Vasopressin in Preeclampsia
A Novel Very Early Human Pregnancy Biomarker and Clinically Relevant Mouse Model


Abstract—Preeclampsia, a cardiovascular disorder of late pregnancy, is characterized as a low-renin hypertensive state relative to normotensive pregnancy. Because other nonpregnant low-renin hypertensive disorders often exhibit and are occasionally dependent on elevated arginine vasopressin (AVP) secretion, we hypothesized a possible use for plasma AVP measurements in the prediction of preeclampsia. Copeptin is an inert prosegment of AVP that is secreted in a 1:1 molar ratio and exhibits a substantially longer biological half-life compared with AVP, rendering it a clinically useful biomarker of AVP secretion. Copeptin was measured throughout pregnancy in maternal plasma from preeclamptic and control women. Maternal plasma copeptin was significantly higher throughout preeclamptic pregnancies versus control pregnancies. While controlling for clinically significant confounders (age, body mass index, chronic essential hypertension, twin gestation, diabetes mellitus, and history of preeclampsia) using multivariate regression, the association of higher copeptin concentration and the development of preeclampsia remained significant. Receiver operating characteristic analyses reveal that as early as the sixth week of gestation, elevated maternal plasma copeptin concentration is a highly significant predictor of preeclampsia throughout pregnancy. Finally, chronic infusion of AVP during pregnancy (24 ng per hour) is sufficient to phenocopy preeclampsia in C57BL/6J mice, causing pregnancy-specific hypertension, renal glomerular endotheliosis, proteinuria, and intrauterine growth restriction. These data implicate AVP release as a novel predictive biomarker for preeclampsia very early in pregnancy, identify chronic AVP infusion as a novel and clinically relevant model of preeclampsia in mice, and are consistent with a potential causative role for AVP in preeclampsia in humans. (Hypertension. 2014;64:00-00.) • Online Data Supplement

Key Words: antidiuretic hormone  ■ models, animal  ■ preeclampsia

Preeclampsia affects 2% to 8% of all pregnancies, ≈300,000 per year in the United States. It causes 10% to 15% of all maternal mortality with close to 100,000 preeclampsia-related annual maternal deaths worldwide.1 Maternal death because of preeclampsia disproportionately affects developing countries with 99% of preeclampsia-related maternal deaths occurring in low- and middle-income countries. In addition, 25% of stillbirths and neonatal deaths in these developing countries are associated with preeclampsia/eclampsia.2 Preeclampsia is known to cause immediate and long-term maternal–fetal morbidities such as fetal growth restriction, maternal–fetal death, and future adult neurological and cardiovascular diseases for mother and child.3–5 Because its pathogenesis is poorly understood, preventive and therapeutic modalities for preeclampsia are elusive. This emphasizes the importance of identifying unifying pathways to predict and treat preeclampsia. One candidate is the arginine vasopressin (AVP) pathway.

Select nonpregnant populations including blacks,9 the elderly,10 and patients with chronic heart11 or renal failure12 exhibit AVP-dependent hypertension.13 These populations are also characterized by low circulating renin–angiotensin system activity. Relative to nonpreeclamptic pregnant women, preeclamptic pregnant women exhibit reduced circulating activity of the renin–angiotensin system.14 This body of literature led us to hypothesize a relationship between AVP hypersecretion and the development of preeclampsia.

AVP exhibits a short biological half-life (5–20 minutes in blood), which complicates the assessment of AVP secretion...
by direct measurement of this hormone. AVP is translated in 1:1 stoichiometric ratio with a small, inactive prosegment copeptin. Copeptin is eliminated primarily by renal excretion and is stable in plasma. Consequently, it is a very useful and reliable biomarker for AVP secretion.\textsuperscript{15} Zulfikaroglu et al\textsuperscript{16} recently documented a late second/early third trimester elevation in circulating copeptin in preeclamptics. Similarly, Foda et al\textsuperscript{17} demonstrated increased maternal copeptin levels in preeclamptics at the time of delivery. The first objective of the current study was to determine if there are differences in first trimester copeptin concentrations between pregnant women who did and did not develop preeclampsia. The second objective of this study was to determine whether chronic AVP infusion during pregnancy is sufficient to induce preeclampsia phenotypes in mice.

