Increased Levels of Copeptin Before Clinical Diagnosis of Preeclampsia

Edwina H. Yeung, Aiyi Liu, James L. Mills, Cui Lin Zhang, Tuija Männistö, Zhaohui Lu, Michael Y. Tsai, Pauline Mendola

Abstract—Copeptin, a surrogate biomarker of vasopressin, has been associated with renal function decline and may serve as a useful early biomarker for preeclampsia. We measured serum copeptin using samples collected longitudinally during pregnancy among unaffected controls (n=136) and cases of preeclampsia (n=169), gestational diabetes mellitus (n=92), gestational hypertension (n=101), and preterm birth (n=86) in the Calcium for Preeclampsia Prevention trial (1992–1995). Preeclampsia and gestational hypertension were defined as having a diastolic blood pressure ≥90 mm Hg on 2 occasions with and without proteinuria, respectively. The risk of pregnancy complications associated with copeptin was estimated by logistic regression adjusting for maternal age, race, body mass index, insurance status, marital status, current smoking, and clinical site. Baseline copeptin levels, at mean 16 weeks of gestation, were associated with increased preeclampsia risk (adjusted odds ratios and 95% confidence interval being 1.55 per log unit: 1.03–2.31) when compared with controls (P=0.03). The association was stronger among cases diagnosed before 37 weeks (1.86; 1.08–3.20) than those diagnosed later (1.45; 0.91–2.32). Copeptin levels rose with increasing gestational age in both cases and controls but remained significantly higher among those who were diagnosed with preeclampsia. Differences in levels of copeptin between cases and controls became more apparent closer to time of diagnosis. No significant associations were found for gestational hypertension without proteinuria, gestational diabetes mellitus, or preterm birth without preeclampsia. Copeptin levels are elevated in pregnant women before diagnosis of preeclampsia with elevation specific to this pregnancy complication rather than hypertension alone. (Hypertension. 2014;64:00-00.) • Online Data Supplement

Key Words: copeptin, human • diabetes, gestational • hypertension, pregnancy-induced • preeclampsia
adding to difficulties in its measurement.\textsuperscript{15} Copeptin has the advantage of remaining in circulation longer and can serve as a potential biomarker of preeclampsia.\textsuperscript{16}

Therefore, we sought to explore the association between copeptin and development of preeclampsia prospectively. To evaluate the potential of copeptin as a specific biomarker of preeclampsia, we also examined its relationship with gestational hypertension, gestational diabetes mellitus (GDM), and preterm birth.

**Methods**

The Calcium for Preeclampsia Prevention (CPEP) trial was a randomized clinical trial (1992–1995), where 4589 nulliparous women with singleton pregnancies were randomized to calcium supplements or placebo.\textsuperscript{17,18} Women were excluded if lost to follow-up or had incomplete information on outcomes (n=283), had a pregnancy loss (n=49), had an infant with chromosomal abnormality (n=1), or had no serum collected at baseline or samples were misdated (n=589).\textsuperscript{19}

We conducted a nested case control study selecting samples from the remaining 3667 women with and without certain pregnancy complications of interest. Case definitions of preeclampsia and gestational hypertension have been previously published.\textsuperscript{18–20} See online-only Data Supplement for details on outcomes and covariates.

**Selection**

All cases of preterm preeclampsia (n=72), GDM (n=88), and preterm birth (n=87 without preeclampsia or diabetes mellitus) were included. In addition, randomly selected cases of term preeclampsia (n=89) and gestational hypertension (n=114) were also included. The control group consisted of 136 women who were normotensive, normoglycemic, and gestational hypertension, GDM (without GDM), 86 GDM (with 10 cases also having term preeclampsia), and 86 preterm deliveries without preeclampsia. This study was exempted from Institutional Review Board review and approval by the National Institutes of Health’s Office of Human Subjects Research because of use of existing de-identified samples.\textsuperscript{19}

**Statistical Analysis**

Crude and adjusted odds ratios and the 95% confidence intervals between copeptin and outcomes of interest were estimated by logistic regression. Measures were grouped into 3 gestational age periods of blood draw as originally designated by the trial.\textsuperscript{17} To ensure that associations remained prospective in nature at later gestational ages, we excluded values measured after the date of diagnosis of preeclampsia or gestational hypertension. We also included only 1 measure per woman for each time window. (online-only Data Supplement).

