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;64:00-00-00.) • 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 annual maternal deaths worldwide. 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. 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. 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, the elderly, and patients with chronic heart or renal failure exhibit AVP-dependent hypertension. 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. 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} Zulfikarolu et al\textsuperscript{16} recently documented a late second/early third trimester elevation in circulating copeptin in preeclampsics. Similarly, Foda et al\textsuperscript{17} demonstrated increased maternal copeptin levels in pre-eclamptics 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 non-pregnant, control, and preeclamptic groups (Table 1). The nonpregnant group had significantly fewer previous pregnancies (lower gravid), 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 gravid, 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>
<th>Nonpregnant (n=33)</th>
<th>Control (n=54)</th>
<th>Preeclampsia (n=50)</th>
<th>( P ) Value for All Groups Compared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>31.4±7.2</td>
<td>29.9±5.2</td>
<td>30.0±5.6</td>
<td>0.47</td>
</tr>
<tr>
<td>Gravida</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>29.6±8.5</td>
<td>30.0±6.7</td>
<td>31.9±9.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Chronic essential hypertension</td>
<td>9.1%</td>
<td>25.9%</td>
<td>20.0%</td>
<td>0.16 (( \chi^2=3.7 ))</td>
</tr>
<tr>
<td>Pre-existing diabetes mellitus</td>
<td>3.0%</td>
<td>20.4%</td>
<td>22.0%</td>
<td>0.05 (( \chi^2=5.9 ))</td>
</tr>
<tr>
<td>History of preeclampsia</td>
<td>0.0%</td>
<td>29.6%</td>
<td>18.0%</td>
<td>0.002 (( \chi^2=12.1 ))</td>
</tr>
<tr>
<td>Race: white, not Hispanic</td>
<td>90.9%</td>
<td>92.6%</td>
<td>90.0%</td>
<td>0.56 (( \chi^2=6.8 ))</td>
</tr>
<tr>
<td>Race: Hispanic</td>
<td>0%</td>
<td>3.7%</td>
<td>4.0%</td>
<td>0.56 (( \chi^2=6.8 ))</td>
</tr>
<tr>
<td>Race: Asian</td>
<td>6.1%</td>
<td>1.8%</td>
<td>0%</td>
<td>0.56 (( \chi^2=6.8 ))</td>
</tr>
<tr>
<td>Race: black</td>
<td>3.0%</td>
<td>1.9%</td>
<td>4.0%</td>
<td>0.56 (( \chi^2=6.8 ))</td>
</tr>
<tr>
<td>Systolic blood pressure 1, mmHg</td>
<td>...</td>
<td>120.4±12.3</td>
<td>129.5±12.3</td>
<td>0.041</td>
</tr>
<tr>
<td>Diastolic blood pressure 1, mmHg</td>
<td>...</td>
<td>69.1±9.8</td>
<td>72.2±5.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Systolic blood pressure 2, mmHg</td>
<td>...</td>
<td>121.0±13.6</td>
<td>124±18.5</td>
<td>0.558</td>
</tr>
<tr>
<td>Diastolic blood pressure 2, mmHg</td>
<td>...</td>
<td>70.7±9.0</td>
<td>70.7±11.3</td>
<td>0.998</td>
</tr>
<tr>
<td>Systolic blood pressure 3, mmHg</td>
<td>...</td>
<td>121.9±17.9</td>
<td>129.2±16.7</td>
<td>0.049</td>
</tr>
<tr>
<td>Diastolic blood pressure 3, mmHg</td>
<td>...</td>
<td>70.7±9.9</td>
<td>76.3±11.6</td>
<td>0.028</td>
</tr>
<tr>
<td>Pregnancy characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>...</td>
<td>38.8±1.7</td>
<td>36.1±3.1</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Mode of delivery: vaginal</td>
<td>...</td>
<td>58.5%</td>
<td>32.7%</td>
<td>(&lt;0.001) (( \chi^2=16.6 ))</td>
</tr>
<tr>
<td>Mode of delivery: C-section</td>
<td>...</td>
<td>11.3%</td>
<td>67.3%</td>
<td>(&lt;0.001) (( \chi^2=16.6 ))</td>
</tr>
<tr>
<td>Mode of delivery: operative vaginal delivery</td>
<td>...</td>
<td>30.2%</td>
<td>0%</td>
<td>(&lt;0.001) (( \chi^2=16.6 ))</td>
</tr>
<tr>
<td>Twin gestation</td>
<td>...</td>
<td>13.0%</td>
<td>22.0%</td>
<td>0.34 (( \chi^2=0.92 ))</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>...</td>
<td>3386.5±644.8</td>
<td>2749.6±800.1</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>1 min APGAR</td>
<td>...</td>
<td>8.0</td>
<td>8.0</td>
<td>0.64</td>
</tr>
<tr>
<td>5 min APGAR</td>
<td>...</td>
<td>9.0</td>
<td>9.0</td>
<td>0.76</td>
</tr>
</tbody>
</table>