**Methods**

The Methods section is available in the online-only Data Supplement.

**Results**

A total of 54 control pregnant, nonpreeclamptic women, 50 pregnant, preeclamptic women, and 33 nonpregnant women were analyzed in this study. A full complement of first, second, and third trimester pregnant plasma samples was not available for each pregnant participant. The numbers of analyzed samples for each trimester were as follows: first trimester, 26 controls and 20 cases; second trimester, 19 controls and 20 cases; and third trimester, 38 controls and 50 cases. Maternal age, body mass index, and percentage of those with chronic essential hypertension were similar among the nonpregnant, control, and preeclamptic groups (Table 1). The nonpregnant group had significantly fewer previous pregnancies (lower gravida), lower percentage of subjects with a history of preeclampsia, and history of pre-existing diabetes mellitus in comparison with the control and preeclamptic pregnant groups. These data may suggest that the nonpregnant cohort represents a cohort at lower risk for preeclampsia. Yet when gravida, history of preeclampsia, and pre-existing diabetes mellitus were compared between control and preeclamptic pregnant women, no statistically significant differences were noted (gravida \( P=0.99 \); history of preeclampsia \( P=0.97 \); pre-existing diabetes mellitus \( P=0.33 \)). In addition, the racial distribution among these groups was also similar and reflective of the Iowa population with a predominantly white populace based on current Iowa census data.\textsuperscript{18} When evaluating the pregnancy characteristics between the control and preeclampsia groups (Table 1), typical differences were observed. The preeclampsia group exhibited a significantly

<table>
<thead>
<tr>
<th>Table 1. Group Characteristics</th>
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<tbody>
<tr>
<td>Group Characteristics</td>
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<tr>
<td>Nonpregnant (n=33)</td>
</tr>
<tr>
<td>Control (n=54)</td>
</tr>
<tr>
<td>Preeclampsia (n=50)</td>
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<tr>
<td>( P ) Value for All Groups Compared</td>
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<tr>
<td>Maternal characteristics</td>
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<tr>
<td>Maternal age, y</td>
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<tr>
<td>Gravida</td>
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<tr>
<td>Body mass index, kg/m(^2)</td>
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<tr>
<td>Chronic essential hypertension</td>
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<tr>
<td>Pre-existing diabetes mellitus</td>
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<tr>
<td>History of preeclampsia</td>
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<tr>
<td>Race: white, not Hispanic</td>
</tr>
<tr>
<td>Race: Hispanic</td>
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<tr>
<td>Race: Asian</td>
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<tr>
<td>Race: black</td>
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<tr>
<td>Systolic blood pressure 1, mmHg</td>
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<td>Diastolic blood pressure 1, mmHg</td>
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<td>Systolic blood pressure 2, mmHg</td>
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<tr>
<td>Systolic blood pressure 3, mmHg</td>
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<tr>
<td>Diastolic blood pressure 3, mmHg</td>
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<tr>
<td>Pregnancy characteristics</td>
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<tr>
<td>Gestational age at delivery, wk</td>
</tr>
<tr>
<td>Mode of delivery: vaginal</td>
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<tr>
<td>Mode of delivery: C-section</td>
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<tr>
<td>Mode of delivery: operative vaginal delivery</td>
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<tr>
<td>Twin gestation</td>
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<tr>
<td>Birth weight, g</td>
</tr>
<tr>
<td>1 min APGAR</td>
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<td>5 min APGAR</td>
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Continuous variables are expressed as mean±SD or median. APGAR indicates Appearance, Pulse, Grimace, Activity, Respiration.
lower gestational age at delivery (36.1±3.1 versus 38.8±3.1 weeks; \( P<0.001 \)), lower birthweight (2749.6±800.1 versus 3386.5±644.8 g; \( P<0.001 \)), and higher blood pressures particularly in the third trimester. These findings are consistent with the known clinical factors associated with preeclampsia: higher rate of preterm delivery, higher rate of twin gestation, and lower birthweight because of vascular causes and earlier delivery.19

