Time Course of Maternal Plasma Volume and Hormonal Changes in Women With Preeclampsia or Fetal Growth Restriction

Sofía P. Salas, Guillermo Marshall, Blanca L. Gutiérrez, Pedro Rosso

Abstract—We tested the hypothesis that women with idiopathic fetal growth restriction (FGR) or preeclampsia (PE) have lower concentrations of some water-retaining hormones, such as aldosterone and estradiol, either preceding or concomitant with the onset of the reduced plasma volume described in these women. Plasma volume and serum concentrations of estradiol, progesterone, and aldosterone were measured serially at monthly intervals in 135 pregnant women from week 10 until term. Twenty-three developed idiopathic FGR, 17 had PE, and 95 remained normotensive and delivered normal-size infants (controls). Changes over time for each variable were studied using mixed models. Maternal age, parity, and weight/height at term were similar in all of the groups. Birth weight, body length, and ponderal index were lower in FGR and PE than in controls. Plasma volume increased throughout pregnancy in controls but was lower in FGR and PE from week 14 to 17 until term. Aldosterone values were lower in PE from week 26 to 29 onwards and in FGR after week 34. Progesterone concentrations were higher in PE than either control or FGR from week 18 to 21 until term. In contrast, FGR pregnancies had reduced progesterone and estradiol concentrations after week 34. Progesterone:estradiol ratio was significantly higher only in the PE group. In mothers with idiopathic FGR or PE, less expansion in plasma volume occurred before alterations in hormonal concentrations. We speculate that the early rise in progesterone may have a pathogenic role in the development of preeclampsia. (Hypertension. 2006;47:203-208.)

Key Words: blood ■ aldosterone ■ preeclampsia ■ estrogen

Preeclampsia (PE) and fetal growth restriction (FGR) are frequent disorders of pregnancy and a leading cause of prenatal morbidity and mortality. In near-term pregnant women with either PE or FGR, we demonstrated lower plasma volume expansion, reduced cardiac output, and an increased total peripheral vascular resistance when compared with normotensive women who gave birth to normal-size infants.1–3 Volume expansion during normal pregnancy seems to be secondary to renal and systemic vasodilatation that would activate the renin–angiotensin–aldosterone system that, in turn, would cause renal sodium and water retention.4–6 Estrogen production may also have a role in plasma volume expansion by stimulating hepatic angiotensinogen synthesis.5,7 According to this proposed pathway for volume expansion, in the present study we tested the hypothesis that women with PE or FGR would have lower serum aldosterone and estradiol concentrations either preceding or concomitant with the onset of the reduced plasma volume. We measured plasma volume and hormonal concentrations in initially healthy pregnant women from weeks 10 to 13 until near term. After delivery, we compared the time course of these changes in control, FGR, and PE women.

Methods

Participants were women attending 2 prenatal clinics of the Southeastern Health Service of Santiago, Chile (Alejandro del Rio and La Granja). These women belonged to a low-income population and were of either European or mixed European and American Indian ancestry, reflecting the general patient population of these clinics. Participants received iron supplementation and standard prenatal care and delivered at the Sótero del Río Hospital, which is affiliated with the Universidad Católica School of Medicine. The Institutional Review Boards of the Southeastern Health Service and the Universidad Católica approved study procedures. Written informed consent was obtained before enrollment. All of the procedures followed the ethical standards for human experimentation established by the Declaration of Helsinki.

Inclusion criteria were a reliable gestational age, a body mass index between 21 and 24 at 10 to 13 weeks and between 25.5 and 28 at term, singleton fetuses, and absence of either chronic medical conditions or obstetric complications. Only women who denied using drugs, alcohol, or tobacco were invited to participate in the study. Maternal height and unclad clothed weight were measured at the first prenatal visit (10 to 13 weeks) and then at monthly intervals until weeks 34 to 37 of gestation. Blood pressure was measured in seated subjects after a 30-minute rest with a mercury sphygmomanometer; the disappearance of sound (phase V) was used for the diastolic reading.8 Blood samples for plasma volume, hematocrit, estradiol, progesterone, and aldosterone determinations were obtained at each

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preterm birth visit between 9:00 AM and 12:00 PM. Plasma volume was measured by the Evan’s Blue dye dilution technique, as described previously.2,3,9 The remaining sera were aliquoted and stored at −70°C for hormone measurements. Serum estradiol, progesterone, and aldosterone concentrations were measured by radioimmunoassay, with the use of commercial kits (Diagnostic Products Corporation). Between- and within-assay coefficients of variability were <10% for each of these determinations.

