

First Trimester Insulin Resistance and Subsequent Preeclampsia: A Prospective Study

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Insulin resistance is implicated in the pathogenesis of preeclampsia, but prospective data are limited. SHBG, a marker of insulin resistance among nonpregnant individuals, has not been studied in detail during pregnancy. We conducted a prospective, nested, case-control study to test the hypothesis that increased insulin resistance, marked by reduced first trimester SHBG levels, is associated with increased risk of subsequent preeclampsia. First trimester SHBG levels were measured in 45 nulliparous women who subsequently developed preeclampsia (blood pressure, $\geq 140/90$ mm Hg; proteinuria, either $\geq 2+$ by dipstick or ≥ 300 mg/24 h, after 20 wk gestation) and in 90 randomly selected normotensive nulliparous controls. Compared with controls, women who developed preeclampsia had significantly reduced first trimester SHBG levels (302 ± 130 vs. 396 ± 186 nmol/liter; $P < 0.01$). Every 100 nmol/liter increase in SHBG was associated with a 31% reduced risk of preeclampsia [odds ratio (OR), 0.69; 95% confidence interval (CI), 0.55, 0.88; $P < 0.01$]. After adjusting for covariates in a multiple logistic regression model, the association between first trimester SHBG and preeclampsia re-

mained significant (per 100 nmol/liter increase; OR, 0.66; 95% CI, 0.47, 0.92; $P = 0.01$). When subjects were stratified by body mass index (lean: body mass index, < 25 kg/m²; overweight: body mass index, ≥ 25 kg/m²), overweight women had lower SHBG levels than lean women (286 ± 156 vs. 410 ± 166 nmol/liter; $P < 0.01$), and within each stratum, women with preeclampsia had lower SHBG levels than their respective controls. In a multivariable analysis, the association between SHBG and preeclampsia strengthened among lean women, such that every 100 nmol/liter increase in serum SHBG was associated with a 55% reduction in the risk of preeclampsia (OR, 0.45; 95% CI, 0.27, 0.77; $P < 0.01$), whereas in overweight women, the association was mitigated (OR, 1.02; 95% CI, 0.62, 1.69; $P = 0.9$). We conclude that increased early pregnancy insulin resistance is independently associated with subsequent preeclampsia. First trimester SHBG levels may be a useful biomarker for preeclampsia, especially among lean women who otherwise would be perceived to be at low risk. (*J Clin Endocrinol Metab* 87: 1563–1568, 2002)

PREECLAMPSIA, WHICH is characterized by pregnancy-induced hypertension and proteinuria, complicates 3–4% of pregnancies and thus is a leading cause of maternal and fetal morbidity and mortality (1). Currently, there are no early gestation screening tests available to predict the occurrence of preeclampsia, and the only effective therapy for established preeclampsia is delivery. Prophylactic strategies, including calcium supplementation and aspirin therapy, have been mostly unsuccessful (2, 3). Novel therapeutic targets, identified preferably during early gestation when there is time for therapeutic modification, are needed for future clinical trials aimed at preventing preeclampsia.

The insulin resistance syndrome is comprised of a cluster of metabolic abnormalities that confer increased risk of diabetes, hypertension, and cardiovascular disease (4). Several features of the insulin resistance syndrome, such as obesity (5), hypertension (6), dyslipidemia (7), systemic inflammation (8), and impaired fibrinolysis (9), are also associated with preeclampsia. In addition, women with polycystic ovary syndrome or gestational diabetes, two disorders characterized by insulin resistance, are at increased risk of preeclampsia (10, 11). Collectively, these data suggest that insulin resistance may contribute to the pathogenesis of preeclampsia (12). Most studies that examined insulin resistance in pre-

eclampsia, however, were cross-sectional or retrospective, and as a result, it remains unclear whether insulin resistance is involved in the pathogenesis of preeclampsia or is a consequence of the disease.

