Insulin Resistance But Not Inflammation Is Associated With Gestational Hypertension

Myles Wolf, Laura Sandler, Ricardo Jimenez-Kimble, Anand Shah, Jeffrey L. Ecker, Ravi Thadhani

Abstract—Hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia, are leading causes of pregnancy-associated morbidity. Although insulin resistance and inflammation contribute to preeclampsia, prospective data regarding mechanisms of gestational hypertension are sparse. We conducted a prospective, nested case-control study to test the hypotheses that insulin resistance, marked by reduced sex hormone–binding globulin (SHBG) levels, and inflammation, marked by increased C-reactive protein levels, are similarly associated with gestational hypertension. We measured first-trimester C-reactive protein and SHBG levels in 51 women who subsequently developed gestational hypertension and 102 randomly selected normotensive pregnant controls. Compared with controls, first-trimester SHBG levels were significantly reduced among women who later developed gestational hypertension (176±73 versus 203±79 nmol/L; P=0.03), but there was no difference in C-reactive protein levels. There was statistically significant interaction among nulliparity, first-trimester SHBG levels, and risk of gestational hypertension, such that increasing SHBG levels were associated with significantly reduced risk of gestational hypertension among nulliparous women (odds ratio, 0.64 per 50-nmol/L increase; 95% confidence interval, 0.46, 0.90; P<0.01) but not among multiparous women. This association remained significant after adjusting for potential confounders (odds ratio, 0.55; 95% confidence interval, 0.31, 0.98; P=0.04). We conclude that insulin resistance, but not inflammation, is an independent risk factor for gestational hypertension among nulliparous women. Furthermore, important mechanistic differences exist in the pathogenesis of gestational hypertension comparing nulliparous and multiparous women. (Hypertension. 2002;40:886-891.)

Key Words: hypertension, gestational ■ insulin resistance ■ prospective studies

Hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia, complicate 5% to 10% of pregnancies.1 Gestational hypertension refers to transient, pregnancy-induced hypertension, whereas preeclampsia is defined as similar new onset hypertension in association with proteinuria.1 Although preeclampsia is widely recognized as a leading cause of maternal and fetal morbidity and mortality, gestational hypertension is often considered a benign condition. However, although maternal end-organ damage is more common in preeclampsia, gestational hypertension is also associated with increased rates of cesarean section, preterm delivery, and small-for-gestational-age babies.2–4 Furthermore, like preeclampsia, gestational hypertension is often the harbinger of future chronic hypertension.5,6 Nonetheless, most studies that examined hypertensive disorders of pregnancy focused primarily on preeclampsia, and as a result, little is known about mechanisms of gestational hypertension. Moreover, whether gestational hypertension and preeclampsia represent different ends of a single pathophysiological spectrum or 2 distinct processes linked coincidentally by hypertension is also unknown.

Insulin resistance and inflammation are associated with essential hypertension and cardiovascular disease.7,8 These factors have also been proposed as potential mechanisms of hypertensive disorders of pregnancy.9–11 Although data support a role for insulin resistance in preeclampsia,12 evidence in gestational hypertension is conflicting and comes mostly from small, cross-sectional or retrospective case-control studies.13–22 Furthermore, although prospective data suggest increased inflammation is associated with the development of preeclampsia, no studies have examined the role of inflammation in the pathogenesis of gestational hypertension.

An increased level of C-reactive protein (CRP) is a sensitive marker of systemic inflammation that is associated with cardiovascular disease23 and preeclampsia.10 Sex hormone–binding globulin (SHBG) is a glycoprotein synthesized by the liver that serves as a carrier protein for circulating estrogen and testosterone.24 Insulin is a potent inhibitor of hepatic SHBG synthesis,25 and thus, reduced SHBG is a marker of hyperinsulinemia26 and insulin resistance.27 Two large prospective studies of healthy women, in which reduced baseline SHBG levels were independently associated with
future type 2 diabetes, validated the clinical relevance of SHBG as a marker of insulin resistance. In pregnancy, reduced first-trimester SHBG levels were independently associated with increased risk of developing preeclampsia. Therefore, the aims of the present study were to test the hypotheses that like preeclampsia, increased first-trimester insulin resistance (marked by reduced SHBG levels) and inflammation (marked by increased CRP levels) are similarly associated with increased risk of gestational hypertension.

**Methods**

We conducted a prospective, nested case-control study. Study subjects were selected from the Massachusetts General Hospital Obstetric Maternal Study (MOMS). The design of this prospective cohort has been described previously. In brief, women who receive prenatal care at Massachusetts General Hospital and its affiliates are offered inclusion in the MOMS cohort during their first prenatal visit (10 to 12 weeks of gestation). Subjects who provide written informed consent allow first-trimester serum samples to be collected and frozen for future research purposes. Details of the prenatal period, outcome of pregnancy, and laboratory data are collected prospectively on all subjects in an electronic medical record that is used to generate the research database. The institution’s committee for the protection of human research subjects approved the study.