Newborns were examined within 12 hours of birth to make anthropometric measurements and to rule out congenital infections and anomalies. Adequacy of birth weight for gestational age was evaluated using a Chilean prenatal growth standard that includes reference charts for newborn gender, maternal parity, and height.10 This same chart was used in our previous studies.1–3 Group assignment was made after delivery as follows: (1) controls: mothers who remained normotensive, had uncomplicated pregnancies, and delivered infants with a body weight and ponderal index above the tenth percentile for gestational age (n=95); (2) FGR: women who were considered infants with a body weight and ponderal index above the tenth percentile (n=23); and (3) PE: women who developed hypertension after week 20 and who were normotensive at 6 weeks postpartum. Hypertension was defined based on blood pressure readings >140/90 mm Hg on ≥2 occasions ≥6 hour apart, plus proteinuria >0.3 g/24 hours.11

**Statistical Analysis**

For time-independent factors, such as anthropometric values, we compared differences in the group means by a 1-way ANOVA followed by a Fisher’s protected least-significant difference test to determine pair-wise differences among group means (Stat View II, Abacus Concepts Inc). Statistical significance was accepted at a level of P<0.05. Linear12 and nonlinear13 mixed-effects models were used to evaluate subject-specific and group average curves for the time-dependent changes. Based on the descriptive analyses, we established for each variable a parametric curve best fitting the changes occurring as pregnancy progressed. An overall comparison

**TABLE 2. Curve Fit Formulas and Coefficients Values for Mean Blood Pressure, Plasma Volume, Aldosterone, Progesterone, and Estradiol**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model Equation</th>
<th>Group</th>
<th>Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>α</td>
</tr>
<tr>
<td>MBP</td>
<td>(y = a + b + \beta t + \gamma t^2 + e)</td>
<td>Control</td>
<td>70.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FGR</td>
<td>82.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE</td>
<td>85.32</td>
</tr>
<tr>
<td>Plasma volume</td>
<td>(y = a + b t + \beta \exp{γ(t−δ)^2} + e)</td>
<td>Control</td>
<td>1908</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FGR</td>
<td>2139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE</td>
<td>2264</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>(y = a + \beta t + (\gamma + \delta) t^2 + e)</td>
<td>Control</td>
<td>244.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FGR</td>
<td>39.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE</td>
<td>44.25</td>
</tr>
<tr>
<td>Progesterone</td>
<td>(y = a + \beta t + (\gamma + \delta) t^2 + e)</td>
<td>Control</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FGR</td>
<td>66.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE</td>
<td>81.82</td>
</tr>
<tr>
<td>Estradiol</td>
<td>(y = a + (\beta + \delta) t + e)</td>
<td>Control</td>
<td>−9.488</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FGR</td>
<td>−7.422</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE</td>
<td>−5.307</td>
</tr>
</tbody>
</table>

The term \(y\) represents the parameter values, term \(t\) in the formula represents the gestational age, and the term \(b\) represents the subject effect in mixed models terminology.
between the 3 study groups was done by testing the parameters of the curves using a Wald-type test. Comparisons among the 3 study groups at different gestational age intervals were performed by using the area under the curve with a Wald test. The S-PLUS statistical software MathSoft (Version 3.3, MathSoft, Inc) was used for these statistical analyses.

**Results**

No significant differences in maternal age, parity, height, and weight at term were observed among groups (Table 1). Gestational age at delivery was lower in PE mothers than in control or FGR mothers. Neonates from the FGR and PE groups had lower birth weight, body length, ponderal index, and head circumference than controls (Table 1). There was no difference in the male:female newborn ratios (controls, 51:44; FGR, 14:9; PE, 5:12; χ² test, P=0.120).

