Abstract—Leptin, an adipocyte-derived hormone, plays an important role in reproduction and angiogenesis. Studies examining leptin in preeclampsia are inconsistent, possibly because of small sample sizes and variability in sampling and outcome. We conducted a nested case–control study to examine associations between serum leptin (measured: 9–26 weeks gestation) and preeclampsia among 430 primiparous preeclamptic women and 316 primiparous normotensive controls from the Danish National Birth Cohort. Median (interquartile range) leptin concentrations were calculated. Associations between leptin and preeclampsia (blood pressure ≥140/90 mm Hg, term preeclampsia (preeclampsia and delivery ≥37 weeks gestation), or preterm preeclampsia (preeclampsia and delivery <37 weeks gestation) were examined using generalized linear models adjusting for body mass index, gestational age at blood draw, maternal age, smoking, and socio-occupational status. As leptin is increased in obese women and the risk of preeclampsia increases with body mass index, we used the Sobel test to examine whether leptin is a mediator of this relationship. After adjustments, leptin concentrations were significantly higher in women with preeclampsia (30.5 [24.6]; P=0.0117) and term preeclampsia (30.4 [24.9]; P=0.0228) compared with controls (20.9 [28.3]). There was no significant difference between preterm preeclampsia (30.6 [23.4]; P=0.2210) and controls. Leptin is a possible mediator of the association between body mass index and preeclampsia (P=0.0276). Leptin concentrations are higher in women with preeclampsia compared with normotensive controls and may mediate some of the relationship between body mass index and preeclampsia. (Hypertension. 2015;65:00-00. DOI: 10.1161/HYPERTENSIONAHA.114.03979.)

Key Words: body mass index • hypertension • leptin • preeclampsia • pregnancy
Although epidemiological studies have shown significant associations between leptin and preeclampsia, some studies have found no association after adjustment for maternal characteristics, including body mass index (BMI).14–20 Indeed serum leptin is increased with obesity,2 and increasing BMI has been shown to be linked with preeclampsia.21,22 However, it is possible that leptin partly mediates this association. This may occur as a result of an increase in placental leptin resistance and a dysregulation of leptin function, which is observed in obese women.23 We aimed to examine the association between serum leptin in early pregnancy (9–26 weeks gestation) and pre-eclampsia defined by severity among 430 preeclamptic women and 316 normotensive controls from the Danish National Birth Cohort (DNBC). In addition, we explored whether leptin mediated the association between maternal BMI and preeclampsia.

Methods

Study Population
This study was part of a previously completed nested case control study of 562 primiparous women with preeclampsia, singleton pregnancies, and no gestational diabetes mellitus and 377 primiparous normotensive controls with singleton pregnancies and no gestational diabetes mellitus selected from the DNBC.24 Our subset has similar characteristics compared with primiparous singleton women in the DNBC. For example, in both groups, most women had a maternal age between 26 and 30 (52.9% versus 52.2%) and a high socio-occupational status (67.5% versus 64.9%). There were slight differences in BMI (normal, 70.6% versus 62.5%) and smoking (25.3% versus 18.8%) due mainly to the larger percentage of preeclamptic women in our cohort who are more likely to have increased BMI and less likely to smoke.25 The DNBC is a longitudinal population-based cohort of 101,033 pregnancies and their offspring. Details on the methods of recruitment, retention, and data collection have been published elsewhere.26 Briefly, between 1996 and 2003, women who were receiving prenatal care were recruited at first prenatal visits by their general practitioners. At the first study visit, pregnancy was confirmed and a blood sample was obtained. Telephone interviews were administered at recruitment (median 16 weeks, range 6–40 weeks), at 30 weeks gestation, and twice after delivery. For this study, cohort members were merged with National Birth Register and National Hospital Discharge Registry via a unique personal code given to citizens of Denmark. Gestational age was determined by last menstrual period reported but corrected with an early ultrasound if the subject reported use of contraception 4 months before conception, had irregular periods, or had an abnormal last menstrual cycle. The DNBC was approved by the Danish Ethics Central Committee.

For this substudy, leptin results were available for 512 cases and 339 controls. Women with a history of hypertension (n=75) and those with samples obtained in the third trimester (n=30) were excluded from the analyses, yielding 430 cases and 316 controls with markers assessed at recruitment dates ranging from 9 to 26 weeks of gestation. The majority of samples in our study were collected in the second trimester (n=675; median 17 weeks). An additional 71 samples were from women recruited in the first trimester (median 12 weeks). The main analyses were conducted pooling these samples together because inclusion of the relatively small number of first trimester samples was not expected to significantly influence the results. Furthermore, median leptin levels were similar in the first and second trimester samples (29.6 [interquartile range, 27.25] versus 24.9 [interquartile range, 26.3]; P=0.5432). This study was approved by the University of Pittsburgh Institutional Review Board and the Danish Data Protection Agency.