As seen in Figure 1A and 1B, maternal plasma copeptin concentration is significantly higher in pregnant women who developed preeclampsia in comparison with control, nonpreeclamptic women in the first, second, and third trimesters. In addition, the trimester-specific copeptin concentrations in preeclamptic women are higher than the plasma copeptin concentration of nonpregnant women presenting to our institution for gynecologic care. The nonpregnant cohort data suggest that the rise in copeptin concentration is both pregnancy- and preeclampsia-specific. These group differences in plasma copeptin concentration is significantly higher in pregnant women who developed preeclampsia in comparison with control, nonpreeclamptic women in the first, second, and third trimesters. In addition, the trimester-specific copeptin concentrations in preeclamptic women are higher than the plasma copeptin concentration of nonpregnant women presenting to our institution for gynecologic care. 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In addition, the trimester-specific copeptin concentrations in preeclamptic women are higher than the plasma copeptin concentra...
Hypertension

October 2014

was increased during pregnancy, but vasopressin had no significant modulatory effect (nonpregnant plus saline, 494±13; nonpregnant plus AVP, 491±21; pregnant plus saline, 546±12; pregnant plus AVP, 516±14 beats per minute; pregnancy P=0.01; infusion P=0.29; infusion×pregnancy interaction P=0.38). Pregnant mice infused with AVP exhibited a gross elevation in protein loss to urine (Figure 3B). This proteinuria was associated with robust renal glomerular endotheliosis (Figure 3C), a pathognomonic finding in preeclampsia.

Chronic AVP infusion during pregnancy also significantly influenced fetal growth and development. Fetus masses on gestational day 18 were suppressed =24% by AVP infusion (Figure 3D and 3E). One of 17 AVP-infused dams underwent preterm labor on gestational day 17. AVP infusion may also increase the rate of spontaneous fetoplacental unit resorption (Figure 3F). In the AVP-infused (n=16) and saline-infused (n=16) pregnancies, similar numbers of fetuses were carried to gestational day 18 (AVP 115 versus saline 117). Yet, there was a trend for increased fetoplacental resorption in the AVP-infused pregnancies (12% versus 6%; P=0.17).

Table 2. Using Trimester-Specific Cutoffs, Maternal Plasma Copeptin Remains Significantly Associated With the Development of Preeclampsia Despite Adjustment of Significant Clinical Covariates

<table>
<thead>
<tr>
<th>Cutoff (pg/mL)</th>
<th>First trimester model (copeptin) {181 pg/mL}</th>
<th>Adjusted Odds Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>First trimester (copeptin)</td>
<td>3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus Maternal Age</td>
<td>First trimester (copeptin)</td>
<td>3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus Body Mass Index</td>
<td>First trimester (copeptin)</td>
<td>3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus Diabetes Mellitus</td>
<td>First trimester (copeptin)</td>
<td>3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus Chronic Essential Hypertension</td>
<td>First trimester (copeptin)</td>
<td>3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus History of Preeclampsia</td>
<td>First trimester (copeptin)</td>
<td>4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus Twin Gestation</td>
<td>First trimester (copeptin)</td>
<td>3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus All Clinical Covariates</td>
<td>First trimester (copeptin)</td>
<td>6.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Second trimester model (copeptin) cutoff=866 pg/mL</td>
<td>3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus Maternal Age</td>
<td>Second trimester (copeptin)</td>
<td>3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus Body Mass Index</td>
<td>Second trimester (copeptin)</td>
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<td>&lt;0.001</td>
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<tr>
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<td>&lt;0.001</td>
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<tr>
<td>Plus History of Preeclampsia</td>
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<td>&lt;0.001</td>
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<tr>
<td>Plus Twin Gestation</td>
<td>Second trimester (copeptin)</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus All Clinical Covariates</td>
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<tr>
<td>Pregnancy</td>
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<tr>
<td>Plus Maternal Age</td>
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<td>&lt;0.001</td>
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<tr>
<td>Plus Body Mass Index</td>
<td>Third trimester (copeptin)</td>
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<td>&lt;0.001</td>
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<td>Plus Diabetes Mellitus</td>
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<td>&lt;0.001</td>
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</table>