The crude preeclampsia analysis was repeated dividing samples into 7 finer categories of gestational age. Mean log values and their SEs were plotted along with P values estimated using Wilcoxon rank-sum test. Finally, differences in copeptin measure by time until diagnosis while maintaining the distribution of participants from each site from among eligible participants.

**Copeptin Measurement**

Women provided a nonfasting blood sample before randomization and twice during follow-up between gestational weeks 26 and 29 and at approximately week 36. Women were randomized before 21 weeks and 6 days of gestation. Serum copeptin was measured by BRAHMS Immunoluminometric Assay (Thermo Scientific, Berlin, Germany) using coated-tubes after the methods of Morgenthaler et al.\textsuperscript{21} Additional details on copeptin measurement can be found in the online-only Data Supplement. Our current analysis with measured copeptin consisted of 136 controls, 71 preterm preeclampsia, 98 term preeclampsia, 78 gestational hypertension (without GDM), 88 GDM (with 10 cases also having term preeclampsia), and 86 preterm deliveries without preeclampsia. This study was exempted from Institutional Review Board review and approval by the National Institutes of Health’s Office of Human Subjects Research because of use of existing de-identified samples.\textsuperscript{19}

**Table 1. Characteristics of Participants by Preeclampsia and Gestational Hypertensive Status in the Calcium for Preeclampsia Prevention Study**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>Preterm PE</th>
<th>Term PE</th>
<th>All PE</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>136</td>
<td>71</td>
<td>98</td>
<td>169</td>
<td>101</td>
</tr>
<tr>
<td>Age, y</td>
<td>20.4 (3.7)</td>
<td>21.0 (4.5)</td>
<td>20.6 (4.5)</td>
<td>20.7 (4.5)</td>
<td>21.6 (5.6)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66.6 (14.3)</td>
<td>72.2 (19.1)*</td>
<td>72.3 (17.1)</td>
<td>72.3 (17.9)</td>
<td>71.7 (17.2)*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5 (5.6)</td>
<td>28.2 (7.2)†</td>
<td>27.6 (5.7)†</td>
<td>27.9 (6.4)†</td>
<td>27.0 (5.7)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>10 (7.4)</td>
<td>6 (8.5)</td>
<td>15 (15.3)</td>
<td>21 (12.4)</td>
<td>10 (9.9)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White non-Hispanic</td>
<td>47 (34.6)</td>
<td>19 (26.8)</td>
<td>32 (32.7)</td>
<td>51 (30.2)</td>
<td>38 (37.6)</td>
</tr>
<tr>
<td>Black</td>
<td>63 (46.3)</td>
<td>37 (52.1)</td>
<td>53 (54.1)</td>
<td>90 (53.3)</td>
<td>47 (46.5)</td>
</tr>
<tr>
<td>Hispanic or other race</td>
<td>26 (19.1)</td>
<td>15 (21.1)</td>
<td>13 (13.3)</td>
<td>28 (16.6)</td>
<td>16 (15.8)</td>
</tr>
<tr>
<td>Less than high school education, n (%)</td>
<td>60 (44.1)</td>
<td>29 (40.8)</td>
<td>46 (46.9)</td>
<td>75 (44.4)</td>
<td>44 (43.6)</td>
</tr>
<tr>
<td>No private insurance, n (%)</td>
<td>118 (86.8)</td>
<td>64 (90.1)</td>
<td>94 (95.9)*</td>
<td>158 (93.5)*</td>
<td>93 (92.1)</td>
</tr>
<tr>
<td>Married, n (%)</td>
<td>30 (22.1)</td>
<td>19 (26.8)</td>
<td>17 (17.3)</td>
<td>36 (21.3)</td>
<td>26 (26.0)</td>
</tr>
<tr>
<td>Male infant, n (%)</td>
<td>63 (46.3)</td>
<td>38 (53.5)</td>
<td>48 (49.0)</td>
<td>86 (50.9)</td>
<td>53 (52.5)</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>3314 (445)</td>
<td>2126 (731)*</td>
<td>3316 (494)</td>
<td>2816 (843)*</td>
<td>3359 (593)</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>39.7 (1.5)</td>
<td>34.8 (2.9)†</td>
<td>39.7 (1.2)</td>
<td>37.6 (3.2)†</td>
<td>39.7 (1.6)</td>
</tr>
<tr>
<td>Randomized to calcium, n (%)</td>
<td>68 (50.0)</td>
<td>40 (56.3)</td>
<td>35 (35.7)*</td>
<td>75 (44.4)</td>
<td>52 (51.5)</td>
</tr>
<tr>
<td>Baseline copeptin, pmol/L</td>
<td>3.8 (2.3)</td>
<td>5.4 (4.7)†</td>
<td>4.9 (4.3)*</td>
<td>5.1 (4.5)*</td>
<td>4.1 (2.9)</td>
</tr>
<tr>
<td>Gestational age of first sample, wk</td>
<td>16.0 (2.6)</td>
<td>15.7 (2.9)</td>
<td>16.0 (2.7)</td>
<td>15.9 (2.8)</td>
<td>16.2 (2.3)</td>
</tr>
<tr>
<td>Gestational age of second sample, wk</td>
<td>27.7 (1.5)</td>
<td>27.6 (1.5)</td>
<td>27.4 (1.6)</td>
<td>27.2 (1.5)</td>
<td>27.5 (1.7)</td>
</tr>
<tr>
<td>Gestational age of third sample, wk</td>
<td>36.5 (0.8)</td>
<td>35.6 (0.9)†</td>
<td>36.4 (0.6)</td>
<td>36.3 (0.7)*</td>
<td>36.5 (0.7)</td>
</tr>
</tbody>
</table>

