Glycogen phosphorylase is a key enzyme in glycogenolysis. Released with myocardial ischemia, blood concentration of glycogen phosphorylase isoenzyme BB (GPBB) is a marker of acute coronary syndromes. Pregnancy imposes metabolic stress, and preeclampsia is associated with cardiac complications. However, plasma GPBB concentration during pregnancy is unknown. This study was conducted to determine maternal plasma GPBB concentration in normal pregnancy and in preeclampsia. Plasma samples from 6 groups (n=396) were studied: nonpregnant and pregnant women with normal term delivery, term and preterm preeclampsia, and term and preterm small-for-gestational-age neonates. GPBB concentration was measured with a specific immunoassay. Placental tissues (n=45) obtained from pregnant women with preterm and term preeclampsia, spontaneous preterm delivery, and normal term delivery were analyzed for potential GPBB expression by immunoblotting. Median plasma GPBB concentration was higher in pregnant women than in nonpregnant women (38.7 versus 9.2 ng/mL; \( P<0.001 \)), which remained significant after adjusting for age, race, and parity. Maternal plasma GPBB concentrations did not change throughout gestation. Cases of preterm (but not term) preeclampsia had higher median plasma GPBB concentrations than gestational age-matched normal pregnancy cases (72.6 versus 26.0 ng/mL; \( P=0.001 \)). Small-for-gestational-age neonates did not affect plasma GPBB concentration. GPBB was detected in the placenta and was less abundant in preterm preeclampsia than in preterm delivery cases (\( P<0.01 \)). There is physiological elevation of plasma GPBB concentration during pregnancy; an increase in maternal plasma GPBB is a novel phenotype of preterm preeclampsia. It is strongly suggested that these changes are attributed to GPBB of placental origin. (Hypertension. 2012;59:00-00.) ● Online Data Supplement

Key Words: small-for-gestational-age neonate ■ labor ■ hypoxia ■ placenta
striction. Furthermore, maternal cardiac complications, such as myocardial infarction and heart failure, are more frequent in cases with preeclampsia. However, the plasma concentrations of GPBB in normal pregnancy and in pregnancy disorders have not yet been studied. We hypothesized that there is a change in plasma GPBB concentration during pregnancy, especially in association with preeclampsia.

The objective of this study was to determine changes in maternal plasma GPBB concentrations for pregnant women with normal term delivery and those with preterm or term preeclampsia.

Methods

Study Design
Plasma samples were collected from the following 6 groups classified according to the clinical circumstances at the time of blood sampling: nonpregnant women (n=33), pregnant women with a normal singleton pregnancy (n=151), patients with term preeclampsia (n=72), patients with term small-for-gestational-age (SGA) neonates and no preeclampsia (n=38), patients with preterm preeclampsia (n=74), and patients with preterm SGA neonates and no preeclampsia (n=28). Three serial blood samples obtained during each trimester throughout gestation (6–14, 15–24, and 37–42 weeks) were available in 32 of 151 women with normal pregnancy. Preeclampsia was diagnosed according to the diagnostic criteria of the American College of Obstetricians and Gynecologists:16 in the presence of hypertension (elevated systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg at least twice, 4 hours to 1 week apart, after the 20th week of gestation) and of proteinuria (≥300 mg in a 24-hour urine collection or 2 random urine specimens containing ≥1+ protein by dipstick or 1 dipstick measurement ≥2+ protein). SGA pregnancies included the following: those of fetal growth restriction fetuses detected by ultrasonography and confirmed SGA neonates after delivery in cases of ≥5 days of blood sampling-to-delivery intervals and SGA neonates at the time of delivery in cases of ≤5 days of intervals. The SGA neonate was defined when its birth weight was below the 10th percentile for gestational age according to the reference range proposed by Alexander et al, and fetal growth restriction was diagnosed when the estimated fetal weight proposed by Hadlock et al was below the 10th percentile for gestational age. A normal pregnancy outcome was defined when patients met the following criteria: (1) no previous abnormal medical and surgical conditions; (2) no obstetric, maternal, or fetal complications during pregnancy; and (3) delivery of a healthy neonate at term whose birth weight was appropriate for gestational age (≥10th percentile and ≤90th percentile). Placental tissues (n=45) were obtained from pregnant women with preterm preeclampsia (n=9), preterm labor and preterm delivery (n=9), term preeclampsia (n=9), and normal term delivery (n=18).