SHBG is a glycoprotein synthesized by the liver that binds circulating estrogens and T. Hepatic SHBG production is inhibited by insulin (13), and thus reduced SHBG levels are a marker of hyperinsulinemia and insulin resistance (14–21). The clinical utility of SHBG measurement as an index of insulin resistance was established by two prospective studies in which reduced SHBG levels were associated with increased risk of future type II diabetes in otherwise healthy women (22, 23). In normal pregnancy, SHBG levels rise steadily during the first and second trimesters, reaching a peak that is 4–6 times the normal nonpregnant range (24, 25). Whether altered early gestation SHBG levels are associated with preeclampsia is unknown. Therefore, we conducted a prospective, nested, case-control study to test the hypothesis that increased insulin resistance, marked by reduced first trimester SHBG levels, is associated with increased risk of subsequent preeclampsia.

Materials and Methods

The Massachusetts General Hospital Obstetric Maternal Study was developed in 1998 for the prospective study of early gestation risk factors for hypertensive disorders of pregnancy. The details of the design of this cohort have been described previously (8). In brief, women who receive

Abbreviations: BMI, Body mass index; CI, confidence interval; CV, coefficient(s) of variation; OR, odds ratio.

TABLE 1. Baseline and delivery characteristics by pregnancy outcome

	Preeclampsia (n = 45)	Controls (n = 90)	P
Baseline characteristics			
Age (yr)	30.5 ± 7	29.6 ± 6	NS
Caucasian (%)	74	78	NS
Smoking (%)	23	20	NS
Gestational age at blood collection (wk)	10.6 ± 2	10.6 ± 2	NS
BMI (kg/m ²)	28.1 ± 6.8	23.1 ± 4	<0.01
Systolic blood pressure (mm Hg)	119 ± 9	111 ± 11	<0.01
Diastolic blood pressure (mm Hg)	73 ± 8	68 ± 8	<0.01
Conception aided by fertility therapy (%) ^a	27	8	<0.01
Delivery characteristics			
Gestational age at delivery (wk)	37.6 ± 3	39.6 ± 2	<0.01
Birth weight (g)	2942 ± 665	3394 ± 739	<0.01
Cesarean (%)	40	20	0.02

NS, Not statistically significant.

^a Hormonal stimulation, *in vitro* fertilization, or intrauterine insemination.

prenatal care at Massachusetts General Hospital and its affiliated health centers are eligible for inclusion in the cohort. After providing informed consent, eligible women have first trimester serum samples collected and frozen at -80°C for future analysis. Baseline and intrapartum data are collected in a computerized record on all participants through the early postpartum period. Pregnancy outcome and other variables are verified using the hospital paper record and the hospital laboratory records.

For this study nulliparous women with singleton gestations resulting in delivery after 20 wk were eligible for inclusion. Forty-five consecutive cases of preeclampsia, defined by blood pressure elevation of 140/90 mm Hg or more after 20 wk gestation in association with proteinuria, either 2+ or more by dipstick or 300 mg/24 h or more in the absence of urinary tract infection, were selected (1). For each case two nulliparous controls were randomly selected. Controls were women who entered the Massachusetts General Hospital Obstetric Maternal Study cohort within 2 wk of each case and who remained normotensive and nonproteinuric throughout pregnancy. Women with a history of diabetes; thyroid, liver, or chronic renal disease; or preexisting chronic hypertension (defined as blood pressure ≥140/90 or need for antihypertensive medications before pregnancy or before 20 wk gestation) were excluded. This institution's human subjects committee approved the study.

Frozen serum was sent on dry ice by overnight courier to Esoterix, Inc. (Calabasas Hills, CA) for assay of SHBG levels. Given the reported association between increased T levels and preeclampsia (26), total T and free T assays were also performed. All samples were handled identically during storage, transport, and processing. In a pilot study we performed first trimester E2 assays on 10 cases of preeclampsia and 10 randomly selected controls. Mean E2 levels did not differ among cases and controls (5.9 ± 0.9 vs. 5.3 ± 0.8 nmol/liter, respectively; *P* = 0.35). Based on these data and similar reports in the literature in which E2 levels did not differ among women with preeclampsia and controls (26, 27), E2 assays were not performed on the remaining study subjects.