Mean (SD) unless specified. Marital status was missing for one woman in the gestational hypertensive group. BMI indicates body mass index; GH, gestational hypertension; and PE, preeclampsia.

*P<0.05, †P<0.01, ‡P<0.001 for tests of difference between groups with pregnancy complications vs the control group.
were explored by pairing cases with control samples of similar gestational age at blood collection. For these analyses, samples measured at time of diagnosis or past time of diagnosis were grouped as 0 weeks from diagnosis. P values between mean levels of copeptin at each of these time points were calculated using Wilcoxon rank-sum test.

Results

Women with preeclampsia were significantly more likely to have higher body mass index and deliver lower birthweight babies (Table 1). Among women with preterm and term preeclampsia, 43 (61%) and 23 (24%) cases were severe, respectively. No significant differences in gestational age for the first and second blood draw by case status were found. In bivariate analyses, greater copeptin levels were significantly \( (P<0.05) \) associated with younger maternal age, black race, having never married, and not having private health insurance and clinical site (data not shown).

The median (interquartile range) of baseline log copeptin levels among preeclampsia cases and controls measured on average at 16 weeks of gestation were 3.85 (2.57–6.30) and 3.15 (2.17–4.72) pmol/L, respectively. Baseline levels were generally higher for term than for preterm cases. Adjustment for covariates slightly attenuated the associations and term preeclampsia risk was no longer significantly elevated, whereas preterm preeclampsia remained associated with copeptin. Repeating analyses excluding HELLP syndrome cases or preeclampsia cases with concurrent GDM did not affect results (data not shown).

Higher copeptin levels were associated with increased risk of preterm birth not affected by preeclampsia; however, the association did not persist after adjustment for race (Table 2). In addition, among those who had SGA without preeclampsia (n=22, some with other complications: 6 with GH, 3 GDM, and 2 preterm), we found no difference in mean baseline levels of copeptin (geometric means of 2.91 for SGA versus 3.21 for non-SGA; \( P=0.46 \)). Neither were differences observed in the risk of SGA \( (P>0.3) \) when compared with the controls at any time during pregnancy. Hence, differences observed for preeclampsia are not because of SGA or preterm birth. Copeptin was not associated with risks of gestational hypertension or GDM. The second and third copeptin measures were also associated with risk of preeclampsia (Table 2).

Because associations became progressively stronger closer to delivery, we investigated more finely divided categories of gestational age (Figure 1) and associations by time to diagnosis (Figure 2). As seen in Figure 1, copeptin levels increased with gestational age among both cases and controls. Copeptin levels were generally higher for cases at each cross-sectional comparison than controls although some of the time points with sparse data did not reach statistical significance and the difference in mean log copeptin levels was smaller for term than for preterm cases. Mean copeptin levels increasingly differed by case, especially after 30 weeks of gestation. Mean levels did not differ by case until closer to time of diagnosis although differences were borderline significant \( (P=0.05) \) among preterm preeclampsia cases for samples taken from 9 to 14 weeks before diagnosis (Figure 2). Samples at or after diagnosis (time 0 in Figure 2) strongly differed among both term and preterm cases.