All women were enrolled at Hutzel Women’s Hospital (Detroit, MI) and provided written informed consent for the collection and use of clinical data and biological materials. Plasma and tissue samples from all patients were retrieved from the Bank of Biological Materials of the Perinatology Research Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, US Department of Health and Human Services. This study was conducted under the ethical standards for human experimentation established in the Declaration of Helsinki. The Institutional Review Boards of Wayne State University and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, US Department of Health and Human Services approved the collection and use of clinical data and biological materials for research purposes.

ELISA for GPBB
Blood samples, collected in EDTA tubes, were centrifuged at 1300 g for 10 minutes at 4°C. The plasma samples were kept at −70°C until assay.

Maternal plasma GPBB concentration was measured with GPBB ELISA kits (Diagenics SE, Essen, Germany), according to the manufacturer’s instructions. The sensitivity of the assay was 1.463 ng/mL, and the coefficients of intra-assay variation and interassay variation were 5.2% and 10.7%, respectively.

Immunoblotting
Villous placental tissue samples were pooled from 5 different sampling sites. An equal amount of placental villous tissues was taken from 5 sampling sites generated by a systematic random sampling method. After trimming the chorionic plate and the basal plate, the pooled samples were flash frozen using liquid nitrogen and kept at −80°C until use. Proteins were extracted from liquid nitrogen–pulverized chorionic villous tissue using a radioimmuno-precipitation assay buffer (Sigma-Aldrich, St Louis, MO) with a proteinase inhibitor mixture (Roche, Basel, Switzerland). Protein lysates were subjected to 4% to 15% SDS-PAGE gel (Bio-Rad, Hercules, CA), electrophoresed under reducing conditions, and followed by electrotransfer onto polyvinylidene difluoride membranes (GE Healthcare Bio-Sciences, Piscataway, NJ). Non-specific binding was blocked for 1 hour at room temperature with 5% nonfat dry milk in tris-buffered saline containing 0.1% Tween 20. After washing, membranes were incubated overnight at 4°C with primary antibodies specific to GPBB (mouse antihuman; SC-81751) and hypoxanthine phosphoribosyltransferase (HPRT; rabbit antihuman; SC-20975; 1:200; Santa Cruz Biotechnology, Inc, Santa Cruz, CA). Horseradish peroxidase–conjugated antino-mouse IgG (7074; 1:1500; Cell Signaling Technology, Inc, Danvers, MA) were used as secondary antibodies. Signals were detected using chemiluminescence (ChemilGlow West kit; Alpha Innotech Corporation, San Leandro, CA), and the densitometric analysis was performed using FluorChem SP densitometry (Alpha Innotech Corporation).

Immunofluorescence Staining
For double-label immunofluorescence staining, 5-μm-thick frozen sections of the placenta were used. These were fixed with 4% paraformaldehyde, permeabilized with 0.25% Triton X-100, and incubated with 5% BSA in PBS for 30 minutes at room temperature. Tissue sections were incubated with anti-GPBB (ab61036; 1:25; rabbit polyclonal; Abcam, Cambridge, MA) and cytokeratin-7 (M7018; 1:1000; mouse monoclonal, DAKO, Carpinteria, CA) in 1% BSA in PBS for 1 hour. Thereafter, the sections were incubated with Alexa Fluor 488 donkey antirabbit IgG (A21206) and Alexa Fluor 594 donkey antimouse IgG (A21203) as secondary antibodies in 1% BSA for 30 minutes and mounted in ProLong Gold antifade reagent with 4’,6-diamidino-2-phenylindole (Invitrogen, Carlsbad, CA). The stained sections were evaluated with a Leica TCS SP5 spectral confocal system (Leica Microsystems, Wetzlar, Germany).

