ORIGINAL RESEARCH

Early Onset Preeclampsia Is Associated With Glycocalyx Degradation and Reduced Microvascular Perfusion

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Background—The endothelial glycocalyx is a vasoprotective barrier between the blood and endothelium. We hypothesized that glycocalyx degradation is present in preeclampsia, a pregnancy-specific hypertensive disorder characterized by endothelial dysfunction and activation.

Methods and Results—We examined the sublingual glycocalyx noninvasively using sidestream dark field imaging in the third trimester among women with normotensive pregnancies (n=73), early (n=14) or late (n=29) onset preeclampsia, or gestational diabetes mellitus (n=21). We calculated the width of the glycocalyx that was permeable to red blood cells (called the *perfused boundary region*, a measure of glycocalyx degradation) and the percentage of vessels that were filled with red blood cells ≥50% of the time (a measure of microvascular perfusion). In addition, we measured circulating levels of glycocalyx components, including heparan sulfate proteoglycans, hyaluronic acid, and SDC1 (syndecan 1), in a subset of participants by ELISA. Repeated-measures ANOVA was performed to adjust for vessel diameter and caffeine intake. Women with early onset preeclampsia showed higher glycocalyx degradation, indicated by a larger perfused boundary region (mean: 2.14 [95% CI, 2.05–2.20]), than the remaining groups (mean: normotensive: 1.99 [95% CI, 1.95–2.02], *P*=0.002; late-onset preeclampsia: 2.01 [95% CI, 1.96–2.07], *P*=0.024; gestational diabetes mellitus: 1.97 [95% CI, 1.91–2.04], *P*=0.004). The percentage of vessels that were filled with red blood cells was significantly lower in early onset preeclampsia. These structural glycocalyx changes were accompanied by elevated plasma concentrations of the glycocalyx components, heparan sulfate proteoglycans and hyaluronic acid, in early onset preeclampsia compared with normotensive pregnancy.

Conclusions—Glycocalyx degradation and reduced microvascular perfusion are associated with endothelial dysfunction and activation and vascular injury in early onset preeclampsia. **(*J Am Heart Assoc*. 2019;8:e010647. DOI: 10.1161/JAHA.118.010647.)**

Key Words: gestational diabetes mellitus • microcirculation • preeclampsia/pregnancy • vascular glycocalyx

Preeclampsia is a leading cause of maternal and fetal morbidity and mortality that affects 2% to 7% of pregnancies.[1,2] This hypertensive pregnancy disorder is diagnosed in women presenting with new-onset hypertension and often proteinuria after 20 weeks gestation.[3] Preeclampsia can also be diagnosed in hypertensive pregnant women without proteinuria who have other signs of severe organ dysfunction.[3] Delivery is the only known cure.

The role of endothelial dysfunction and endothelial cell activation in vascular disease has been increasingly recognized,[4] and many of these postulates have been studied in preeclampsia. Endothelial dysfunction, which has been associated with impaired vasodilation resulting from a decrease in nitric oxide bioavailability or activity,[4] has been documented in preeclampsia, using both circulating markers of endothelial injury and vascular reactivity studies.[5–7] Endothelial activation

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Accompanying Tables S1 through S5 and Figure S1 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.010647

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Clinical Perspective

What Is New?

- Early onset preeclampsia is associated with structural and functional damage of the glycocalyx, a layer of proteoglycans and glycoproteins that lines the vascular endothelium and protects endothelial cells from endothelial activation.

What Are the Clinical Implications?

- Endothelial activation, which is the result of adhesion of white blood cells and platelets, and proinflammatory and procoagulation effector molecules may contribute to vascular injury in preeclampsia.
- Noninvasive glycocalyx measurements, using sidestream dark field imaging, offer a unique opportunity to assess endothelial activation in real time, which may provide insight into the pathophysiology of pregnancy complications.

is the result of endothelial expression of cell-surface adhesion molecules that assist in recruitment and attachment of circulating leukocytes to the vessel wall, ultimately leading to vascular inflammation and atherosclerosis.[4] Evidence of endothelial activation in preeclampsia includes upregulated levels of proinflammatory cytokines, such as TNF-α (tumor necrosis factor α) and IL-6 (interleukin 6),[8] as well as upregulation of cell-surface adhesion molecules.[9] However, little is known about the glycocalyx, a layer of proteoglycans and glycoproteins that lines the vascular endothelium. The glycocalyx protects endothelial cells from endothelial activation, including adhesion of white blood cells and platelets, and proinflammatory and procoagulation effector molecules.[10]

Recent advances allow for measurements of glycocalyx degradation noninvasively using a hand-held camera that captures video of red blood cells (RBCs) passing through small vessels under the tongue.[11] Commercially available software estimates the depth to which RBCs can penetrate the glycocalyx.[11] Healthy glycocalyx is relatively impermeable to RBCs and other circulating factors, whereas degradation creates gaps that allow RBCs to penetrate further into the glycocalyx.[11] Glycocalyx damage, as measured by greater permeability of the glycocalyx by RBCs,[11] is expressed as an increase in the perfused boundary region (PBR). Studies to date have shown that the PBR increases with diabetes mellitus[12] and reduced renal function[13] and in those patients with white matter lesions, which are indicative of small vessel disease.[14] In HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count; a particularly severe form of preeclampsia), serum levels of SDC1 (syndecan 1), a protein core of proteoglycans that forms glycocalyx, are higher than in gestational age–matched normotensive pregnancies.[15] However, these markers have not been studied in the context of different subtypes of preeclampsia. In addition, comparative in vivo measurements of glycocalyx in small sublingual vessels using sidestream dark field imaging have not been performed previously in preeclampsia.

The present study tests the hypothesis that glycocalyx degradation is enhanced in women with preeclampsia compared with normotensive pregnant women. In addition, we posited that glycocalyx degradation would be more pronounced in early preeclampsia (<34 gestational weeks) than in late preeclampsia (≥34 gestational weeks). Compositional and dimensional changes of glycocalyx were examined by using in vivo sidestream dark field imaging of the sublingual vasculature and by measuring circulating levels of glycocalyx components, including SDC1, heparan sulfate proteoglycans (HSPG), and hyaluronic acid. Microvascular perfusion was assessed by measuring the percentage of vessels that were filled with RBCs ≥50% of the time.