Table 2. Cross-Sectional Associations Between Log Copeptin Levels and Risk of Pregnancy Complications, Calcium for Preeclampsia Prevention Study

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>n*</th>
<th>Crude OR (95% CI)</th>
<th>P Value</th>
<th>Adjusted OR (95% CI)†</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline copeptin (&lt;22 wk GA)</td>
<td>vs 136 controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclampsia (all cases)</td>
<td>158</td>
<td>1.61 (1.11–2.33)</td>
<td>0.01‡</td>
<td>1.55 (1.03–2.31)</td>
<td>0.03‡</td>
</tr>
<tr>
<td>Preterm PE (diagnosis &lt;37 wk)</td>
<td>63</td>
<td>1.73 (1.06–2.83)</td>
<td>0.03‡</td>
<td>1.86 (1.08–3.20)</td>
<td>0.03‡</td>
</tr>
<tr>
<td>Term PE (diagnosis ≥37 wk)</td>
<td>95</td>
<td>1.58 (1.03–2.44)</td>
<td>0.04‡</td>
<td>1.45 (0.91–2.32)</td>
<td>0.12</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>101</td>
<td>1.20 (0.78–1.86)</td>
<td>0.41</td>
<td>1.33 (0.83–2.13)</td>
<td>0.24</td>
</tr>
<tr>
<td>GDM</td>
<td>92</td>
<td>0.87 (0.56–1.36)</td>
<td>0.55</td>
<td>1.23 (0.72–2.12)</td>
<td>0.45</td>
</tr>
<tr>
<td>Preterm birth without PE</td>
<td>86</td>
<td>1.83 (1.14–2.94)</td>
<td>0.01‡</td>
<td>1.46 (0.87–2.46)</td>
<td>0.15</td>
</tr>
<tr>
<td>Second copeptin (22–32 wk GA)</td>
<td>vs 115 controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclampsia (all cases)</td>
<td>144</td>
<td>2.07 (1.33–3.21)</td>
<td>0.001‡</td>
<td>2.06 (1.28–3.31)</td>
<td>0.003‡</td>
</tr>
<tr>
<td>Preterm PE (diagnosis &lt;37 wk)</td>
<td>57</td>
<td>2.59 (1.47–4.57)</td>
<td>0.001‡</td>
<td>2.76 (1.45–5.10)</td>
<td>0.001‡</td>
</tr>
<tr>
<td>Term PE (diagnosis ≥37 wk)</td>
<td>87</td>
<td>1.70 (1.05–2.76)</td>
<td>0.03‡</td>
<td>1.68 (0.99–2.86)</td>
<td>0.05</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>92</td>
<td>1.32 (0.83–2.11)</td>
<td>0.24</td>
<td>1.37 (0.83–2.28)</td>
<td>0.22</td>
</tr>
<tr>
<td>GDM</td>
<td>81</td>
<td>1.10 (0.69–1.75)</td>
<td>0.69</td>
<td>1.25 (0.72–2.16)</td>
<td>0.43</td>
</tr>
<tr>
<td>Third copeptin (33–38 wk GA)</td>
<td>vs 110 controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclampsia (all cases)</td>
<td>99</td>
<td>2.64 (1.58–4.40)</td>
<td>0.0002‡</td>
<td>2.52 (1.42–4.50)</td>
<td>0.002‡</td>
</tr>
<tr>
<td>Preterm PE (diagnosis &lt;37 wk)</td>
<td>14</td>
<td>5.16 (1.87–14.27)</td>
<td>0.0002‡</td>
<td>8.56 (1.97–37.24)</td>
<td>0.004‡</td>
</tr>
<tr>
<td>Term PE (diagnosis ≥37 wk)</td>
<td>85</td>
<td>2.37 (1.38–4.07)</td>
<td>0.0002‡</td>
<td>2.40 (1.28–4.50)</td>
<td>0.007‡</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>77</td>
<td>1.23 (0.75–2.03)</td>
<td>0.41</td>
<td>1.53 (0.85–2.73)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; GA, gestational age; GDM, gestational diabetes mellitus; OR, odds ratio; and PE, preeclampsia.

