Aberrant Synthesis, Metabolism, and Plasma Accumulation of Circulating Estrogens and Estrogen Metabolites in Preeclampsia

Implications for Vascular Dysfunction

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Abstract—Estrogens and estrogen metabolites have important functions in cardiovascular and other physiology, yet the patterns of estrogen synthesis, metabolism, and the individual plasma profile of estrogens and estrogen metabolites during human pregnancy as well as in preeclampsia remain undetermined. We performed liquid chromatography mass spectrometry on plasma samples from normotensive pregnant women (normP; n=8), women with mild (mPE; n=8), and severe (sPE; n = 8) preeclampsia at labor. Compared with normP, estrone was lower in sPE, whereas plasma level of estradiol-17β was significantly lower in women with mPE and sPE. Estriol was lower in sPE, but not in mPE. Although 2-hydroxyestrone was lower in mPE and sPE, 4-hydroxyestrone was high in sPE. 16-α-hydroxyestrone was higher in mPE, but not in sPE. 2-hydroxyestradiol in women with mPE and sPE were lower compared with normP. Compared with 2-methoxyestrone in normP, levels were lower in sPE. 3-methoxyestrone and 4-methoxyestrone were unchanged. 2-methoxyestradiol was lower in mPE and sPE; however, 4-methoxyestradiol was low only in sPE. Compared with normP, 16-keto-estradiol-17β levels were significantly higher in sPE, whereas 16-epi-estriol and 17-epi-estriol were lower in women with sPE. Our findings show that preeclampsia is characterized by aberrant synthesis, metabolism, and accumulation of estrogens and estrogen metabolites that are likely to be associated with alterations in vascular function. These results underscore the need to investigate the functional vascular and other physiology of estrogens and estrogen metabolites in the pathophysiology of preeclampsia. (Hypertension. 2013;61:480-487.)

Key Words: estrogens ■ estrogen metabolites ■ placenta ■ preeclampsia ■ pregnancy ■ steroid synthesis and metabolism

Preeclampsia is a hypertensive disorder of pregnancy that affects 5% to 8% of pregnancies, thus remaining a significant cause of maternal and fetal morbidity and mortality, as well as greater susceptibility and earlier onset of future cardiovascular disease in both mother and baby.1-3 Although the pathogenesis of preeclampsia remains elusive, its pathophysiology is characterized by impairment of several of the normal maternal cardiovascular adaptations seen during pregnancy.4,5

We and others have demonstrated that regulation of normal maternal cardiovascular adaptation during pregnancy is mediated, in part, by the primary estrogens, estrone, estradiol-17β, and estriol, which are synthesized by the uteroplacental unit using circulating steroid precursors from both the maternal and fetal adrenal glands.6,7 Evidence also supports a role for these primary estrogens in preeclampsia8-10; however, whether primary estrogens can be useful biomarkers for maternal and fetal well being in adverse pregnancies, including preeclampsia, has been a subject of controversy. Several studies support evidence that serum and urinary primary estrogens may be useful for screening for adverse pregnancy outcomes,8 whereas others contend that measurement of these estrogens is of little value.11 Nevertheless, the levels and plasma profile of circulating primary estrogens in preeclampsia are, at best, unclear, and this is further complicated by the lack of information on estrogen metabolites, thus hindering our comprehensive understanding of their role(s) in its pathophysiology.

Primary estrogens are converted in the uteroplacental unit by cytochrome P450s (CYP450s) into multiple hydroxylated metabolites defined by the position of hydroxylation, such as 2-hydroxyestrone, 4-hydroxyestrone, 16-α-hydroxyestrone, 2-hydroxyestradiol, and 4-hydroxyestradiol (Figure 1).12 Hydroxylated primary estrogens, especially the catecholestrogens (hydroxylated estrogens at the carbon 2 and 4 positions), undergo enzymatic O-methylation by catechol-O-methyltransferase (COMT) to form methoxyestrogens, such as 2-methoxyestrone, 3-methoxyestrone, 4-methoxyestrone, and 4-methoxyestradiol (Figure 1).12 Other metabolites of primary estrogens formed from enzymatic pathways include 16-ketosteradiol-17β, 16-epi-estriol, and 17-epi-estriol (Figure 1). There is increasing strong support for the concept that regulation of

