

## ORIGINAL ARTICLE

## Soluble Endoglin and Other Circulating Antiangiogenic Factors in Preeclampsia

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## ABSTRACT

**BACKGROUND**

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Alterations in circulating soluble fms-like tyrosine kinase 1 (sFlt1), an antiangiogenic protein, and placental growth factor (PlGF), a proangiogenic protein, appear to be involved in the pathogenesis of preeclampsia. Since soluble endoglin, another antiangiogenic protein, acts together with sFlt1 to induce a severe preeclampsia-like syndrome in pregnant rats, we examined whether it is associated with preeclampsia in women.

**METHODS**

We performed a nested case-control study of healthy nulliparous women within the Calcium for Preeclampsia Prevention trial. The study included all 72 women who had preterm preeclampsia (<37 weeks), as well as 480 randomly selected women — 120 women with preeclampsia at term (at ≥37 weeks), 120 women with gestational hypertension, 120 normotensive women who delivered infants who were small for gestational age, and 120 normotensive controls who delivered infants who were not small for gestational age.

**RESULTS**

Circulating soluble endoglin levels increased markedly beginning 2 to 3 months before the onset of preeclampsia. After the onset of clinical disease, the mean serum level in women with preterm preeclampsia was 46.4 ng per milliliter, as compared with 9.8 ng per milliliter in controls ( $P<0.001$ ). The mean serum level in women with preeclampsia at term was 31.0 ng per milliliter, as compared with 13.3 ng per milliliter in controls ( $P<0.001$ ). Beginning at 17 weeks through 20 weeks of gestation, soluble endoglin levels were significantly higher in women in whom preterm preeclampsia later developed than in controls (10.2 ng per milliliter vs. 5.8 ng per milliliter,  $P<0.001$ ), and at 25 through 28 weeks of gestation, the levels were significantly higher in women in whom term preeclampsia developed than in controls (8.5 ng per milliliter vs. 5.9 ng per milliliter,  $P<0.001$ ). An increased level of soluble endoglin was usually accompanied by an increased ratio of sFlt1:PlGF. The risk of preeclampsia was greatest among women in the highest quartile of the control distributions for both biomarkers but not for either biomarker alone.

**CONCLUSIONS**

Rising circulating levels of soluble endoglin and ratios of sFlt1:PlGF herald the onset of preeclampsia.

**P**REECLAMPSIA, CHARACTERIZED BY HYPERTENSION and proteinuria after 20 weeks of gestation, complicates 3 to 5 percent of pregnancies and results in substantial maternal and neonatal complications and deaths.<sup>1,2</sup> It seems to be precipitated by the release of circulating factors from the placenta that induce endothelial dysfunction.<sup>3,4</sup>

Soluble fms-like tyrosine kinase 1 (sFlt1) (also known as soluble vascular endothelial growth factor [VEGF] receptor 1 [sVEGFR1]), a circulating antiangiogenic protein that sequesters the proangiogenic proteins placental growth factor (PlGF) and VEGF, is increased before the onset of clinical disease in the circulation of women with preeclampsia. Circulating levels of sFlt1 correlate with the severity of preeclampsia and proximity to the onset of hypertension or proteinuria.<sup>5-11</sup> Serum free PlGF and free VEGF levels are decreased before the development of preeclampsia.<sup>5,12-15</sup> Overexpression of sFlt1 in pregnant rats results in a preeclampsia-like phenotype.<sup>8</sup> Furthermore, anti-VEGF therapy in patients with cancer has been associated with hypertension, proteinuria, and the reversible posterior leukoencephalopathy syndrome, which are hallmarks of preeclampsia and eclampsia.<sup>16-18</sup> Therefore, an imbalance in circulating angiogenic factors may be associated with vascular endothelial dysfunction and the maternal syndrome of preeclampsia.<sup>19</sup>

Endoglin, a coreceptor for transforming growth factor  $\beta$ 1 and  $\beta$ 3 (TGF- $\beta$ 1 and TGF- $\beta$ 3, respectively), is highly expressed on cell membranes of vascular endothelium and syncytiotrophoblasts.<sup>20,21</sup> Placental endoglin is up-regulated in preeclampsia, releasing soluble endoglin into the maternal circulation.<sup>22</sup> Soluble endoglin is an antiangiogenic protein that may inhibit TGF- $\beta$ 1 signaling in vasculature.<sup>22,23</sup> In one study, overexpression of soluble endoglin in rodents by means of adenoviral vectors led to increased vascular permeability and induced modest hypertension without significant proteinuria.<sup>22</sup> Adenoviral-mediated overexpression of both sFlt1 and soluble endoglin caused severe vascular damage, nephrotic-range proteinuria, severe hypertension, a syndrome similar to the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), and fetal growth restriction.<sup>22</sup> Thus, soluble endoglin and sFlt1, two antiangiogenic proteins operating through sepa-

rate mechanisms, may combine to produce endothelial dysfunction and severe preeclampsia.

We performed a nested case-control study within the Calcium for Preeclampsia Prevention (CPEP) trial<sup>24</sup> to determine gestational patterns of circulating soluble endoglin in women with normal pregnancies and in those with preeclampsia. We hypothesized that serum soluble endoglin levels would be elevated before the onset of clinical preeclampsia and would correlate with the severity of the disease and proximity to the onset of clinical manifestations.

## METHODS

### PARTICIPANTS AND SPECIMENS

The CPEP trial was a randomized, double-blind trial conducted from 1992 to 1995 in healthy nulliparous women with singleton pregnancies to evaluate the effects of daily supplementation with calcium or placebo on the incidence and severity of preeclampsia.<sup>24,25</sup>

Of the 4589 women enrolled in the CPEP trial, we excluded 333 who had incomplete information about outcomes, whose pregnancy ended before 20 weeks, or who had a stillbirth<sup>5</sup>; 102 with no serum specimen before enrollment; and 524 whose pre-enrollment serum specimen may have been dated inaccurately, since the date on the specimen label was more than 2 days after the date recorded by the research nurse. Among the remaining 3630 women, 2469 were normotensive throughout pregnancy and delivered infants who were appropriately sized or large for gestational age (controls), 225 were normotensive but delivered small-for-gestational-age infants, 651 had gestational hypertension, 72 had preeclampsia before 37 weeks of gestation (preterm preeclampsia), and 213 had preeclampsia at or after 37 weeks (term preeclampsia). We randomly selected 120 from each group except the group with preterm preeclampsia; we included all 72 women in this group. We analyzed all serum specimens obtained before labor or delivery. Calcium supplementation did not affect levels of angiogenic factors (data not shown).