Statistical Analysis
Statistical analysis was performed using SPSS version 15.0 (SPSS Inc, Chicago, IL). For continuous variables, after distributions were determined for normality using Kolmogorov-Smirnov tests, the Kruskal-Wallis ANOVA was used with the Mann-Whitney U test or 1-way ANOVA with post hoc analysis. For categorical variables, proportions were compared using the χ² test or Fisher exact test. For related variables, the Friedman test and the Wilcoxon signed rank test were performed to examine the change in maternal GPBB concentrations throughout gestation. Medians and ranges or interquartiles were calculated for continuous variables, and frequencies and percentages were reported for categorical variables. ANCOVA and logistic regression analysis were conducted for comparison of maternal GPBB concentrations to adjust for maternal age, racial disparity, parity, infant sex, and gestational age at blood sampling. All P values were 2-sided, and a value of <0.05 was considered statistically significant.
Results

GPBB Plasma Concentration in Pregnancy and Preeclampsia

The analysis of plasma GPBB concentration in this study (Tables 1 and 2) included 396 women. Table 1 shows the demographics and clinical characteristics of the study groups of nonpregnant women and pregnant women with normal term delivery. Maternal age, racial distribution, and parity were different between nonpregnant women and pregnant women with normal term delivery \((P<0.01, \text{for each})\). The

### Table 1. Demographics of the Study Population of Nonpregnant Women and Pregnant Women With Normal Term Delivery

<table>
<thead>
<tr>
<th>Group</th>
<th>Nonpregnant Women ((N=33))</th>
<th>Normal Term Birth ((N=151))</th>
<th>(P)</th>
<th>Cases for the Longitudinal Analysis ((N=32))</th>
<th>Cases of Additional Term No Labor at Blood Sampling ((N=25))</th>
<th>Cases of Term in Labor at Blood Sampling ((N=65))</th>
<th>Preterm Control Cases for Preeclampsia ((N=29))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y*</td>
<td>26 (18–49)</td>
<td>23 (15–40)</td>
<td>0.005</td>
<td>20.5 (15–31)</td>
<td>27 (15–40)</td>
<td>23 (16–35)</td>
<td>23 (15–34)</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>39.4</td>
<td>90.7</td>
<td>93.8</td>
<td>93.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>45.5</td>
<td>2.6</td>
<td>0.0</td>
<td>0.0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.0</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>15.2</td>
<td>6.0</td>
<td>6.3</td>
<td>6.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Para (nullipara), %</td>
<td>63.6</td>
<td>31.8</td>
<td>&lt;0.001</td>
<td>56.3</td>
<td>8.0</td>
<td>33.8</td>
<td>20.7</td>
</tr>
<tr>
<td>Gestational age at blood sampling, wk*</td>
<td>39.4 (37.0–42.0)</td>
<td>39.7 (38.0–42.0)</td>
<td>39.1 (37.0–41.9)</td>
<td>39.7 (37.0–41.9)</td>
<td>39.3 (37.6–41.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery, wk*</td>
<td>39.4 (37.0–42.0)</td>
<td>39.7 (38.0–42.0)</td>
<td>39.1 (37.0–41.9)</td>
<td>39.7 (37.0–41.9)</td>
<td>39.3 (37.6–41.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant sex (male), %</td>
<td>49.0</td>
<td>50.0</td>
<td>64.0</td>
<td>46.2</td>
<td>51.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA neonates, %</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean delivery, %</td>
<td>29.8</td>
<td>31.3</td>
<td>100.0</td>
<td>3.1</td>
<td>27.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SGA indicates small-for-gestational age.
*Data show median (range).