Methods

Deidentified data were deposited on the Open Science Framework website.[16]

Participants

A convenience sample of pregnant women in the third trimester were recruited through the Mayo Clinic Department of Obstetrics and Gynecology during prenatal appointments or following admission to the Family Birth Center. Normotensive women had no history of gestational hypertension or preeclampsia in prior pregnancies and did not develop these conditions during the index pregnancy. Preeclampsia was diagnosed based on established criteria[3]: (1) hypertension after 20 weeks of gestation, defined as blood pressure ≥140/90 mm Hg, and (2) proteinuria (defined as ≥300 mg of protein in a 24-hour urine specimen and/or protein/creatinine ratio of 0.3 and/or 1+ (30 mg/L) dipstick urinalysis in the absence of urinary tract infection). In the absence of proteinuria, the diagnosis of preeclampsia was confirmed (1) if any of the following laboratory abnormalities are present: thrombocytopenia <100 000/μL, serum creatinine >1.1 mg/dL or its doubling, or elevated liver function tests (AST [aspartate aminotransferase] and ALT [alanine aminotransferase] >2 times the upper limit of normal); or (2) in the presence of pulmonary edema or cerebral or visual symptoms.[3] Preeclampsia was categorized as *early onset* when diagnosed <34 weeks gestation or as *late onset* when diagnosed ≥34 weeks gestation. Heparin can alter the glycocalyx; therefore, preeclamptic women who were receiving heparin were excluded. A group of women with gestational diabetes mellitus (GDM) was also included because GDM is an important preeclampsia risk factor,[17,18] and endothelial dysfunction is hypothesized to contribute to the pathophysiology of GDM.[19]

GDM was diagnosed using the 2-step approach outlined in the American Diabetes Association guidelines.[20] Women completed a 3-hour 100-g oral glucose tolerance test if plasma glucose concentrations were ≥140 mg/dL 1 hour after a 50-g glucose load test. GDM was diagnosed in women with at least 2 abnormal values (fasting: ≥95 mg/dL; 1 hour: ≥180 mg/dL; 2 hours: ≥155 mg/dL; 3 hours: ≥140 mg/dL).

The Mayo Clinic Institutional Review Board approved the study (no. 2104-05). All participants provided written informed consent before participating. Participant characteristics, pregnancy outcomes, and medications administered to hospital inpatients for 24 hours before the glycocalyx test were abstracted from the medical record. Medical records were reviewed by 2 observers to confirm pregnancy diagnoses (V.D.G., T.L.W.).

Noninvasive Glycocalyx Measurements

Measurements were performed after a 10-minute rest period. Images of RBCs flowing through sublingual vessels were obtained by sidestream dark field imaging (CapiScope Handheld Video Capillaroscopy System; KK Technologies). The camera is placed under the tongue and uses green light–emitting diodes to detect hemoglobin in RBCs (Figure 1A). Participants are asked not to apply pressure to the tongue when holding the camera to maintain normal blood flow. Automated software (GlycoCheck; GlycoCheck BV) records video clips when the image is stable and in focus. As described previously,[11] the software identifies all measurable microvessels that are <30 μm in diameter based on contrast between the RBCs and the background. Measurements were made every 10 μm along each vessel. The participant held the camera stable while frames were recorded and then shifted the camera to a new location to view different vessels. This process was repeated until data on 3000 vascular segments were obtained (1–5 minutes). The software performs quality checks on each video clip to identify vascular segments that are in focus and have sufficient blood flow for analysis. For each valid vascular segment, the program calculates the vessel diameter (median width of the RBC column) and the

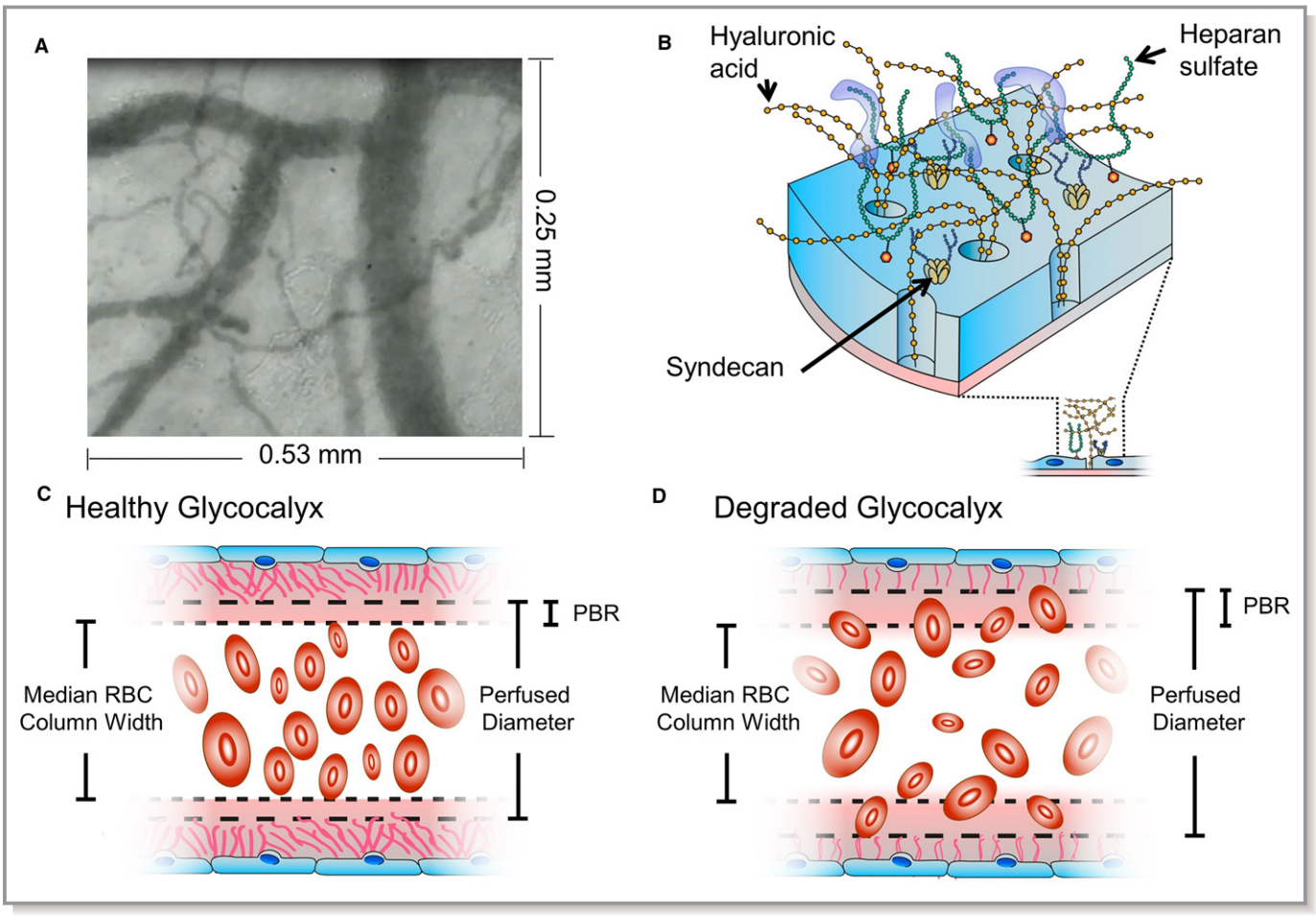

Figure 1. Glycocalyx measurements. **A**, The noninvasive camera records live video of RBCs moving through sublingual vessels. **B**, Schematic illustration of glycocalyx structure. **C**, The healthy glycocalyx is relatively impermeable to RBCs, resulting in a small PBR. **D**, Glycocalyx damage allows RBCs to penetrate further into the glycocalyx, increasing PBR width. PBR indicates perfused boundary region; RBC, red blood cell.

width of the portion of the glycocalyx that is permeable to RBCs (PBR; Figure 1). Glycocalyx degradation allows RBCs to penetrate further into the glycocalyx, and this can be detected by a higher PBR value. The program also assesses perfusion by calculating the percentage of vessels in each size category that are filled with RBCs. PBR is calculated using validated methods.[14,21–23] Measurements are accurate to 0.05 μm.[11] Previous studies in humans and animals have validated this technique.[13,14,21–26] Sublingual and retinal glycocalyx dimensions were both reduced in patients with type 2 diabetes mellitus, suggesting that sublingual glycocalyx degradation may reveal systemic effects.[27] For each woman, glycocalyx measurements were obtained for 22 vessel-size categories (median RBC column width of 3–25 μm). Each participant completed 3 trials separated by 5-minute rest periods. We found no evidence of systematic differences in results across trials; therefore, the results of the 3 trials were averaged. Participants provided information on food and caffeine intake on the test day and medication use for 24 hours before the test.

