</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>5 (14.7)</td>
<td>2 (4.4)</td>
<td>0.133</td>
</tr>
<tr>
<td>Previous GH, n (%)</td>
<td>1 (2.9)</td>
<td>1 (2.2)</td>
<td>1.000</td>
</tr>
<tr>
<td>Previous PE, n (%)</td>
<td>5 (14.7)</td>
<td>2 (4.4)</td>
<td>0.133</td>
</tr>
<tr>
<td>Family history of CVD, n (%)</td>
<td>26 (76.5)</td>
<td>34 (75.6)</td>
<td>1.000</td>
</tr>
<tr>
<td>BMI at 11–13 wk, kg/m²</td>
<td>28.5 (7.3)</td>
<td>23.1 (4.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>SBP at 11–13 wk, mmHg</td>
<td>119 (11)</td>
<td>112 (10)</td>
<td>0.012</td>
</tr>
<tr>
<td>DBP at 11–13 wk, mmHg</td>
<td>76 (9)</td>
<td>68 (9)</td>
<td>0.003</td>
</tr>
<tr>
<td>MAP at 11–13 wk, mmHg</td>
<td>90 (9)</td>
<td>83 (8)</td>
<td>0.001</td>
</tr>
<tr>
<td>UtA-Doppler at inclusion, PI</td>
<td>1.22 (0.91)</td>
<td>0.71 (0.16)</td>
<td>0.004</td>
</tr>
<tr>
<td>Gestation at delivery, wk</td>
<td>37 (4)</td>
<td>39(1)</td>
<td>0.000</td>
</tr>
<tr>
<td>Cesarean delivery, n (%)</td>
<td>23 (68)</td>
<td>13 (29)</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>2775 (1030)</td>
<td>3544 (425)</td>
<td>0.000</td>
</tr>
<tr>
<td>Birth centile, g</td>
<td>35 (29)</td>
<td>52 (28)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD) unless otherwise indicated. Comparisons were made with unpaired \(t\) test for continuous data (which were all normally distributed) and \(\chi^2\) test for categorical data. BMI indicates body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; GH, gestational hypertension; MAP, mean arterial pressure; PE, preeclampsia; PI, pulsatility index; SBP, systolic blood pressure; and UtA, uterine artery.

*Nonpregnant controls.
†Multiple comparisons with univariate ANOVA.
Table 2. Clinical Characteristics According to Study Group and Measurement

<table>
<thead>
<tr>
<th>Variables</th>
<th>NC</th>
<th>PE</th>
<th>NP</th>
<th>P*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>22</td>
<td>34</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, wk‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.8 (2.7)</td>
<td>28.9 (7.4)</td>
<td>24.1 (4.4)</td>
<td>0.002</td>
<td>0.020</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>115 (8)</td>
<td>137 (17)</td>
<td>110 (10)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>77 (7)</td>
<td>87 (12)</td>
<td>68 (9)</td>
<td>0.006</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). Comparisons were made with multivariate ANOVA (all data were normally distributed). BMI indicates body mass index; DBP, diastolic blood pressure; MAP, mean arterial pressure; NC, nonpregnant controls; NP, women with normal pregnancy; PE, women with preeclampsia; and SBP, systolic blood pressure.

*PE versus NP.
†PE versus NC.
‡Gestational and postpartum duration.

In preeclampsia, the vasoconstrictive effect of endothelin-1 may be counteracted by a strong NO-mediated vasodilatation.27 Endothelium-derived oxygen species, which are abundant in preeclampsia,29 induce further vasoconstriction in precontracted ophthalmic arteries and thus may contribute to further progression of diseases involving altered blood flow and vascular tone, such as hypertension.30 These opposing mechanisms in the perivascular retinal tissue may lead to arteriolar stiffening and decreased AA, which is possibly compensated for by increased MAP.15

In preeclampsia, we did not find significant differences in baseline vessel diameters compared with NP and NC, with adjustment for confounders, including MAP. This is in contrast to the results by Lupton et al.5 which were corrected but not statistically adjusted for MAP. A possible explanation may be the different methods used for diameter measurement and that static retinal vessel analysis strongly correlates with BP.31

Table 3. Parameters of Flicker Response According to Study Group and Measurement

<table>
<thead>
<tr>
<th>Variables</th>
<th>NC</th>
<th>PE</th>
<th>NP</th>
<th>P*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>22</td>
<td>34</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>art BD, MU</td>
<td>119.1 (17.6)</td>
<td>114.1 (14.8)</td>
<td>117.8 (18.1)</td>
<td>1.000</td>
<td>0.693</td>
</tr>
<tr>
<td>art FID, %</td>
<td>3.9 (2.1)</td>
<td>4.6 (3.1)</td>
<td>4.9 (2.4)</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>MAC, %</td>
<td>−3.5 (1.5)</td>
<td>−0.9 (0.7)</td>
<td>−3.3 (1.5)</td>
<td>0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>AA, %</td>
<td>7.4 (2.9)</td>
<td>4.9 (2.4)</td>
<td>8.1 (3.3)</td>
<td>0.008</td>
<td>0.037</td>
</tr>
<tr>
<td>ven BD, MU</td>
<td>147.2 (20.9)</td>
<td>139.6 (21.7)</td>
<td>141.8 (23.2)</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>ven FID, %</td>
<td>3.9 (1.7)</td>
<td>5.8 (2.4)</td>
<td>4.9 (2.2)</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). Comparisons were made with multivariate ANOVA (all data were normally distributed), adjusted for age, body mass index, mean arterial pressure, arteriolar baseline diameter, and family history of cardiovascular disease, unless otherwise indicated. AA indicates arteriolar diameter; art, arteriolar; BD, baseline diameter; FID, flicker-induced dilatation; MAC, maximum arteriolar constriction; MU, measuring units (correlate to micrometer for a Gullstrand’s normal eye); NC, nonpregnant controls; NP, women with normal pregnancy; PE, women with preeclampsia; and ven, venular.