Table 2 shows the curve fit formulas and coefficient values for mean blood pressure, plasma volume, aldosterone, progesterone, and estradiol. We consider that 2 curves are significantly different at all of the gestational time points when the coefficients of the models are significantly different. The best-fit model for mean blood pressure was quadratic (Figure 1A; Table 2). When the coefficients of the fitted model for the 3 groups were compared using Wald test, significant differences were found among the 3 groups ($\chi^2=15.6$ with 6 degrees of freedom; $P=0.016$). During the first trimester, blood pressure was similar in all of the groups; this variable exhibited a moderate decline during the second trimester in all of the groups and increased in the third trimester in women who developed PE (Figure 1A; Table 3). The best-fit model for plasma volume was a logistic curve (Figure 1B; Table 2). When the coefficients of the fitted model were compared, significant differences were found among the 3 groups ($\chi^2=72.4$ with 8 degrees of freedom; $P<0.001$). Plasma volume increased progressively from early pregnancy until 34 to 37 weeks in controls, when the highest mean value was observed. In the FGR and PE groups, plasma volume was significantly lower than in controls by weeks 14 to 17 of pregnancy and remained lower than controls throughout pregnancy (Figure 1B; Table 3). The best-fit models for aldosterone and progesterone were quadratic, whereas for estradiol it was linear (Figure 2; Table 2). When the coefficients of the fitted model were compared, significant differences were found among the 3 groups ($\chi^2=0.054$, and significant differences were found for progesterone ($P<0.001$) and estradiol ($P<0.005$). During the first half of pregnancy, aldosterone values increased similarly in all of the groups. As of weeks 22 to 25 of gestation, values continued to increase in controls but remained rather constant in FGR and PE groups (Figure 2A). Aldosterone concentrations were significantly lower in PE than in controls by weeks 26 to 29 (Table 3) and after week 34 in FGR mothers (536±50 versus 439±46 pg/mL; $P<0.05$). Progesterone values were higher in PE than in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control GA, Weeks</th>
<th>Control Value</th>
<th>FGR Value</th>
<th>PE Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma volume, mL</td>
<td>14–17</td>
<td>2609±35</td>
<td>2356±2*</td>
<td>2325±78*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone, ng/mL</td>
<td>18–21</td>
<td>54.4±3.2</td>
<td>47.3±2.4</td>
<td>69.4±3.9*</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Aldosterone, pg/mL</td>
<td>26–29</td>
<td>490±44</td>
<td>465±62</td>
<td>364±51*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Progesterone/Estradiol</td>
<td>26–29</td>
<td>8.6±0.6</td>
<td>8.2±0.6</td>
<td>12.0±1.6*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Estradiol, ng/mL</td>
<td>&gt;34</td>
<td>23.6±2.5</td>
<td>17.4±1.4*</td>
<td>17.9±1.2*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>&gt;34</td>
<td>69.4±1.5</td>
<td>70.6±2.7</td>
<td>79.8±4.2*</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

GA indicates gestational age, FGR, fetal growth restriction; PE, preeclampsia; MBP, mean blood pressure.

Values are expressed as means±standard error of the mean.

*Differences with respect to control group.

†Differences between FGR and PE groups, Wald type test based on the area under the curve.
control or FGR mothers from weeks 18 to 21 of pregnancy onward and were lower in FGR than in controls by weeks 30 to 33. Serum estradiol concentrations were similar in all of the groups until week 34; thereafter, estradiol values were lower in FGR and PE mothers when compared with controls (Figure 2; Table 3). The best-fit model for the progesterone:estradiol ratio was quadratic (data not shown). The progesterone:estradiol ratio fell in the 3 groups until weeks 18 to 21; thereafter, values remained rather constant in control and FGR mothers but were statistically significantly higher in the PE group (Table 3). Table 3 shows a summary of the gestational age at first occurrence of significant intergroup differences in some selected parameters, according to the area under the curve using a Wald test.

In the PE group, 9 newborns were normal size (3194±96 g), and 8 had FGR (2312±203 g). Comparison between these 2 subgroups of PE mothers indicated that, near term, PE mothers with normal-size infants had a significantly higher plasma volume than those with FGR (3102±107 versus 2733±93 mL; P<0.020). However, we observed no significant differences in estradiol (18.5±1.7 versus 17.3±2.0 ng/mL), progesterone (265±26.7 versus 266±30 ng/mL), or aldosterone (368±73 versus 249±31 pg/mL) in preeclamptic women with normal-size or FGR newborns, respectively.

Discussion

This longitudinal study demonstrated that pregnant mothers who subsequently develop PE or idiopathic FGR have lower plasma volume by the beginning of the second trimester of gestation. Lower plasma volume anteceded changes in estradiol or aldosterone concentrations. Additionally, the present data indicate that mothers who develop PE have elevated progesterone values before the clinical onset of the disease.

We have reported previously that near-term pregnant women with PE or FGR have reduced plasma volume that is caused by a limited expansion and not by a reduced prepregnancy plasma volume. Newborns in the FGR group had a reduced ponderal index, suggesting the presence of an asymmetrical type of growth restriction. The clinical onset of this type of FGR has been established at weeks 27 to 30 of gestation. In the present study, we demonstrated that the reduced volume expansion occurred by weeks 14 to 17 of pregnancy, before the clinical onset of either disease, thus confirming previous findings. Using transvaginal Doppler ultrasound of the uteroplacental circulation, it was observed that women who developed complications of pregnancy, such as PE, premature delivery, or FGR, exhibited differences in uterine and umbilical artery Doppler blood flow indices as early as 12 to 16 weeks, when compared with women with uneventful pregnancy outcome. This similarity suggests a possible causal relationship between reduced plasma volume and abnormal uteroplacental blood flow. In addition, a recent study demonstrated an enhanced microvascular response preceding the clinical onset of PE by several weeks, suggesting that alteration in vascular tone is an early event, at least in PE.