Gestational hypertension was defined as blood pressure ≥140/90 mm Hg that first appeared after 20 weeks of gestation and that resolved within 6 weeks postpartum in the absence of proteinuria (i.e., urine protein <2+ by dipstick and <300 mg/24 hours). Members of the cohort who remained normotensive throughout pregnancy and who delivered after 37 weeks of gestation were eligible controls. Women with preexisting diabetes or chronic hypertension (blood pressure ≥140/90 mm Hg or antihypertensive medication use before or during early pregnancy) and those who developed gestational diabetes or preeclampsia were excluded. Because blood pressure normally decreases during the second trimester of pregnancy, to avoid misclassifying women with chronic hypertension as normotensive, only women who had normal blood pressure measurements recorded before 14 weeks of gestation were eligible. Based on these criteria, all 51 eligible cases of gestational hypertension in the MOMS cohort were included in this study. From the pool of eligible controls, 102 women were randomly selected by using random number–generating software. We estimated, based on a prior study that this sample of 51 cases and 102 controls would generate 90% power to detect a standardized difference in mean SHBG levels (>0.5 in CRP levels).

Frozen first-trimester serum samples were thawed and assayed for SHBG and CRP. Because estradiol stimulates and testosterone inhibits SHBG synthesis, these sex hormones were also measured. All samples were handled identically by dedicated laboratory personnel from the time of blood collection, through the sample storage and assay process. Batched samples consisting of a random mix of cases and controls were assayed by laboratory personnel blinded to pregnancy outcome. Sex hormone and SHBG assays were performed using Diagnostic Products Corporation test kits. SHBG was measured using an immunometric assay with inter- and intra-assay coefficients of variation (CV) of <8%. Estradiol was measured using a microparticle enzyme immunoassay with inter- and intra-assay CVs of <10%. Testosterone was measured using a solid-phase radioimmunoassay with inter- and intra-assay CVs of <10%. The free androgen index was calculated according to the following formula: total testosterone (nmol/L) × 100/SHBG (nmol/L). CRP was measured using a high-resolution N-latex CRP mono-assay (Quest Diagnostics Inc) with inter- and intra-assay CVs of <6%. All women underwent routine screening for gestational diabetes using the nonfasting, 50-g oral glucose–loading test between 26 and 28 weeks of gestation. One-hour postloading plasma glucose levels were measured using standard glucose oxidase assays with an inter- and intra-assay CV of <2%.

Data were analyzed using STATA statistical software (STATA Corporation). Continuous variables were analyzed using 2-sample t tests or Wilcoxon rank-sum test; categorical variables, using Fisher’s exact test. Logistic regression was used to adjust for confounders. Because nulliparity is a powerful independent risk factor for gestational hypertension, we tested for interaction between nulliparity and the exposure variables of interest (SHBG and CRP), and selected covariates (baseline blood pressure and body mass index, estradiol and testosterone), as has been suggested. Continuous variables are reported as mean±SD unless otherwise stated. Probability values <0.05 were considered statistically significant.

**Results**

Baseline and delivery characteristics of all subjects according to pregnancy outcome are presented in the Table. The mean gestational age at the time of the first prenatal visit when serum was sampled was between 10 and 11 weeks and was no different between cases and controls. Baseline characteristics were similar among cases and controls, except women who later developed gestational hypertension displayed increased blood pressure at enrollment and were more likely to be nulliparous and non-Hispanic. There was no significant difference in cesarean section rates, fetal birth weight, or gestational age at delivery among women with gestational hypertension compared with controls.

The distributions of first-trimester sex hormone profiles and CRP, according to pregnancy outcome, are presented in Figure 1. Compared with controls, women who subsequently developed gestational hypertension displayed reduced first-trimester SHBG (176±73 versus 203±79 nmol/L; P=0.03). In contrast, when comparing cases to controls, there were no significant differences in levels of estradiol (5033±2595 versus 6105±4112 pmol/L), total testosterone (median, 1.4

<table>
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<tr>
<th>Baseline and Delivery Characteristics According to Pregnancy Outcome</th>
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<tr>
<td><strong>Gestational Hypertension</strong> (n=51)</td>
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<td><strong>Baseline characteristics</strong></td>
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<td>Age, y</td>
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<tr>
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<td>Nulliparous, %</td>
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<td>Body mass index, kg/m²</td>
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Continuous variables are reported as mean±SD.
versus 1.5 nmol/L), free androgen index (median, 0.8 versus 0.7), or CRP (median, 1.8 versus 2.3 mg/L). One-hour postloading plasma glucose levels measured during the screening test for gestational diabetes at 26 to 28 weeks of gestation were significantly increased among women who subsequently developed gestational hypertension compared with controls (6.6±1.4 versus 6.1±1.2 mmol/L; \( P = 0.02 \)).