*PE versus NP.
†PE versus NC.
‡Significant to preeclampsia during pregnancy (paired t test: P=0.042).
§Significant to preeclampsia during pregnancy (paired t test: P=0.015).

Preeclampsia, the vasoconstrictive effect of endothelin-1 may be counteracted by a strong NO-mediated vasodilatation.27 Endothelium-derived oxygen species, which are abundant in preeclampsia,29 induce further vasoconstriction in precontracted ophthalmic arteries and thus may contribute to further progression of diseases involving altered blood flow and vascular tone, such as hypertension.30 These opposing mechanisms in the perivascular retinal tissue may lead to arteriolar stiffening and decreased AA, which is possibly compensated for by increased MAP.15

In preeclampsia, we did not find significant differences in baseline vessel diameters compared with NP and NC, with adjustment for confounders, including MAP. This is in contrast to the results by Lupton et al.5 which were corrected but not statistically adjusted for MAP. A possible explanation may be the different methods used for diameter measurement and that static retinal vessel analysis strongly correlates with BP.31

In preeclampsia, postpartum arteriolar baseline diameter was increased. This may reflect the vasoconstriction seen before preeclampsia.5 In contrast, despite the full recovery of diameter dimensions, MAC was still diminished postpartum. These results demonstrate microvascular changes in biomechanical properties in preeclampsia, supposedly linked with autoregulatory and compensatory mechanisms, which partly resolve postpartum. Furthermore, RFR was equally distributed between women with and without previous gestational hypertensive disorders within PE and NP group (data not shown), supporting the 2-year resolution hypothesis of preeclampsia, which poses that vascular changes which still occur after preeclampsia seem to have recovered 2 years later.32

After preeclampsia, the risk of CVD is increased,33 and there is a relationship between high-normal BP and later CVD14 and obesity.25 Altered retinal microvascular function is closely associated with cardiovascular risk factors and may predict to some extent future CVD or may be of some use for first trimester risk assessment.11,19

One limitation of the study may be that PE group consisted of women with predominantly mild early- and late-onset preeclampsia (85.3%), with potentially less impact on retinal vessel dynamics, and women with severe preeclampsia. The latter have different pathophysiological mechanisms and may be under-represented. Although the numbers are small, we did not find significant differences in RFR between
women with early- and late-onset preeclampsia, as well as severe and mild preeclampsia. 67.6% of subjects were treated with methyldopa, which could be argued as another potential limitation because mean PI of the central retinal artery drops after methyldopa use in preeclampsia.13 In our opinion, the alpha adrenergic effect of methyldopa can be excluded because retinal arterioles lack sympathetic innervation22 and exhibit autoregulation over a wide range of perfusion pressure changes.12 Furthermore, we found no differences in RFR within PE group between women with and without antihypertensive medication. Finally, because it is not possible to measure perfusion pressure while performing Dynamic Vessel Analyzer examination, we cannot rule out that it partially influences the results. Furthermore, we did not measure retinal neuronal activity or the influence of retinal vasoactive factors and are therefore unable to clarify whether altered RFR in preeclampsia is attributable to reduced retinal neurovascular coupling or changes in retinal biochemical properties. Although preeclampsia may arise from a maternal predisposition to metabolic syndrome or EDF,37-39 it is uncertain whether altered RFR in this study is a cause or consequence of preeclampsia based on unavailable prepregnancy data. Retinal flicker analysis might be able to unmask preexisting differences in preeclamptic women, who already had higher—although not hypertensive—BP and BMI in early gestation. RFR could serve as a novel predictive marker of preeclampsia, which needs to be clarified in further studies.

Perspectives
To the best of our knowledge, this is the first study that has examined retinal flicker response in preeclampsia and normal pregnancy. Unchanged retinal FID may support a preserved autoregulatory competence of the microvasculature, and the diminished retinal AA, mainly because of the absence of MAC, may indicate commenced retinal microvascular dysfunction in preeclamptic women during pregnancy and postpartum. The mechanisms responsible for the altered retinal vascular flicker response in preeclampsia are currently unclear, and future studies are needed.

Acknowledgments
We cordially thank Marie-Theres Gläßler for the accurate analysis and dedicated management of the database, Catrin Schinköth and Andrea Schröter for the organization and recruitment of patients, Carolin Reichmacher and Evelyn Jung for the compilation of the tables, and Pierre Kallenbach for the technical setup. We also thank Prof Christoph Lees (London) for kindly prereviewing the article, and we are very grateful for his suggestions.