### Table 2. Demographics of the Study Population of Cases With Preeclampsia and SGA Neonates

<table>
<thead>
<tr>
<th>Group</th>
<th>Control ((N=122))</th>
<th>Preeclampsia ((N=72))</th>
<th>SGA ((N=38))</th>
<th>Control ((N=33))</th>
<th>Preeclampsia ((N=74))</th>
<th>SGA ((N=28))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>90.2</td>
<td>83.3</td>
<td>92.1</td>
<td>93.9</td>
<td>82.4</td>
<td>92.9</td>
</tr>
<tr>
<td>White</td>
<td>3.3</td>
<td>6.9</td>
<td>7.9</td>
<td>0.0</td>
<td>9.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.8</td>
<td>4.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Others</td>
<td>5.7</td>
<td>5.6</td>
<td>0.0</td>
<td>6.1</td>
<td>8.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Para (nullipara), %</td>
<td>34.4</td>
<td>62.5</td>
<td>34.2</td>
<td>21.2</td>
<td>52.7</td>
<td>35.7</td>
</tr>
<tr>
<td>Gestational age at blood sampling, wk*</td>
<td>39.1 (37.0–41.9)</td>
<td>38.7 (37.0–42.4)</td>
<td>39.1 (37.1–41.6)</td>
<td>30.1 (20.3–36.9)</td>
<td>30.3 (22.6–36.7)</td>
<td>33.6 (20.3–36.7)</td>
</tr>
<tr>
<td>Gestational age at delivery, wk*</td>
<td>39.6 (37.0–42.0)</td>
<td>38.7 (37.0–42.4)</td>
<td>39.3 (37.1–41.6)</td>
<td>39.6 (37.6–42.0)</td>
<td>30.9 (22.9–36.7)</td>
<td>33.8 (20.9–36.9)</td>
</tr>
<tr>
<td>Infant sex (male), %</td>
<td>50.8</td>
<td>44.4</td>
<td>26.3</td>
<td>51.5</td>
<td>51.4</td>
<td>17.9</td>
</tr>
<tr>
<td>SGA neonates, %</td>
<td>0.0</td>
<td>44.4</td>
<td>100.0</td>
<td>0.0</td>
<td>50.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Cesarean delivery, %</td>
<td>30.3</td>
<td>45.8</td>
<td>26.3</td>
<td>27.3</td>
<td>83.8</td>
<td>17.9</td>
</tr>
</tbody>
</table>

SGA indicates small-for-gestational age.
*Data are median (range).
†Term controls \((n=122)\) for cases with term preeclampsia or term SGA neonates consisted of cases of term no labor \((n=57)\) and those of term in labor at blood sampling \((n=65)\).
‡Preterm controls \((n=33)\) for cases with preterm preeclampsia or preterm SGA neonates, matched with gestational age at blood sampling, consisted of preterm control cases for preterm preeclampsia \((n=29)\) and 4 cases for longitudinal study (second drawn blood samples \([n=4]\)).
group of pregnant women with normal term delivery included: (1) 32 cases whose blood samples were drawn serially for the longitudinal analysis; (2) 65 cases presenting with term labor; (3) 25 cases presenting with term no labor; and (4) 29 normal term delivery cases whose blood samples were collected before 37 weeks of gestation as preterm controls for patients with preterm preeclampsia. Table 2 illustrates the demographics and clinical characteristics of the study populations including cases with preeclampsia or SGA neonates and their gestational age-matched controls both in term and preterm gestations. There were significant differences in parity, gestational age at blood sampling, gestational age at delivery, infant sex, the rates of SGA neonates, and Cesarean delivery among groups both in term and preterm gestations ($P$<0.05, for each).

The median GPBB plasma concentration was significantly higher in pregnant women with a normal pregnancy outcome ($n$=151) than in nonpregnant women ($n$=33; median: 38.7 ng/mL, range: 2.8–825.4 ng/mL versus median: 9.2 ng/mL, range: 1.5–61.8 ng/mL; $P$<0.001), which remained significant after adjusting for maternal age, race, and parity (Figure 1). In addition, an analysis of plasma samples serially obtained during each trimester from 32 women with a normal pregnancy outcome, whose blood samples were drawn in the absence of labor, demonstrated no differences in the maternal plasma GPBB concentration as a function of advancing gestation (first trimester: median: 26.4 ng/mL, range: 8.3–155.0 ng/mL; second trimester: median: 39.5 ng/mL, range: 6.0–205.5 ng/mL; and third trimester: median: 27.5 ng/mL, range: 6.8–88.5 ng/mL; Figure 2).