SHBG was measured using an immunoradiometric assay that has an intraassay coefficient of variation (CV) less than 4%, and an interassay CV less than 7.8%. The sensitivity of the SHBG assay is 2 nmol/liter. Total T was measured by a specific RIA after extraction in hexane-ethyl acetate and column chromatography. The intra- and interassay CV are less than 8.1% and 8.5%, respectively. The free T concentration was determined by the product of the fraction of free T, measured by equilibrium dialysis, and the total T concentration. The lower limit of detection for the assay is 0.1 pmol/liter. The free T assay has an intraassay CV less than 6.9%, and an interassay CV less than 9.4%.

To assess glucose tolerance, participants underwent a 50-g oral glucose-loading test between 24–28 wk gestation. This routine prenatal test is used to screen for gestational diabetes. In the nonfasting state subjects consumed 50 g oral glucose. Glucose levels in 1 h postloading plasma samples were determined using standard glucose oxidase assays with intra- and interassay CV less than 2%.

Analyses were performed with SAS (SAS Institute, Inc., Cary, NC) and STATA (STATA Corp., College Station, TX) statistical packages. Continuous variables were compared using two-sample *t* tests or Wil-

TABLE 2. Androgens and indices of insulin resistance by pregnancy outcome

	Preeclampsia (n = 45)	Controls (n = 90)	P
T (nmol/liter)	2.91 ± 2.43	2.78 ± 3.40	NS
Free T (pmol/liter)	13.19 ± 15.96	12.15 ± 19.09	NS
SHBG (nmol/liter)	302 ± 130	396 ± 186	<0.01
Glucose (mmol/liter) ^a	6.8 ± 1.4	6.2 ± 1.3	0.03

NS, Not statistically significant.

^a One hour after 50-g oral glucose load at 24–28 wk gestation.

coxon rank-sum test, and categorical variables were compared using Fisher's exact test. Logistic regression was used to adjust for potential confounding variables. In addition, to control for the effects of obesity, we performed a stratified analysis by body mass index (BMI; lean: BMI, <25 kg/m²; overweight: BMI, ≥25 kg/m²). Similar stratified analyses have been used to examine the effects of insulin resistance, independent of obesity, in polycystic ovary syndrome and in studies that related SHBG as a marker of insulin resistance and future type II diabetes (19, 22, 28). Two-tailed *P* < 0.05 was considered statistically significant. Results are reported as the mean ± sd.

Results

Baseline and delivery characteristics are presented in Table 1. Women who subsequently developed preeclampsia displayed significantly increased baseline BMI and systolic and diastolic blood pressures and were more likely to have undergone fertility treatment. Women with preeclampsia delivered smaller birth weight babies at younger gestational ages and were more likely to deliver by cesarean.

First trimester SHBG, total T, free T, and 1 h postloading glucose levels are displayed in Table 2. Compared with women with normotensive pregnancies, women who subsequently developed preeclampsia had significantly reduced SHBG levels. There was no difference in total or free T levels among cases and controls. Mean 1 h postloading plasma glucose levels were significantly higher among women who developed preeclampsia compared with women with normotensive pregnancies. The correlation between first trimester SHBG and 1 h postloading glucose levels was 0.2 (*P* = 0.03). BMI was negatively correlated with SHBG (*r* = -0.3; *P* < 0.01). Increasing gestational age at the time of SHBG sampling was positively correlated with SHBG (*r* = 0.4; *P* < 0.01).

The association between SHBG and risk of preeclampsia

TABLE 3. Indices of insulin resistance stratified by BMI and pregnancy outcome

	Lean (BMI <25)		Overweight (BMI ≥25)	
	Preeclampsia (n = 19)	Controls (n = 68)	Preeclampsia (n = 26)	Controls (n = 19)
BMI (kg/m ²)				
Mean	22.2	21.6	32.0	28.9
Range	18.7–24.9	17.7–24.9	25.3–47.3	25.2–40.7
SHBG (nmol/liter)	343 ± 126	428 ± 172	278 ± 129	298 ± 193
Glucose (mmol/liter) ^a	6.4 ± 1.6	6.1 ± 1.3	7.0 ± 1.4	6.7 ± 0.8

^a One hour after 50-g oral glucose load at 24–28 wk gestation.