Reliability Study

A subset of women who were willing to return 24 hours later completed a second test, using identical procedures, 24 hours after the first test.

Blood Samples

Samples obtained by venipuncture were drawn into EDTA-coated vacutainers and centrifuged. Plasma was stored at −80°C. Concentrations of 3 vascular glycocalyx components, SDC1, HSPG, and hyaluronic acid, were measured in duplicate using commercially available ELISAs. The average of duplicate values was used for analysis.

Statistical Analysis

Detailed statistical methods are described in table and figure legends. Reliability of noninvasive glycocalyx measurements was assessed by calculating intraclass correlation coefficients to assess the trial-to-trial (trials 1–3) or day-to-day (days 1 and 2) reliability for individual vessel-size categories, as well as composite measures. Participant demographics in the 4 groups of participants were compared using 1-way ANOVA (least significance difference test for post hoc analyses) or the Kruskal–Wallis test (Mann–Whitney test for post hoc analyses). The effect of test conditions, including time of day, fasting for >4 hours, caffeine intake in the 6 hours before the test, patient-reported intake of any medications in the previous 24 hours, and treatment with clinical medications to induce labor (misoprostol, oxytocin) or to treat signs of preeclampsia (antihypertensive medications, magnesium sulfate, betamethasone) were examined by repeated-measures ANOVA with group as a between-subjects factor and vessel-size category as a within-subjects factor (5–16 μm). Noninvasive glycocalyx measurements among vessels with a median RBC column width of 5 to 16 μm were compared using repeated-measures ANOVA, with group as a between-subjects factor, vessel size as a within-subjects factor (5–16 μm), and caffeine intake as a covariate. Repeated-measures ANOVAs did not include interaction terms. Composite PBR values were evaluated by ANOVA after adjusting for caffeine intake. Concentrations of SDC1, HSPG, and hyaluronic acid were compared using the Kruskal–Wallis test (Mann–Whitney for post hoc analyses). Correlations between gestational age and other variables were identified using Pearson or Spearman correlation coefficients, according to the data distribution. Interactive line graphs for noninvasive glycocalyx measurements were created using a free online tool.[28] Analyses were performed using SPSS for Windows (v25.0; IBM SPSS). This was an exploratory study because published data using this technique in pregnant women were not available. There was no a priori power calculation.

Results

Reliability Study

Fifteen pregnant women were included in the reliability study at a median gestational age of 33 weeks (range: 27–39 weeks). Characteristics of the reliability study population are presented in Table S1. Two more women completed the reliability measurements but were excluded because of unstable preeclampsia requiring changes in medications during the study. To assess the reliability of glycocalyx measurements for the population of pregnant women, we examined trial-to-trial and day-to-day reliability for each of the 22 vessel-size categories (based on the median RBC column width [3–25 μm]) and for composite PBR measurements for vessels in different size categories calculated by software (5–25, 5–9, 10–19, and 20–25 μm). Trial-to-trial reliability for PBR was poor for all 22 size categories on both days 1 and 2 (Table S2). When 3 trials were averaged, however, day-to-day reliability was moderate to excellent for vessels between 9 and 18 μm but poor for smaller and larger vessels. Larger vessels were uncommon in pregnant women, with many size categories having <5 vessel segments. Each size category >16 μm accounted for <0.5% of all vessel segments among participants in the reliability study (Figure S1), and similar results were observed in the larger cohort described below (n=137; Figure 2). Vessels with a median RBC column width of 9 μm showed poor reliability for measurements of the percentage of vessel segments that

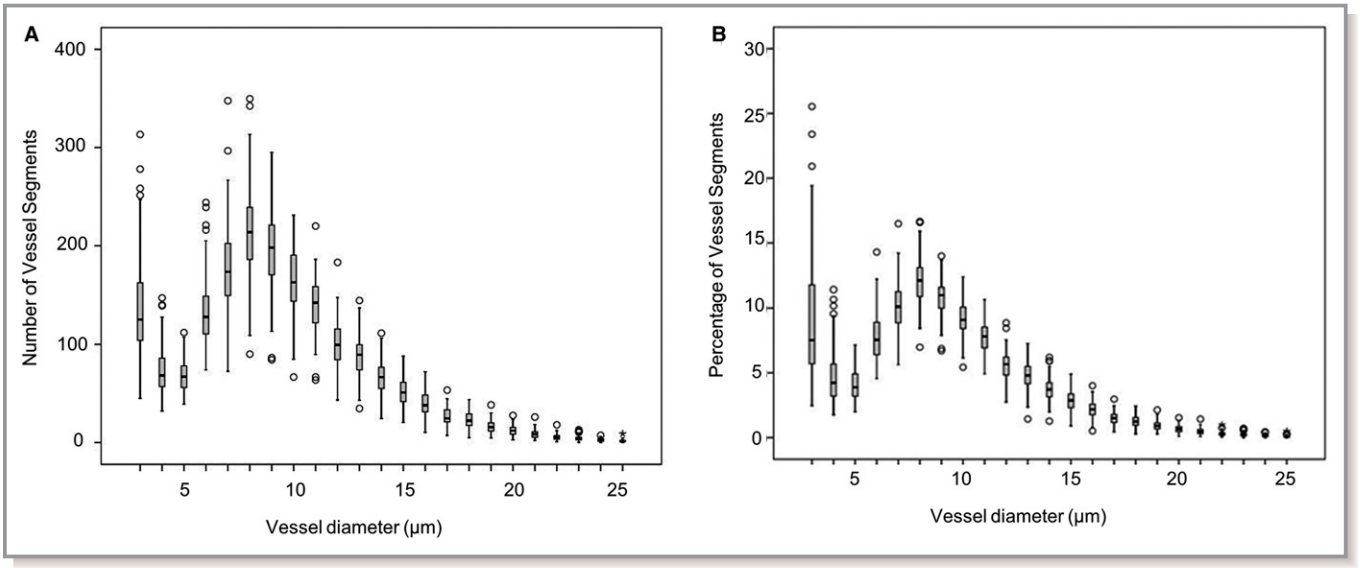

Figure 2. Vessel segments in each size category among pregnant women. Pooled data from 73 normotensive pregnant women, 21 women with gestational diabetes mellitus, 29 women with late-onset preeclampsia, and 14 women with early onset preeclampsia. Values for the number (**A**) and percentage (**B**) of segments in each size category are the average of 3 trials.

were filled with RBCs (data not shown). Based on these data, we developed a new composite PBR measure for pregnant women that included vessels with a median RBC column width of 10 to 16 μm; that measure showed good day-to-day reliability among pregnant women in the third trimester (Table S3). Day-to-day reliability for software composite measures, based on the average of 3 trials, was moderate to good for each composite measure (Table S3) and was also presented to facilitate comparisons with previously published studies in other populations.