Figure 3 displays the comparison of maternal plasma GPBB concentration according to the presence or absence of labor at term because it was shown that labor itself is associated with intermittent perfusion and oxidative stress of the placenta. The comparison was conducted in patients at term with labor at the time of blood sampling ($n$=65) and those without labor at the time of blood sampling ($n$=57: last drawn samples of cases for the longitudinal analysis [n=32] and cases of additional term no labor at blood sampling [n=25]; Table 1). There was no statistically significant difference in maternal plasma GPBB concentration between patients with and without labor (median: 62.6 ng/mL, range: 2.8–825.4 ng/mL versus median: 40.5 ng/mL, range: 5.0–163.9 ng/mL; $P$=0.157).

Figure 1. Plasma glycogen phosphorylase isoenzyme BB (GPBB) concentration in pregnant women. Median GPBB plasma concentration was higher in pregnant women with a normal pregnancy outcome ($n$=151) than in nonpregnant women ($n$=33) ($P$<0.001). Plasma GPBB concentrations were shown as median and interquartile ranges.

Figure 2. Maternal plasma glycogen phosphorylase isoenzyme BB (GPBB) concentration as a function of gestational age. There was no difference in the maternal plasma GPBB concentration as a function of advancing gestation.

Figure 3. Comparison of maternal plasma glycogen phosphorylase isoenzyme BB (GPBB) concentration according to the presence or absence of labor. Maternal plasma GPBB concentration tended to be higher in patients in labor at term ($n$=65) than in those not in labor at term ($n$=57), but the difference was not statistically significant ($P$=0.157). Maternal plasma GPBB concentrations were shown as median and interquartile ranges. TNL indicates term not in labor; TIL, term in labor.
Maternal plasma GPBB concentration was higher in patients with preterm preeclampsia (n=74; median: 72.6 ng/mL, range: 1.6–428.8 ng/mL) than in gestational age–matched pregnant women with a normal pregnancy outcome (n=33; median: 26.0 ng/mL, range: 6.8–205.5 ng/mL) and in patients with preterm SGA neonates and no preeclampsia (n=28; median: 15.9 ng/mL, range: 1.7–468.9 ng/mL; \( P<0.01 \), for each), which remained statistically significant after adjusting for maternal age, gestational age at blood draw, race, infant sex, and parity (Figure 4A). Maternal plasma GPBB concentration was not affected in cases of preterm SGA gestation without preeclampsia. There was no difference in this concentration in the following types of cases: term pregnancy with preeclampsia (n=72; median: 39.2 ng/mL, range: 2.7–311.9 ng/mL); SGA gestation and no preeclampsia (n=38; median: 67.2 ng/mL, range: 3.3–158.4 ng/mL); and normal pregnancy (n=122; median: 45.5 ng/mL, range: 2.8–825.4 ng/mL; Figure 4B). Additionally, preterm preeclamptic patients had a significantly higher median plasma GPBB concentration than preeclamptic patients at term (\( P<0.05 \)).