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 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 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.

Receiver operating characteristic curves for each trimester were constructed to interrogate if this elevated plasma copeptin concentration was predictive of the development of preeclampsia. Furthermore, optimal copeptin concentration cutoffs were determined from these curves. As seen in Figure 2A and 2B, the receiver operating characteristic curves demonstrated significant areas under the curve (AUC) in the first trimester (AUC=0.90±0.05; *P*<0.0001), second trimester (AUC=0.90±0.06; *P*<0.0001), and third trimester (AUC=0.78±0.05; *P*<0.0001). These data indicate that maternal plasma copeptin concentration is strongly predictive of the development of preeclampsia in all 3 trimesters with clinically significant sensitivities and specificities. Copeptin prediction of preeclampsia demonstrates very high negative predictive values at all ranges of clinically significant preeclampsia risk and high positive predictive values in those who would be at higher risk of developing preeclampsia (Figure 2C).

Furthermore, we determined if clinically significant and regression-identified covariates would alter the association of preeclampsia and copeptin concentration at particular trimesters. Logistic regression models were constructed with the diagnosis of preeclampsia as the dependent variable. Participants were dichotomized according to being above or below the receiver operating characteristic curve–determined cutoff for a particular trimester (Figure 2B). Models were generated using the status of being above or below the cutoff as an independent variable while controlling for significant covariates such as maternal age, body mass index, diabetes mellitus, chronic essential hypertension, history of preeclampsia, and twin gestation. Copeptin concentration remained the only variable in all the models that continued to be significantly associated with preeclampsia (Table 2). These clinical data in total suggest that the robust elevation in copeptin concentration occurs early in the first trimester and remains elevated throughout pregnancy despite potential confounding effects of clinically significant obstetric and vascular covariates.

Finally, to examine a causal role for AVP in the pathogenesis of preeclampsia, we tested the sufficiency of chronic AVP infusion throughout gestation to induce the major preeclampsia phenotypes in wild-type C57BL/6J mice. AVP infusion (24 ng per hour) had no effect on systolic blood pressure in nonpregnant female mice, but caused a robust elevation in systolic blood pressure in pregnant mice (Figure 3A; Figure S1 in the online-only Data Supplement). Heart rate
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%; \( P=0.17 \)).

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).

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>First trimester model (copeptin) cutoff=811 pg/mL</th>
<th>Adjusted Odds Ratio</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester (copeptin)</td>
<td>3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First trimester (copeptin) plus maternal age</td>
<td>3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First trimester (copeptin) plus body mass index</td>
<td>3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First trimester (copeptin) plus diabetes mellitus</td>
<td>3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First trimester (copeptin) plus chronic essential hypertension</td>
<td>3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First trimester (copeptin) plus history of preeclampsia</td>
<td>4.5</td>
<td>0.001</td>
</tr>
<tr>
<td>First trimester (copeptin) plus twin gestation</td>
<td>3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First trimester (copeptin) plus all clinical covariates</td>
<td>6.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Second trimester model (copeptin) cutoff=866 pg/mL</th>
<th>Adjusted Odds Ratio</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second trimester (copeptin)</td>
<td>3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Second trimester (copeptin) plus maternal age</td>
<td>3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Second trimester (copeptin) plus body mass index</td>
<td>3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Second trimester (copeptin) plus diabetes mellitus</td>
<td>3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Second trimester (copeptin) plus chronic essential hypertension</td>
<td>3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Second trimester (copeptin) plus history of preeclampsia</td>
<td>3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Second trimester (copeptin) plus twin gestation</td>
<td>3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Second trimester (copeptin) plus all clinical covariates</td>
<td>7.1</td>
<td>0.015</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Third trimester model (copeptin) cutoff=758 pg/mL</th>
<th>Adjusted Odds Ratio</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Third trimester (copeptin)</td>
<td>2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Third trimester (copeptin) plus maternal age</td>
<td>2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Third trimester (copeptin) plus body mass index</td>
<td>2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Third trimester (copeptin) plus diabetes mellitus</td>
<td>2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Third trimester (copeptin) plus chronic essential hypertension</td>
<td>2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Third trimester (copeptin) plus history of preeclampsia</td>
<td>2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Third trimester (copeptin) plus twin gestation</td>
<td>2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Third trimester (copeptin) plus all clinical covariates</td>
<td>2.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
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.20 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.21–23 Additional studies have examined first trimester circulating interleukin-1β (IL-1β),24 high-sensitivity C-reactive protein,25 and pregnancy-associated plasma protein-A,25 but these 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 predictive (AUC=0.74) of preeclampsia, but the requirements of performing both an assay and a UAD analysis are not practical for clinical screening, particularly in developing countries.22 An elevated UAD pulsatile index (UAD-PI) alone in the first trimester is strongly correlated with the development of preeclampsia. Poon et al26,27 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,28 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.