Participants

In total, 139 pregnant women completed the study. The analysis included 73 women with normotensive pregnancies, 21 women with GDM, and 43 women with preeclampsia (early, n=14; late, n=29). Two women with preeclampsia were excluded from the analysis because of extreme trial-to-trial variability in noninvasive glycocalyx measurements. Three women in each preeclampsia group also had GDM.

Age, gravidity, parity, and fetal sex did not differ between women with normotensive pregnancies and those with GDM, early onset preeclampsia, or late-onset preeclampsia (Table 1). The median gestational age at the time of the glycocalyx test was lower in women with early onset preeclampsia compared with the other groups. Women with early and late-onset preeclampsia had higher blood pressures than women in the other 2 groups. Gestational age at delivery, delivery type, birth weight, and Apgar scores differed among groups because of earlier deliveries and worse outcomes among women with early onset preeclampsia.

Test Conditions

Controlled test conditions are not possible in women with preeclampsia because of clinical instability. These patients often deliver soon after diagnosis to prevent worsening symptoms and adverse pregnancy outcomes. The potential impact of fasting, caffeine use, patient medication use, administration of clinical medications that are used to induce labor (misoprostol, oxytocin) or to manage symptoms of preeclampsia (eg, antihypertensive medications, magnesium sulfate), and time of day were examined (Table S4). None of these factors was significantly associated with changes in PBR. Caffeine intake was the only factor that significantly affected the percentage of vessel segments that were filled with RBCs. Among normotensive pregnant women, those who had consumed caffeine in the 6 hours before the glycocalyx measurement had a higher percentage of vessel segments that were filled with RBCs (P=0.011; Figure 3). PBR did not differ between women who had consumed caffeine and those who had not (P=0.118). There was no relationship between noninvasive glycocalyx measurements and gestational age in the third trimester (P>0.05 for all).

Noninvasive Glycocalyx Measurements

We performed a repeated-measures ANOVA to determine whether any relationship between pregnancy outcome and glycocalyx degradation was consistent across different size categories. Women with early onset preeclampsia had increased glycocalyx degradation, as indicated by a higher PBR, than normotensive pregnant women (P=0.002; Table S5, Figure 4) or women with GDM (P=0.004) or late onset preeclampsia (P=0.024). Microvascular perfusion, as

Table 1. Participant Characteristics

Variable	Normotensive (n=73)	GDM (n=21)	Late-Onset PE (n=29)	Early Onset PE (n=14)	*P* Value
Age, y, mean±SD	29.8±4.5	30.3±4.2	28.9±4.8	29.6±5.7	0.740
Race (% white)	66 (93.0)	20 (95.2)	26 (92.9)	14 (100.0)	0.763
Gravidity, median (IQR)	2 (1–2)	2 (1–4)	2 (1–3)	1 (0–1)	0.589
Parity, n (%)					
0	43 (58.9)	11 (52.4)	19 (65.5)	7 (50.0)	0.722
≥1	30 (41.1)	10 (47.6)	10 (34.5)	7 (50.0)	
At GlycoCheck test					
Gestational age, wk, median (IQR)	39 (35–41)*	39 (37–39)	38 (37–39)	31 (30–33)	<0.001
Systolic BP, mm Hg, mean±SD	111±12	118±17	141±13	141±13	<0.001
Diastolic BP, mm Hg, mean±SD	67±9	73±14	83±11	88±13	<0.001
Proteinuria, median (IQR)†	NA	NA	710 (460–1530)	660 (544–1543)	
At delivery					
Gestational age, wk, median (IQR)	41 (40–41)	39 (38–40)	38 (37–39)	33 (31–34)	<0.001
Preeclampsia with severe features, n (%)	NA	NA	18 (62)	11 (100)	<0.001
Superimposed preeclampsia, n (%)	NA	NA	5 (17.2)	3 (21.4)	0.741
Delivery type					
Vaginal, n (%)	61 (83.6)	18 (85.7)	18 (62.1)	5 (35.7)	<0.001
Cesarean section, n (%)	12 (16.4)	3 (14.3)	11 (37.9)	5 (64.3)	
Systolic BP, mm Hg, mean±SD	121±10	125±12	145±17	147±15	<0.001
Diastolic BP, mm Hg, mean±SD	76±9	77±12	93±8	84±12	<0.001
Fetal sex, n (% male)	42 (57.5)	10 (47.6)	13 (44.8)	5 (35.7)	0.377
Birth weight, g, mean±SD	3643±474	3281±519	3083±618	1699±809	<0.001
Apgar, 1 min, median (IQR)	8 (8–9)	9 (8–9)	7 (7–8)	6 (4–7)	<0.001
Apgar, 5 min, median (IQR)	9 (9–9)	9 (9–9)	9 (8–9)	8 (8–9)	<0.001

Normally distributed numerical data (mean±SD) were analyzed with 1-way ANOVA and the least significant differences post hoc test. Numerical data that are not normally distributed (median [IQR]) were analyzed with a Kruskal–Wallis test and a Mann–Whitney post hoc test. Categorical data (n [%]) were analyzed with a χ^2 test. BP indicates blood pressure; GDM, gestational diabetes mellitus; IQR, interquartile range; NA, not assessed; PE, preeclampsia.

*Seventeen women completed glycocalyx testing between 27 and 34 weeks of gestation.

†Twenty-four–hour proteinuria was measured (early, n=8; late, n=9), or estimated from the protein creatinine ratio (early, n=5; late, n=20) or the protein osmolality ratio (early, n=1).

indicated by the percentage of vessel segments filled with RBCs, was lower in women with early onset preeclampsia compared with normotensive pregnant women (*P*=0.045) or women with GDM (*P*=0.018) or late-onset preeclampsia (*P*=0.024). Both analyses were adjusted for caffeine intake and included vessel-size categories of 5 to 16 μm. Results were not different when analyses were restricted to vessel-size categories with good reliability and high counts (10–16 μm; data not shown).

The new composite measure of vessels with a median RBC column width of 10 to 16 μm was also examined (PBR 10–16 μm; Table 2). Women with early onset preeclampsia had lower PBR values, indicating higher glycocalyx degradation, compared with women with late-onset preeclampsia (*P*=0.023), GDM (*P*=0.004), or normotensive pregnancies (*P*=0.002) after adjusting for caffeine intake. The percentage of vessels segments that were filled with RBCs (RBC 10–16 μm) was also lower in women with early onset preeclampsia compared with the remaining groups after adjusting for caffeine intake (late-onset preeclampsia: *P*=0.017; GDM: *P*=0.010; normotensive pregnancy: *P*=0.021). Gestational age did not correlate with PBR 10 to 16 μm (*r*=0.211, *P*=0.859) or RBC 10 to 16 μm (*r*=0.335, *P*=0.727), suggesting that differences in women with early onset preeclampsia were not due to gestational age. The 4 composite measures calculated by the software also showed differences between normotensive women and women with early onset preeclampsia after adjustment for caffeine intake (Table 2). Differences between women with early onset preeclampsia and those with late-onset preeclampsia or GDM were observed for some composite measures calculated by the GlycoCheck software.