Subgroup analysis was performed to determine the relationship between maternal plasma GPBB concentration and the severity of preeclampsia. Eighty-two percent (59 of 72) of term preeclamptic patients and 96% (71 of 74) of preterm preeclamptic patients had severe symptoms and signs of preeclampsia based on the criteria of the American College of Obstetricians and Gynecologists.\(^{16}\) There was no significant difference in plasma GPBB concentration according to the severity of preeclampsia both in term preeclamptic cases (mild preeclampsia: median: 21.0 ng/mL, range: 6.1–147.9 ng/mL versus severe preeclampsia: median: 39.5 ng/mL, range: 2.7–311.9 ng/mL; \( P=0.959 \)) and preterm preeclamptic cases (mild preeclampsia: median: 37.1 ng/mL, range: 18.1–72.4 ng/mL versus severe preeclampsia: median: 72.8 ng/mL, range: 1.6–428.8 ng/mL; \( P=0.331 \)). However, cases of preterm severe preeclampsia had a higher median plasma GPBB concentration than cases of term severe preeclampsia (\( P<0.05 \)). When subgroup analysis in the context of pre-eclampsia was conducted to determine whether there was an additional effect of SGA birth on maternal plasma GPBB concentration, preterm preeclamptic patients who delivered SGA neonates (n=37; median: 63.0 ng/mL, range: 1.6–263.7 ng/mL) and those who delivered appropriate-for-gestational-age neonates (n=37; median: 72.8 ng/mL, range: 2.4–428.8 ng/mL) had higher median maternal plasma GPBB concentrations than gestational age–matched pregnant women with a normal pregnancy outcome (n=33; median: 26.0 ng/mL, range: 6.8–205.5 ng/mL; \( P<0.05 \), for each). However, there was no difference in maternal plasma GPBB concentration between SGA neonates and appropriate-for-gestational-age neonates of preterm preeclamptic patients. There were also no differences in maternal plasma GPBB concentration among term patients with preeclampsia who delivered SGA neonates (n=32; median: 36.1 ng/mL, range: 2.7–308.0 ng/mL), appropriate-for-gestational-age neonates (n=40; median: 45.0 ng/mL, range: 3.2–311.9 ng/mL), and gestational age–matched pregnant women with a normal pregnancy outcome (n=122; median: 45.2 ng/mL, range: 3.2–311.9 ng/mL).

### Placental Expression of GPBB

The significant increase of GPBB concentration in normal pregnant women and particularly in women with preterm preeclampsia, which is characterized by uteroplacental hypoxia, led us to examine whether GPBB is expressed in the placenta and whether there is a difference in GPBB expression in the placenta according to the presence or absence of preeclampsia and gestational age at delivery. When we conducted immunoblotting using the placental protein lysates of 45 cases (Table S1, available in the online Data Supple-
ment, please see http://hyper.ahajournal.org). GPBB was readily detected in the villous placenta of all cases (Figure 5A). Densitometric analysis of immunoblotting data showed a significant decrease in the expression of GPBB in preterm preeclampsia cases (n=9; median GPBB/HPRT ratio: 0.59; range: 0.06–1.00) than in cases of preterm labor and delivery (n=9; median GPBB/HPRT ratio: 1.18; range: 0.61–2.64; P<0.01), whereas there was no difference in placental GPBB expression between term preeclampsia cases (n=9; median GPBB/HPRT ratio: 1.10; range: 0.62–1.54) and normal term delivery cases (n=18; median GPBB/HPRT ratio: 1.05; range: 0.53–4.01; Figure 5B). Accordingly, placental GPBB in preterm preeclampsia cases was less abundant than in term preeclampsia cases (P<0.01). Labor itself did not change the GPBB expression in the placentas of cases delivered at term, which was similar to the result in maternal plasma GPBB concentration (Figure 5B). Immunofluorescence staining further demonstrated distinct GPBB immunoreactivity in the syncytiotrophoblast and villous cytotrophoblast (Figure 5C).

**Discussion**

This study reports profiles of plasma GPBB concentration in normal pregnancy and in pregnancy complicated with preeclampsia for the first time. The novel findings of this study are: (1) there is a physiological increase in plasma GPBB concentration during pregnancy; (2) preterm (but not term) preeclampsia is characterized by a further increase in plasma GPBB concentration; (3) GPBB is readily detected in the placenta, and its abundance is decreased in preterm preeclampsia; (4) SGA gestation does not affect plasma GPBB concentration; and (5) there was no difference in maternal plasma GPBB concentration between women with and without labor at term.

