The etiology of preeclampsia is one of the major unsolved mysteries in obstetrics. Because vascular changes are a prominent feature of this condition, numerous investigations during the past decade have studied the possibility that preeclampsia is causally linked to an imbalance in the formation of prostacyclin (PGI₂), a vasodilator, and thromboxane A₂ (TXA₂), a vasoconstrictor.¹⁻⁸ The results of these studies, however, have been conflicting and confusing. One problem is that assessment of eicosanoid formation was usually performed after the onset of symptoms and, in some instances, after initiation of treatment. Thus, it is difficult, if not impossible, to determine whether changes in PGI₂ or TXA₂ are the cause or the result of the disease (or even its therapy). Prospective studies have been carried out in an attempt to circumvent the problem, but the number of women

Context An imbalance in vasodilating (prostacyclin [PGI₂]) and vasoconstricting (thromboxane A₂ [TXA₂]) eicosanoids may be important in preeclampsia, but prospective data from large studies needed to resolve this issue are lacking. Because most trials using aspirin to reduce TXA₂ production have failed to prevent preeclampsia, it is critical to determine whether eicosanoid changes occur before the onset of clinical disease or are secondary to clinical manifestations of preeclampsia.

Objective To determine whether PGI₂ or TXA₂ changes occur before onset of clinical signs of preeclampsia.

Design, Setting, and Participants Multicenter prospective study from 1992 to 1995 of subjects from the placebo arm of the Calcium for Preeclampsia Prevention Trial. Women who developed preeclampsia (n = 134) were compared with matched normotensive control women (n = 139).

Main Outcome Measures Excretion of urinary metabolites of PGI₂ (PGI-M) and TXA₂ (TX-M) as measured from timed urine collections obtained prospectively before 22 weeks', between 26 and 29 weeks', and at 36 weeks' gestation.

Results Women who developed preeclampsia had significantly lower PGI-M levels throughout pregnancy, even at 13 to 16 weeks' gestation (long before the onset of clinical disease); their gestational age-adjusted levels were 17% lower than those of controls (95% confidence interval [CI], 6%-27%; P = .005). The TX-M levels of preeclamptic women were not significantly higher overall (9% higher than those of controls; 95% CI, −3% to 23%; P = .14). The ratio of TX-M to PGI-M, used to express relative vasoconstricting vs vasodilating effects, was 24% higher (95% CI, 6%-45%) in preeclamptic women throughout pregnancy (P = .007).

Conclusions Our results show that reduced PGI₂ production, but not increased TXA₂ production, occurs many months before clinical onset of preeclampsia. Aspirin trials may have failed because an increase in thromboxane production is not the initial anomaly. Future interventions should make correcting prostacyclin deficiency a major part of the strategy to balance the abnormal vasoconstrictor-vasodilator ratio present in preeclampsia.

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who developed preeclampsia was small, limiting the power of these studies to detect differences.

Despite the lack of definitive evidence that thromboxane (Tx) levels are elevated in preeclampsia, aspirin, which reduces levels of Tx, has been used in preeclampsia prevention trials. The results generally have been disappointing.

To determine whether alterations in production of PGI₂ and TxA₂ are important in the pathophysiology of preeclampsia, we conducted a prospective study using the 2294 women enrolled in the placebo group of the National Institute of Child Health and Human Development Calcium for Pre-eclampsia Prevention Trial (CPEP). This cohort provided a sufficiently large number of women who later developed preeclampsia to detect abnormalities in TxA₂ or PGI₂ production long before onset of clinical symptoms and signs.

**METHODS**

**CPEP Trial Design**

CPEP methods have been published in detail elsewhere. Between April 1992 and March 1995, nulliparous women between 11 and 21 gestational weeks were screened, and follow-up continued until September 30, 1995. Those who were diagnosed as having conditions that could markedly influence the study endpoints, affect the absorption or metabolism of calcium, make the use of calcium inappropriate, or impede compliance were dropped. Eligible women then underwent a single-blind test of compliance with a placebo. Those who took at least 75% of the tablets were enrolled if they: (1) had blood pressures consistently below 135/85 mm Hg; (2) had a dipstick test for urinary protein with negative or trace results; and (3) were between 13 and 21 weeks pregnant determined by ultrasonography.

Subjects were randomly assigned to receive either calcium supplements or placebo tablets. For this analysis, only women who received the placebo were included to eliminate any possible effect of calcium on eicosanoid production. Other than prenatal vitamins, acetaminophen, and a non–calcium-containing antacid, which were provided to participants, women were instructed not to take vitamin supplements, analgesics, or antacids. The study protocol was approved by the institutional review boards at the 5 participating medical centers, and all women gave written informed consent.