* Four term PE cases and 22 gestational hypertension cases also had GDM.
† Adjusted for age, race, body mass index, current smoking, married, private insurance, and clinical site.
‡ P<0.05.
Copeptin levels had slightly stronger associations with severe than with mild preeclampsia (Table S1 in the online-only Data Supplement), but confidence intervals overlapped. Reclassification of definitions for hypertension, which include systolic blood pressure $\geq 140$ mmHg as a diagnostic criterion, also did not alter findings (Table S1). Finally, among 56 women with measured soluble fms-like tyrosine kinase-1 and placental growth factor before 22 weeks (42 of whom were preeclampsia cases), neither soluble fms-like tyrosine kinase-1 ($r=0.10; P=0.45$) or placental growth factor ($r=-0.08; P=0.54$) was correlated with copeptin measures nor significant correlations were observed at later gestational age (data not shown).

**Discussion**

Copeptin is a biomarker of arginine vasopressin, which acts on multiple systems to increase blood pressure and water retention. As a potential biomarker of preeclampsia, we prospectively evaluated copeptin levels among women who consequently developed preeclampsia and other pregnancy complications. Higher copeptin concentrations, measured before recognition of clinical disease, were associated with increased preeclampsia risk. Levels of copeptin increased across gestation regardless of preeclampsia case status but differed more markedly by case status closer to the time of diagnosis. Copeptin was not associated with GDM or gestational hypertension apart from preeclampsia, suggesting that it was specific to development of preeclampsia.

Vasopressin secretion and metabolism are altered in normal pregnancy, with a rise in secretion in early pregnancy. Vasopressinase produced by the placenta increases dramatically during pregnancy, leading to increased vasopressin clearance through cleavage of vasopressin. In fact, vasopressin is cleared 3 to 4× faster after midgestation but plasma levels are maintained, suggesting increased production rates. Our observation that copeptin increases during gestation supports copeptin as a good biomarker for vasopressin secretion.

Vasopressin function is regulated by 3 receptor subtypes. Although the V1a receptor function is suspected to play a role in hypertension, studies have not been able to find strong evidence. Rather, decline in kidney function has more recently been found to be associated with vasopressin via measurement of copeptin levels in large epidemiological studies of nonpregnant individuals. Multiple animal studies suggest that vasopressin's link with kidney function is causal; for instance, blocking vasopressin V1a or V2 receptors protects the deterioration of kidney function in rat models for nephropathy. Conversely, administration of desmopressin, a powerful V2-receptor agonist, in rats and humans increases glomerular filtration rate and urinary albumin excretion.
observations, it would seem women with gestational hypertension (ie, those who avoid kidney damage despite elevated blood pressure) do not overly secrete vasopressin above and beyond that of normal pregnancy. This once again confirms the differences in pathophysiology of the 2 conditions. Among women who come to be diagnosed with preeclampsia, copeptin may serve as an early marker of the possible effect on renal function.

Studies measuring vasopressin directly in human pregnancy have not been conclusive. Risberg et al²⁹ found nonsignificantly higher levels of plasma vasopressin in weeks 12 and 24 of gestation and lower concentrations among mild preeclampsia cases at 36 weeks. Yet another study observed decreases in pregnancy, possibly because of vasopressinase metabolism.²⁸ Our findings using copeptin as a more stable biomarker of vasopressin provide a more accurate picture of vasopressin in pregnancy and in association with preeclampsia.

Few studies have measured copeptin with regards to preeclampsia.²⁹⁻³² The first study observed significant differences in copeptin levels between 64 preeclampsia cases and 32 normotensive controls, but authors provided no information on time of preeclampsia diagnosis and cases may have been diagnosed before blood sampling.¹³ Three other studies have also observed increased maternal copeptin among preeclampsia cases.²⁹,³⁰,³² Santillan et al³² prospectively observed a significant difference in copeptin levels beginning in the first trimester between 20 preeclampsia cases and 26 controls. They also observed using an animal model that infusion of vasopressin during pregnancy in mice leads to the clinical phenotypes observed in preeclampsia.³² Our findings remain novel with regards to investigating the prospective association in a larger sample of preeclampsia cases and including other pregnancy complications.

Vasopressin affects glucose homeostasis.¹¹ Epidemiological evidence have observed associations between elevated copeptin levels and increased risk of incident type 2 diabetes mellitus.¹³,¹⁶,³⁴ Despite these findings, our study showed no association between copeptin and risk of GDM. A lack of association has also been observed in another study conducted at delivery.³⁵ We add to these findings that copeptin levels do not differ before diagnosis of GDM.