GPBB is abundant in normal tissues of the brain and myocardium,1 and the placenta turned out to be an additional source of the enzyme. Although pregnancy is clearly a physiological state, the median plasma GPBB concentration in pregnant women with normal term delivery was almost 3 times higher than the cutoff value (10 ng/mL) used for the diagnosis of acute coronary syndromes in nonpregnant pa-
Preeclampsia is a pregnancy-specific syndrome characterized by hypertension and proteinuria, and occurs in 5% to 8% of all pregnancies worldwide. Hypertensive disorders in pregnancy are associated with the development of acute myocardial infarction during pregnancy. Pregnancy can also change cerebrovascular blood flow, such as a decrease in the mean blood flow of the middle and posterior cerebral arteries, but does not affect cerebrovascular autoregulation; and the blood-brain barrier remains intact in healthy pregnant women. Therefore, it is less likely that the increase in the plasma GPBB concentration during normal pregnancy is solely attributed to the GPBB release from the heart or the brain. Instead, additional GPBB release from the placenta would be a reasonable expectation.

Hypertensive disorders in pregnancy are associated with the abberations of cardiovascular functions, such as hyperdynamic ventricular function and low intravascular blood volume. Moreover, Ladner et al. have reported that essential hypertension and preeclampsia are associated with the development of acute myocardial infarction during pregnancy. Melchiorre et al. also have reported that ~20% of patients with preeclampsia at term show evident myocardial damage. In addition, increased blood-brain barrier permeability and brain edema are found in both preeclamptic and eclamptic patients. However, no patient of the current study was diagnosed with acute myocardial infarction or brain damage during the index pregnancy.

Preeclampsia developing before 37 weeks of gestation (preterm preeclampsia) is a more severe and complicated maternal phenotype of preeclampsia than that developing at term (term preeclampsia) in general. The long-term risk of mortality from the following cardiovascular causes, the proportion of SGA neonates, and the concentrations of maternal serum liver enzymes are higher in preterm preeclamptic patients than in term preeclamptic patients. Huppertz proposed recently that these features largely composed the phenotype of early onset but not late-onset preeclampsia. Our observation of a significant increase in maternal plasma GPBB concentration in preterm preeclampsia, but not in term preeclampsia, further supports the opinion that preeclampsia can be subclassified according to the gestational age at disease onset.

Pregnancies presenting SGA neonates and preeclampsia have been reported to share common features of shallow placentation and uteroplacental insufficiency. However, the maternal manifestation of these conditions differs, and some investigators have proposed that they are biologically different disease entities. In the present study, an additional effect of SGA birth on maternal GPBB concentration in preeclampsia cases was not found, which suggests that the increased maternal GPBB concentration is mainly related to preeclampsia. This finding was consistent with that from the analysis of GPBB concentration according to SGA births among normotensive pregnant women. Although an SGA pregnancy is one of the indicators for severity of preeclampsia and is commonly associated with such disease, an SGA gestation alone was less likely to affect maternal GPBB concentration.

Previous studies of placental metabolism in preeclampsia have shown abnormalities in glycogen metabolism. Bloxam et al. demonstrated impaired glycolysis in the placentas of women with preeclampsia by showing decreased concentrations of pyruvate and lactate but not glycogen and glucose. Arkwright et al. have shown abnormalities in glycogen metabolism. Previous studies of placental metabolism in preeclampsia have shown decreased concentrations of pyruvate and lactate but not glycogen and glucose. Arkwright et al. have shown abnormalities in glycogen metabolism. Arkwright et al. have shown abnormalities in glycogen metabolism.