Although 87 women (30 with preterm preeclampsia, 52 with term preeclampsia, 4 controls, and 1 who was normotensive and had an infant who was small for gestational age) had been included in a previous study,<sup>5</sup> aliquots not thawed

previously were used to conduct new analyses of angiogenic factors. Of the 552 women, 41 (7.4%) had one serum specimen available for analysis, 119 (21.6%) had two, 333 (60.3%) had three, 56 (10.1%) had four, and 3 (0.5%) had five. Since three or fewer specimens were available for most women, we analyzed the data largely in a cross-sectional manner using all available specimens within intervals of gestational age and according to the time before the onset of preeclampsia. Within these 3-to-5-week intervals, the numbers of women and specimens were generally equal.

Analyses according to the severity of preeclampsia were conducted within larger intervals (21 through 32 or 33 through 42 weeks of gestation). In these intervals of gestational age, when more than one specimen existed per woman, the earliest was used. After the onset of clinical disease, specimens were available from 40 of the 72 women in whom preterm preeclampsia developed and from 32 of the 120 in whom term preeclampsia developed. In paired comparisons, specimens were matched within 1 week of gestational age at the time of collection of the specimen. Although specimens were obtained from 32 women with term preeclampsia after the clinical onset of the condition, only 16 matched controls were found for these women.

Preeclampsia was defined as hypertension (i.e., diastolic blood pressure of at least 90 mm Hg on two occasions 4 to 168 hours apart) and proteinuria, characterized as one of the following: urine dipstick results of at least 1+ (30 mg per deciliter) on two occasions 4 to 168 hours apart; a protein:creatinine ratio of at least 0.35; urine dipstick results of at least 2+ (100 mg per deciliter); or a 24-hour urine specimen containing at least 300 mg of protein. Severe preeclampsia was defined as the HELLP syndrome, eclampsia, or preeclampsia with either severe hypertension (diastolic blood pressure of  $\geq 110$  mm Hg) or severe proteinuria (urinary protein excretion of  $\geq 3.5$  g per 24 hours or urine dipstick results of  $\geq 3+$  [300 mg per deciliter]). Gestational hypertension was defined as hypertension without proteinuria.<sup>24,25</sup> The onset of preeclampsia or gestational hypertension was considered to be the time of the first elevated blood-pressure or urinary-protein measurement leading to diagnosis. A small-for-gestational-age infant had a birth weight below the

10th percentile according to U.S. tables of birth weight for gestational age that take into account race, parity, and sex of the infant.<sup>26</sup>

Because the study used specimens that were collected as part of the CPEP trial and could not be linked to identifiable women, the Office of Human Subjects Research of the National Institutes of Health granted the study an exemption from the requirement for review and approval by the institutional review board.

#### PROCEDURES

Specimens were randomly ordered for analysis, and assays were performed by personnel who were unaware of the outcome of the pregnancy. Enzyme-linked immunosorbent assays (ELISAs) for human soluble endoglin, sFlt1, and free PlGF were conducted in duplicate with the use of commercial kits (R&D Systems). These assays have been validated by recovery studies from the serum of pregnant women. Minimal detectable levels in the assays for soluble endoglin, sFlt1, and PlGF were 7 pg per milliliter, 5 pg per milliliter, and 7 pg per milliliter, respectively, and interassay coefficients of variation in our laboratory were 12 percent, 13 percent, and 5 percent, respectively. We previously reported on sFlt1 and PlGF in preeclampsia<sup>5</sup>; in the present study we sought to compare these proteins with soluble endoglin using their ratio. The ratio of sFlt1:PlGF is an index of antiangiogenic activity that reflects both increased sFlt1 and decreased PlGF in women in whom preeclampsia develops and predicts preeclampsia more reliably than either protein alone.<sup>27,28</sup>

#### STATISTICAL ANALYSIS

Chi-square tests were used for the comparison of categorical variables, and t-tests were used for the comparison of continuous variables. Although the arithmetic mean levels of angiogenic proteins are reported in the text and figures, statistical testing was conducted after logarithmic transformation. All P values are two-tailed. The Wilcoxon rank-sum test was also applied to comparisons within the gestational-age and weeks-before-preeclampsia intervals and provided P values indicating similar significance. Risk was calculated with the use of odds ratios, multivariable analyses were performed with logistic-regression analysis, and log-likelihood ratios were examined to assess model fit.

**Table 1. Characteristics of Controls, Normotensive Women with Small-for-Gestational-Age Infants, and Women with Gestational Hypertension, Preterm Preeclampsia, or Term Preeclampsia at Enrollment in the CPEP Trial and Characteristics of Their Infants and Serum Specimens.\***

Characteristic	Controls (N=120)†	Women Who Were Normotensive and Delivered Small-for-Gestational-Age Infants (N=120)	Women with Gestational Hypertension (N=120)	Women with Preterm Preeclampsia (N=72)	Women with Term Preeclampsia (N=120)
			P Value	P Value	P Value
<b>Women</b>					
Age — yr	21.0±4.3	21.3±4.8		21.0±4.6	21.6±4.8
Height — cm	163.5±6.1	160.4±7.1	<0.001	163.2±6.5	161.6±6.7
Weight — kg	69.4±16.8	60.4±13.2	<0.001	73.4±19.4	73.3±19.2
Body-mass index‡	25.9±5.9	23.4±4.5	<0.001	27.4±6.5	28.0±6.5
Systolic blood pressure — mm Hg	105±8	105±9		108±9	110±7
Diastolic blood pressure — mm Hg	59±7	58±8		60±8	62±7
Primigravida — no. (%)	96 (80.0)	91 (75.8)		81 (67.5)	96 (80.0)
Gestational age at enrollment — days	124±16	123±17		122±17	122±19
Gestational age at delivery — days	276±12	275±10		276±16	279±9
Current smoker — no. (%)	20 (16.7)	34 (28.3)	0.03	8 (6.7)	11 (9.2)
Calcium treatment — no. (%)	62 (51.7)	62 (51.7)		52 (43.3)	58 (48.3)
Private insurance — no. (%)	11 (9.2)	14 (11.7)		12 (10.0)	8 (6.7)
Ever married — no. (%)	26 (21.7)	30 (25.0)		21 (17.5)	20 (16.7)
Race or ethnic group — no. (%)§					
White, non-Hispanic	50 (41.7)	53 (44.2)		31 (25.8)	25 (20.8)
White, Hispanic	18 (15.0)	19 (15.8)		11 (9.2)	19 (15.8)
Black	52 (43.3)	47 (39.2)		77 (64.2)	73 (60.8)
Other or unknown	0	1 (0.8)		1 (0.8)	3 (2.5)
<b>Infants</b>					
Birth weight — g	3308±532	2539±287	<0.001	3218±635	3270±485
Delivery at <37 wk — no. (%)	10 (8.3)	10 (8.3)		9 (7.5)	0
Small for gestational age (<10th percentile) — no. (%)	NA	120 (100.0)		13 (10.8)	18 (15.0)
<b>Specimens</b>					
Freezer storage —70°C — yr	10.7±0.7	10.9±0.8	<0.001	10.6±0.7	10.7±0.7

\* Enrollment in the CPEP trial was at 13 to 21 weeks of gestation. Plus-minus values are means ±SD. P values are given only for significant differences in comparison with the controls. NA denotes not applicable.  
 † The controls were women who were normotensive during pregnancy and who delivered infants that were appropriately sized or large for gestational age.  
 ‡ Body-mass index is the weight in kilograms divided by the square of the height in meters.  
 § Race was self-determined. P values for race are for the distribution across the four categories.