Composite measurements of the PBR and the percentage of vessel segments that were filled with RBCs were highly

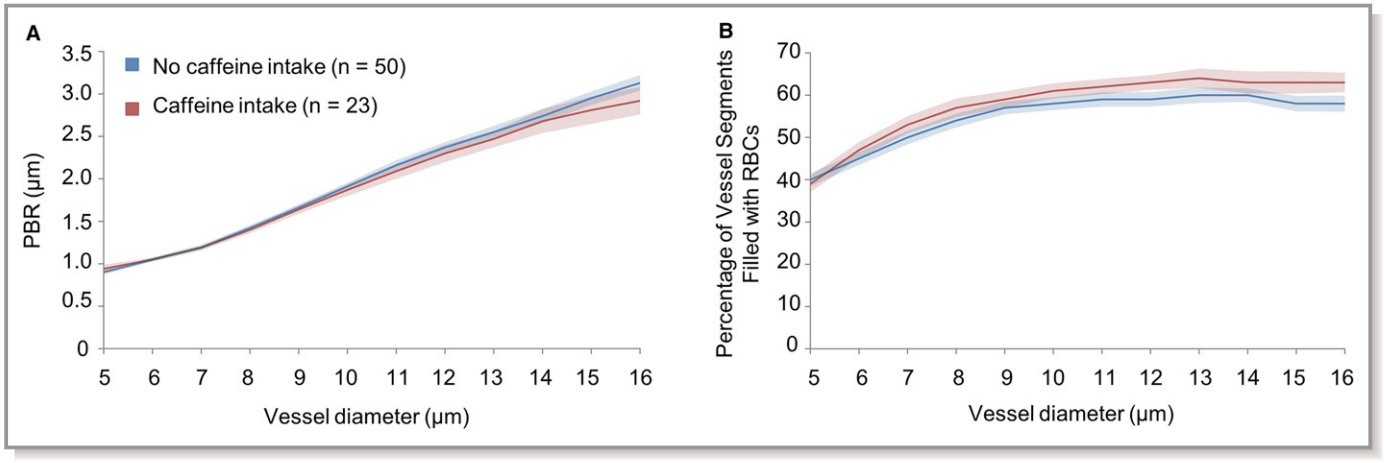

Figure 3. Effect of caffeine intake on noninvasive glycocalyx measurements in normotensive pregnant women. PBR (**A**) and the percentage of vessel segments that were filled with RBCs (**B**) were compared from 23 women who had caffeine in the 6 hours before the test and 50 women who did not have caffeine. Data were analyzed by repeated-measures ANOVA with caffeine intake (yes vs no) as a between-subjects factor and vessel size as a within-subjects factor. Vessel size included 12 categories (5–16 µm), based on the median width of the RBC column. The main effect of caffeine intake was not statistically significant for PBR (mean: caffeine: 1.946 [95% CI, 1.888–2.005]; no caffeine: 2.003 [95% CI, 1.963–2.043]; F=2.501, *df*=1, *P*=0.118). There was a significant main effect of caffeine on the percentage of segments filled with RBCs (mean: caffeine: 0.579 [95% CI, 0.560–0.599]; no caffeine: 0.548 [95% CI, 0.535–0.562]; F=6.806, *df*=1, *P*=0.011). Post hoc analyses were performed with a least significant differences test. PBR indicates perfused boundary region; RBC, red blood cell.

correlated (PBR 10–16 µm and RBC 10–16 µm: r=0.803, P<0.001; PBR 5–25 µm and RBC 5–25 µm: r=0.745, P<0.001).

Glycocalyx Components in Plasma

HSPG concentrations were higher in women with early onset preeclampsia compared with normotensive women (P=0.002; Table 3) and women with GDM (P=0.003). Women with early onset preeclampsia had also higher concentrations of hyaluronic acid than normotensive pregnant women (P=0.046). Gestational age was not correlated with HSPG (ρ=0.147, P=0.345) or hyaluronic acid (ρ=0.267, P=0.083), whereas SDC1 was correlated with gestational age (ρ=0.454, P=0.002; Figure 5). No differences in SDC1 were observed

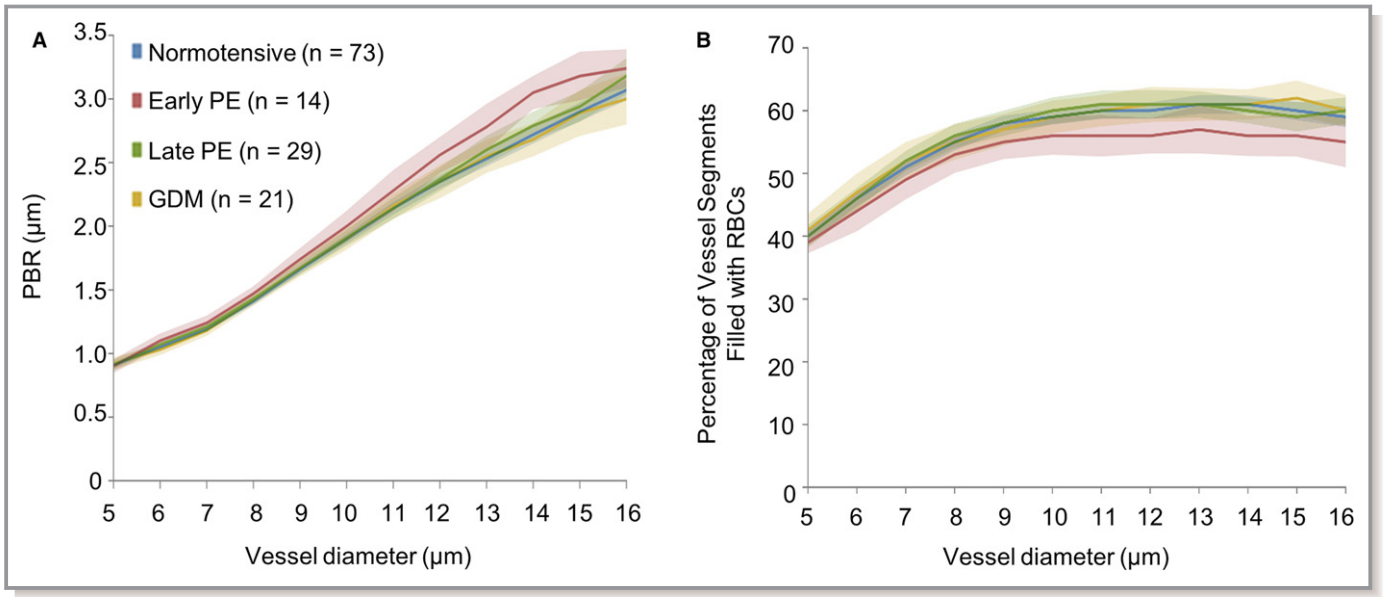

Figure 4. Relationship between pregnancy outcome and noninvasive glycocalyx measurements. PBR (**A**) and the percentage of vessel segments that were filled with RBCs (**B**) for vessel sizes 5 to 16 µm, among women with different pregnancy outcomes after adjusting for caffeine intake. See statistical results in Table S5. GDM indicates gestational diabetes mellitus; PBR indicates perfused boundary region; PE, preeclampsia; RBC, red blood cell.