Preeclampsia Definition
Women with preeclampsia were identified by a positive report of preeclampsia at the postnatal interview and confirmed by an International Classification of Diseases (ICD) code and discharge diagnoses in the National Hospital Discharge Registry of 637.03, 637.04, 637.09, 637.19 (ICD-8) or D014 to D015 (ICD-10). Preeclampsia was determined if a woman had either systolic or diastolic blood pressure ≥140/90 mm Hg measured twice with an interval of 6 hours and the presence of proteinuria (≥0.3 g/24 hours or ≥1 urine dipstick measured twice with an interval of 4 hours). A chart abstraction study within the DNBC shows that compared with a chart review using American College of Obstetrics and Gynecology (ACOG) criteria, the Danish National Discharge Registry yields a highly specific diagnosis of preeclampsia (99%).26 Preeclampsia is a heterogeneous syndrome.27 For example, early and late onset preeclampsia are suggested subtypes with different pathophysiological pathways and clinical presentation.28 In this analysis, we further classified preeclampsia resulting in either a term birth (≥27 weeks gestation) or a preterm birth (<37 weeks of gestation) as separate outcomes. Preeclampsia with preterm birth was used as a proxy for severity and early onset.

Leptin Measurement
Whole blood samples obtained at the first study visit were mailed to the Statens Serum Institute in Copenhagen and were separated and stored at −80°C. The average time from collection to processing was 28 hours. Leptin was measured in duplicate with an in-house assay using the multiplex low-cyrometric assay system Luminex MultiAnalyte Profiling Technology (LabMap; Luminex Corporation, Austin, TX). The calibration curve was calculated by the Bio-Plex 3.0 software (BioRad). A 5-parameter logistic regression equation was used to determine leptin concentrations. The working range for leptin was assessed from the precision profile and defined as the concentration range where the coefficient of variation was <20%.29

Maternal Characteristics
During the first study interview, women reported the following: gravidity/parity, occupation, cigarette use during pregnancy, prior medical conditions, prior spontaneous abortions, and prepregnancy weight and height. Maternal age was self-reported at delivery and grouped as <25, 25–29, 30–34, and 35+. Socio-occupational status was based on a women’s job classification or education. High status was assigned to women in management or jobs that required >4 years of post-high school education. Mid status was assigned to those with office, service, or skilled manual workers or women in the military. Low status included unskilled or unemployed women. Pre-pregnancy BMI was determined using reported height and weight at the first study interview and was categorized as underweight or normal (<25), overweight (≥25 and <30), or obese (≥30).

Statistical Analyses
Baseline variables, including gestational age at blood draw, maternal age, BMI, smoking, and sociooccupational status, were compared between all preeclamptic cases and normotensive controls. In addition, we compared rates of preterm birth between cases and controls. Logistic regression was used to examine associations between variables and preeclampsia. A P value <0.05 was used to determine statistical significance. The median and interquartile range for leptin was calculated for preeclampsia, preeclampsia defined by gestational age of delivery, and normotensive controls. Distributions were compared by Wilcoxon rank-sum test. To determine whether leptin concentrations and preeclampsia were significantly associated, P values were calculated using generalized linear models. As leptin is not normally distributed, the logarithm of leptin with base 2 was used (this represents a doubling of intensity). Additionally, leptin was dichotomized (median) and logistic regression was used to calculate odds ratio (ORs; as an estimate for relative risk), and 95% confidence interval (CI). Dichotomizing by the median is 1 method to analyze biomarkers with limits of detection.30 However, we chose to also examine the data in the continuous models to determine whether there was consistency among both modeling approaches. Maternal age, gestational age at blood draw, BMI, smoking, and socio-occupational status were included in all regression models. Finally, we explored whether leptin mediated the relationship between BMI and preeclampsia using a modified version of the Sobel test for binary outcomes.31 Statistical significance was determined as a P value <0.05. Although adequately
powered for our main analyses, a post hoc power analysis reveals that our power was reduced (60%) to detect significant differences in leptin levels between preterm preeclampsia and normotensive controls (based on calculated medians). All analyses were conducted using SAS V9.2 (Cary, NC).