Given the strong association between nulliparity and gestational hypertension, we tested for interaction between nulliparity and other exposures and the risk of developing gestational hypertension. There was no interaction between nulliparity and CRP, blood pressure, body mass index, estradiol, testosterone, or free androgen index. The test for interaction between nulliparity and SHBG, however, was statistically significant (\( P = 0.04 \)), suggesting different associations between SHBG and risk of gestational hypertension among nulliparous versus multiparous women. Therefore, in subsequent analyses, nulliparous and multiparous women were examined separately.

There were 86 nulliparous women in the study, 40 of whom developed gestational hypertension. In contrast, only 11 of the 67 multiparous women developed gestational hypertension (odds ratio [OR], 4.4; 95% confidence interval [CI], 1.9 to 10.6; \( P < 0.01 \)). Although a significant association between reduced SHBG and gestational hypertension was observed in the crude univariate analysis (Figure 1), this association was primarily owing to nulliparas. Specifically, the difference in mean first-trimester SHBG levels among cases and controls was significant in the nullipara stratum (175±64 versus 218±75 nmol/L; \( P < 0.01 \)) but not in the multipara stratum (179±100 versus 192±81 nmol/L; \( P = \text{NS} \)). In contrast, there were no differences comparing the parity-stratified distributions of estradiol, testosterone, free androgen index, and CRP with their respective unstratified distributions (data not shown).

The differential risk of developing gestational hypertension among nulliparas compared with multiparas, expressed as a function of first-trimester SHBG levels, is presented in Figure 2. As shown in Figure 2, nulliparous women were at increased risk for gestational hypertension compared with multiparas women across the full spectrum of SHBG. However, there was a statistically significant association between SHBG levels and risk of gestational hypertension only among nulliparous women. Among nulliparas, each 50-nmol/L increase in SHBG was associated with a 36% reduced risk of gestational hypertension (OR, 0.64; 95% CI, 0.46 to 0.90; \( P < 0.01 \)). Among multiparas, the same increment in SHBG
was not associated with gestational hypertension risk (OR, 0.90; 95% CI, 0.61 to 1.35; \( P = \text{NS} \)). In a logistic regression model including only nulliparous women and adjusted for age, race, baseline body mass index and blood pressure, smoking, gestational age at the time of serum sampling, estradiol and testosterone, each 50-nmol/L increase in SHBG was associated with a 45% reduced risk of developing gestational hypertension (OR, 0.55; 95% CI, 0.31 to 0.98; \( P = 0.04 \)). Baseline mean arterial blood pressure was the only other independent correlate of gestational hypertension (OR, 1.14 per unit increase; 95% CI, 1.05 to 1.23; \( P < 0.01 \)). Increased CRP was not associated with gestational hypertension when included in the multivariable models.

### Discussion

In this prospective study, we identified an association between first-trimester insulin resistance, marked by reduced SHBG levels, and subsequent gestational hypertension. Interestingly, this association was driven almost entirely by nulliparous women, whereas among multiparas, no link was detected. In contrast, we found no association between inflammation and gestational hypertension. These are among the first prospective data that support the hypothesis that insulin resistance contributes to the pathogenesis of gestational hypertension. At the same time, the data suggest that important mechanistic differences exist in the pathogenesis of gestational hypertension comparing nulliparous versus multiparous women.

SHBG is a glycoprotein synthesized by the liver that mediates the balance between free (biologically active) and bound (biologically inactive) testosterone and estrogens. Hepatic SHBG synthesis is stimulated primarily by estradiol and thyroid hormone, and it is inhibited by insulin. Importantly, the inhibitory effects of insulin predominate in situations when there are competing stimuli for hepatic SHBG production, such as pregnancy, when both estradiol and insulin levels increase progressively. Furthermore, SHBG levels exhibit minimal diurnal variability and, unlike other markers of insulin resistance, minimal variability when comparing fasting and postprandial levels. These properties render SHBG a unique marker of insulin resistance that is especially useful in nonfasting states, including obstetric prenatal care. Indeed, cross-sectional studies indicate that reduced SHBG levels are associated with gestational diabetes, a disorder known to be associated with increased insulin resistance.