**Specimen Collection**

All women participating in the CPEP trial were asked to provide 24-hour urine specimens at enrollment (13 to 21 weeks), between 26 and 29 weeks', and at 36 weeks' gestation. When a woman was found to have developed hypertension or proteinuria, another specimen was collected if possible. All samples were frozen, sent to the central CPEP repository, and stored at −70°C. Specimens were rejected for the study if they contained visible blood (n = 3), if the total volume was less than 400 mL or the collection period was less than 10 hours (n = 22), or if rupture of membranes occurred before collection was complete, unless the specimen was collected by catheter (n = 11) (see “Diagnosis of Preeclampsia” section). In all, 11% of specimens from preeclamptic women and 6% of specimens from controls were rejected.

**Diagnosis of Preeclampsia**

Women were evaluated for preeclampsia by a trained staff member every 4 weeks through the 29th week of gestation, every 2 weeks through the 35th week, and weekly thereafter. Medical records of all outpatient visits and hospitalizations were reviewed for blood pressure and urine protein. Women were not asked to perform any self-diagnosis. A preliminary diagnosis of preeclampsia was made by the principal investigator after reviewing the medical data. The final diagnosis was determined by 3 researchers working independently, assisted by a computer algorithm. All women with a preliminary diagnosis were considered potential cases; those in whom the diagnosis was not confirmed were dropped.

Preeclampsia was defined as pregnancy-associated hypertension (diastolic blood pressure ≥ 90 mm Hg on 2 occasions 4 to 168 hours apart) and pregnancy-associated proteinuria (≥ 300 mg of protein in a 24-hour urine specimen, or 2 urine specimens 4 to 168 hours apart containing ≥ 1+ protein by dipstick measurement, or a single urine specimen with a protein-creatinine ratio of ≥ 0.35 or containing ≥ 2+ protein by dipstick). Collection of samples via catheter was mandatory after rupture of membranes or in the presence of vaginitis. Severe preeclampsia was defined as preeclampsia with a diastolic blood pressure of at least 110 mm Hg or severe proteinuria (≥ 3.5 g per 24 hours or ≥ 3+ by dipstick on 2 occasions). Eclampsia and HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) were also counted as severe preeclampsia.

**Control Selection**

Controls were randomly selected from women with no preliminary diagnosis of preeclampsia or any related disorder (gestational hypertension or proteinuria). Also, controls were frequency-matched to cases (using random numbers) by study center and date of last menstrual period within 1 calendar year. Subjects were matched on study center because the preeclampsia rates differed significantly between centers, perhaps due to differences in the pathophysiology of the disease. In addition, the centers may have used slightly different procedures for collecting, preparing, and storing specimens. The rationale for matching by last menstrual period was to ensure that the duration of storage of specimens would be comparable in cases and controls. One control was selected per case by random number.

Because women did not always provide specimens on schedule, specimens collected before 154 days were classified as “early (baseline),” between 154 and 237 days as “middle,” and after 237 days as “late.” To avoid potential problems with deterioration of urine specimens over time, samples were selected...
EICOSANOIDS AND PREECLAMPSIA

Table. Subject Characteristics at Enrollment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 134)</th>
<th>Controls (n = 139)</th>
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</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>21 (5)</td>
<td>20 (4)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163 (7)</td>
<td>162 (7)</td>
</tr>
<tr>
<td>Weight, kg†</td>
<td>75 (21)</td>
<td>65 (14)</td>
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<tr>
<td>Body mass index, kg/m²†</td>
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<td>25 (5)</td>
</tr>
<tr>
<td>Upper-arm circumference, cm†</td>
<td>20 (5)</td>
<td>27 (4)</td>
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<tr>
<td>Blood pressure, mm Hg†</td>
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<td></td>
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<tr>
<td>Systolic</td>
<td>110.9 (8.0)</td>
<td>106.9 (8.5)</td>
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<tr>
<td>Diastolic</td>
<td>62.7 (7.3)</td>
<td>60.0 (7.2)</td>
</tr>
<tr>
<td>Week of gestation</td>
<td>16.9 (2.6)</td>
<td>17.3 (2.3)</td>
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<td>Previous abortion, %</td>
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<td>16</td>
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<tr>
<td>Race, %</td>
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<td>13</td>
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<tr>
<td>Black including black</td>
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<td>53</td>
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<tr>
<td>Hispanic</td>
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<td>Other or unknown</td>
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<td>1</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>8</td>
<td>6</td>
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<tr>
<td>Highest grade of school</td>
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<td>11 (2)</td>
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<tr>
<td>completed</td>
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<td></td>
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<tr>
<td>Never married, %</td>
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<td>76</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SD) unless noted otherwise. †P < .001 by the Wilcoxon 2-sample test.