To our knowledge, our study is the largest study to date investigating copeptin levels during pregnancy with risk of multiple pregnancy complications. Prospective measurements allowed for investigations into the effect of gestational age and associations in relation to timing of diagnosis. The inclusion of only healthy primiparas may preclude generalizability of results for cases of recurrent preeclampsia. Also, we did not have sufficient numbers of cases of SGA without preeclampsia (n=22) to rule out an independent contribution of SGA completely. In addition, despite the prospective nature of the study, we cannot decipher whether elevation in vasopressin secretion was secondary to endothelial dysfunction caused by placental disease because angiogenic factors were measured at the same time as copeptin. Our limited observation that copeptin was not correlated with measured angiogenic factors suggests that copeptin may provide additional information. A good biomarker for preeclampsia remains elusive and even angiogenic factors, such as soluble endoglin and soluble fms-like tyrosine kinase-1, both minimally affect prediction when examined against other clinical risk factors.³⁶⁻³⁹

**Perspectives**

Copeptin levels are elevated before diagnosis of preeclampsia. Levels progressively increase throughout pregnancy among normal pregnant women but significantly higher levels were observed only among women who develop preeclampsia and not for cases of gestational hypertension or GDM. Our observations add to the understanding of the role of vasopressin on the pathophysiology of preeclampsia.

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**Disclosures**

None.

**References**

What Is New?

- Maternal serum levels of copeptin, a biomarker for vasopressin, rise before clinical diagnosis of preeclampsia. Early pregnancy levels did not differ for other pregnancy complications, such as gestational diabetes mellitus or gestational hypertension without proteinuria, or for preterm birth not complicated by PE, suggesting that copeptin may serve as a specific biomarker for preeclampsia. Copeptin levels also rose as gestational age increased. This observation agrees with what has been shown for levels of vasopressin during pregnancy.

What Is Relevant?

- Because copeptin serves as a stable biomarker for vasopressin, our findings indicate that vasopressin may be dysregulated in cases of preeclampsia before its clinical diagnosis. Copeptin is associated with declining renal function in nonpregnant populations and this supports the notion that vasopressin is important in development of preeclampsia.

Summary

We investigated whether maternal serum copeptin, a biomarker for the antidiuretic hormone arginine vasopressin, differed in preg-

novelty complicated by preeclampsia, gestational hypertension, and gestational diabetes mellitus when compared with uncomplicated pregnancies using stored samples from the Calcium for Preeclampsia Prevention trial (1992–1995). Baseline copeptin levels at mean 16 weeks of gestation were positively associated with increased risk of preeclampsia even after accounting for maternal age, race, body mass index, insurance status, marital status, current smoking, and clinical site. Associations were stronger among cases diagnosed before 37 weeks of gestation, and no associations were found for gestational diabetes mellitus or gestational hypertension. Our findings demonstrate that copeptin levels are elevated in pregnant women before diagnosis of preeclampsia with elevation specific to this pregnancy complication rather than hypertension alone.
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INCREASED LEVELS OF COPEPTIN BEFORE CLINICAL DIAGNOSIS OF PREECLAMPSIA
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Supplemental Methods

Outcome definitions
Trained staff members measured blood pressure as detailed previously1 and urinary protein excretion by strict protocol every 4 weeks from entry until week 29 of gestation, every 2 weeks from week 29 until week 35 and every week thereafter until delivery.2 Case definitions of PE and gestational hypertension have been previously published.2-4 Preeclampsia was defined as hypertension with diastolic blood pressure (DBP) ≥90 mmHg on 2 occasions (4 to 168 hours apart) and proteinuria, defined in one of four ways (i.e., urine dipstick results of at least 1+ (30 m/dL) on 2 occasions (4 to 168 hours apart); a protein:creatinine ratio ≥0.35; urine dipstick results of at least 2+ (100 mg/dL); or a 24-hour urine specimen containing ≥300 mg of protein). Preterm preeclampsia was defined as being diagnosed before 37 weeks gestation and term preeclampsia refers to diagnoses at or after 37 weeks gestation. Gestational hypertension used the same DBP cutoffs as above with no proteinuria. Severe preeclampsia was defined as either severe hypertension (DBP ≥110 mmHg) or severe proteinuria (urinary protein excretion of ≥3.5 g per 24 hours or urine dipstick results of ≥3+[300 mg/dl]). HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) and eclampsia cases were analyzed with severe preeclampsia. GDM was defined using the American Diabetes Association 1997 criteria.5 Preterm birth was defined as delivery before 37 weeks’ gestation.

