0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(12):6239–6243

doi: 10.1210/jc.2004-0548

Preeclampsia and Future Cardiovascular Disease: Potential Role of Altered Angiogenesis and Insulin Resistance

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Altered angiogenesis and insulin resistance are associated with preeclampsia and cardiovascular disease (CVD), and women with preeclampsia appear to be at increased risk of future CVD. We hypothesized that these factors are detectable in asymptomatic postpartum women with a history of preeclampsia and may represent pathophysiological mechanisms bridging preeclampsia and future CVD. We measured fasting insulin, glucose, vascular endothelial growth factor, and its circulating inhibitor, soluble fms-like tyrosine kinase (sFlt-1) in 29 normotensive women with a history of preeclampsia and 32 women with prior normotensive pregnancies at 18.0 ± 9.7 months postpartum. The homeostasis model of insulin resistance ($HOMA_{IR}$) [(insulin [microunits per milliliter] × glucose [millimoles per liter])/22.5] was calculated. Compared with women with normal pregnancies, women with prior preeclampsia had significantly increased levels of sFlt-1 (41.6 ± 6.7 *vs.* 30.4 ± 10.2; $P < 0.01$) and median $HOMA_{IR}$ (2.8 *vs.* 1.9; $P = 0.04$). Membership in the upper quartile of either sFlt-1 or $HOMA_{IR}$ was associated with prior preeclampsia (odds ratio 5.7; 95% confidence interval 1.7, 20.0; $P < 0.01$), and all five women in the upper quartiles of both sFlt-1 and $HOMA_{IR}$ had a history of preeclampsia. Women with a history of preeclampsia demonstrate altered expression of angiogenesis-related proteins and increased $HOMA_{IR}$ more than 1 yr postpartum. These factors may contribute to their risk of future CVD. (*J Clin Endocrinol Metab* 89: 6239–6243, 2004)

PREECLAMPSIA AFFECTS APPROXIMATELY 5% of pregnancies and is associated with significant maternal and fetal morbidity (1). Although hypertension and proteinuria associated with preeclampsia typically resolve soon after delivery, women with a history of preeclampsia appear to be at significantly increased risk of developing cardiovascular disease (CVD) in later years (2). Preeclampsia and CVD share several risk factors, including insulin resistance, obesity, diabetes, and inflammation (3). These likely contribute to the endothelial dysfunction of preeclampsia and are also markers for early CVD. Therefore, preeclampsia likely represents a precursor of CVD, and pregnancy provides a risk-stratifying stress test for future disease. Examining postpartum women with a history of preeclampsia offers a unique approach to *in vivo* investigation of early mechanisms of human CVD decades before clinical disease.

Alterations in angiogenesis during early pregnancy contribute to the incomplete remodeling of uterine spiral arterioles and abnormal placental vascular development that are central to the pathogenesis of preeclampsia (4). Altered placental vascular development is thought to lead to the elaboration of soluble factors of placental origin that induce the maternal preeclampsia syndrome (5). Soluble fms-like tyrosine kinase (sFlt-1) is a secreted splice variant of Flt-1 that antagonizes vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) by preventing their binding to their target tissue receptors Flt-1 and kinase insert domain-containing receptor (6). It has been recently shown that increased sFlt1 occurs as early as 5–6 wk before the onset of clinical symptoms of preeclampsia (7), whereas significant reductions in PlGF are detectable as early as the first trimester in women who subsequently develop preeclampsia (8). Furthermore, administration of exogenous sFlt1 to pregnant rats leads to reduced PlGF, hypertension, proteinuria, and glomerular endothelial injury, suggesting an important pathogenic role for angiogenic factors in preeclampsia (6). Because alterations in angiogenesis and insulin resistance are

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Abbreviations: BMI, Body mass index; BP, blood pressure; CV, coefficient of variation; CVD, cardiovascular disease; $HOMA_{IR}$, homeostasis model of insulin resistance; MOMS, MGH Obstetric Maternal Study; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase; VEGF, vascular endothelial growth factor.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

also associated with CVD (9), we hypothesized that these alterations would be evident in postpartum women with a history of preeclampsia before the development of hypertension or CVD.