Figure 2. Maternal plasma copeptin concentration is significantly predictive of the development of preeclampsia in all 3 trimesters of pregnancy. Receiver operating characteristic curves illustrate performance of maternal plasma copeptin in each trimester to identify pregnancies that will eventually develop preeclampsia (area under the curve [AUC]). Trimester-specific cutoffs were calculated to maximize the true-positive rate (sensitivity) while minimizing the false-positive rate (1-specificity). AUC values from all 3 trimesters are statistically significant at P<0.0001 (A and B). Furthermore, first trimester maternal plasma copeptin concentrations exhibit clinically significant positive and negative predictive values (C).
Discussion
The current study supports the novel concept of a strong relationship between AVP/copeptin and preeclampsia. First, AVP secretion, assessed via maternal plasma copeptin, is grossly elevated in the first few weeks of human pregnancies that eventually develop preeclampsia. Second, maternal plasma copeptin is significantly predictive of the development of preeclampsia, regardless of clinical covariates, at least as early as the sixth week of pregnancy. Third, chronic infusion of AVP during pregnancy in wild-type C57BL/6J mice is sufficient to induce all of the major maternal and fetal phenotypes associated with human preeclampsia, including pregnancy-specific hypertension. Together, these findings support the use of AVP/copeptin measurements as a novel very early pregnancy predictive biomarker for the late pregnancy development of preeclampsia. Furthermore, these findings are consistent with a causal role for AVP in the pathogenesis of preeclampsia.

The determination that AVP hypersecretion predicts preeclampsia as early as the sixth week of pregnancy represents a major advance in the prediction of this disorder. Currently, antiangiogenic factors such as sFLT-1 and endoglin are elevated only as early as 12 weeks before the diagnosis of preeclampsia. Follow-up analyses of sFLT-1, endoglin, and other antiangiogenic factors suggest that testing characteristics of these factors are poor in application to clinical practice. Additional studies have examined first trimester circulating antiangiogenic factors such as sFLT-1, endoglin, and placental growth factor in the pathogenesis of preeclampsia, they have also been investigated in the first trimester as possible predictors of preeclampsia. In conjunction with uterine artery Doppler (UAD) analyses, these antiangiogenic factors have been shown to be poor to moderately predictive of preeclampsia. Given the promise of antiangiogenic markers such as sFLT-1, endoglin, and placental growth factor in the pathogenesis of preeclampsia, they have also been investigated in the first trimester as possible predictors of preeclampsia.

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The current study supports the novel concept of a strong relationship between AVP/copeptin and preeclampsia. First, AVP secretion, assessed via maternal plasma copeptin, is grossly elevated in the first few weeks of human pregnancies that eventually develop preeclampsia. Second, maternal plasma copeptin is significantly predictive of the development of preeclampsia, regardless of clinical covariates, at least as early as the sixth week of pregnancy. Third, chronic infusion of AVP during pregnancy in wild-type C57BL/6J mice is sufficient to induce all of the major maternal and fetal phenotypes associated with human preeclampsia, including pregnancy-specific hypertension. Together, these findings support the use of AVP/copeptin measurements as a novel very early pregnancy predictive biomarker for the late pregnancy development of preeclampsia. Furthermore, these findings are consistent with a causal role for AVP in the pathogenesis of preeclampsia.

The determination that AVP hypersecretion predicts preeclampsia as early as the sixth week of pregnancy represents a major advance in the prediction of this disorder. Currently, antiangiogenic factors such as sFLT-1 and endoglin are elevated only as early as 12 weeks before the diagnosis of preeclampsia. Follow-up analyses of sFLT-1, endoglin, and other antiangiogenic factors suggest that testing characteristics of these factors are poor in application to clinical practice. Additional studies have examined first trimester circulating antiangiogenic factors such as sFLT-1, endoglin, and placental growth factor in the pathogenesis of preeclampsia, they have also been investigated in the first trimester as possible predictors of preeclampsia. In conjunction with uterine artery Doppler (UAD) analyses, these antiangiogenic factors have been shown to be poor to moderately predictive of preeclampsia. Given the promise of antiangiogenic markers such as sFLT-1, endoglin, and placental growth factor in the pathogenesis of preeclampsia, they have also been investigated in the first trimester as possible predictors of preeclampsia. In conjunction with uterine artery Doppler (UAD) analyses, these antiangiogenic factors have been shown to be poor to moderately predictive of preeclampsia. Given the promise of antiangiogenic markers such as sFLT-1, endoglin, and placental growth factor in the pathogenesis of preeclampsia, they have also been investigated in the first trimester as possible predictors of preeclampsia.