Table 2. Composite Variables for Glycocalyx Assessment

Variable	Normotensive (n=73)	GDM (n=21)	Late-Onset PE (n=29)	Early Onset PE (n=14)
Suggested composite variables for pregnant women				
PBR 10–16	2.520 (2.469–2.572)*	2.495 (2.399–2.591)*	2.555 (2.473–2.636)*	2.721 (2.604–2.838)
RBC 10–16	0.598 (0.587–0.610)*	0.609 (0.587–0.631)*	0.604 (0.585–0.622)*	0.564 (0.537–0.591)
Composite variables calculated by the GlycoCheck software				
PBR 5–25	2.441 (2.392–2.490)*	2.448 (2.356–2.539)*	2.461 (2.384–2.539)*	2.612 (2.500–2.723)
RBC 5–25	0.548 (0.537–0.558)*	0.549 (0.529–0.568)	0.549 (0.533–0.566)*	0.518 (0.495–0.542)
PBR 5–9	1.243 (1.225–1.261)	1.263 (1.230–1.296)	1.261 (1.233–1.290)	1.283 (1.242–1.323)
PBR 10–19	2.706 (2.651–2.762)*	2.690 (2.587–2.793)*	2.746 (2.658–2.834)	2.872 (2.746–2.998)
PBR 20–25	3.092 (2.992–3.192)*	3.101 (2.915–3.287)*	3.086 (2.928–3.244)*	3.426 (3.199–3.653)

Data are presented as mean (95% CI). All variables were adjusted for caffeine intake. RBC composites denote composite values for the percentage of vessel segments that are filled with RBCs. Each composite variable was calculated as the median value of the averages of each vessel-size category within the specified range (ie, 5–9 includes vessels with a median RBC column width of 5, 6, 7, 8, and 9 μm). Data were analyzed by univariate ANOVA (fixed factor: group; covariate: caffeine intake; post hoc test: least significant differences). GDM indicates gestational diabetes mellitus; PBR, perfused boundary region; PE, preeclampsia; RBC, red blood cell.
*$P<0.05$ in comparison to early PE.

among the groups before or after the adjustment for gestational age.

Discussion

The results of our study indicate greater glycocalyx degradation and lower microvascular perfusion among women with early onset preeclampsia compared with normotensive pregnant women. Glycocalyx degradation, as measured by higher PBR values, was consistently observed for both composite vessel measures and in analyses that included individual vessel-size categories. Compared with normotensive pregnant women, women with early onset preeclampsia also had higher plasma concentrations of 2 glycocalyx components: HSPG and hyaluronic acid. This suggests that markers of glycocalyx degradation are present in systemic circulation at the time of disease in women with early onset preeclampsia. These findings appear to be specific to early onset preeclampsia, as glycocalyx degradation was not present in women with either late-onset preeclampsia or GDM.

Our study provides new evidence of dimensional and compositional glycocalyx changes in preeclampsia. It has been increasingly recognized that preeclampsia is a heterogeneous disease, with the different clinical subtypes (eg, early versus late) reflecting distinct underlying pathological mechanisms.[29] Women with early preeclampsia commonly have severe features, including thrombocytopenia, impaired liver function tests, new development of renal insufficiency, pulmonary edema, and new onset of cerebral or visual disturbances.[3] Our data indicate that glycocalyx degradation is present in early but not late preeclampsia, suggesting that the resultant endothelial activation may contribute to differences in clinical presentation between these disease subtypes.

The mechanisms responsible for endothelial activation in preeclampsia are multifactorial and include upregulation of several potent mediators of endothelial cell dysfunction,[30] and, most important, reduced nitric oxide bioavailability.[4] Of note, nitric oxide prevents leukocyte recruitment and endothelial cell activation through inhibition of the transcription factor NF-κB (nuclear factor κB), which mediates the

Table 3. Effect of Pregnancy Outcome on Plasma Glycocalyx Components

Variable	Normotensive (n=43)	GDM (n=10)	Late-Onset PE (n=11)	Early Onset PE (n=11)
Gestational age, wk	39 (33–40)*	37 (33–39)*	38 (36–39)*	33 (30–34)
HSPG, pg/mL	1984 (1679–2618)*	1566 (1398–1839)*	3228 (2294–3697)	2952 (2177–3572)
HA, ng/mL	654 (585–719)*	611 (550–667)	754 (668–836)	757 (642–991)
SDC1, ng/mL	161 (70–1109)	124 (86–450)	125 (47–1872)	189 (81–365)

Data were skewed and are presented as median (interquartile range). Groups were compared with a Kruskal–Wallis test (Mann–Whitney post hoc test). One outlier was excluded. GDM indicates gestational diabetes mellitus; HA, hyaluronic acid; HSPG, heparan sulfate proteoglycans; PE, preeclampsia; SDC1, syndecan 1.
*$P<0.05$ compared with early onset PE.

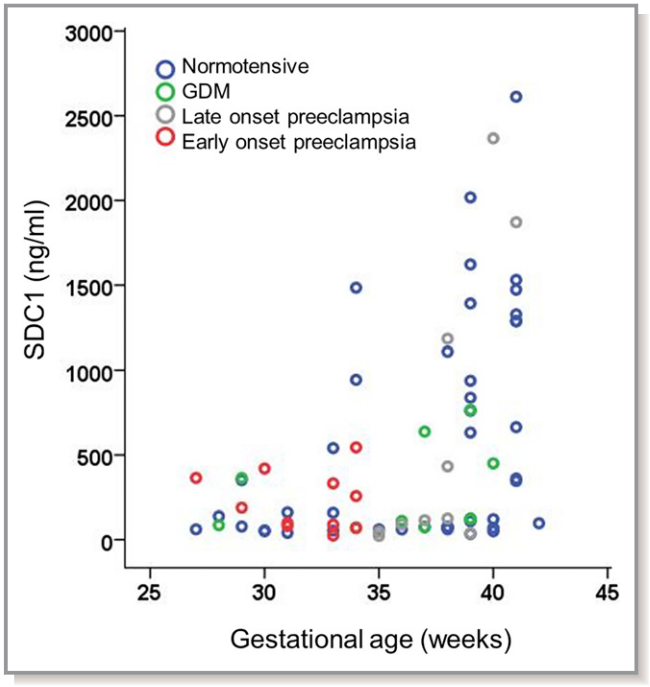

Figure 5. Plasma SDC1 (syndecan 1) concentrations in women with normotensive pregnancies, early and late-onset preeclampsia, and GDM. SDC1 was correlated with gestational age (ρ=0.454, *P*=0.002, Spearman correlation coefficient; 1 outlier was excluded from this analysis). GDM indicates gestational diabetes mellitus.