Insulin resistance has been proposed as a pathogenic mechanism of gestational hypertension and preeclampsia. However, data in favor of this hypothesis are limited and conflicting. For example, some studies report an association between insulin resistance and gestational hypertension but not preeclampsia, whereas others report the opposite. Still others suggest that insulin resistance contributes to both disorders. There are several potential explanations for these discrepancies. First, across different studies, the criteria used to define gestational hypertension and preeclampsia were nonuniform, thereby increasing the likelihood of outcome misclassification. Second, the cross-sectional design of many of these studies might limit their comparability. For example, although normal pregnancy is characterized by a progressive increase in insulin resistance, the differential pattern of progression in gestational hypertension and preeclampsia relative to normal gestation and relative to each other is unknown. Therefore, studying women at different stages of their disease could lead to disparate, yet not necessarily incompatible, conclusions. Third, gestational hypertension likely represents a heterogeneous disorder with multiple contributing causes. Small studies conducted in different specific populations are therefore prone to inconsistent results. Even within this single study, we identified important differences when nulliparous and multiparous women were considered separately. This observation emphasizes the necessity of detailed clinical phenotyping when studying the hypertensive disorders of pregnancy, as has been suggested.

Inflammation is an important mechanism of cardiovascular disease that is detectable years before overt disease manifests clinically. In pregnancy, inflammation has been proposed as a potential mechanism of preeclampsia. In the present study, we found no association between first-trimester CRP levels and subsequent gestational hypertension. This is in contrast to a prior study in the same cohort in which increased first-trimester CRP levels were associated with subsequent preeclampsia. This discrepancy suggests that important mechanistic differences exist in the pathogenesis of the specific types of hypertensive disorders of pregnancy. However, because this is one of the first studies to examine inflammation in gestational hypertension, it is also possible that insufficient sample size or chance may have precluded us from showing a difference in CRP levels that truly exists between gestational hypertension and normotensive women. Additional studies are therefore needed to verify these results.

The strength of the present study is its prospective, nested case-control design. This approach maximized our statistical power and minimized cost. More importantly, the prospective design ensured nonbiased data collection, and the case-control approach allowed for meticulous clinical phenotyping of pregnancy outcome by using research-specific criteria. Furthermore, SHBG results are in accordance with those of a prior study in which a similar design was used to examine the role of insulin resistance in preeclampsia within the same source population. In that study, which was restricted to nulliparous women, reduced first-trimester SHBG levels were likewise independently associated with subsequent preeclampsia. Interestingly, the magnitude of the association derived from the multivariable models was similar in these 2 studies. We conclude that among nulliparous women, insulin resistance is a common risk factor for both gestational hypertension and preeclampsia, and thus, the 2 disorders appear to be not only clinically but also etiologically related.

We cannot exclude the possibility that certain cases of gestational hypertension might have in fact been cases of early preeclampsia in which proteinuria had not yet appeared. This is unlikely, however, because <50% of women who manifest pregnancy-induced hypertension remote from term subsequently develop proteinuria. Moreover, in this study, in which all women were screened for proteinuria at each prenatal visit and the mean gestational age of delivery among...
cases was 39.6 weeks, such misclassification is even less likely to have affected the results.

It is important to note that there were few multiparous cases of gestational hypertension included in this study, and understanding mechanistic differences between nulliparous and multiparous gestational hypertension was not the primary hypothesis to be tested. Therefore, it is possible that the absence of an association of insulin resistance with gestational hypertension among multiparas was owing to insufficient statistical power or chance. Interestingly, a study that examined risk of hypertensive disorders of pregnancy according to early pregnancy waist circumference, another marker of insulin resistance, likewise reported a more substantial effect among nulliparas compared with multiparas. Additional dedicated studies are needed to confirm these observations.

Perspectives

Hypertensive disorders of pregnancy are common and morbid complications of pregnancy with mechanisms that are largely unknown. There are currently no early diagnostic tests capable of identifying women at increased risk, and as a consequence of our limited understanding of the biology of these diseases, no effective preventive strategies have been identified. The results of this study of gestational hypertension analyzed alongside similar studies of preeclampsia support the hypothesis that among nulliparous women, insulin resistance contributes to increased risk of both hypertensive disorders of pregnancy. Furthermore, these studies highlight the potential for developing early-pregnancy diagnostic tests capable of stratifying risk for hypertensive disorders of pregnancy and, thereby, identifying high-risk women for future interventional studies. Yet, despite these modest advances, many important questions remain unanswered. For example, how hypertensive disorders of pregnancy differ mechanistically between nulliparous and multiparous women is unclear. Furthermore, whether increasing insulin sensitivity (pharmacologically or through lifestyle modification) in high-risk insulin resistant women before or during early pregnancy might improve maternal and fetal outcome is exciting but unknown. Likewise, why individual insulin-resistant women develop gestational hypertension and others preeclampsia or gestational diabetes is also unknown and worthy of further investigation. Perhaps inflammation is one intermediate factor that contributes to the differentiation of the insulin-resistant phenotype into individual clinical syndromes during pregnancy. Undoubtedly, other known and yet to be identified maternal and fetal-placental factors contribute to the complex pathogenesis of hypertensive disorders of pregnancy.

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References

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