Subjects and Methods

The population for the current study was derived from independent recruiting sites at Massachusetts General Hospital (MGH) and the Magee-Womens Hospital (MWH). Women were eligible for recruitment in the MGH component of the study if they had previously enrolled in the MGH Obstetric Maternal Study (MOMS), a prospective pregnancy cohort study of risk factors for preeclampsia and gestational diabetes that has been described in detail (10). More than 60% of all women who receive prenatal obstetric care at the main MGH campus or its affiliated neighborhood health centers enroll in MOMS and thus the cohort includes a socioeconomically and ethnically diverse group of women. For the MWH component, study participants had already given permission to be contacted for additional preeclampsia-related research studies after having similarly participated in a pregnancy cohort study of risk factors for preeclampsia at MWH. Like MOMS, this study consists of a broad cross-section of women recruited from private obstetrical practices (~50%) and the university's prenatal outpatient clinic (~50%), which serves a mostly low-income, racially diverse population. Consecutive subjects who developed preeclampsia during their first completed (nulliparous) pregnancy and participants with normotensive pregnancies within the same timeframe were mailed a letter cosigned by their primary caregivers and the investigators in which they were offered an opportunity to opt out of the current study. Women who chose not to opt out were contacted by the investigators and offered inclusion.

In total, we examined 29 normotensive women with a history of preeclampsia during their first completed (nulliparous) pregnancy (18 from MGH and 11 from MWH) and 32 normotensive women (18 from MGH and 14 from MWH) at 18.0 ± 9.7 months postpartum in the General Clinical Research Centers at the Massachusetts Institute of Technology and the MWH. The study was approved by the MGH and MWH human research committees, and all subjects provided written informed consent. At both sites, preeclampsia was defined according to research criteria as hypertension (>140/90 mm Hg) and proteinuria (either 2+ or greater by dipstick or 300 mg or greater per 24 h) that first appeared after 20 wk of gestation and resolved within 12 wk postpartum (1). Because preeclampsia often presents near term and other disorders can lead to preterm delivery, to prevent misclassification of pregnancy outcome, all women in the normal pregnancy group had delivered at term (>38 wk). At both sites, women with current pregnancy; diabetes; or a history of gestational diabetes, chronic hypertension, proteinuria, or serum creatinine greater than 1.0 mg/dl were excluded.

Subjects underwent a history and physical examination and a urine pregnancy test. Blood was collected on the morning after an overnight fast for measurement of insulin, glucose, free VEGF, and sFlt-1. Samples were processed immediately, stored at −80 C for no longer than 18 months and were thawed only for the current study. Glucose was measured using standard glucose oxidase assays with intra- and interassay coefficients of variation (CVs) less than 2%. Insulin levels were measured using the Linco's RIA (Linco Research, St. Charles, MO), which does not cross-react with proinsulin. The intra- and interassay CVs were less than 8%. Commercial ELISA kits were used for sFlt-1 and free VEGF (R&D Systems, Minneapolis, MN). The intra- and interassay CVs for sFlt-1 and VEGF were 3.5 and 5.6 and 8.1 and 10.9, respectively. All samples were run in duplicate by technicians blinded to pregnancy outcome.

Univariate comparisons between the pregnancy outcome groups were performed using two-sample *t* tests, Wilcoxon rank sum test, or Fisher exact test as appropriate. Due to rightward skewing of the VEGF and homeostasis model of insulin resistance ($HOMA_{IR}$) distributions, these variables were analyzed after natural log transformations. Pearson correlation coefficients between markers of angiogenesis, $HOMA_{IR}$, and covariates were calculated. Analysis of covariance was used to examine the association between sFlt-1 and preeclampsia adjusted for potential confounding. Logistic regression was used to calculate odds ratios for having had prior preeclampsia given levels of postpartum markers and adjust for potential confounding.

Results

Postpartum characteristics are presented in Table 1 according to pregnancy outcome. Although not statistically significant, women in the preeclampsia group were more likely than women in the normal pregnancy group to have been evaluated at the clinical research centers within 2 wk of their last menstrual period. Mean blood pressure (BP) and body mass index (BMI) were significantly increased in women with a history of preeclampsia, but there were no differences in race, contraception use, smoking, or lactation status. Women with a history of preeclampsia were more likely to have a first-degree relative with a history of ischemic CVD: coronary artery disease, myocardial infarction, or cerebrovascular disease. Among all women, postpartum BMI was strongly correlated (r = 0.9) with their prepregnancy BMI.