induction of the cellular adhesion molecules.[30,31] Therefore, endothelial activation in preeclampsia may represent a missing link between endothelial dysfunction and vascular disease,[4] the latter demonstrated by reduction in capillary density (ie, rarefaction) in skin[32,33] and by the presence of acute atherosis of the placental blood vessels in preeclampsia, which is similar to early stage atherosclerosis.[34]

Recent advances allow for the measurements of glycocalyx degradation noninvasively, using a hand-held camera that captures videos of RBCs passing through small vessels under the tongue.[11] Commercially available software estimates the depth to which RBCs can penetrate the glycocalyx.[11] Although healthy glycocalyx is relatively impermeable to RBCs and other circulating factors, degradation creates gaps that allow RBCs to penetrate further into the glycocalyx.[11]

In the current study, dimensional glycocalyx changes were assessed noninvasively in the sublingual vasculature, whereas glycocalyx degradation was examined by measuring plasma concentrations of the glycocalyx components HSPG, hyaluronic acid, and SDC1. Proteoglycans form the backbone of the endothelial glycocalyx and are frequently classified by the types of glycosaminoglycan chains that are bound to them.[10] Heparan sulfate is the most common type of glycosaminoglycan chain that binds proteoglycans in the endothelial glycocalyx.[10] Hyaluronic acid is also common.[35] Noncovalent interactions between hyaluronic acid and small proteoglycans are important for maintaining the structural stability and organization of the endothelial glycocalyx.[35] SDC1 is a type I transmembrane HSPG. Whereas noninvasive sublingual measurements reflect maternal vascular glycocalyx degradation, circulating concentrations of glycocalyx components may reflect a combination of maternal vascular and placental glycocalyx sources. The placenta is hypothesized to be a major source of SDC1 in pregnancy[36]; however, placental-staining studies suggest that the placenta does not secrete hyaluronic acid directly into the intervillous space.[37] In contrast, hyaluronic acid released from infarcted areas or fibrin deposits in the placenta may contribute to elevated circulating concentrations in preeclampsia.[37,38] Plasma HSPG and hyaluronic acid concentrations were elevated in women with early onset preeclampsia in the present study compared with normotensive pregnant women; however, there were no differences in SDC1 concentrations after adjusting for gestational age. Although serum SDC1 concentrations were elevated in women with HELLP syndrome,[15] lower concentrations of plasma SDC1 have been reported before the onset of preeclampsia (20 weeks) and at the time of preeclampsia compared with normotensive pregnant women.[36] These divergent results may be explained by the small sample sizes of existing studies. Alternatively, differences between women with and without preeclampsia may depend on preeclampsia subtype, and the proportion of women with different subtypes may vary among studies. Larger studies examining circulating glycocalyx components in women with different types of preeclampsia are needed.

Previous studies suggest that glycocalyx degradation may occur in other tissues among women with preeclampsia. A study of black South African women reported higher urinary concentrations of HSPG and chondroitin sulfate proteoglycans among preeclamptic women, compared with normotensive pregnant women.[39] Urinary HSPG excretion was correlated with 24-hour urinary protein excretion.[39] The structure and composition of the syncytiotrophoblast glycocalyx is abnormal in severe preeclampsia.[40] Taken together, these studies and our data suggest that glycocalyx dysregulation is present in multiple vessel beds; this suggestion is consistent with a systemic nature of preeclampsia.

Implications for Noninvasive Glycocalyx Measurements

The reliability study suggests that investigators should pool multiple trials when performing noninvasive glycocalyx measurements, to reduce measurement error and to improve reliability. Although the automated analysis program offers several composite measures (ie, PBR 5–25, 5–9, 10–19, and 20–25 μm), the size categories included in these measures may need to be adjusted for different populations. Compared

with previous studies in men,[41] the vessel-size distribution in pregnant women appears to shift downward. Although counts in young men peaked at 10 μm,[41] counts in pregnant women peaked at 8 μm (Figure 2). Studies should control for caffeine intake, which significantly influences the percentage of vessels filled with RBCs. Previous reports examining the effects of oral caffeine on microvascular perfusion in different tissue beds have yielded conflicting results. No significant differences in microvascular perfusion in the hand were observed following a small dose or oral caffeine[42]; however, small differences that the study was not powered to detect cannot be ruled out.[43] Oral caffeine may reduce microvascular blood flow in the ocular fundus.[44] Although gestational age did not affect noninvasive glycocalyx measurements among women in the third trimester in the present study, studies characterizing longitudinal changes in glycocalyx degradation throughout pregnancy are needed.

Future studies should examine whether glycocalyx degradation in women with early onset preeclampsia is present before pregnancy or precedes the onset of disease. If so, the vascular glycocalyx may be a new preeclampsia-prevention target. Follow-up studies should also determine whether vascular glycocalyx degradation in early onset preeclampsia is correlated with glycocalyx degradation in other tissues, such as placental trophoblast cells and the renal glomerulus. Finally, future studies should examine whether glycocalyx degradation in women with early onset preeclampsia persists for months or years after delivery. A recent meta-analysis concluded that vascular dysfunction, as measured by flow-mediated dilation, persists for at least 3 years following a preeclamptic pregnancy.[5] If these changes are accompanied by glycocalyx degradation and reduced microvascular perfusion, treatments that target the vascular glycocalyx may aid in preventing cardiovascular disease in women who have had preeclampsia.

Conclusions

Early onset preeclampsia is associated with signs of structural and functional glycocalyx damage. We propose that noninvasive glycocalyx measurements, using sidestream dark field imaging, offer a unique opportunity to assess endothelial activation in real time. The potential role of this methodology in identifying women with increased cardiovascular risks based on their histories of pregnancy complications needs to be determined.

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Disclosures

None.

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Supplemental Material

Table S1. Participant characteristics and pregnancy outcomes for reliability study.

Variable	Repeatability study participants (n=15)
Age (years)	31.9 ± 3.5
Race(% white)	15 (100.0%)
Gravida	2 (1-3)
Parity	
0	7 (46.7%)
≥1	8 (53.3%)
Glycocheck test	
Gestational age (weeks)	33 (29-34)
Delivery	
Gestational age (weeks)	39 (35-40)
Pregnancy outcome	
Normotensive*	10 (66.7%)
Subsequent preeclampsia†	1 (6.7%)
Preeclampsia	1 (6.7%)
Superimposed preeclampsia	1 (6.7%)
Subsequent GH†	2 (13.3%)
Delivery type	
Vaginal	11 (73.3%)
C-section	4 (26.7%)
Systolic blood pressure (mmHg)	129±13
Diastolic blood pressure (mmHg)	81±10
Fetal sex (% male)	10 (66.7%)
Birthweight (g)	3354 ± 1001
Apgar (1 minute)	8 (8-9)
Apgar (5 minutes)	9 (9-9)

Values are presented as mean ± SD, median (interquartile range) or n (%). 10 women had normotensive pregnancies, one

* Two women had a history of preeclampsia, but were normotensive during the pregnancy examined.

† Patient developed the outcome after completing the reliability study

g, grams; GH, gestational hypertension.

Table S2. Trial-to-trial and day-to-day reliability for PBR among vessels in each size category.