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maternal cardiovascular adaptation during pregnancy is partly mediated by estrogen metabolites.\textsuperscript{13,14} Estrogen metabolites may also play a role in preeclampsia because pregnant mice deficient in COMT, an enzyme that catalyzes the methylation of catecholestrogens to methoxyestrogens, exhibit a preeclampsia-like phenotype.\textsuperscript{15} However, the metabolism of estrogens is very complex and necessitates that a plethora of other metabolites of estrogens be properly accounted for to achieve a more comprehensive knowledge of their potential collective contributions to the pathogenesis of preeclampsia.

Therefore, the objective of this study was to compare plasma levels of total primary estrogens and estrogen metabolites in normotensive pregnant women (normP) compared with women with mild (mPE) and severe (sPE) preeclampsia. We hypothesized that preeclampsia, a disease characterized by widespread cardiovascular dysfunction, is associated with aberrant synthesis, metabolism, and accumulation of estrogens and estrogen metabolites. In this report, we also present a working hypothesis that 16-keto-estradiol-17β may be a possible interconversion metabolite between estradiol-17β and estriol through either the sequential actions of CYP450s and a hydrogenase or the sequential actions of a dehydrogenase and deoxygenase. 17β-hydroxysteroid dehydrogenase (17β-HSD), cytochrome P450s (CYP450s), and catechol-O-methyltransferase (COMT).

Methods

Subjects
All subjects signed a written informed consent, and all study-related procedures and protocols were approved by the Institutional Review Boards at the University of Wisconsin and Meriter Hospital, Madison, WI. The women were recruited at the time of admission to Labor and Delivery.

Inclusion Criteria
The normP control group included pregnant women with no pre-existing medical diseases or antenatal complications. Preeclampsia criterion for inclusion was based as described in Creasy and Resnik’s Maternal-Fetal Medicine: Principles and Practice, 6th Edition Chapter 35. Mean (SD) gestational ages at enrollment for normP, mPE, and sPE women were 38.9±0.49, 36.6±3.02, and 35.5±3.5 weeks, respectively. Twenty-four-hour total urine protein was measured only in women who showed ≥1+ on urine dipstick and elevated blood pressure on 2 separate assessments >4 hours apart.

Exclusion Criteria
Subjects were excluded if they exhibited any of the following: lupus, antiphospholipid antibody syndrome, and placental abruption. In addition, subjects were also excluded if they exhibited chorioamnionitis or meconium-stained fluids, respectively, which are signs of antenatal and delivery complications.

Sample Collection
Blood samples were collected from normP (n=8), mPE (n=8), and sPE (n=8) subjects via venipuncture of the brachial vein, prepared, centrifuged, and plasma aliquots were stored at –80°C, until quantitative measurement.

Liquid Chromatography–Tandem Mass Spectrometry
Plasma levels of estrogens and estrogen metabolites were measured by a sensitive, specific, and precise high-performance liquid
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chromatography–tandem mass spectrometry method using selected reaction monitoring for measuring the absolute quantities of total (conjugated and unconjugated) estrogens and estrogen metabolites, as previously described, and performed at Primera Analytical Solutions Corp, Princeton, NJ. The unit for the calculated level of estrogens and estrogen metabolites was pg/mL.

Statistical Analysis

Data are presented as means±SEM unless noted. The difference in mean values between experimental groups was determined by 1-way ANOVA (SigmaPlot 12 Statistical Software) followed by post hoc Student-Newman-Keuls multiple pairwise comparisons. Level of significance was established a priori at \( P<0.05 \).