An elevated UAD pulsatile index (UAD-PI) alone in the first trimester is strongly correlated with the development of pre-eclampsia. Poon et al. demonstrated that UAD-PI coupled with maternal history and aneuploidy markers in the first trimester can be predictive of preeclampsia with AUC=0.96. In and of itself, UAD-PI has an AUC of 0.91. Although UAD-PI may be a powerful, predictive tool, reliable measurement requires substantial training for sonographers through verified programs, such as the Fetal Medicine Foundation, to decrease significant interassay variability. Such training may not be available in all hospital settings, again with the most susceptible populations in developing countries having the least access. In comparison, we report that first trimester plasma copeptin measurement, which could be easily simplified for point-of-care testing, is independently highly predictive of preeclampsia much earlier in pregnancy, with clinically significant sensitivity, specificity, negative predictive value, and positive predictive value. Clearly, there is utility in finding a simple, high-fidelity predictor of preeclampsia as early in pregnancy as possible, and copeptin represents exactly this type of simple, powerful, and individually predictive biomarker.
Modeling preeclampsia in animals is difficult, both because of a lack of spontaneous preeclampsia in most species and a general lack of understanding of the early pregnancy events that initiate this disorder. One common powerful model of preeclampsia is the reduced uterine perfusion pressure model, which involves physical constriction of the uterine arteries.\(^{29}\) This model is effective to emulate the late pregnancy vascular dysfunction that is typical of preeclampsia by modeling uterine artery vasoconstriction. Similarly, viral delivery of sFLT-1 during pregnancy in mice results in many of the phenotypes associated with preeclampsia.\(^{30}\) Although both methods effectively model the late pregnancy pathogenesis of preeclampsia, neither intervention can recapitulate the early pregnancy–initiating stimuli that cause sFLT-1 elevation and subsequent vascular dysfunctions. One genetic mouse model of preeclampsia (the BPH/5 model) has been documented, which may help inform the search for the early pregnancy pathogenic processes.\(^{31,32}\) The primary criticism of the BPH/5 model is the presence of a baseline hypertension that models chronic hypertension as opposed to pregnancy-induced elevations in blood pressure. The AVP infusion model of preeclampsia model demonstrates a pregnancy-specific hypertension, a phenotype that is not associated with any of these other models. This finding represents a major breakthrough in the modeling of preeclampsia. Our study strongly supports the clinical and translational relevance of this new model, because the model was based on our observation that AVP secretion is chronically and robustly elevated throughout preeclamptic human pregnancies. Further studies into the mechanisms that cause elevated AVP secretion may lead to the discovery of even earlier pregnancy biomarkers.

Our human prediction and murine model data lead us to hypothesize that AVP production, secretion, steady state, or action at various receptors may represent novel and rational therapeutic targets for the prevention and treatment of preeclampsia. Pregnancy mechanisms that mediate preeclampsia such as immune dysfunction, endothelial and vascular dysfunction, oxidative stress, and angiogenesis\(^{33}\) must be initiated by an earlier event or signal. AVP is known to interact with essentially all of these processes.\(^{34,35}\) Therefore, we contend that AVP hypersecretion represents an initiating event/signal (Figure 4).

AVP induces expression of the vascular endothelial growth factor at nanomolar concentrations in human vascular smooth muscle cells\(^{36}\) and human mesangial cells.\(^{37}\) Because AVP and vascular endothelial growth factor act to increase vascular resistance and may, therefore, contribute to reduced uterine blood flow in preeclampsia, one may hypothesize a reflexive and protective induction of sFLT-1, because sFLT-1 functions to reduce vascular endothelial growth factor signaling.\(^{38}\) Excessive subsequent induction of sFLT-1 then contributes to the pathogenesis of preeclampsia through its antiangiogenic effects.