## RESULTS

## CHARACTERISTICS OF THE WOMEN

Severe preeclampsia occurred in 44 of 72 women with preterm preeclampsia (61.1%) and 30 of 120 women with term preeclampsia (25.0%). Consistent with published literature, women with preeclampsia or gestational hypertension had greater body-mass index, higher blood pressure at enrollment, were more often black, and were less likely to smoke than normotensive women with appropriately sized or large-for-gestational-age infants (Table 1).<sup>1,2</sup> Women who were normotensive during pregnancy and delivered small-for-gestational-age infants were smaller in stature and more likely to smoke than were women in the control group. Specimens from women with small-for-gestational-age infants or preterm preeclampsia had been stored slightly longer at  $-70^{\circ}\text{C}$  than had specimens from women in the control group.

## LEVELS OF SOLUBLE ENDOGLIN

We first confirmed that serum levels of soluble endoglin were altered in women with clinical signs of preeclampsia. Among 40 paired specimens, mean soluble endoglin levels were elevated in women with preterm preeclampsia as compared with matched controls (46.4 ng per milliliter vs. 9.8 ng per milliliter,  $P<0.001$ ). Findings were similar among 16 specimens from women with term preeclampsia and matched controls (31.0 ng per milliliter and 13.3 ng per milliliter, respectively;  $P<0.001$ ).

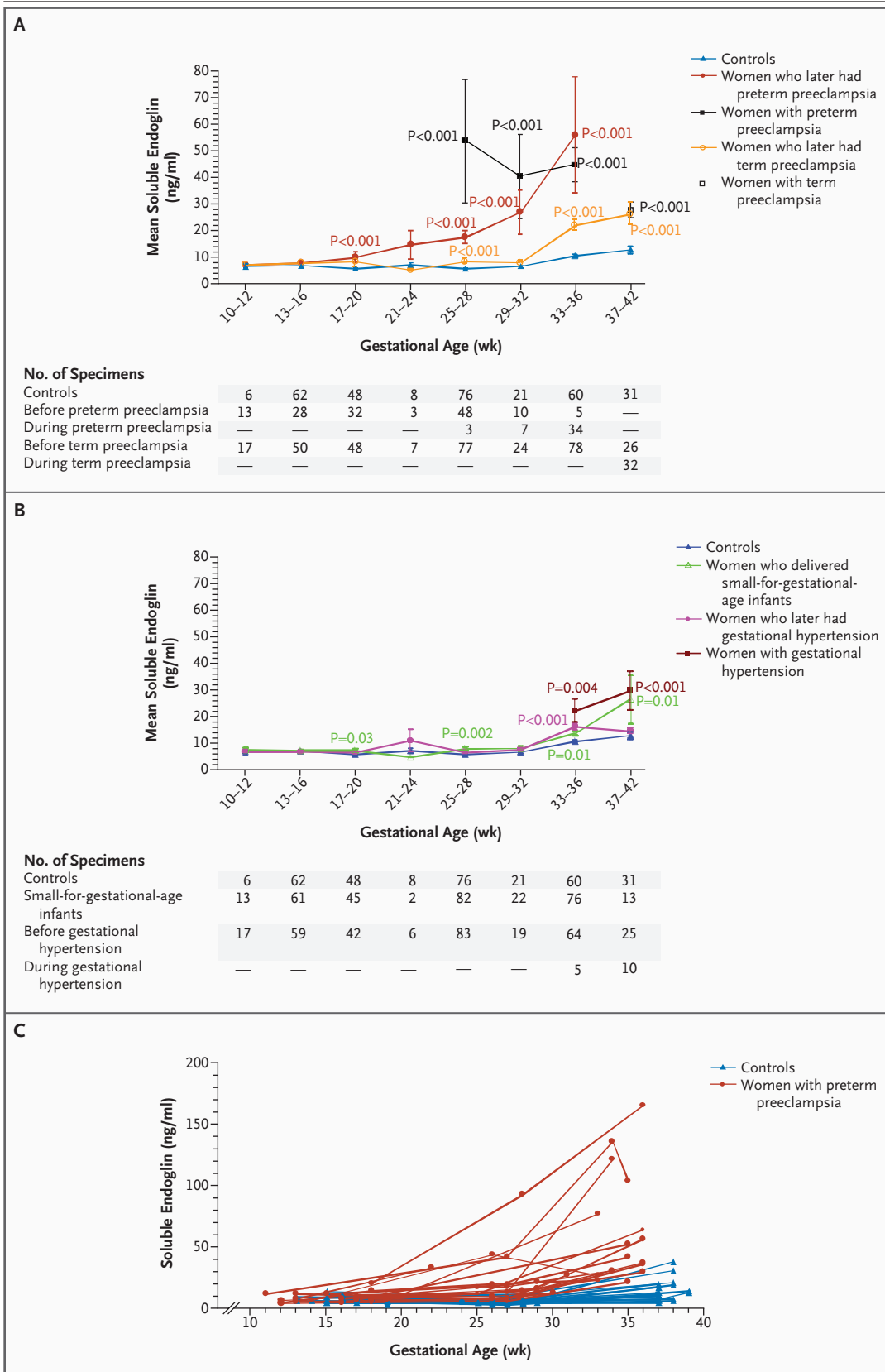
To evaluate gestational patterns, we performed a cross-sectional analysis within 4-week intervals of gestational age. Levels of soluble endoglin among controls were stable until 33 through 36 weeks of gestation and then increased by an average of 0.69 ng per milliliter per week until labor or delivery (Fig. 1A). In specimens obtained before the onset of preeclampsia from women in whom preterm preeclampsia subsequently developed, soluble endoglin began to rise at 17 weeks through 20 weeks of gestation, to 10.2 ng per milliliter, compared with 5.8 ng per milliliter in controls ( $P<0.001$ ), and had a steep rise at 33 through 36 weeks. Among women in whom term preeclampsia developed, soluble endoglin levels increased slightly beginning at 25 weeks through 28 weeks, to 8.5 ng per milliliter, as compared with 5.9 ng per milliliter in controls ( $P<0.001$ ),