Results

Characteristics of the Subjects

Compared with normP, sPE, but not mPE, women had significantly higher body weight and body mass index (Table). Per inclusion criteria, both women with mPE and sPE demonstrated higher than normal systolic and diastolic blood pressures. Moreover, women with sPE had significantly higher systolic and diastolic blood pressure compared with mPE women (Table). Per inclusion criteria, compared with normP women who had negative dip stick protein levels, both women with mPE and sPE had higher protein in their urine. In addition, women with sPE had significantly higher proteinuria compared with mPE women. Gestational ages at time of admission were not significantly different among the women.

Plasma Profile of Primary/Classical Estrogens and Estrogen Metabolites in Preeclampsia

NormP and mPE women had similar levels of plasma estrone (4503±740 and 5341±1255 pg/mL, respectively; Figure 2A); however, women with sPE had significantly lower level of plasma estrone (1163±162 pg/mL; Figure 2A). Estradiol-17β levels were lower in women with mPE (5326±213 pg/mL) compared with levels in normP (9441±626 pg/mL; Figure 2B). Moreover, plasma estradiol-17β in women with sPE (1975±162 pg/mL) was lower compared with both normP and mPE (Figure 2B). Levels of 16-keto-estradiol-17β in normP and mPE women (861±22 and 652±229 pg/mL, respectively) were not different, whereas levels in sPE women (1894±100 pg/mL) were significantly higher (Figure 2C). Plasma levels of estriol were similar between normP women and women with mPE (2599±162 and 2589±203 pg/mL, respectively); however, women with sPE had significantly lower estriol (1163±34 pg/mL; Figure 2D).

Levels of plasma 2-hydroxyestrone in normP and mPE were 315±43 pg/mL and 215±22 pg/mL, respectively (Figure 3A);
however, levels in sPE were significantly lower (138±21 pg/mL; Figure 3A). Compared with normP (129±28 pg/mL), levels of plasma 4-hydroxyestrone were increased in mPE and sPE (180±10 and 202±10 pg/mL, respectively; Figure 3B). Plasma level of 16-α-hydroxyestrone in mPE women (6781±286 pg/mL) was significantly higher compared with normP women (3346±1354 pg/mL) and sPE women (4227±856 pg/mL; Figure 3C). We observed that plasma level of 2-hydroxyestradiol in women with mPE was significantly lower compared with levels in normP women (Figure 3D). In addition, the levels of plasma 2-hydroxyestradiol in women with sPE (170±25 pg/mL) was significantly lower compared with both normP (502±26 pg/mL) and mPE women (231±7 pg/mL; Figure 3D).

Plasma levels of 2-methoxyestrone were similar between normP women and mPE (769±45 and 723±109 pg/mL, respectively); however, women with sPE had significantly lower 2-methoxyestrone (530±26 pg/mL; Figure 4A). Plasma levels of 3-methoxyestrone (168±16, 187±56, and 132±6 pg/mL, respectively) and 4-methoxyestrone (26±9.0, 36±5.9, and 21±5.7 pg/mL, respectively) were significantly lower in women with sPE compared with both normP and mPE women. In addition, the levels of plasma 2-methoxyestradiol in women with sPE (170±25 pg/mL) was significantly lower compared with both normP (502±26 pg/mL) and mPE women (231±7 pg/mL; Figure 4D).

**Figure 3.** Products of hydroxylation: plasma levels of 2-hydroxyestrone, 4-hydroxyestrone, 16-α-hydroxyestrone and 2-hydroxyestradiol, in normotensive pregnant control women (normP; n=8), women with mild preeclampsia (mPE; n=8), and women with severe preeclampsia (sPE; n=8). *Significantly different (P<0.001) in levels compared with normP. †Significantly different (P<0.001) in levels compared with mPE. ‡Significant increase (P<0.001) in levels compared with normP.