was examined in greater detail. In a simple logistic regression model, every 100 nmol/liter rise in serum SHBG was associated with a 31% reduced risk of preeclampsia [odds ratio (OR), 0.69; 95% confidence interval (CI), 0.55, 0.88; $P < 0.01$]. Next, SHBG levels were adjusted for age, race, smoking, BMI, baseline systolic and diastolic blood pressures, androgen levels, fertility treatment, and gestational age at the time of SHBG sampling. In this multivariable model, every 100 nmol/liter increase in serum SHBG was independently associated with a 34% reduced risk of preeclampsia (OR, 0.66; 95% CI, 0.47, 0.92; $P = 0.01$), similar to the unadjusted analysis. First trimester BMI (OR, 1.14/U increase; 95% CI, 1.04, 1.24; $P < 0.01$), systolic blood pressure (OR, 1.06/U increase; 95% CI, 1.02, 1.11; $P < 0.01$), and fertility treatment (OR, 4.0; 95% CI, 1.2, 13.4; $P = 0.02$) were the only other variables independently associated with preeclampsia.

To further understand the effects of BMI on the relationship between SHBG and preeclampsia, subjects were stratified by BMI (lean: BMI, <25 kg/m²; overweight: BMI, ≥25 kg/m²). Overall, SHBG levels were lower (286 ± 156 vs. 410 ± 166 nmol/liter; $P < 0.01$) and 1 h postloading glucose levels were higher (6.9 ± 1.2 vs. 6.2 ± 1.4 mmol/liter; $P < 0.01$) among overweight women compared with lean women. Table 3 lists the indexes of insulin resistance stratified by BMI and pregnancy outcome. Within both the lean and overweight strata, SHBG levels were lower, and 1 h postloading glucose levels were higher among women who developed preeclampsia compared with their respective controls. The largest stratum-specific difference in SHBG levels was among lean women, in whom SHBG levels were 20% lower in cases compared with controls.

The probability of preeclampsia among lean and overweight women expressed as a function of increasing SHBG levels is presented in Fig. 1. The difference in maximum likelihood estimates indicated that the slopes of the probability functions among lean ($\beta = -0.35$) and overweight ($\beta = -0.08$) women were significantly different ($P < 0.01$). After adjusting for covariates, the association between SHBG and preeclampsia strengthened among lean women, such that every 100 nmol/liter increase in serum SHBG was associated with a 55% reduced risk of preeclampsia (OR, 0.45; 95% CI, 0.27, 0.77; $P < 0.01$). In contrast, there was no longer an association between SHBG and risk of preeclampsia in overweight women (OR, 1.02; 95% CI, 0.62, 1.69; $P = 0.9$). In the adjusted multivariable model, the difference in maximum likelihood estimates between the lean and overweight strata

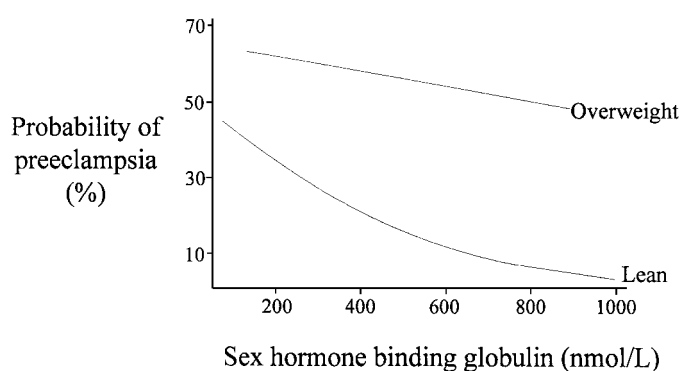


FIG. 1. Probability of preeclampsia as a function of SHBG levels among lean (BMI, <25 kg/m²) and overweight (BMI, ≥25 kg/m²) women.

remained significantly different ($P = 0.02$). For all analyses when women who conceived spontaneously were analyzed separately from women who conceived with the assistance of fertility treatment, the results were unchanged (data not shown).

Discussion

In this prospective study of nulliparous women we identified a significant association between first trimester insulin resistance and subsequent risk of preeclampsia. This association was independent of elevated blood pressure, BMI, and fertility treatment, which are established risk factors for preeclampsia (5, 29, 30). Although insulin resistance has been implicated in the pathogenesis of preeclampsia, until now, most of the evidence in support of this hypothesis was derived from cross-sectional and retrospective studies. To date, SHBG levels have not been examined prospectively in preeclampsia.