Data were collected, on the average, 150 days before diagnosis.

FIGURE 1 shows the distribution and mean levels of PGI-M, Tx-M, and Tx-M/PGI-M ratio values in preeclampsia cases and controls in the baseline, middle, and late time periods. Levels of PGI-M were significantly lower in the preeclampsia group in the baseline period. Levels of Tx-M were significantly higher in the preeclampsia group in the late period. The vasoconstrictor-vasodilator ratio (Tx-M/PGI-M) was significantly higher in the preeclampsia group at both the baseline and late periods (P = .04 and P = .01, respectively) and of borderline significance at the middle period (P = .10).

It is important to note that it is not possible to take into account several important factors in this crude analysis, namely, gestational age strongly influences both PGI-M and Tx-M levels, and crude data do not reflect changes over time in individual women. Therefore, the mixed model was used to account for the effect of gestational age and longitudinal changes in values from the same subject.

The preeclampsia and control groups’ PGI-M levels are shown by 4-week intervals in Figure 2A. The mixed model demonstrates that the preeclampsia group had lower PGI-M levels at virtually all intervals. The preeclampsia group had an overall decrease of 17% (95% confidence interval [CI], 6%–27%) in PGI-M levels (P = .005) compared with the control group. In particular, the preeclampsia group had a significantly lower level (P = .03) at weeks 13 to 16, long before the onset of clinical disease.

Levels of Tx-M are shown in Figure 2B. Contrary to what has been reported in the literature, the preeclampsia group had significantly lower levels (P = .01) of Tx-M in the earliest time period (13-16 weeks). This may have been a chance
event, because the preeclampsia group had higher Tx-M levels in each subsequent time interval. Over the entire study period, the preeclampsia group had a median Tx-M level 9% higher (95% CI, −3 to 23%) than controls, but the difference was not significant (P = .14). If one wishes to argue that the early levels were a statistical accident, or that changes in Tx-M occur later in pregnancy, it can be said that beyond the baseline period (gestational age greater than 21 weeks) the preeclampsia group had a 15% higher median Tx-M (95% CI, 1%-31%) than controls. The difference was significant.
As shown in Figure 2, B, Tx-M levels clearly increased more rapidly in the preeclampsia group. The preeclampsia group had a consistently higher Tx-M/PGI-M ratio after 16 weeks, indicating a predominant vasoconstrictor effect (Figure 2, C). The preeclampsia group showed an overall increase of 24% (95% CI, 6-45%) compared with the control group ($P = .007$).

Next we compared women with severe preeclampsia with the other women in the preeclampsia group. Levels of PGI-M and Tx-M and the Tx-M/PGI-M ratio were not significantly different in the baseline, middle, or late time periods or overall using the mixed procedure.

**Samples Collected After Onset**

Many studies have reported eicosanoid values obtained after onset of clinical preeclampsia. To determine whether such values reflect the preclinical state, we also examined Tx-M and PGI-M levels in 51 specimens obtained after onset of clinical preeclampsia. Levels of TxM were significantly higher ($P < .001$) in specimens collected after rupture of membranes ($n = 28$) (age-adjusted mean, 2208 pg/mg of creatinine) than in those collected before ($n = 23$) (age-adjusted mean, 735 pg/mg of creatinine). Women treated with magnesium sulfate ($n = 20$) also had significantly higher levels ($P < .001$) than women who were untreated at the time of collection ($n = 31$) (1998 vs 1097 pg/mg of creatinine, respectively). The group that had rupture of membranes had a significantly increased Tx-M/PGI-M ratio (1.1) compared with those who had intact membranes (0.7) ($P = .018$). Those who were treated with magnesium sulfate had a significantly higher ratio (1.7) than those who were untreated (0.6) ($P = .002$). Results for PGI-M were inconsistent; magnesium sulfate treatment was associated with lower PGI-M levels if membranes were ruptured and higher levels if membranes were intact.

It should be emphasized that there could have been major differences in the clinical condition of the treated and untreated groups (eg, clinicians are more...

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**Figure 2. Changes in Eicosanoid Levels in Preeclampsia Cases and Controls**

(A) Prostacyclin metabolite (PGI-M) levels. B, Thromboxane metabolite (Tx-M) levels. C, Ratio of vasoconstricting (Tx-M) to vasodilating (PGI-M) eicosanoids. The percentage by which cases differed from controls is shown for each gestational age. The arrows indicate whether case values were higher or lower than control values. CI indicates confidence interval.
likely to treat severe cases and those in whom delivery is not imminent) and that samples were available for only a small proportion of study subjects. These findings illustrate the difficulty of using samples obtained after onset of clinical disease.