**Figure 4.** Products of O-methylation: plasma levels of 2-methoxyestrone, 3-methoxyestrone, 4-methoxyestrone, 2-methoxyestradiol, and 4-methoxyestradiol in normotensive pregnant control women (normP; n=8), women with mild preeclampsia (mPE; n=8), and women with severe preeclampsia (sPE; n=8). *Significantly different (P<0.001) in levels compared with normP. †Significantly different (P<0.001) in levels compared with mPE.
20±2.7 pg/mL, respectively) were not significantly different among normP, mPE, and sPE (Figure 4B and 4C). Plasma levels of 2-methoxyestradiol in women with mPE (1813±133 pg/mL) were significantly lower compared with levels in normP (2186±156 pg/mL; Figure 4D). In addition, the levels of plasma 2-methoxyestradiol in women with sPE (982±55 pg/mL) were significantly lower compared with both normP and mPE (Figure 4D). NormP and mPE did not have different levels of 4-methoxyestradiol (903±168 and 1008±30 pg/mL, respectively; Figure 4E). However, women with sPE had lower 4-methoxyestradiol (393±18 pg/mL) compared with normP and mPE (Figure 4E).

The plasma levels of 16-epi-estradiol (1104±39 pg/mL) and 17-epi-estradiol (189±48 pg/mL) were different in sPE compared with normP (2268±457 and 627±58 pg/mL, respectively) and mPE women (2958±124 and 521±16 pg/mL, respectively; Figure 4A and 4B).

Discussion

Our findings demonstrate that preeclampsia, especially sPE, is characterized by aberrant synthesis, metabolism, and levels of estrogens and estrogen metabolites. Although plasma levels of these estrogens and estrogen metabolites suggest aberrant synthesis and metabolism, they are particularly specific and distinct and, therefore, potentially useful in our understanding of the pathophysiology of preeclampsia and exploration of better clinical management and outcomes.

In normal pregnancy, plasma levels of estrone, estradiol-17β, and estriol are increased, and parallel some of the increases in uteroplacental blood flow as well as development of an extensive uteroplacental vascular bed. During this time, the placenta is the main source of these estrogens using circulating steroid precursors from the maternal uterine compartment, adrenal glands, as well as fetal adrenal glands. Placental biosynthesis of primary estrogens during pregnancy is complex and results from interaction and interdependence of separate maternal, placental, and fetal systems that individually do not possess the necessary enzymatic capabilities to make these critical estrogens. Because the placenta does not express 17α-hydroxylase, the obligatory synthesis of C19-steroid precursors is not possible for estrogen synthesis. Therefore, the primary estrogens are synthesized from C-19 precursors synthesized from the maternal and fetal adrenal glands. It is important to note that at or near term, half of placental estrone and estradiol-17β is derived from C-19 precursors from maternal adrenal glands, whereas the other half is derived from the fetal adrenal zone. By contrast, over 90% of placental estradiol is derived from C-19 precursors from the fetal adrenal zone, whereas only 10% is derived from maternal sources at or near term. Our findings are consistent with studies, albeit controversial, by other investigators that estrone, estradiol-17β, and estriol may be low in sPE. However, it is important to note that only estradiol-17β was found to be low in both mPE and sPE. Estrone and estradiol-17β have been demonstrated to be potent vasodilators in the uterine and systemic vascular beds. As sPE is characterized by impaired uterine and systemic vascular vasodilatory responsiveness, our findings suggest that aberrant activities of uteroplacental aromatase, 17β-hydroxysteroid dehydrogenase, placental sulfatase, and fetal 16α- and 17α-hydroxylase may be culprits in the pathophysiology of this disease. Low levels of estrone, estradiol-17β, and estriol in sPE observed in this study may also be a result of reduced circulating steroid precursors from the maternal uterine compartment, adrenal glands, as well as fetal adrenal glands. However, previous studies have shown that preeclampsia is not associated with low levels of C-19 steroid precursors, including androstenedione and dehydroepiandrosterone.