Furthermore, AVP is known to be affected by, and acts upon, multiple immune cells. The Redman and Sargent\(^{39}\) model of the pathogenesis of preeclampsia holds that poor immunoregulation leads to immune rejection of the placenta. This immune rejection leads to downstream poor placentation and placental vascular dysfunction leading to the phenotype of preeclampsia. Natural killer cells are in direct contact with the placenta and are likely to play a critical role in the tolerance of the placenta as a significant source of interferon-\(\gamma\) and tumor necrosis factor-\(\alpha.\)\(^ {40,41}\) Interferon-\(\gamma\) and tumor necrosis factor-\(\alpha\) are elevated in preeclamptic women with a concurrent decrease in IL-10 and IL-4 production. This creates a milieu rich in cytokines that drive a T helper 1 T-cell response promoting a placental cytotoxic response leading to poor placentation. The production of AVP is stimulated by proinflammatory cytokines, including IL-6, IL-1\(\beta\), interferon-\(\gamma\), and tumor necrosis factor-\(\alpha\), which are affected by preeclampsia.\(^{35,42}\) Further, Johnson et al\(^ {43,44}\) demonstrated that AVP replaces the IL-2 help requirement for production of interferon-\(\gamma\) by lymphocytes. These processes are important in the development of the immune phenotype of preeclampsia.\(^ {39}\) Future studies to understand the complex interactions among AVP, immunologic dysregulation, angiogenesis, and vascular dysfunction are clearly needed to understand the early pregnancy mechanisms that initiate preeclampsia. These mechanisms can identify additional molecular targets to treat and, more importantly, prevent preeclampsia.

The current study has benefitted from high-quality clinical data and biosamples from the University of Iowa Maternal-Fetal Tissue Bank and Women’s Health Tissue Repository. Furthermore, the study was appropriately powered to evaluate our desired outcomes. One weakness of our study is the predominantly white population of our sample. As aforementioned, the racial distribution in this cohort is consistent with the current population of the state of Iowa. Although the relationship of copeptin and preeclampsia is robust after clinical covariate adjustment, we are not appropriately powered to analyze potential variance because of race. Numerous additional human and animal translational studies are also required to identify the sites and causes of AVP hypersecretion, to identify...
the tissue sites of action and receptors involved, and to isolate the critical timeframe for AVP hypersecretion to elicit various preeclampsia phenotypes. Such knowledge will identify the specific targets and timing at which interference with AVP signaling could prevent or treat preeclampsia. Future, large-scale clinical studies confirming the prediction of preeclampsia by copeptin and investigating the safety and effectiveness of interference with AVP signaling during pregnancy are required, as are studies to investigate further improvements in predictive power of copeptin in combination with other predictors of preeclampsia.

**Perspectives**

We posit that a very early pregnancy role for AVP in preeclampsia supports the novel concept that the central nervous system, and more specifically the neurohypophysis, is critically involved in the pathogenesis of preeclampsia. This challenges and expands the current understanding of preeclampsia as a second trimester disease of the vasculature and immune system. Measurement of AVP release in the first few weeks of pregnancy holds great promise as a novel diagnostic tool to predict the development of preeclampsia, and the inhibition of AVP release or action may represent a novel and rational therapeutic approach in preventing and treating preeclampsia. Finally, these data identify AVP infusion as a new, simple, and clinically relevant means of modeling preeclampsia in rodents.

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**Disclosures**

M.K. Santillan and J.L. Grobe currently hold a provisional patent application for the treatment of preeclampsia. No direct financial or other interests of interference with AVP signaling during pregnancy are required, as are studies to investigate further improvements in predictive power of copeptin in combination with other predictors of preeclampsia.

**References**


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Vasopressin in Preeclampsia: A Novel Very Early Human Pregnancy Biomarker and Clinically Relevant Mouse Model


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Vasopressin in Preeclampsia: A Novel Very-Early Human Pregnancy Biomarker and Clinically-Relevant Mouse Model.