Postpartum markers of angiogenesis and insulin resistance are presented in Table 2 and Fig. 1. Women with a history of preeclampsia demonstrated significantly increased sFlt-1 levels, compared with normotensive women ($P < 0.01$). There was a trend toward increased VEGF levels among women with prior preeclampsia ($P = 0.06$). Although

TABLE 1. Postpartum characteristics according to pregnancy outcome

	Preeclampsia (n = 29)	Normotensive (n = 32)	*P*
Age (yr)	33.7 ± 5.8	30.7 ± 7.1	0.08
Race (% Caucasian)	86	84	0.6
Months postpartum	18.0 ± 10	18.0 ± 10	1.0
Smoking (% current)	7	3	0.3
BMI (kg/m^2)	29.2 ± 7.8	25.0 ± 5.8	0.02
Systolic BP (mm Hg)	111 ± 10	105 ± 8	0.01
Diastolic BP (mm Hg)	73 ± 10	68 ± 7	0.04
Oral or sc contraception (%)	31	34	0.8
Lactation (% current)	3	6	0.6
Menstrual cycle phase (%)			0.07
Within 2 wk of menses	66	37	
Between 2 and 4 wk after menses	17	44	
Unknown last menses	17	19	
Family history of CVD (%)[a]	45	16	0.05

Continuous variables are reported as mean ± SD or median (interquartile range) as appropriate.

[a] Family history data was available in 47 of 61 women. Family history of CVD is defined as coronary artery disease, myocardial infarction, or cerebrovascular disease in any first-degree relative.

TABLE 2. Markers of angiogenesis and insulin resistance

	Preeclampsia (n = 29)	Normotensive (n = 32)	*P*
sFlt-1 (pg/ml)	41.6 ± 6.7	30.4 ± 10.2	<0.01
VEGF (pg/ml)	424 (184, 628)	218 (149, 401)	0.06
Fasting glucose (mg/dl)	81 ± 7	80 ± 6	0.5
Fasting insulin (μU/ml)	14 (9, 18)	9.7 (7.3, 14.5)	0.05
$HOMA_{IR}$[a]	2.8 (1.7, 4.0)	1.9 (1.5, 2.9)	0.04

Continuous variables are reported as mean ± SD or median (interquartile range) as appropriate.

[a] $HOMA_{IR}$ = [insulin (μU/ml) × glucose (mmol/liter)]/22.5.

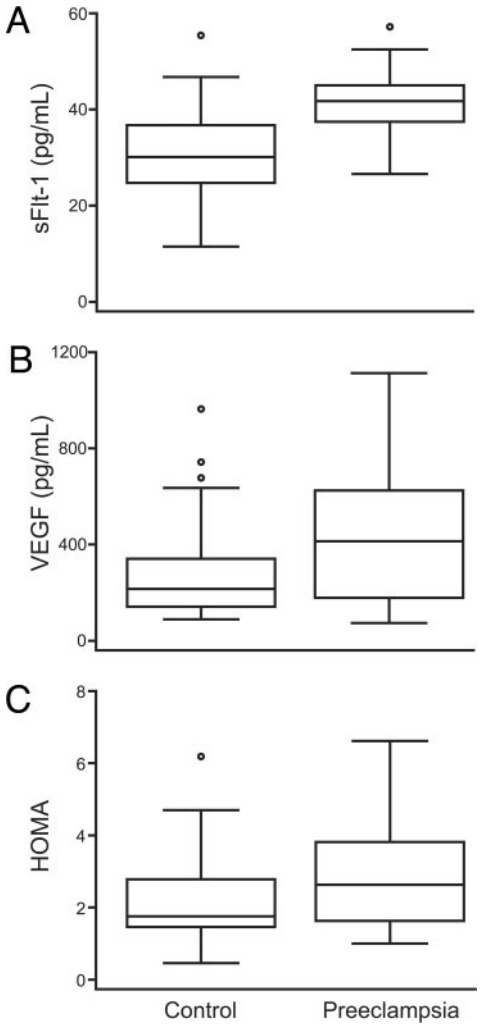

FIG. 1. Box plots demonstrating the distributions of sFlt-1 (A), VEGF (B), and $HOMA_{IR}$ (C) among normal women and women with a history of preeclampsia. *Boxes* denote the interquartile range with the *upper* and *lower horizontal edges* representing the 75th and 25th percentiles, respectively. The *central horizontal lines* represent the medians. The *vertical whiskers* above and below the boxes represent the range of outlying data points up to 1.5 times the interquartile range, and the *circles beyond the whiskers* represent severe outliers. *P* values for the univariate comparisons are presented in Table 2.

there were no differences in fasting glucose levels, women with preeclampsia demonstrated significantly increased $HOMA_{IR}$ levels ($P = 0.04$). When the pregnancy outcome groups' sFlt-1 and $HOMA_{IR}$ levels were compared separately within each individual recruiting site, the results were unchanged (data not shown).