We report herein, for the first time, that the plasma level of 16-keto-estradiol-17β is significantly higher in women with sPE. The concomitant higher levels of 16-keto-estradiol-17β, with low levels of estradiol-17β and estriol, as well as structural and kinetic evaluations led us to the novel hypothesis that this metabolite may be an interconversion metabolite between estradiol-17β and estriol (see Figure 1). Therefore, our observations suggest that elevated levels of 16-keto-estradiol-17β may be used as a predictive biomarker in preeclampsia, indicating levels of both estradiol-17β and estriol.

The present findings also show, for the first time, that plasma levels of 2-hydroxyestrone, 4-hydroxyestrone, 16-α-hydroxyestrone, and 2-hydroxyestradiol levels are altered in preeclampsia. The low level of both 2-hydroxyestrone and 2-hydroxyestradiol in women with mPE and sPE suggests low or aberrant activity of CYP1A1, CYP1A2, and CYP3A4 that primarily hydroxylate estrogens in the C-2 position to form catecholestrogens. We report that levels of 4-hydroxyestrone are increased only in sPE compared with normal pregnant women, whereas levels of 16-α-hydroxyestrone are increased in only mPE, but not in sPE. The differential changes in estrogen or estrogen metabolite concentrations between mPE versus sPE points to the notion that mPE and sPE may represent a class of related diseases with different pathogenesis or pathophysiology. Nevertheless, these data suggest that formations of hydroxylated estrogens, which have been demonstrated to possess several uteroplacental vascular effects, including vasodilatory
activities,\textsuperscript{27} induction of endothelial cell proliferation,\textsuperscript{13,28} generation of prostacyclin,\textsuperscript{29} and synergistic effects with nitric oxide,\textsuperscript{14} are highly dysregulated in preeclampsia that may contribute to the reduced uterine perfusion in preeclampsia.

Enzymatic $O$-methylation of catecholestrogens by COMT forms several methoxyestrogens.\textsuperscript{12} Preeclampsia is also associated with low activity of COMT in human placentas,\textsuperscript{4} and pregnant COMT-deficient mice exhibit a preeclampsia-like phenotype.\textsuperscript{15} In support of this hypothesis, we show herein that apart from 2-methoxyestradiol, other physiologically relevant COMT-derived metabolites, including 2-methoxyestrone and 4-methoxyestradiol, are also decreased in sPE, but not mPE. Apart from low activity of COMT, low levels of methoxyestrogens in preeclampsia may also be a result of low levels of circulating catecholestrogen precursors as well as the primary estrogens, as discussed above. Nevertheless, as methoxyestrogens induce various positive vascular effects via vasoactive and intracellular molecules that have been implicated in preeclampsia, such as nitric oxide, prostacyclin, endothelin-1, cyclic nucleotides, hypoxia-inducible factor 1, and adhesion molecules, our findings suggest that the effects of COMT in preeclampsia could be more critical than previously thought and necessitates further investigation. Many compounds with catechol structures are substrates for COMT, including the vasoactive catecholamines epinephrine and norepinephrine. We have also previously proposed that this property of COMT also points to the potential relevance of the convergence of the sympathetic catecholamine system and estrogen metabolism systems in the dysregulation of vascular responsiveness in preeclampsia.\textsuperscript{30}

We noted, for the first time, that 16-epi-estriol and 17-epi-estriol are both significantly decreased in sPE, but only 17-epi-estriol was low in both preeclampsia types, suggesting that in addition to low estriol levels, epimerization metabolism of estriol may also be aberrant in preeclampsia. 16-epi-estriol has been demonstrated to possess strong anti-inflammatory effects without profound immunosuppressive or glycogenic activities.\textsuperscript{31,32} 17-epi-estriol possesses negative effects on inflammatory and adhesion by suppressing tumor necrosis factor-$\alpha$-induced and nitric oxide–mediated vascular cell adhesion molecule 1 expression.\textsuperscript{33} Thus, as preeclampsia is associated with abnormal increased expression of adhesion molecules and increased levels of proinflammatory cytokines that induce alterations in vascular vasodilatory responsiveness,\textsuperscript{14} our findings suggest the first possibility that low 16-epi-estriol and 17-epi-estriol may potentially contribute to perturbations in inflammatory and impaired adhesion responses in preeclampsia.