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SUPPLEMENTAL METHODS

Biosample and Clinical Data Acquisition:

Coded maternal plasma with annotated clinical patient information were obtained through the University of Iowa IRB-approved (IRB# 200910784) Maternal Fetal Tissue Bank (MFTB). In this biorepository, pregnant women are prospectively recruited from the beginning of their prenatal care. MFTB inclusion criteria include any women ≥ 18 years old receiving prenatal care at the University of Iowa Hospitals & Clinics who are English-speaking. The MFTB exclusion criteria include human immunodeficiency virus or hepatitis C positive women. Women who enroll into the MFTB provide a maternal blood sample for research whenever they have clinically indicated blood draws throughout pregnancy. All maternal blood in the MFTB is uniformly processed. Maternal whole blood is collected in Acid Citrate Dextrose-A (ACD-A) Vacutainer tubes (Becton Dickenson) and centrifuged at 1300 g. Maternal plasma and the buffy coat are isolated, aliquoted, and stored at -80°C. Maternal and neonatal clinical data obtained by the MFTB are obtained via data extraction from the electronic medical record using standardized data extraction forms. Extracted clinical data are routinely monitored for accuracy and completeness by two of the authors (MKS and DAS). Additional data are also extracted by bioinformatics collaborators from the University of Iowa Institute for Clinical and Translational Science who are able to query the central electronic medical record database. For comparisons to nonpregnant patients, clinical data and patient plasma was obtained through the University of Iowa Women’s Health Tissue Repository (IRB# 201010769, 201202714) focused on recruiting women who seek gynecologic care. These biorepositories are run by two of the authors (DAS and MKS) ensuring uniform sample processing and clinical data extraction to maintain high fidelity clinical data and samples for all the biobanks. The human study was IRB approved as the use of these deidentified and coded samples and clinical data from these biorepositories were deemed non-human subjects research (IRB # 201405808) by the University of Iowa IRB. Further elaboration of the mechanisms of this bank and the quality assurance measures of the biosamples and clinical data have been recently reported.

Cohort Assembly:

Inclusion criteria for preeclampsia cases included women who delivered at UIHC, were enrolled in the MFTB, and carried the diagnosis of preeclampsia at the time of delivery. The diagnosis and classification of cases of mild preeclampsia, severe preeclampsia and chronic hypertension with superimposed preeclampsia were based on the standard American College of Obstetrics and Gynecology (ACOG) definitions for analysis with a minimal clinical presentation of elevated blood pressures of greater than 140/90 at least 2 occasions 6 hours apart and proteinuria. These cases were identified by cross-referencing the MFTB database with the bioinformatics query of mild and severe preeclampsia and chronic hypertension with superimposed preeclampsia ICD-9 codes (642.4x, 642.5x, 642.7x, 642.9x) of bank participants at the time of delivery. The electronic medical record of each potential case was evaluated to confirm the diagnosis of preeclampsia by the ACOG definitions. Maternal age-matched plasma samples and corresponding clinical data for the control population were obtained by utilizing the MFTB database. Control pregnancies were pregnant women who did not develop...
preeclampsia. The gestational age at the time of the collection of the samples was
classified by trimesters: first trimester (< 13 completed gestational weeks), second
trimester (13-26 completed gestational weeks), and third trimester (>26 weeks). To
compare pregnant and non-pregnant plasma copeptin concentrations, a cohort of
clinical data and plasma from non-pregnant, age-matched patients was assembled.
These patients were recruited from the gynecologic clinics at our institution. A sample
of blood was obtained at the time of recruitment which was processed in the same way
as the maternal blood for plasma isolation. Control and case assignment was
performed by a trained high risk obstetrician (MKS).