The events that lead to plasma volume expansion, although incompletely understood, are most likely triggered by a primary fall in systemic vascular tone. In turn, this would lead to a compensatory activation of volume-restoring mechanisms, such as renal vasodilatation and activation of the renin–angiotensin–aldosterone system, both of which occur before full placentation. Placental estrogen may also play a role in volume expansion through hepatic stimulation of angiotensinogen synthesis and by activation of NO synthase activity. For these reasons, changes in the renin–angiotensin–aldosterone system and in estrogen concentrations are considered primary modulators of plasma volume expansion during pregnancy.
If this proposed pathway for plasma volume regulation is correct, we could expect that women who develop FGR or PE would have lower serum aldosterone and estradiol concentrations either preceding or concomitant with the onset of the reduced plasma volume. In contrast, the present study indicates that aldosterone and estradiol concentrations in PE and FGR mothers were within the range of values observed in controls at the time when inadequate volume expansion was detected and were altered afterward. Thus, other factors not explored in the present study may contribute to the inadequate volume expansion observed in these pregnancies, including changes in atrial natriuretic peptide, relaxin, and NO, among others. Because in the present study all 3 of the groups had acceptable body mass index and similar weight at term, this confounding variable can be ruled out as a possible explanation.

There were distinct differences in estradiol and progesterone concentrations among control, FGR, and PE mothers. The relative fall in estradiol concentrations observed in FGR and PE pregnancies during late gestation might reflect an altered metabolic function of the fetoplacental unit, most likely caused by the reduced uteroplacental blood flow described in these pregnancies. Thus, the lower estradiol concentration seems to be a consequence rather than a cause of the reduced plasma volume expansion. Progesterone concentrations were increased in PE mothers starting at the second trimester and were reduced in FGR mothers. In addition, around midpregnancy, the progesterone:estradiol ratio was elevated in PE but not in FGR mothers. We are fully aware that a limitation of the present study is the lack of consideration for hormonal circadian rhythms. Some changes in the dynamics (eg, circadian amplitude and/or phase) may have taken place and could not have been assessed with the design used. This is particularly relevant for progesterone, a hormone that has a known circadian rhythm with highest values between 2:00 PM and 8:00 PM and 8:00 PM and 2:00 AM. We intended to counteract this possible confounding variable by obtaining samples in the morning, at a time of the day when progesterone values are not increased. Other investigators have observed that circulating progesterone concentrations are increased in hypertensive women who subsequently develop PE and in normotensive women around week 27, before the development of PE. Several lines of evidence suggest that these changes might have a role in the development of PE. Placental progesterone production is elevated by ≈50% in PE, and this excess of progesterone inhibits the production of the vasodilator prostacyclin by normal placentas to a rate characteristic of PE. It has been shown that the messenger RNA expression level of the progesterone receptor is elevated in placentas from women with PE, without changes in estradiol receptors, suggesting a functional progesterone predominance in PE. The possible role of estrogen and progesterone in endothelium-dependent relaxations has been evaluated in vitro using coronary arteries obtained from ovariectomized female dogs supplemented with hormones. In this model, it was shown that an excess of progesterone relative to estradiol antagonizes the stimulatory effects of estrogen on endothelium-dependent responses associated with the production of NO. Taken altogether, these data suggest that elevated progesterone relative to estradiol can impair vasodilatation. Therefore, it is tempting to speculate that higher progesterone concentration might have a causal role in both vasospasm and in the imbalance between thromboxane and prostacyclin described in PE. Although several authors, including us, have postulated that PE and FGR share a common pathophysiologial mechanism, these distinct differences do not support this idea.

**Perspectives**

Three main conclusions may be drawn from the present study. First, it confirms previous findings that demonstrate that plasma volume either fails to increase or falls before the clinical onset of both PE and FGR. Second, the present data do not support the idea that estradiol and aldosterone are the most important modulators of plasma volume expansion during pregnancy, because plasma volume values were reduced in FGR and PE mothers ≥15 weeks before these hormones were altered. Third, high progesterone concentrations were observed early in pregnancy in women who later developed PE. We speculate that additional research in the mechanisms of progesterone metabolism during normal and abnormal pregnancy may provide a better understanding of the pathogenesis of PE.

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**References**