In summary, our findings provide the first complete evidence that preeclampsia is associated with aberrant synthesis, metabolism, and distinct levels of 15 estrogens and estrogen metabolites, therefore suggesting important roles for a plethora of estrogens and estrogen metabolites in the pathogenesis of preeclampsia. These findings also necessitate prospective longitudinal studies covering early, mid, and late trimesters to further assess and validate whether these estrogens and estrogen metabolites can be useful biomarkers in our understanding of the pathophysiology of preeclampsia, early prediction, and identification. Therefore, we propose a working hypothesis that reduced activities of uteroplacental aromatase, cytochrome P450s, catechol-$O$-methyltransferase, and fetal 16-$\alpha$ and 17-$\alpha$ hydroxylase activities leading to low levels of primary estrogens and estrogen metabolites may cause low availabilities and activities of several local molecular mediators of numerous normal uteroplacental adaptations during pregnancy, thereby triggering some of the pathophysiology and clinical manifestations of preeclampsia (Figure 6).

![Figure 6](http://hyper.ahajournals.org/) Possible connections between preeclampsia and the synthesis and metabolism of estrogens and estrogen metabolites. ET indicates Endothelin; HIF-1$\alpha$, hypoxia-inducible factor 1; IL-6, interleukin 6; IL-8, interleukin 8; NO, nitric oxide; PG1, prostacyclin; PI GF, placental growth factor; ROS, reactive oxygen species; sFlt1, soluble Fms-like tyrosine kinase 1; sEng, soluble endoglin; TNF$\alpha$, tumor necrosis factor-alpha; TXA2, thromboxane; and VEGF, vascular endothelial growth factor.
Perspectives
Evidence is increasing that the multiple and diverse pathways by which primary estrogens are converted to multiple metabolites may contribute to numerous cardiovascular functions attributed to these estrogens in several vascular beds. The connection between estrogen metabolites and preeclampsia was first noted when the activity COMT was shown to be lower in the placentas of patients with sPE and that pregnant mice deficient in COMT exhibit a preeclampsia-like phenotype. However, the diverse pathways that extensively convert estrogens to multiple metabolites are extremely complex. This necessitates the importance to investigate a plethora of other functional estrogen metabolites to achieve a more comprehensive understanding of their contributions, if any, in preeclampsia and other hypertensive diseases. We show herein that aberrant synthesis and metabolism of estrogens and estrogen metabolites in preeclampsia may suggest a connection and provide insight into several previously unknown and underappreciated critical links between the clinical pathophysiology of the disease with the cardiovascular and other functional physiology of these estrogens and estrogen metabolites.

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Disclosures
None.

References
Novelty and Significance

What Is New?

- Preeclampsia is characterized by distinct aberrant synthesis and metabolism of estrogens and estrogen metabolites, including estrone, estradiol-17β, 16-keto-estradiol-17β, estriol, 2-hydroxyestrone, 4-hydroxyestrone, 16-α-hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone, 3-methoxyestrone, 4-methoxyestrone, 2-methoxyestradiol, 4-methoxyestradiol, 16-epiestriol, and 17-epi-estriol.

What Is Relevant?

- Preeclampsia is a hypertensive disorder of pregnancy with an elusive pathogenesis and affects 5% to 8% of pregnancies, thus remaining a significant cause of maternal and fetal morbidity and mortality, as well as greater susceptibility and earlier onset of future cardiovascular disease in both mother and baby.

Summary

Our findings demonstrate that preeclampsia is characterized by specific and distinct aberrant synthesis, metabolism, and plasma accumulation of 15 individual estrogens and estrogen metabolites, and suggest the need to investigate the functional vascular and other physiology of these estrogens and estrogen metabolites in the pathophysiology of preeclampsia.
Aberrant Synthesis, Metabolism, and Plasma Accumulation of Circulating Estrogens and Estrogen Metabolites in Preeclampsia Implications for Vascular Dysfunction
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