Covariates
Information was collected at baseline using standardized questionnaires for self-reported age, race/ethnicity, smoking status, education, insurance status, and marital status. BMI was calculated based on measured weight and height at the screening visit. Gestational age was verified by ultrasound at entry. Delivery information was abstracted from medical records. In a subgroup of participants, data regarding levels of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor were available from previous analyses.

**Copeptin measurement**

Samples from the CPEP trial have been stored at -70°C for 17-21 years. Almost all samples (98%) were unthawed. The limit of detection using the BRAHMS Immunoluminometric Assay (Thermo Scientific, Berlin, Germany) was 0.4 pmol/l and the intra- and inter- coefficients of variation were <5% and <10%, respectively. In total, 1465 serum samples had copeptin measured in Germany due to availability of the coated tubes necessary for the assay. Hence, copeptin measurements underwent an additional freeze-thaw due to the shipment but this was uniform across all specimens and previous testing suggest little effect of thawing on copeptin levels. Samples for 36 of the gestational hypertensive cases (32%) lacked sufficient volume. As a result, the current analysis consisted of 136 controls, 71 preterm PE, 98 term PE, 78 gestational hypertension (without GDM), 88 GDM (with 10 cases also having term PE), and 86 preterm deliveries without PE.

**Expanded Statistical Methods**

Copeptin data were normalized by log transformation. Five outlying copeptin values greater than three standard deviations above the mean were deemed implausible and removed from analysis. None of these outliers occurred in baseline samples. Analysis of variance (ANOVA) and chi-square tests were used to compare maternal and infant characteristics by case status for continuous and categorical measures, respectively. Pearson correlations between copeptin and sFlt-1 and placental growth factor (PIGF) were also estimated. The bivariate association between copeptin and risk factors for PE (i.e., maternal age, race, BMI, education, marital status, private insurance, current smoking, and male infant), gestational age at collection and clinical site was assessed by linear regression. Odds ratios for the associations were adjusted for age (continuous), race (African American, Caucasian, Hispanic/Other), BMI (continuous), current smoking (yes/no), married (yes/no), private insurance (yes/no), and clinical site (Albuquerque, Birmingham, Cleveland, Portland, Memphis). All analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC).

**Supplemental References**


Table S1. Risk of PE by severity and by using contemporary definitions per log unit increase in baseline copeptin measured before 22 weeks gestation

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>N</th>
<th>Crude OR (95% CI)</th>
<th>p-value</th>
<th>Adjusted OR (95% CI)*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preeclampsia (all cases)</td>
<td>158</td>
<td>1.61 (1.11-2.33)</td>
<td><strong>0.01</strong></td>
<td>1.55 (1.03-2.31)</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>Severe PE†</td>
<td>61</td>
<td>1.85 (1.11-3.09)</td>
<td><strong>0.02</strong></td>
<td>1.95 (1.11-3.42)</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>Mild PE</td>
<td>97</td>
<td>1.52 (1.00-2.30)</td>
<td><strong>0.05</strong></td>
<td>1.42 (0.90-2.23)</td>
<td>0.13</td>
</tr>
<tr>
<td>Severe Gestational Hypertension</td>
<td>44</td>
<td>1.55 (0.85-2.81)</td>
<td>0.15</td>
<td>1.52 (0.78-2.96)</td>
<td>0.21</td>
</tr>
<tr>
<td>Mild Gestational Hypertension</td>
<td>114</td>
<td>1.64 (1.10-2.43)</td>
<td><strong>0.01</strong></td>
<td>1.58 (1.03-2.43)</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Severe Proteinuria</td>
<td>24</td>
<td>1.63 (0.81-3.29)</td>
<td>0.17</td>
<td>1.77 (0.79-3.97)</td>
<td>0.16</td>
</tr>
<tr>
<td>Mild Proteinuria</td>
<td>134</td>
<td>1.63 (1.10-2.41)</td>
<td><strong>0.01</strong></td>
<td>1.52 (0.99-2.33)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Adjusted for age, race, BMI, current smoking, married, private insurance, and clinical site
† 12 severe PE cases met criteria for both severe hypertension and proteinuria
‡ Updated definition of PE based on both systolic and diastolic blood pressure cutoffs (140/90 mmHg). Six normal controls had gestational hypertension after reclassification. Five cases of preterm PE and 14 term cases were added (from previously preterm, GDM, gestational hypertension, gestational proteinuria groups.)