Disclosures
None.

References


Novelty and Significance

**What Is New?**

- This is the first study that reveals differences in the dynamic behavior of the retinal microvasculature under the influence of flickering light in women with preeclampsia and with normal pregnancies.
- A reduced retinal arteriolar diameter amplitude as a result of an absent flicker-induced arteriolar constriction occurs during preeclampsia and postpartum.

**What Is Relevant?**

- Preeclampsia remains a major cause of maternal and fetal morbidity and mortality. The disorder is associated with macrovascular endothelial dysfunction and future cardiovascular risk.

- Advanced knowledge of microvascular reaction patterns during preeclampsia enables an understanding of microvascular autoregulatory capabilities and the pathogenesis of preeclampsia.

**Summary**

Preeclampsia is associated with commenced microvascular dysfunction and preserved autoregulative competence of the retinal microvasculature.
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Altered retinal flicker response indicates microvascular dysfunction in women with preeclampsia.

Andreas Brückmann, MD\textsuperscript{1,2}, Christin Seeliger, MD\textsuperscript{1}, Thomas Lehmann, PhD\textsuperscript{3}, Ekkehard Schleußner, MD\textsuperscript{1}, Dietmar Schlembach, MD\textsuperscript{1,4}

\textsuperscript{1} Department of Obstetrics, Jena University Hospital Jena, Friedrich-Schiller-University, Germany
\textsuperscript{2} Department of Prenatal Diagnosis and Preventive Medicine, GesaTal Medical Center, Erfurt, Germany
\textsuperscript{3} Institute for Medical Statistics, Jena University Hospital Jena, Friedrich-Schiller-University, Germany
\textsuperscript{4} Clinic of Obstetrics, Vivantes Hospital Berlin-Neukoelln, Germany

\textbf{Corresponding author}
Dr Andreas Brückmann
Talstr. 5
99089 Erfurt
Email: andreas.brueckmann@drmb.de
Telephone: +49-361-66343838
FAX: +49-361-66343840
Supplementary Methods

Retinal Flicker Response

Retinal flicker response was measured after 1% tropicamide was admitted to dilate the pupil of the left eye, since both eyes provide equivalent measures and mydriasis was reached after approximately 20-30 min, while the patients rested in a dark, temperature controlled room. The Dynamic-Vessel-Analyzer comprises a fundus camera (FF-450-plus, Carl-Zeiss-Meditec, Jena, Germany) equipped with an optoelectric liquid crystal display (LCD) shutter, a digital video camera and a personal computer with analyzing software. The shutter modulates a luminance flicker frequency of 12.5 Hz, which is within the range of maximum human visual sensitivity, with a bright to dark ratio of at least 25:1. The arteriolar and venular vessel diameters are expressed as measuring units, which correlate to \( \mu m \) for a Gullstrand’s normal eye.

References


| Table S1. Correlations of retinal flicker response with clinical variables |
|--------------------------|-------------|--------------|----------|
| Variables                | MAC Correlation Coefficient | \( P \)     | AA Correlation Coefficient | \( P \) |
| Age                      | 0.18         | 0.012        | -0.24    | 0.013     |
| Nulliparous              | -0.09        | 0.385        | 0.18     | 0.091     |
| Current smoking          | 0.12         | 0.215        | -0.10    | 0.290     |
| Previous GH              | -0.06        | 0.513        | 0.08     | 0.410     |
| Previous PE              | 0.19         | 0.056        | -0.19    | 0.054     |
| Family history of CVD    | -0.07        | 0.492        | 0.15     | 0.037     |
| Body mass index          | 0.26         | 0.009        | 0.02     | 0.853     |
| Mean arterial pressure   | 0.50         | 0.000        | -0.25    | 0.017     |
| Art Baseline diameter    | 0.002        | 0.982        | -0.12    | 0.202     |
| Uterine artery Doppler   | 0.189        | 0.022        | -0.170   | 0.039     |

MAC, maximum arteriolar constriction; AA, arteriolar amplitude; GH, gestational hypertension; PE, preeclampsia; CVD, cardiovascular disease; Art, arteriolar.
Figure S1. Flow chart of women enrolled in this study, including 22 non-pregnant controls. Five were excluded from final analysis and 16 were lost of postpartum follow up.

**Figure S2.** Timeline of retinal vessel analysis. First tropicamide was admitted to dilate the pupil of the left eye. After 20-30 min rest to reach complete mydriasis, the retinal vessels were visualized and marked with the DVA as shown in figure 1. That automatically activates the continuous vessel diameter measurement cycle of 350 sec, consisting of a baseline diameter acquisition and 3 consecutive flicker and relaxation periods.

**Figure S3.** Maximum arteriolar constriction after flicker-induced dilatation at study inclusion, adjusted for age, body mass index, mean arterial pressure, arteriolar baseline diameter and family history of cardiovascular disease. Multiple comparisons were made with univariate ANOVA, * significant to preeclampsia (P<0.002).