**Possible Confounding Factors**

To determine whether the results could be due to underlying differences in the 2 groups, particularly differences in initial blood pressure, we adjusted for factors in which the groups differ significantly (Table). Adjusting for baseline systolic and diastolic blood pressure, weight, body mass index, and arm circumference, either individually or together, did not change the results. Levels of PGI-M remained significantly lower in preeclamptic women for all adjustments ($P<.02$ for all). Similarly, the Tx-M/PGI-M ratio remained significantly higher in the preeclamptic patients ($P<.03$ for all). No adjustments produced a significant association between preeclampsia and Tx-M levels. Thus, the association between preeclampsia and PGI-M was not explained by underlying differences in the study groups.

**CONCLUSION**

Preeclampsia remains a major cause of morbidity and mortality in pregnancy. Preventive efforts using aspirin or calcium supplements have produced mixed, but generally disappointing, results.11,17-21 Although interest in the role of eicosanoids is high, research has been hampered by the lack of prospectively collected data for large numbers of affected women.

This is by far the largest prospective study of preeclampsia and eicosanoids performed to date. Because of the large number of specimens available over the course of pregnancy, we were able to document changes in PGI$_2$, Tx, and Tx/PGI$_2$ ratios many months before the onset of signs in women who would eventually develop preeclampsia.

Women who developed preeclampsia had significantly lower PGI$_2$ production and higher Tx$_A$/PGI$_2$ ratios, indicative of vasoconstriction beginning in the second trimester of pregnancy and persisting throughout. Women who developed preeclampsia did not have significantly higher Tx levels overall, yet they did have significantly higher Tx levels if only the latter part of pregnancy (after 21 weeks) is considered, suggesting that the rise in Tx levels could be a secondary event.

Our findings suggest that a deficiency in PGI$_2$ production is an early and important event in women who develop preeclampsia. This is associated with an early increase in the vasoconstrictor-vasodilator (Tx$_A$/PGI$_2$) ratio. While it is not yet clear whether PGI$_2$ deficiency is the primary cause of preeclampsia, our results indicate that endothelial dysfunction may be a critical factor because of the role of the endothelium in PGI$_2$ production.

Our findings may explain the failure of trials of low-dose aspirin to prevent preeclampsia. These trials assumed that platelet hyperactivity, mediated by excess Tx, produced preeclampsia. Low-dose aspirin is known to reduce Tx production by selective inhibition of platelet eicosanoid production with sparing of PGI$_2$ production by the vascular endothelium. Our data show that decreased PGI$_2$, rather than increased Tx, is the earlier, probably more important, eicosanoid disruption of preeclampsia, and Tx elevation is a later, perhaps secondary event. Thus, it is not surprising that reducing Tx did not prevent preeclampsia.

Previous studies of prostacyclin and thromboxane metabolites in preeclampsia have produced inconsistent results. While several studies reported significantly lower PGI$_2$ levels in preeclamptic subjects,1-5 the only long-term prospective study6 found no difference. For Tx$_A$, some studies found no difference between patients with and without preeclampsia,1-3,6-8 while others have found significantly higher levels in preeclamptic patients.7,8 Reasons for these discordant results are easy to find. The problems with collecting data after onset of symptoms have been noted; indeed, our own analysis of samples collected after rupture of membranes and treatment produced uninformative results. The problem with low power in some prospective studies has also been noted previously and could account for the difference between their findings and ours.

In summary, this large, prospective study demonstrated that, in women who develop preeclampsia, PGI$_2$ production is significantly decreased and the vasoconstrictor-vasodilator ratio is significantly increased many months before signs appear. Clinically, these findings are important because they suggest that the primary therapeutic strategy for preventing the complications of preeclampsia should be restoring PGI$_2$ homeostasis; reducing Tx production might be a secondary goal to assist in reducing the elevated vasoconstrictor-vasodilator ratio. Long-term infusion of PGI$_2$ has proved useful in treating endothelial dysfunction and vasoconstriction in primary pulmonary hypertension and pulmonary complications of connective tissue disorders.22,23 Future trials should explore the use of PGI$_2$ or PGI$_2$ analogs in the prevention and treatment of preeclampsia.

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**Acknowledgment:** We thank Patricia Moyer, BS, of the National Institute of Child Health and Human Development for technical support.

**REFERENCES**


5. Yamaguchi M, Mori N. 6-Keto prostaglandin F$_1$, thromboxane B$_2$, and 13,14-dihydro-15-keto prostaglandin F concentrations of normotensive and pre-


It is more than likely that if men were ever to lose the appetite for meaning which we call thinking, and cease to ask unanswerable questions, they would lose not only the ability to produce those thought-things which we call works of art but also the capacity for asking all the unanswerable questions upon which every civilization is founded.

—Hannah Arendt (1906-1975)