**Figure 1 (facing page). Mean ( $\pm$ SE) Levels of Soluble Endoglin According to Gestational Age.**

The controls were women who were normotensive during pregnancy and who delivered appropriately sized or large-for-gestational-age infants. The P values given are for the comparisons, after logarithmic transformation, with specimens from controls obtained during the same gestational-age interval. Panel A shows the mean serum soluble endoglin levels before and after the onset of clinical preeclampsia according to the weeks of gestation. The difference, after logarithmic transformation, between the specimens obtained at 25 through 28 weeks from women who already had clinical preeclampsia that began before 37 weeks of gestation and those who later had preterm preeclampsia was also significant ( $P=0.03$ ). One specimen obtained at 21 through 24 weeks and one at 37 through 42 weeks after the onset of preterm preeclampsia, with values of 33.5 ng per milliliter and 14.5 ng per milliliter, respectively, are not shown. Panel B shows the mean serum soluble endoglin levels before and after the onset of gestational hypertension, according to the weeks of gestation, among women who were normotensive during pregnancy and who delivered small-for-gestational-age infants and among the controls. Panel C shows longitudinal plots of the mean soluble endoglin levels in individual women according to the weeks of gestation. A total of 20 women in whom preterm preeclampsia developed and who had a serum specimen obtained after the onset of clinical disease and 20 controls with three or more serum specimens were randomly selected. Controls were selected from among women who had a specimen collected at the latest gestational ages.

and increased greatly beginning at 33 weeks through 36 weeks.

Women in whom gestational hypertension later developed had significantly higher levels of soluble endoglin at 33 through 36 weeks than did controls (Fig. 1B), but levels were lower than those among women with subsequent term preeclampsia both at 33 through 36 weeks ( $P=0.006$ ) and at 37 through 42 weeks ( $P=0.01$ ). After the onset of gestational hypertension, levels were similar to those in women who had clinical signs of term preeclampsia. Among women who were normotensive during pregnancy and had small-for-gestational-age infants, soluble endoglin levels increased slightly beginning at 17 through 20 weeks, to 7.2 ng per milliliter, as compared with 5.8 ng per milliliter in controls ( $P=0.03$ ), with a large increase at 37 through 42 weeks, to 26.5 ng per milliliter as compared with 12.9 ng per milliliter in controls ( $P=0.01$ ). Although soluble endoglin levels at 33 through 36 weeks in women who were normotensive during pregnancy and



had small-for-gestational-age infants were lower ( $P<0.001$ ) than those in the group with term preeclampsia, at 37 through 42 weeks there were no significant differences between the two groups.

Longitudinal levels of soluble endoglin in individual women increased in controls during late gestation. The levels increased in women with preterm preeclampsia earlier and to a greater extent (Fig. 1C).

Levels of soluble endoglin in specimens from women in whom preterm preeclampsia later developed, as compared with matched controls, increased with proximity to the onset of signs of preeclampsia, beginning 9 through 11 weeks before onset (Fig. 2A). A similar pattern but with less magnitude was observed for women with term preeclampsia, beginning 12 through 14 weeks before onset (Fig. 2B).

#### RATIOS OF sFlt1:PIGF

We performed cross-sectional analyses of the ratio of sFlt1:PIGF with the use of serum from the samples analyzed for soluble endoglin (Fig. 3A). In controls, the sFlt1:PIGF ratio was greatest at 10 through 12 weeks of gestation, decreased until 29 through 32 weeks, and then rose slowly until term. In women in whom preeclampsia developed, the pattern was similar to that of soluble endoglin levels. The sFlt1:PIGF ratio was greater beginning at 17 weeks through 20 weeks among women in whom preterm preeclampsia subsequently developed and at 25 through 28 weeks among those in whom term preeclampsia developed than in the controls.

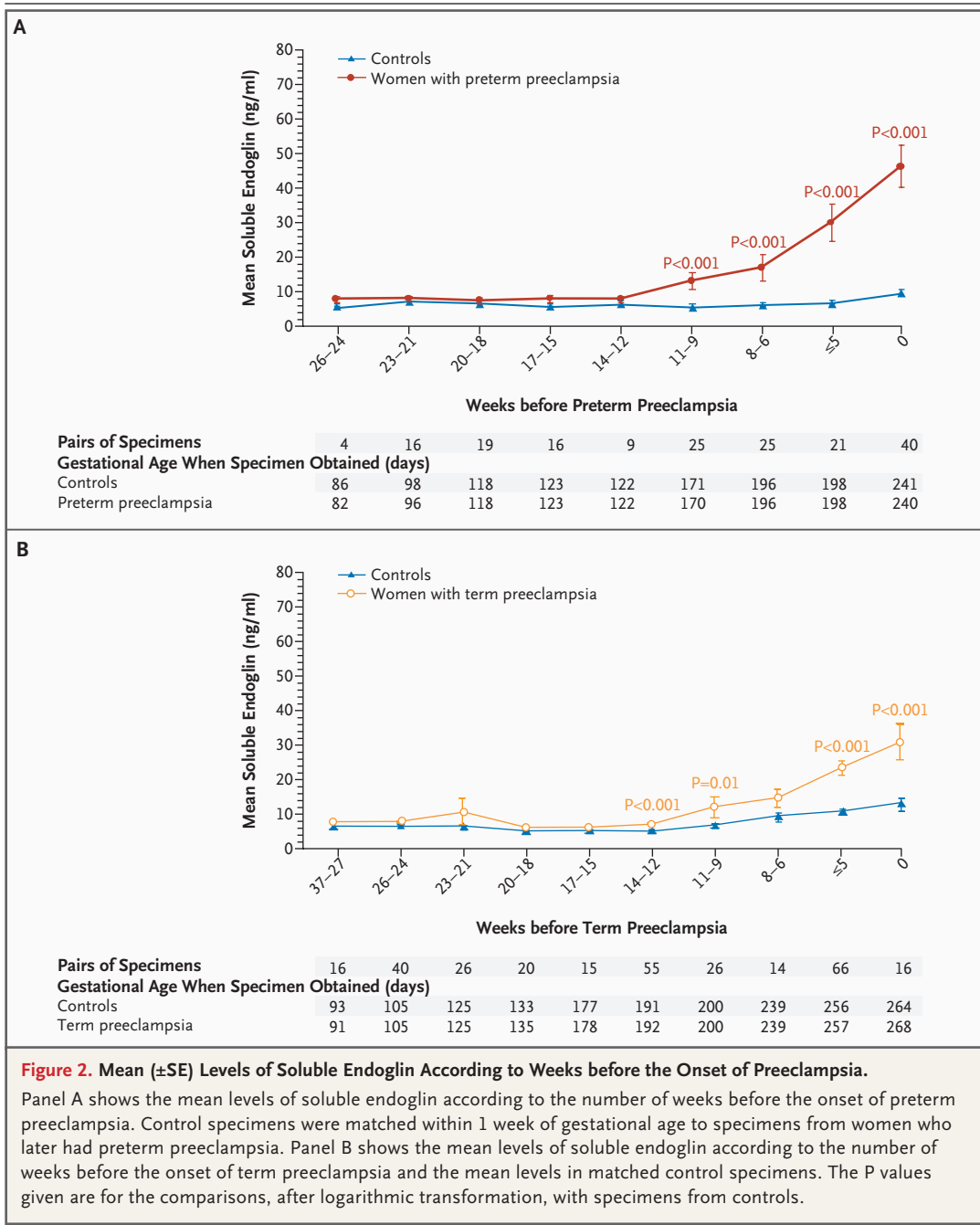
Women with subsequent gestational hypertension did not differ significantly from controls except at 33 through 36 weeks, when ratios were significantly greater (Fig. 3B); ratios were lower than those among women in whom term preeclampsia later developed at 33 through 36 weeks ( $P=0.006$ ) and 37 through 42 weeks ( $P=0.004$ ). Ratios after the onset of gestational hypertension were greater than those among the controls but lower than those among women with clinical onset of term preeclampsia ( $P=0.02$ ). Among women who were normotensive during pregnancy and who delivered small-for-gestational-age infants, the sFlt1:PIGF ratio did not differ significantly from that in controls and was lower at 33 through 36 weeks ( $P<0.001$ ) and at 37 through 42 weeks ( $P=0.01$ ) than the ratios among