Figure 3. Infusion of vasopressin during pregnancy is sufficient to cause the cardinal phenotypes of preeclampsia in mice. A, Systolic blood pressure on gestational day (GD) 15 and 16. Nonpregnant mice infused with saline (n=15) or arginine vasopressin (AVP) at 24 ng/h (n=5) for 15 to 16 d, and pregnant mice infused with saline (n=16) or AVP (n=11). *P<0.05 vs all other groups. B, Total protein lost to urine per day on GD17. Pregnant mice infused with saline (n=15) or AVP (n=11). *P<0.05 vs saline. C, Average GD18 fetus mass (g). *P<0.05 vs saline. D, Electron micrographs of renal cortex, illustrating glomerular endotheliosis. Left panels are from a saline-infused animal, which had a glomerular basement membrane thickness within normal limits (thin white arrow). Right panels are from animals that received vasopressin infusion (24 ng/h). Redundant endothelial cell membrane is present (thick black arrow), and basement membranes are moderately to markedly thickened with electron-dense material (thick white arrow). E, Photographs of representative GD18 fetuses from mice infused with saline (117 fetuses from 16 pregnancies) or AVP at 24 ng/h (115 fetuses from 16 pregnancies). *P<0.05 vs saline. F, Photographs of fetoplacental units in uterus on GD18 during 24 ng/h AVP infusion. White arrows indicate resorbed fetoplacental units.
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. 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 features associated with preeclampsia.

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. 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 preeclampsic 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 must be initiated by an earlier event or signal. AVP is known to interact with essentially all of these processes. 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 and human mesangial cells. 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. 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 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-γ and tumor necrosis factor-α. Interferon-γ and tumor necrosis factor-α 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-1β, interferon-γ, and tumor necrosis factor-α, which are affected by preeclampsia. Further, Johnson et al52 demonstrated that AVP replaces the IL-2 help requirement for production of interferon-γ by lymphocytes. These processes are important in the development of the immune phenotype of preeclampsia. 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 addressing the inhibition of AVP for the treatment of preeclampsia. No direct financial or other professional relationships exist for the authors.

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**Novelty and Significance**

**What Is New?**

- Maternal plasma copeptin predicts late pregnancy onset of preeclampsia at least as early as the sixth week of pregnancy, regardless of clinical covariates.

- Infusion of vasopressin during pregnancy is sufficient to induce the cardinal phenotypes of preeclampsia in mice.

**What Is Relevant?**

- Current diagnostic biomarkers for preeclampsia are largely limited to the second trimester, shortly before the onset of clinical symptoms.

**Summary**

Copeptin represents a novel diagnostic biomarker for the prediction of preeclampsia and is predictive far earlier in pregnancy than all other known biomarkers. Chronic vasopressin infusion represents a new, rational, clinically relevant method to model preeclampsia in mice. These findings are consistent with a role for the neurohypophysis in the very early pregnancy pathogenesis of preeclampsia.
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 reported¹.

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 proteinuria². 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 (LNP EP, 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.