Procedures:
All maternal plasma copeptin concentrations were measured in duplicate using a
commercial enzyme-linked immunosorbent assay (ELISA) specific for human copeptin
(USCN Life Science, Inc, Houston, TX). The assay was performed according to the
manufacturer’s instructions. The minimum detectible dose of human copeptin for this
assay was 5.4 pg/mL. The intra-assay coefficient of variation is < 10% and the inter-
assay coefficient of variation is < 12%. To examine if renal function or AVP degradation
throughout pregnancy affected copeptin concentration, plasma Cystatin C (Sigma-
Aldrich, St. Louis, MO) and vasopressinase (LNPep, USCN Life Science, Inc, Houston,
TX) were measured in duplicate in all samples utilizing commercial ELISA kits. Cystatin
C is a potent marker of renal function that is uniform across multiple populations and is
not altered by muscle mass, meat consumption, physical activity, and overall health
status in comparison to serum creatinine and glomerular filtration rate (GFR). Given the
superiority of cystatin C in estimating renal function in multiple studies, the 2012 KDIGO
(Kidney Disease: Improving Global Outcomes) guideline statements suggest using
cystatin C to estimate glomerular filtration rate\(^3\), \(^4\). For these reasons, cystatin-C was
used to estimate renal function in this study.

Animal Studies:
Wildtype C57BL/6J male and female mice were obtained from the Jackson
Laboratories, and maintained on standard chow (Teklad 7013) at standard room
temperature (22°C) with a 12:12 light:dark cycle. Virgin female mice were implanted
with subcutaneous osmotic minipumps (Alzet Model # 1004) to deliver AVP (24 ng/hr)
or saline vehicle. Three days after implantation, mice were individually mated for a
single overnight period. Blood pressure was assessed by tail-cuff (Visitech, as
previously \(^5\)) for three weeks preceding mating, and through gestational day (GD) 16.
Urine was collected using single-mouse sized metabolic cages (Nalgene, as previously
\(^6\)) overnight on GD17 and GD18, and protein content was assessed using
commercially-available BCA assay kit (Pierce). Mice were sacrificed on GD18, and
organs and fetuses were harvested for subsequent analyses. Electron microscopic
examinations of maternal kidneys were performed by the University of Iowa Department
of Pathology using a JEOL JEM-1011 Transmission Electron Microscope. All
procedures were approved by the University of Iowa Animal Care and Use Committee
(protocols 1211239 and 1311213). A nonpregnant cohort of female mice was
processed in the same way.
**Statistical Analyses:**

A major aim of this study was to determine if there were differences in first-trimester copeptin concentrations between pregnant women who did and did not develop preeclampsia and if these predicted the development of preeclampsia. Using the smallest effect size in late gestation, maternal plasma copeptin concentrations from Zulfikaroglu et al. between control (310 pg/mL) and mild preeclamptics (620 pg/mL) with the largest reported standard deviation of 180 pg/mL, power of 80% and $\alpha = 0.05$, only 7 participants per group were required. However, in order to account for a parsimonious, mixed effects regression model of 3 variables, a minimum of 30 samples per group was necessary.

All statistical analyses were performed with SigmaPlot 12.0 software (Systat Software, Inc, California) and confirmed using SAS 9.1 software (SAS Institute Inc, Cary, NC). Stepwise regression was used to identify potential confounding variables. Logistic regression models were constructed using regression identified and clinically significant confounding variables. Receiver operating characteristic curves were constructed for regression diagnostics. Trimester specific cutoff values were determined by graphing the sensitivity and specificity for each trimester. The cutoff was determined at the intersection of these curves. Positive and negative predictive values were also calculated. In addition, chi square or Fisher exact test was utilized for categorical variables. For continuous variables, the Student’s t-test or ANOVA was utilized. If criteria for normality were not met, Mann-Whitney test or ANOVA on Ranks was utilized. All variables were tested at significance level of 0.05.
SUPPLEMENTAL REFERENCES


SUPPLEMENTAL DATA

Figure S1. Induction of hypertension during pregnancy with AVP infusion. (A) Timeline of procedures. (B) Systolic blood pressure (SBP) increased over time during pregnancy in C57BL/6J mice infused with arginine vasopressin (AVP, 24 ng/hr), achieving a significant increase over baseline (pre-pregnancy) values by gestational days 15-16. In contrast, saline-infused dams exhibited a significant reduction in SBP during gestational days 8-12, which returned to baseline levels by gestational days 15-16. These data are calculated and redrawn from the same mice and recordings as presented in Figure 3A. Saline n=16, AVP n=11. * P<0.05 vs saline-infused, † P<0.05 vs baseline.