women in whom term preeclampsia later developed. As compared with matched controls, the ratio increased beginning 9 through 11 weeks before preterm preeclampsia (Fig. 3C), as with soluble endoglin levels, but only 5 weeks or less before term preeclampsia (Fig. 3D).

#### RELATIONSHIP OF THE SOLUBLE ENDOGLIN LEVEL AND THE sFlt1:PIGF RATIO TO THE SEVERITY OF PREECLAMPSIA

At 21 through 32 weeks of gestation, women in whom severe preterm preeclampsia later developed had significantly higher soluble endoglin levels than did women in whom mild preterm preeclampsia developed (22.1 ng per milliliter vs. 13.5 ng per milliliter,  $P=0.007$ ), but their sFlt1:PIGF ratios were not significantly greater (28.4 vs. 24.4,  $P=0.10$ ). Women who had subsequent preterm preeclampsia and small-for-gestational-age infants had greater abnormalities in the level of angiogenic factors than those who subsequently had preterm preeclampsia and appropriately sized infants (25.0 ng per milliliter vs. 15.6 ng per milliliter for soluble endoglin,  $P=0.009$ ; 47.9 vs. 17.2 for the sFlt1:PIGF ratio,  $P<0.001$ ). Values of soluble endoglin and sFlt1:PIGF ratios were substantially lower in controls (6.1 ng per milliliter and 2.8, respectively) than in all the preterm-preeclampsia subtypes described above ( $P<0.001$  for all comparisons).

Later in pregnancy, at 33 through 42 weeks, the levels of soluble endoglin did not differ significantly between women in whom severe term preeclampsia subsequently developed and those in whom mild term preeclampsia developed (27.9 ng per milliliter and 20.3 ng per milliliter, respectively;  $P=0.06$ ), nor did the ratios of sFlt1:PIGF (31.4 and 26.2, respectively;  $P=0.06$ ). However, these factors did differ significantly between women who subsequently had term preeclampsia with small-for-gestational-age infants and those who had term preeclampsia without small-for-gestational-age infants (39.8 ng per milliliter and 19.2 ng per milliliter for soluble endoglin, respectively;  $P<0.001$ ; 57.4 and 22.2 for sFlt1:PIGF, respectively;  $P<0.001$ ). Soluble endoglin values and sFlt1:PIGF ratios obtained at 33 through 42 weeks were substantially lower in controls (11.4 ng per milliliter and 9.7 ng per milliliter, respectively) than in all the term-preeclampsia subtypes described above ( $P<0.001$  for all comparisons). Two women with preterm HELLP

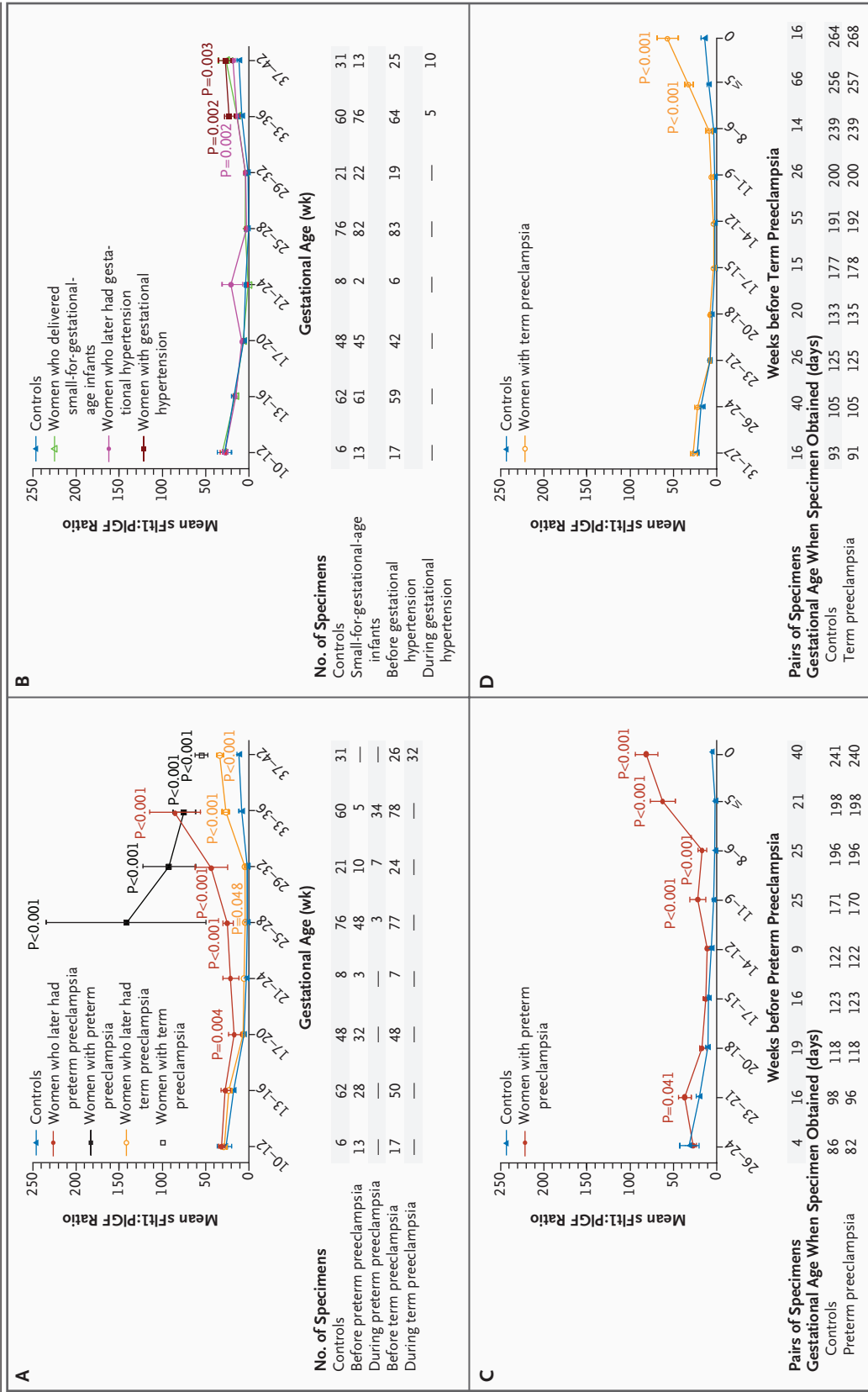


syndrome had higher soluble endoglin levels and a greater sFlt1:PlGF ratio than did controls and women with preterm preeclampsia without the HELLP syndrome.