Results
Table 1 compares characteristics between all women with pre-eclampsia and normotensive controls. The majority of women in our cohort were between the ages of 26 and 30, had a BMI <25, did not smoke during pregnancy, had a high socio-occupational status, and delivered a term infant. When we compared baseline variables between cases and controls, we found that overweight women (OR, 1.9; 95% CI, 1.3–2.8; P<0.0006), obese women (OR, 4.1; 95% CI, 2.4–7.4; P<0.0001), and women with low socio-occupational status (OR, 2.8; 95% CI, 1.1–7.1; P=0.0263) had a higher odds of preeclampsia. Women who smoked at the time of enrollment were significantly less likely to have preeclampsia (OR, 0.6; 95% CI, 0.3–0.9; P=0.0158). In addition, preeclamptic women were significantly more likely to have a preterm infant <37 weeks gestation (OR, 6.8; 95% CI, 3.7–12.7; P<0.0001) and <34 weeks gestation (OR, 4.2; 95% CI, 1.5–10.9; P=0.0038).

Compared with normotensive controls (n=316; median 20.9 [interquartile range, 28.3]), leptin concentration levels were higher in women with preeclampsia (n=430; 30.5 [24.6]; P<0.0001). Results were similar for women with preterm preeclampsia (n=91; 30.6 [23.4]; P=0.0192) and term preeclampsia (n=339; 30.4 [24.9]; P<0.0001). After adjustments for maternal age, gestational age at blood draw, BMI, smoking, and socio-occupational status, there was a significant association between leptin and preeclampsia (P=0.0117) where each unit increase in leptin increased the log odds of having preeclampsia versus being normotensive (Table 2). Results were similar in women with term preeclampsia (P=0.0228). Leptin was not significantly associated with preterm preeclampsia (P=0.2210). Median leptin concentrations in early pregnancy were higher among women with preeclampsia, resulting in a preterm birth <34 weeks (41.0 [23.1]) as compared with controls, although the sample size of these cases was limited (n=27) and multivariate models did not show a significant association (P=0.7646). We conducted our analysis in second trimester samples only (n=675) and found that leptin remained significantly associated with preeclampsia (P=0.0061). Similarly, if we examine a smaller gestational age range between 15 and 18 weeks (n=308), before the diagnosis of preeclampsia and when the majority of samples were collected, leptin remains significantly associated with preeclampsia (P=0.0332).

Dichotomized models examining associations between elevated leptin (median) and preeclampsia yielded similar results (Table 3). Elevated leptin increased the odds of preeclampsia (OR, 1.4; 95% CI, 1.0–2.0). Results were similar for preterm (OR, 1.8; 95% CI, 1.1–3.0) and term preeclampsia (OR, 1.4; 95% CI, 1.0–2.0).

In our cohort, lean women (18.9 [23.1]) had lower median leptin levels than overweight or obese women (41 [3.7]). Furthermore, increasing BMI was significantly associated with increased leptin levels independent of gestational age at blood draw, maternal age, smoking, or socio-occupational status (P<0.0001). We evaluated whether leptin may mediate the relationship between BMI and preeclampsia using the Sobel test. Leptin was a potential mediator of BMI and preeclampsia (P=0.0276) and accounted for 19.6% of the total effect.

Table 1. Comparison of Baseline and Pregnancy Outcome Variables Between Preeclamptic Women and Normotensive Controls

<table>
<thead>
<tr>
<th>Outcome Data</th>
<th>Controls, N (%)</th>
<th>Cases*, N (%)</th>
<th>PValue†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at sampling, mean=SD</td>
<td>16.9±3.5</td>
<td>16.4±3.5</td>
<td>0.7827</td>
</tr>
<tr>
<td>Maternal age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤25</td>
<td>67 (21.2)</td>
<td>119 (27.7)</td>
<td>Reference</td>
</tr>
<tr>
<td>26–30</td>
<td>183 (57.9)</td>
<td>209 (48.6)</td>
<td>0.0160</td>
</tr>
<tr>
<td>31–35</td>
<td>59 (18.6)</td>
<td>88 (20.5)</td>
<td>0.4422</td>
</tr>
<tr>
<td>≥36+</td>
<td>7 (2.2)</td>
<td>14 (3.3)</td>
<td>0.8076</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>245 (77.5)</td>
<td>253 (58.8)</td>
<td>Reference</td>
</tr>
<tr>
<td>≥25 and &lt;30</td>
<td>55 (17.4)</td>
<td>108 (25.1)</td>
<td>0.0006</td>
</tr>
<tr>
<td>≥30</td>
<td>16 (5.1)</td>
<td>69 (16.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smoking in pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>246 (77.9)</td>
<td>350 (81.4)</td>
<td>Reference</td>
</tr>
<tr>
<td>Yes, past</td>
<td>25 (7.9)</td>
<td>44 (10.2)</td>
<td>0.4203</td>
</tr>
<tr>
<td>Yes, current</td>
<td>45 (14.2)</td>
<td>36 (8.3)</td>
<td>0.0158</td>
</tr>
<tr>
<td>Socio-occupational status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>209 (66.1)</td>
<td>270 (62.9)</td>
<td>Reference</td>
</tr>
<tr>
<td>Mid</td>
<td>101 (32.0)</td>
<td>137 (31.9)</td>
<td>0.7609</td>
</tr>
<tr>
<td>Low</td>
<td>6 (1.9)</td>
<td>22 (5.1)</td>
<td>0.0263</td>
</tr>
</tbody>
</table>