SHBG is a glycoprotein synthesized by the liver that binds circulating E2 and T (17). SHBG mediates the balance between inactive, bound sex hormones and biologically active, free sex hormones (17). Clinical and *in vitro* studies indicate that E2 and thyroid hormone are the principal stimuli for hepatic SHBG secretion, whereas insulin, PRL, androgens, and GH suppress SHBG (13–15, 31, 32). Increasing insulin levels suppress hepatic SHBG secretion even in the face of increasing E2 levels (13), an observation that is especially pertinent during pregnancy, when insulin, E2, and SHBG levels increase markedly (13, 24, 25, 33).

In studies of nonpregnant women, SHBG levels correlate inversely with glucose tolerance (18), insulin levels (19), and insulin resistance as determined by the euglycemic-hyperinsulinemic clamp (16). In each of these studies the correlations were independent of BMI and body fat distribution. The link between SHBG and insulin resistance has important clinical implications. In two separate prospective studies, reduced SHBG levels were independently associated with increased incidence of type II diabetes in otherwise healthy women (22, 23). In pregnancy, women with gestational diabetes displayed markedly lower SHBG levels compared with women without gestational diabetes (34). Furthermore, when insulin sensitivity is increased pharmacologically, SHBG levels rise (20, 21). Collectively, these data support a

direct, physiological link between insulin resistance and SHBG *in vivo*. Importantly, unlike other markers of insulin resistance, SHBG is reliable in the nonfasting state (35), and it exhibits minimal diurnal variation (36). These features render SHBG a unique marker of insulin resistance that is especially useful in clinical situations when fasting blood samples are not routinely collected, such as during prenatal obstetric care.

During the first trimester of pregnancy, SHBG levels increase 3- to 5-fold above the normal range in healthy menstruating women (24, 25). This early gestation increase in SHBG levels mirrors the contemporaneous increase in E2 levels, which rise almost 20-fold during the first trimester alone (24, 25). E2 levels continue to rise through the end of pregnancy such that by delivery, levels reach greater than 100 times the normal, nonpregnant, early follicular phase range (24). In contrast, SHBG peaks at levels 4–6 times the normal nonpregnant range within 24 wk gestation and thereafter remain constant through the duration of pregnancy (24, 25). Insulin resistance and insulin levels also increase progressively during normal gestation, but the greatest increment occurs during the second half of pregnancy (33, 37). This physiological increase in insulin resistance during the third trimester may prevent further increases in SHBG levels that otherwise would be expected in the setting of progressive increases in E2 levels. Indeed, in a third trimester study, women with gestational diabetes were more insulin resistant and had significantly reduced SHBG levels compared with normoglycemic controls despite similar E2 and thyroid hormone levels (34).

Although insulin resistance is associated with preeclampsia, the majority of evidence comes from cross-sectional and retrospective studies. For example, in studies that examined surrogate markers of insulin resistance, women with established preeclampsia displayed elevated levels of glucose (38), uric acid (39), triglycerides (40), leptin (41), and plasminogen-activating inhibitor-1 (9) and reduced high density lipoprotein levels (40). In other cross-sectional studies, women with established preeclampsia had higher fasting and postglucose loading insulin levels and lower insulin sensitivity than controls (39, 42). Two prospective studies support an association between insulin resistance and subsequent preeclampsia (43, 44). Sowers *et al.* (43) showed that among African-American women, fasting insulin levels were significantly increased at 20 wk gestation in those who ultimately developed preeclampsia. In a large prospective study involving more than 3600 women, Joffe *et al.* (44) reported that increasing deciles of glucose levels during the 50-g oral glucose-loading test were associated with increased risk of subsequent preeclampsia. In the same study, however, the relative risk of preeclampsia among women with abnormal glucose tolerance or gestational diabetes compared with women with normal glucose tolerance was not significantly increased. Furthermore, in both studies subjects were examined during the second trimester, a time when the pathological changes of preeclampsia and the physiological insulin resistance of pregnancy may already be established (37, 45). Finally, neither study examined the independent association between insulin resistance and preeclampsia among lean and overweight women.