**EFFECTS OF SMOKING ON CIRCULATING ANGIOGENIC PROTEINS**

Since smoking is associated with a reduced risk of preeclampsia,<sup>29,30</sup> we examined whether smok-

ing was associated with levels of angiogenic factors in controls. Levels of sFlt1 were lower throughout gestation in women who reported smoking during pregnancy or having quit smoking after the last menstrual period but before enrollment in the study than in women who reported that they had never smoked or had quit before the last menstrual period (1112 pg per milliliter vs. 1412 pg per milliliter at 10 through 20 weeks,  $P=0.03$ ;



**Figure 3.** Mean sFlt1:PIGF Ratios According to Weeks of Gestation and Weeks before the Onset of Preeclampsia.

Panel A shows the mean sFlt1:PIGF ratios before and after the onset of clinical preeclampsia according to the weeks of gestation among the same women depicted in Figure 1A. The difference, after logarithmic transformation, between the specimens obtained at 25 through 28 weeks from women who already had clinical preeclampsia that began before 37 weeks of gestation and those who later had preterm preeclampsia was also significant ( $P=0.04$ ). One specimen obtained at 21 through 24 weeks and one at 37 through 42 weeks after the onset of preterm preeclampsia, with values of 164.1 and 13.0, respectively, are not shown. Panel B shows the mean sFlt1:PIGF ratios according to the weeks of gestation before and after the onset of gestational hypertension in the same specimens shown in Figure 1B; Panel C shows the mean sFlt1:PIGF ratios in the same specimens shown in Figure 2A according to the number of weeks before the onset of preterm preeclampsia. Panel D shows the mean sFlt1:PIGF ratios in the same specimens shown in Figure 2B according to the number of weeks before the onset of term preeclampsia.

978 pg per milliliter vs. 1543 pg per milliliter at 21 through 32 weeks,  $P < 0.001$ ; and 2119 pg per milliliter vs. 2823 pg per milliliter at 33 through 42 weeks,  $P = 0.02$ ). As compared with women who reported not smoking during pregnancy, those who reported smoking had lower soluble endoglin levels (5.7 ng per milliliter vs. 6.8 ng per milliliter,  $P = 0.004$ ) and higher PlGF levels (200 pg per milliliter vs. 139 pg per milliliter,  $P = 0.007$ ), but only at 10 through 20 weeks.

#### MULTIVARIABLE ANALYSIS OF SOLUBLE ENDOGLIN LEVELS AND sFlt1:PlGF RATIOS AND THE RISK OF PREECLAMPSIA

We computed adjusted odds ratios and 95% confidence intervals (CIs) for preeclampsia in the highest quartile of the distribution of control soluble endoglin levels with respect to the lower three quartiles after adjustment for race or ethnic group, body-mass index, and gestational age at specimen collection (Table 2). Substantial increases in the risk of preterm and term preeclampsia were observed only close to the onset of disease in quartile 4 as compared with the other three quartiles, at 21 through 32 weeks (adjusted odds ratio, 9.4; 95% CI, 4.3 to 20.7) and at 33 through 42 weeks (adjusted odds ratio, 7.0; 95% CI, 3.4 to 14.4), respectively. Similarly, women in the highest quartile of the ratio of sFlt1:PlGF had a substantially increased risk of preterm preeclampsia at 21 through 32 weeks and term preeclampsia at 33 through 42 weeks. Large increases in the risk of preeclampsia with a small-for-gestational-age infant at 21 through 32 weeks and at 33 through 42 weeks were associated with the highest quartile of soluble endoglin or the ratio of sFlt1:PlGF.

Levels of soluble endoglin and the sFlt1:PlGF ratios tended to track together, with correlation coefficients among controls, among women with subsequent preterm preeclampsia, and among women with subsequent term preeclampsia of 0.38, 0.59, and 0.53, respectively. Nevertheless, in multivariable models that included both log-transformed soluble endoglin levels and log-transformed sFlt1:PlGF ratios, as well as race or ethnic group, body-mass index, and gestational age at specimen collection, soluble endoglin levels and the ratios of sFlt1:PlGF were each associated with preeclampsia. The adjusted odds ratio for preterm preeclampsia in specimens obtained at 21 through 32 weeks was 16.2 (95% CI, 3.8 to 68.9) for a one-unit increase of soluble endoglin on the logarithmic scale adjusted for log sFlt1:

PlGF and 2.7 (95% CI, 1.4 to 5.2) for a one-unit increase of sFlt1:PlGF on the logarithmic scale adjusted for log soluble endoglin. Corresponding values for term preeclampsia at 33 through 42 weeks were 5.4 (95% CI, 2.2 to 13.3) and 2.2 (95% CI, 1.4 to 3.5), respectively. Furthermore, the log-likelihood test showed a significant improvement ( $P < 0.001$ ) in the fit of the multivariable model when both the sFlt1:PlGF ratios and soluble endoglin levels were included, as compared with either one alone.

Because experimental data indicated an interaction between the soluble endoglin and sFlt1 pathways,<sup>22</sup> we performed the quartile analysis (Table 2) using the ratio of sFlt1 plus soluble endoglin to PlGF as a measure of the balance between antiangiogenic and proangiogenic proteins. Large increases in the risk of preterm preeclampsia (adjusted odds ratio, 6.1; 95% CI, 2.4 to 15.4) and the risk of preeclampsia and a small-for-gestational-age infant (adjusted odds ratio, 8.1; 95% CI, 2.6 to 24.8) were observed at 13 through 20 weeks of gestation for women in the highest quartile as compared with the lower three quartiles. Still larger increases in the risk of these conditions were observed at 21 through 32 weeks (adjusted odds ratio, 16.0; 95% CI, 6.7 to 38.0; and adjusted odds ratio, 18.5; 95% CI, 6.1 to 55.4, respectively).