*Cases include all women with preeclampsia.  †P values were calculated using multivariate logistic regression models.

Table 2. Associations Between Leptin Concentrations and Preeclampsia Subtypes

<table>
<thead>
<tr>
<th>Leptin Concentration</th>
<th>Preterm Preeclampsia</th>
<th>Term Preeclampsia</th>
<th>Estimate (β)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/mL</td>
<td>All Preeclampsia</td>
<td>30.5 (24.6)</td>
<td>30.4 (24.9)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Preterm preeclampsia</td>
<td>30.6 (23.4)</td>
<td>30.4 (24.9)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Term preeclampsia</td>
<td>30.6 (23.4)</td>
<td>30.4 (24.9)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*P<0.01.  †P<0.05.
Table 3. Associations Between Elevated Leptin Dichotomized Above the Median and Preeclampsia Subtypes

<table>
<thead>
<tr>
<th>Leptin Concentration</th>
<th>Outcome Group</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All preeclampsia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin elevated&gt;median</td>
<td>246 (57.2)</td>
<td>127 (40.1)</td>
</tr>
<tr>
<td>Preterm preeclampsia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin elevated&gt;median</td>
<td>48 (59.3)</td>
<td>127 (40.1)</td>
</tr>
<tr>
<td>Term preeclampsia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin elevated&gt;median</td>
<td>174 (56.6)</td>
<td>127 (40.1)</td>
</tr>
</tbody>
</table>

Odds ratios were calculated by logistic regression. Models were adjusted for maternal body mass index, gestational age at blood draw, maternal age, smoking, and socio-occupational status. CI indicates confidence interval.

Discussion

Our results demonstrate that serum leptin concentrations measured in early pregnancy are significantly higher in women with preeclampsia compared with normotensive controls after adjusting for known confounding factors, including BMI. Furthermore, examining leptin in a smaller gestational age range before 20 weeks when preeclampsia would be diagnosed shows similar results. Therefore, leptin may be elevated in women who will subsequently develop preeclampsia. Our results are consistent with several small studies conducted in the third trimester that have found elevated maternal leptin in preeclamptic women compared with healthy pregnant controls.1-5 In contrast, a longitudinal examination of leptin in 71 preeclamptic women and 71 age-, parity-, and BMI-matched controls reported lower leptin levels at 18 weeks of gestation in women who developed subsequent preeclampsia.22 A study of 126 preeclamptic women found that first trimester free leptin index was significantly elevated compared with 289 controls (P<0.001).33 The largest study to date was nested within a study of pregnancy outcomes, which included 12,804 births in Norway.34 This study included 256 cases of preeclampsia and 607 controls and reported that umbilical cord leptin levels were significantly higher in women with preeclampsia compared with controls after adjustment for gestational age.

Leptin is suggested to play a role in angiogenesis, immunomodulation, and fatty acid metabolism in early placentation.1 Reduced placental perfusion is hypothesized to increase placental expression of leptin, which may increase nutrient delivery to the fetus.5 Studies show that leptin released from the placenta can stimulate system A amino acid transport,35,36 possibly influencing fetal growth. Thus, leptin may be a coping mechanism for reduced placental perfusion and a marker of placental insufficiency. Alternatively, an increase in maternal leptin expression may be a result of other stimuli. Leptin has been shown to play a role in immunity,16 although its function is not completely understood. As an altered immune response is one pathway which may lead to preeclampsia,17 it is possible that inflammatory stimuli or immune dysfunction could alter maternal leptin expression. Once increased, leptin may have direct effects on the development of preeclampsia. In pregnant rats, leptin has been shown to increase blood pressure.35 Leptin has also been shown to be correlated with systolic and diastolic blood pressure in pregnant women, independent of BMI.37 Although 98.4% of placental leptin may be released into the maternal circulation,38 in our study, we cannot determine the proportion of placental leptin measured in maternal serum. As placental leptin expression and placental leptin protein are increased in preeclampsia and correlate with circulating levels,39,40 it is suggested that the placenta contributes substantially to serum leptin concentrations. Additionally, leptin is reported to increase throughout pregnancy and then drastically reduces postpartum.41 However, one study found that placental leptin expression is similar in obese and lean women, suggesting that adiposity may increase circulating leptin concentrations during obesity.