The association we identified between first trimester SHBG levels and preeclampsia in the unstratified univariate and multivariable analyses supports the hypothesis that insulin resistance contributes to the pathogenesis of preeclampsia. In the stratified analysis, the independent association between first trimester SHBG levels and preeclampsia strengthened in lean women, but was mitigated in overweight women. One possible interpretation of this observation is that insulin resistance contributes to the pathogenesis of preeclampsia only in lean women. Alternatively, insulin resistance may contribute to the pathogenesis of preeclampsia in all women, but in overweight women it is but one of several contributing factors, including inflammation (8). Our data suggest the latter possibility. Overweight women displayed significantly reduced SHBG levels compared with lean women, and as shown in Fig. 1, there was a trend toward reduced risk of preeclampsia as SHBG levels increased, even among overweight women. Furthermore, whereas SHBG levels were lower among overweight women who developed preeclampsia than their respective controls, the difference was markedly smaller than the difference within the lean stratum. Therefore, we may have had limited power to identify a significant, independent effect of insulin resistance within the overweight category of women. We conclude that insulin resistance itself, whether in association with obesity or not, is linked to increased risk of preeclampsia. Furthermore, reduced SHBG levels may be especially helpful in identifying lean insulin-resistant women who otherwise would not be considered to be at high risk for preeclampsia.

The results of this study differ from those of a prior cross-sectional study in which SHBG levels were somewhat lower, although not statistically significant, among cases of preeclampsia compared with controls (26). In that study Acromite *et al.* (26) examined women at 37 wk gestation, a time when the physiological insulin resistance of normal pregnancy peaks and thus may reduce the difference in SHBG levels among normotensive and preeclamptic women. Our T data also differ from Acromite's findings. Whereas Acromite *et al.* (26) showed that third trimester free and total T levels were elevated among women with preeclampsia, in this study there was no difference in first trimester androgen levels. Longitudinal studies examining how androgens vary during pregnancy may further elucidate the pathogenesis of preeclampsia.

This study has certain limitations. First, we measured SHBG levels at one time point in pregnancy. This is the first study, however, to prospectively examine SHBG in detail in preeclampsia. In addition, we adjusted SHBG levels for gestational age at the time of the blood sampling to reduce the variability in SHBG levels for which duration of pregnancy accounts. Second, we were unable to measure E2 levels in all participants. There is no evidence, however, that E2 levels differ between preeclampsia and normal pregnancy (26, 27), and a pilot study within our population supported this. Third, since we collected nonfasting serum samples that were drawn as part of routine prenatal care, we were not able to correlate SHBG levels with first trimester fasting insulin or glucose levels. Sampling fasting blood, however, is not part of routine prenatal care, and if a nonfasting marker such as SHBG proves useful, it would have widespread use. Fur-

thermore, SHBG samples were obtained from all subjects during their first prenatal visit, long before case or control status was ascertained. No subjects were instructed about the study before their visit, and none was instructed to fast. Therefore, the timing of the subject's last meal was randomly and probably equally distributed among cases and controls. We expect that such a random distribution would impede, rather than facilitate, identification of a statistically significant difference among cases and controls, further validating our results.

The strength of this study is that we identified evidence of increased insulin resistance in the first trimester among women who subsequently developed preeclampsia, long before preeclampsia became clinically evident. This temporal relationship supports the hypothesis that insulin resistance may be in the "causal pathway" of preeclampsia. Furthermore, as the timing of this study precedes most of the physiological insulin resistance of pregnancy (37), it is possible that the excess insulin resistance we detected in cases may have been present at baseline, predating pregnancy. This point has important therapeutic implications. First, it is tempting to consider that improving insulin sensitivity in high risk women before and during early pregnancy may reduce the risk of preeclampsia. Second, if preeclampsia is associated with increased insulin resistance at baseline, then it represents a potentially modifiable risk factor for the excess long-term cardiovascular risk observed in women with a history of preeclampsia (46). Further intrapartum and postpartum studies are needed to expand our understanding of how insulin resistance contributes to the pathogenesis of preeclampsia so that potential strategies to reduce the risk of preeclampsia may be appropriately designed.

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