Finally, we examined the risk among women with high or low (highest or lower quartiles) levels of soluble endoglin, ratios of sFlt1:PlGF, or both. We used women with low levels of soluble endoglin and low sFlt1:PlGF ratios as the reference group and adjusted for race or ethnic group, body-mass index, and gestational age at the time of specimen collection. Women with high levels of a single biomarker usually had only small elevations in the risk of preterm or term preeclampsia in specimens obtained at 21 through 32 weeks or at 33 through 42 weeks (Fig. 4). However, among women with high levels of both, the risk of preeclampsia was high (adjusted odds ratio at 21 through 32 weeks, 31.6; 95% CI, 10.7 to 93.4; and adjusted odds ratio at 33 through 42 weeks, 30.8; 95% CI, 10.8 to 87.6). Most women in whom preterm preeclampsia developed had high levels of both biomarkers.

## DISCUSSION

Our findings are consistent with a role for soluble endoglin in the pathogenesis and prediction

**Table 2.** Odds Ratios for Preterm or Term Preeclampsia or Preeclampsia with a Small-for-Gestational-Age Infant According to Quartile of Soluble Endoglin Level, sFlt1:PIGF Ratio, and the Ratio of (sFlt1 + Soluble Endoglin):PIGF.\*

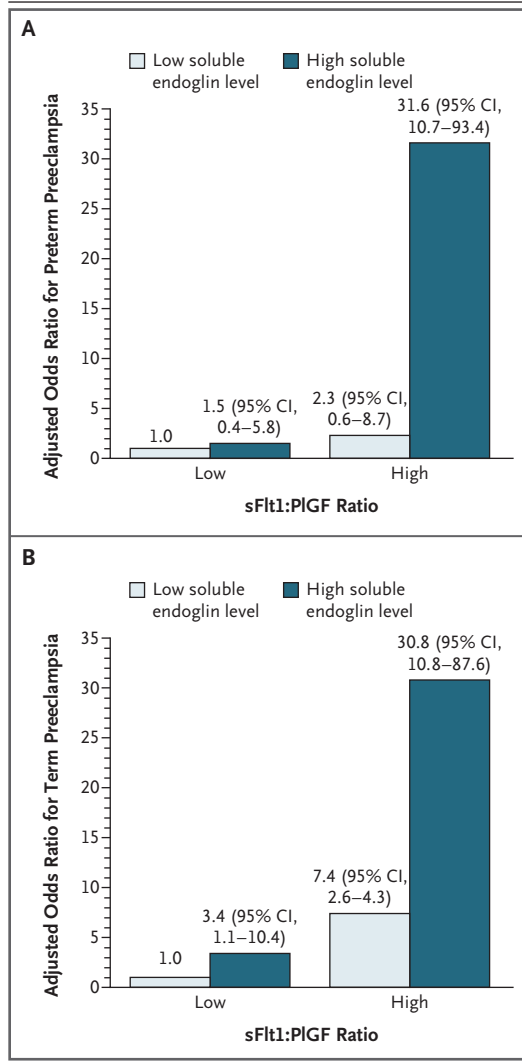
Quartile	No. of Controls	Preterm Preeclampsia		Term Preeclampsia		Preeclampsia and Small-for-Gestational-Age Infants	
		No. of Women	Odds Ratio (95% CI)	No. of Women	Odds Ratio (95% CI)	No. of Women	Odds Ratio (95% CI)
<b>Soluble endoglin</b>							
13–20 Wk of gestation							
Quartile 4: >7.9 ng/ml	27	23	2.2 (1.1–4.6)	25	1.1 (0.6–2.3)	13	1.7 (0.7–4.1)
Quartile 1–3: ≤7.9 ng/ml	83	35	1.0	73	1.0	22	1.0
21–32 Wk of gestation							
Quartile 4: >7.2 ng/ml	27	43	9.4 (4.3–20.7)	45	2.6 (1.4–4.8)	25	8.7 (3.4–21.9)
Quartile 1–3: ≤7.2 ng/ml	78	15	1.0	59	1.0	9	1.0
33–42 Wk of gestation							
Quartile 4: >13.6 ng/ml	22	5	—	62	7.0 (3.4–14.4)	14	40.7 (6.7–246.1)
Quartile 1–3: ≤13.6 ng/ml	67	0	—	37	1.0	2	1.0
<b>sFlt1:PIGF ratio</b>							
13–20 Wk of gestation							
Quartile 4: >16.9	28	20	2.5 (1.0–6.0)	30	1.9 (0.9–4.2)	12	2.0 (0.7–5.5)
Quartile 1–3: ≤16.9	82	38	1.0	68	1.0	23	1.0
21–32 Wk of gestation							
Quartile 4: >3.4	27	44	12.6 (5.3–30.3)	39	1.7 (0.9–3.2)	26	9.9 (3.8–26.0)
Quartile 1–3: ≤3.4	78	14	1.0	65	1.0	8	1.0
33–42 Wk of gestation							
Quartile 4: >11.9	22	5	—	69	12.3 (5.5–27.2)	15	73.5 (7.9–679.9)
Quartile 1–3: ≤11.9	67	0	—	30	1.0	1	1.0
<b>Ratio of (sFlt1 + soluble endoglin):PIGF</b>							
13–20 Wk of gestation							
Quartile 4: >110.7	27	28	6.1 (2.4–15.4)	33	2.4 (1.1–5.4)	19	8.1 (2.6–24.8)
Quartile 1–3: ≤110.7	83	30	1.0	65	1.0	16	1.0
21–32 Wk of gestation							
Quartile 4: >18.6	26	48	16.0 (6.7–38.0)	52	3.1 (1.7–5.8)	29	18.5 (6.1–55.4)
Quartile 1–3: ≤18.6	79	10	1.0	52	1.0	5	1.0
33–42 Wk of gestation							
Quartile 4: >61.9	22	5	—	66	8.3 (4.0–17.3)	16	—
Quartile 1–3: ≤61.9	67	0	—	33	1.0	0	—

\* Controls were women who were normotensive during pregnancy and who delivered appropriately sized or large-for-gestational-age infants. Odds ratios were adjusted for gestational age, race or ethnic group (non-Hispanic whites, Hispanic whites or other or unknown, or blacks), and body-mass index. Quartiles were determined on the basis of specimens from the controls. Specimens were obtained from all women before the onset of clinical signs of preeclampsia. For the odds ratios shown in the highest quartile (quartile 4), the reference category was the lower three quartiles. Dashes denote situations in which odds ratios could not be computed, since all specimens from women with preeclampsia were in the highest quartile.

of preeclampsia. Serum levels of soluble endoglin rose during the last two months of normal pregnancy. Levels of soluble endoglin rose earlier and more steeply in women in whom preeclampsia developed, reaching a peak at the onset of clinical disease. Elevations in soluble endoglin were particularly pronounced — therefore, potentially most useful for prediction — among women in whom preterm preeclampsia developed or women in whom preeclampsia developed and

**Figure 4. Adjusted Odds Ratios for Preterm (Panel A) or Term (Panel B) Preeclampsia According to sFlt1:PIGF Ratios and Soluble Endoglin Levels.**