As leptin has been indicated to be a possible marker for early onset preeclampsia (<34 weeks),41 we examined associations between leptin and preeclampsia subtypes. We found that leptin concentrations were significantly higher in term preeclampsia but not preterm preeclampsia. The effect sizes and median leptin levels were similar for term and preterm cases. Among 27 cases of preterm preeclampsia <34 weeks gestation, no significant associations with leptin were observed. We were underpowered to examine the associations with preterm preeclampsia. Larger cohorts with well-defined subtypes are needed to examine these associations.

Obesity is associated with an increased risk of mild and severe forms of preeclampsia,22 as well as hyperleptinemia.23 The role of obesity in the pathogenesis of preeclampsia is not clear. It has been suggested that increased leptin, inflammation, and metabolic markers may lead to preeclampsia in obese women.22 We found that leptin could be a possible mediator of BMI and preeclampsia. However, it only accounted for a small percent of the total effect. This suggests that other factors such as increased inflammation in addition to leptin may play a role in obesity-related preeclampsia. Obesity may lead to dysregulation in leptin function that results in maternal disease. Placental leptin resistance is present in maternal obesity because of syncytiotrophoblast downregulation of leptin receptor during states of maternal hyperleptinemia.23 Alternatively, an increase in leptin during obesity may have direct effects on inflammation and blood pressure. The relationship between BMI, leptin, and preeclampsia is likely complex. Future work is needed to explore these relationships.

We obtained data from a large well-defined cohort and were able to adjust for several known risk factors for preeclampsia. This is one of the largest studies examining relationships between serum leptin and preeclampsia. We did not have data on time of diagnosis of preeclampsia. Therefore, some women in our study may have had preeclampsia at the time of blood sampling. However, our results were the same when we examined a smaller gestational age window between 15 and 18 weeks before when preeclampsia would have been diagnosed by a clinician. Still, we cannot rule out the presence of subclinical disease at the time of blood sampling. Diagnostic codes to classify women as having severe or mild preeclampsia were not available for all women in the DNBC. We used gestational age of delivery as a proxy for severe disease and disease onset. This is a common approach,13 but may have limited our ability to assess the role of leptin in preeclampsia severity. As the placenta grows during pregnancy, placental-derived products may increase, and thus, leptin levels may...
vary by the gestational age of sampling. However, we did not find significant differences in gestational age at sampling between cases and controls. We relied on self-reported BMI, which may be lower than true BMI and may bias results of our mediation analysis. Although the in-house assay used to measure leptin was previously validated, a coefficient of variation was not calculated specifically for our study.

**Perspectives**

Our observational study suggests that serum leptin levels measured in early pregnancy are elevated in women with pre-eclampsia compared with normotensive controls. Leptin may be a useful biomarker for predicting preeclampsia and could be used clinically as a screening tool in early pregnancy. However, additional studies are needed before assessing its clinical utility. First, these results need to be replicated in an independent cohort. Studies using both circulating and placental expression of leptin would be useful to gain more insight into the role of leptin in preeclampsia pathogenesis. Additionally, further prospective studies with larger samples of women recruited before or early in gestation are needed to confirm whether this relationship is temporal. Finally, the relationship between leptin, BMI, and preeclampsia requires further examination.

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**Disclosures**

None.

**References**


**Novelty and Significance**

- **What Is New?**
  - This is the largest study to examine serum leptin in early pregnancy (9–26 weeks gestation) and its relationship to preeclampsia defined by severity.

- **What Is Relevant?**
  - Serum leptin measured in early pregnancy (9–26 weeks gestation) is elevated in preeclampsia.

**Summary**

Elevated maternal serum leptin measured in early pregnancy is associated with preeclampsia.
Serum Leptin Measured in Early Pregnancy Is Higher in Women With Preeclampsia Compared With Normotensive Pregnant Women
Brandie D. Taylor, Roberta B. Ness, Jørn Olsen, David M. Hougaard, Kristin Skogstrand, James M. Roberts and Catherine L. Haggerty

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