We found limitations in our study. Because postpartum blood samples were unavailable, maternal GPBB concentration during the postpartum period could not be examined. The change in post-delivery GPBB concentration could be a key piece of data in support of our hypothesis: that increased GPBB concentration during pregnancy originates from placcental tissue. Another limitation is that the blood samples and placental samples were not obtained from the same pregnant women, and analyses using blood and placental samples from the same pregnant women would have been more relevant for the determination of placentical origin of peripheral blood GPBB. In this study, there were differences of racial distribution and parity for each of the analyses such as the following: (1) nonpregnant women versus pregnant women with a normal pregnancy; (2) preeclampsia versus SGA gestation versus controls in preterm gestation; and (3) preeclampsia versus SGA gestation versus controls in term gestation. Although the differences in plasma GPBB concentration remained significant after adjusting for these confounding factors, future large-scale studies are needed to address this issue.
Perspectives
Maternal plasma GPBB concentration is increased during pregnancy, and its robust increase is a novel phenotype of preeclampsia. The findings herein strongly suggest that maternal plasma GPBB can be a useful marker in the detection of early onset (preterm) preeclampsia. Uteroplacental hypoxia precedes the clinical symptoms of preeclampsia, and a future study on the predictive value of plasma GPBB concentration as a marker of preeclampsia or other obstetric complications, such as fetal death in asymptomatic pregnant women (ie, during the midtrimester), would be desirable.

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We are grateful to the patients who agreed to participate in our study and to the nurses, laboratory staff, and clinicians who made this work possible.

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Disclosures
None.

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Glycogen Phosphorylase Isoenzyme BB Plasma Concentration Is Elevated in Pregnancy and Preterm Preeclampsia

JoonHo Lee, Roberto Romero, Zhong Dong, Deug-Chan Lee, Yi Dong, Pooja Mittal, Tinnakorn Chaiworapongsä, Sonia S. Hassan and Chong Jai Kim

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GLYCOGEN PHOSPHORYLASE ISOENZYME BB PLASMA CONCENTRATION IS ELEVATED IN PREGNANCY AND PRETERM PREECLAMPSIA

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Short title: Glycogen Phosphorylase Isoenzyme BB in Pregnancy
Supplemental Table

Table S1. Demographics for immunoblotting and densitometric analysis of the placentas for the study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal Term No Labor N=9</th>
<th>Normal Term In Labor N=9</th>
<th>Term Preeclampsia N=9</th>
<th>Preterm Birth† N=9</th>
<th>Preterm Preeclampsia N=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)*</td>
<td>24 (20-38)</td>
<td>22 (19-28)</td>
<td>20 (18-35)</td>
<td>21 (18-37)</td>
<td>20 (16-33)</td>
</tr>
<tr>
<td>Race (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>77.8</td>
<td>77.8</td>
<td>88.9</td>
<td>66.7</td>
<td>66.7</td>
</tr>
<tr>
<td>White</td>
<td>11.1</td>
<td>22.2</td>
<td>11.1</td>
<td>11.1</td>
<td>22.2</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>11.1</td>
</tr>
<tr>
<td>Others</td>
<td>11.1</td>
<td>0.0</td>
<td>0.0</td>
<td>22.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Para (Nullipara) (%)</td>
<td>0.0</td>
<td>22.2</td>
<td>55.6</td>
<td>44.4</td>
<td>55.6</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)*</td>
<td>39.1</td>
<td>39.3</td>
<td>39.0</td>
<td>31.0</td>
<td>33.6</td>
</tr>
<tr>
<td></td>
<td>(39.0-40.0)</td>
<td>(38.6-39.9)</td>
<td>(37.0-42.1)</td>
<td>(28.4-34.1)</td>
<td>(23.9-35.0)</td>
</tr>
<tr>
<td>Baby gender (Male) (%)</td>
<td>44.4</td>
<td>33.3</td>
<td>33.3</td>
<td>66.7</td>
<td>55.6</td>
</tr>
<tr>
<td>SGA (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cesarean delivery (%)</td>
<td>100.0</td>
<td>0.0</td>
<td>44.4</td>
<td>55.6</td>
<td>44.4</td>
</tr>
</tbody>
</table>

*, median (range)  
†, Preterm birth controls without preeclampsia for preterm preeclampsia cases, who were delivered at preterm after preterm labor or preterm prelabor rupture of membranes developed