Panel A shows the adjusted odds ratios and 95% CIs for preterm preeclampsia after adjustment for race or ethnic group (non-Hispanic white, Hispanic white or other or unknown, or black), body-mass index, and gestational age at specimen collection in serum obtained at 21 to 32 weeks of gestation with respect to the reference group of specimens from women with low values for soluble endoglin and low sFlt1:PIGF ratios. High values are values for the highest quartile of the distribution of control specimens. Low values are values for the three lower quartiles. The numbers of specimens among women with high values for both measures, high sFlt1:PIGF ratios but low soluble endoglin values, low sFlt1/PIGF ratios but high soluble endoglin values, or low values for both measures were as follows: 11, 16, 16, and 62, respectively, among controls, and 39, 5, 4, and 10, respectively, among women who later had preterm preeclampsia. A formal test for interaction between the sFlt1:PIGF ratios and soluble endoglin levels with the use of these cutoff points was significant ( $P=0.02$ ). Panel B shows the adjusted odds ratios and 95% CIs for term preeclampsia in serum obtained at 33 to 42 weeks of gestation with respect to the reference group of specimens from women with low values for soluble endoglin and low sFlt1:PIGF ratios. The numbers of specimens among women with high values for both measures, high sFlt1:PIGF ratios but low values of soluble endoglin, low sFlt1:PIGF ratios but high values of soluble endoglin, or low values of both measures were as follows: 9, 13, 13, and 54, respectively, among controls, and 51, 18, 11, and 19, respectively, among women who later had term preeclampsia. A formal test for interaction between the sFlt1:PIGF ratios and soluble endoglin levels with the use of these cutoff points was not significant ( $P=0.79$ ). In both panels, specimens were obtained from all women before the onset of clinical signs of preeclampsia.



who had a small-for-gestational-age infant. Women with soluble endoglin levels in the highest quartile of the control distribution at 21 through 32 weeks had an increased risk of preterm preeclampsia, women with soluble endoglin levels in the highest quartile of the control distribution at 33 through 42 weeks had an increased risk of term preeclampsia, and at both intervals had an increased risk of preeclampsia with a small-for-gestational-age infant.

The data can be interpreted to imply that soluble endoglin levels and the sFlt1:PIGF ratio both contribute to the pathogenesis of preeclampsia. Although the gestational pattern of the soluble endoglin level tended to parallel the trajectory of the sFlt1:PIGF ratio, multivariate analysis indicated that each was associated with preeclamp-

sia. Indeed, a composite measure incorporating all three molecules — the ratio of (sFlt1+soluble endoglin):PIGF — was more strongly predictive of preeclampsia than were individual biomarkers. Furthermore, analyses of soluble endoglin levels and sFlt1:PIGF ratios with the use of cutoff points suggested that an interaction did exist, and formal testing for modification of effects was significant with respect to preterm preeclampsia. Elevations of soluble endoglin alone did not seem to be sufficient for the development of the syndrome. In women with term preeclampsia, soluble endoglin levels were significantly higher beginning about 3 months before hypertension or proteinuria developed, whereas the sFlt1:PIGF ratio rose much closer to the onset of clinical disease. The women who were normotensive during

pregnancy and had a small-for-gestational-age infant were characterized by small, early, sustained elevations in soluble endoglin without an increase in the sFlt1:PlGF ratio. Together these observations suggest that both elevated levels of soluble endoglin and increased sFlt1:PlGF ratios may best predict the onset of preeclampsia. Consistent with renal-biopsy studies,<sup>31,32</sup> gestational hypertension seemed to be a mild form of term preeclampsia, with similar elevations of soluble endoglin but smaller increases in the sFlt1:PlGF ratio.

Serum sFlt1 levels were lower in smokers than in nonsmokers throughout normal pregnancy, confirming the findings of others,<sup>11,33,34</sup> and levels of soluble endoglin were lower and PlGF higher at 10 through 20 weeks. We speculate that smoking may protect against preeclampsia through effects of nicotine on angiogenic proteins.<sup>35,36</sup> Moreover, the rise in sFlt1 and soluble endoglin levels during the last 2 months of normal pregnancy may explain the increasing incidence of preeclampsia with advancing gestational age.

In summary, circulating levels of soluble endoglin increase markedly beginning 2 to 3 months before the onset of preeclampsia, accompanied by increases in the sFlt1:PlGF ratio. Taken together with experimental evidence in rodents,<sup>22</sup> these data suggest that circulating soluble endoglin and sFlt1, each of which causes endothelial

dysfunction by a different mechanism, may both contribute to the syndrome of preeclampsia. Prospective longitudinal studies are needed to assess whether these biomarkers can predict the imminent onset of clinical disease.

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Drs. Maynard and Karumanchi report being named coinventors on multiple provisional patents that have been filed by Beth Israel Deaconess Medical Center for the diagnosis and treatment of preeclampsia. These patents have been nonexclusively licensed to several companies. Dr. Karumanchi reports having served as a consultant to Abbott, Beckman Coulter, and Johnson & Johnson. Dr. Thadhani reports being named a coinventor on a provisional patent filed by the Massachusetts General Hospital on the role of sex hormone-binding globulin and PlGF for the prediction of preeclampsia that has been licensed to several companies and having served as a consultant to Abbott, Beckman Coulter, and Johnson & Johnson. Dr. Sachs reports having served as a consultant to Johnson & Johnson. No other potential conflict of interest relevant to this article was reported.

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#### APPENDIX

The following were members of the CPEP Study Group: *University of Alabama at Birmingham* — J.C. Hauth, R. Goldenberg, B.S. Stofan; *University of New Mexico at Albuquerque* — L.B. Curet, G.M. Joffe, V. Dorato; *University of Tennessee at Memphis* — B.M. Sibai, S.A. Friedman, B.M. Mercer, T. Carr; *Case Western Reserve University at MetroHealth Medical Center, Cleveland* — P.M. Catalano, A.S. Petruilis, L. Barabach; *Oregon Health Sciences University, Portland* — C. Morris, S.-L. Jacobson, K. McCracken; the *Emmes Corp., Rockville, MD* — J.R. Esterlitz, M.G. Ewell, D.M. Brown; *National Institute of Child Health and Human Development* — R.J. Levine, R. DerSimonian, J.D. Clemens, M.A. Klebanoff, E.G. Raymond, J.G. Rigau-Perez, H. Shifrin; *National Heart, Lung, and Blood Institute* — J.A. Cutler, D.E. Bild; and *Data and Safety Monitoring Board* — M. Lindheimer, C. Begg, T. Chalmers, M. Druzin, R. Sokol.

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**CORRECTION**

**Soluble Endoglin and Other Circulating  
Antiangiogenic Factors in Preeclampsia**

Soluble Endoglin and Other Circulating Antiangiogenic Factors in Preeclampsia . On page 1003, in Figure 4B, the 95% confidence interval of the adjusted odds ratio for term preeclampsia among women with a low value of soluble endoglin but a high sFlt1:PlGF ratio should have been "2.6–21.3," not "2.6–4.3" as printed.