Vessel size (μm)*	ICC for PBR		
	Day 1 (Trials 1 to 3)	Day 2 (Trials 1 to 3)	Day1 Average - Day2 Average
3	poor	poor	poor
4	poor	poor	poor
5	poor	poor	poor
6	poor	poor	poor
7	poor	poor	poor
8	poor	poor	poor
9	poor	poor	moderate
10	poor	poor	moderate
11	poor	poor	moderate
12	poor	poor	excellent
13	poor	poor	good
14	poor	poor	moderate
15	poor	poor	moderate
16	poor	poor	moderate
17	poor	poor	moderate
18	poor	poor	moderate
19	poor	poor	poor
20	poor	poor	poor
21	poor	poor	poor
22	poor	poor	poor
23	poor	poor	poor
24	poor	poor	poor
25	poor	poor	poor

ICC, intraclass correlation coefficient; PBR, perfused boundary region. *Vessel size categories, in μm, are defined by the median width of the red blood cell column. Categories for ICCs: < 0.5 poor; 0.5 to 0.75 moderate, 0.75 to 0.9 good; >0.9 excellent. Day 1 and day 2 measurements were performed approximately 24 hours apart in 15 women in the 3rdtrimester of pregnancy.

Table S3. Day-to-day reliability for PBR composite measures.

Vessel size categories (μm)*	ICC (Day 1 Average – Day 2 Average)	
	ICC	Category
5-25	0.788	Good
5-9	0.527	Moderate
10-19	0.868	Good
20-25	0.622	Moderate
10-16	0.836	Good

ICC, intraclass correlation coefficient; PBR, perfused boundary region. *Vessel size categories are defined by the median width of the RBC column (μm). Within each category, values for each day were computed as the average of three trials. Each composite measure was then calculated as the average of the PBR values for all vessels in the included size categories. n=15 women in the 3rd trimester.

Table S4. Test Conditions.

Variable	Normotensive (n = 73)	GDM (n = 21)	Late Onset PE (n = 29)	Early Onset PE (n = 14)
Caffeine intake	23 (31.5%)	4 (19.0%)	6 (20.7%)	3 (21.4%)
Fasted for at least 4 hours	18 (24.7%)	9 (42.9%)	8 (27.6%)	1 (7.1%)
Misoprostol	13 (17.8%)	5 (23.8%)	16 (55.2%)	0 (0%)
Oxytocin	10 (13.7%)	4 (19.0%)	5 (17.2%)	0 (0%)
Active labor	4 (5.6%)	1 (5.0%)	4 (13.8%)	0 (0%)
Epidural	3 (4.1%)	1 (4.8%)	4 (13.8%)	0 (0%)
Anti-hypertensive medications	0 (0%)	1 (4.8%)	7 (24.1%)	6 (42.9%)
Magnesium sulfate	0 (0%)	0 (0%)	9 (31%)	5 (35.7%)
Betamethasone	0 (0%)	0 (0%)	4 (13.8%)	4 (28.6%)
Acetaminophen	2 (2.7%)	2 (9.5%)	11 (37.9%)	8 (57.1%)
Thyroid medications	4 (5.5%)	1 (4.8%)	1 (3.4%)	1 (7.1%)
Glyburide	0 (0%)	8 (38.1%)	1 (3.4%)	1 (7.1%)

Data are presented as n (%). Caffeine intake was recorded as positive if women had consumed food, drinks or medications containing caffeine in the six hours prior to non-invasive glycocalyx measurements. Medications were recorded as positive if women had taken the medication within 24 hours of the non-invasive glycocalyx measurements. No statistical comparisons were performed.

Table S5. Effect of GDM and PE on non-invasive glycocalyx measurements.

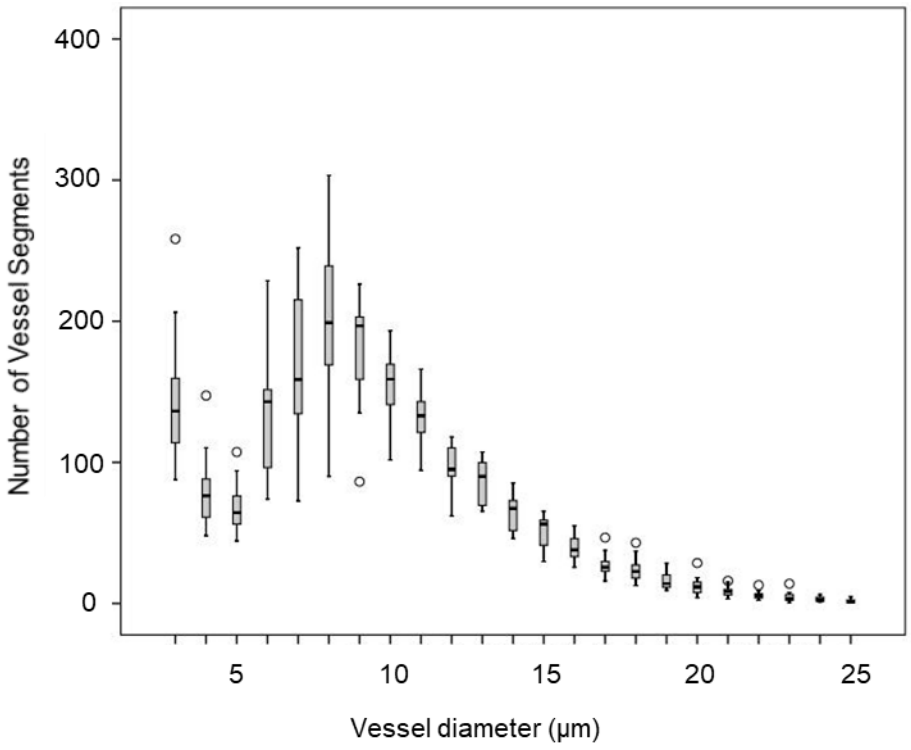
Group	PBR Mean† (95% CI)	Percentage of vessel segments filled with RBCs Mean† (95% CI)
Normotensive (n = 73)	1.989* (1.954-2.024)	0.556* (0.546-0.566)
GDM (n = 21)	1.974* (1.909-2.038)	0.567* (0.547-0.586)
Late onset PE (n = 29)	2.014* (1.959-2.069)	0.563* (0.546-0.580)
Early onset PE (n = 14)	2.124 (2.045-2.203)	0.529 (0.506-0.553)

Data were analyzed by repeated measures ANOVA (Between-subjects factor: group, Within-subjects factor: vessel size, Covariate: caffeine intake, Post-hoc test: least significant differences). Vessel size included 12 categories (5-16 μm), based on the median width of the RBC column. PBR: F = 3.600, df = 3. Percentage of vessel segments filled with RBCs: F = 2.255, df = 3.

CI, confidence interval; df, degrees of freedom; PBR, perfused boundary region; PE, preeclampsia; RBC, red blood cells.

*p<0.05 compared to women with early onset PE; †Adjusted for caffeine intake.

Figure S1. Number of vessel segments for each size category among women in the reliability study.



Average number of vessel segments for six trials (three trials per day), n = 15 women in the third trimester.