Levels of VEGF were linearly correlated with $HOMA_{IR}$ ($r = 0.32$; $P = 0.01$) and BMI ($r = 0.31$; $P = 0.01$). Both BP ($r = 0.40$; $P < 0.01$) and BMI ($r = 0.66$; $P < 0.01$) correlated with $HOMA_{IR}$. In contrast, no variables correlated with sFlt-1 levels. Using analysis of covariance with sFlt-1 as the dependent variable and pregnancy outcome as the primary predictor variable, no additional variables added to the models (age, race, postpartum duration, smoking, BP, BMI, contraceptive use, lactation status, menstrual cycle phase, or family history of cardiovascular disease) appreciably altered the association between increased sFLt-1 and prior preeclampsia, which remained statistically significant ($P < 0.01$) in all models.

There was no association between menstrual cycle phase and sFlt-1, VEGF, or $HOMA_{IR}$. Nonetheless, because Flt-1 expression might be increased in the menstrual phase of the cycle (11), we reanalyzed the difference in mean sFlt-1 levels between the pregnancy outcome groups after excluding women who were evaluated at the clinical research centers on d 1–5 of their cycle. After excluding these 10 women, there remained a statistically significant increase in sFlt-1 levels among the preeclampsia group, compared with the normal pregnancy group (41.6 ± 6.7 *vs.* 30.8 ± 10.3 pg/ml, $P < 0.01$). As noted, adjusting for menstrual cycle phase did not mitigate the association between increased sFLt-1 and prior preeclampsia.

Next, we examined the likelihood of having had prior preeclampsia according to postpartum levels of insulin resistance and angiogenesis. The odds ratio of having had prior preeclampsia among women in the upper quartile (>75th percentile) of the $HOMA_{IR}$ distribution *vs.* the remaining women (<75th percentile) was 4.9 (95% confidence interval 1.2, 23.8; $P = 0.01$). The odds ratio for preeclampsia comparing the upper quartile (>75th percentile) of sFlt-1 *vs.* the remaining women (<75th percentile) was also 4.9 (95% confidence interval 1.2, 23.8; $P = 0.01$), but different groups of women constituted the upper quartiles of the $HOMA_{IR}$ and sFlt-1 distributions. Only five women were in the upper quartiles of both and all five had a history of preeclampsia. When women who were in either the upper $HOMA_{IR}$ or the upper sFlt-1 quartiles (n = 27) were compared with women in the lower quartiles of each (n = 34), the odds ratio of having had preeclampsia increased to 5.7 (1.7, 20.0; $P < 0.01$). Adjusting for differences in age, race, postpartum duration, BP, BMI, smoking, lactation status, or contraceptive use did not appreciably alter the observed association among increased postpartum sFlt-1, $HOMA_{IR}$, and prior preeclampsia.

Discussion

Seemingly healthy postpartum women with a history of preeclampsia demonstrated significantly increased levels of sFlt-1 and $HOMA_{IR}$, compared with women with prior normotensive pregnancies. Whereas postpartum sFlt-1 levels were 50-fold lower than intrapartum levels (6, 8), the 37% increase among postpartum women with prior preeclampsia, along with their significantly increased levels of $HOMA_{IR}$, suggests that altered angiogenesis and insulin resistance may

represent baseline maternal factors that contribute to the development of both preeclampsia and future CVD. Furthermore, although the placenta represents the predominant source of sFlt-1 during pregnancy (6), our data suggest an additional contribution from maternal sources, perhaps endothelial or mononuclear cells that are known to express VEGF receptors (12). Genetic differences influencing Flt-1 gene splicing might explain the differences between the groups at baseline, and phenotypic amplification might occur with aging, predisposing to CVD, and in states of metabolic stress such as pregnancy, predisposing to preeclampsia.

Studies during pregnancy (6) suggest that the increased concentrations of sFlt-1 we observed should be associated with decreased levels of free VEGF. Instead, there was a trend toward increased free VEGF levels in the preeclampsia group. Furthermore, a negative correlation between sFlt-1 and VEGF was not observed, and the relative molar concentrations of sFlt-1 were at least 20-fold lower than VEGF, suggesting that sFlt-1 does not meaningfully influence circulating levels of free VEGF in the basal postpartum state. Prior studies of patients with established CVD also found increased levels of circulating VEGF, but its role in atherogenesis remains controversial (13–18). Some studies suggest a beneficial effect of enhanced angiogenesis in coronary and peripheral vascular disease models (19), but recent data suggest that increased VEGF could actually worsen vascular disease by promoting neoangiogenesis in atherosclerotic plaques (20). Nonetheless, our results are mostly in agreement with prior cross-sectional clinical studies (13–18) and extend the results from populations with prevalent CVD back to a population at high risk of CVD years before its development.

Women with a history of preeclampsia also demonstrated increased $HOMA_{IR}$. Insulin resistance is associated with CVD; prospective studies during pregnancy suggest that it is associated with increased risk of preeclampsia, and previous postpartum studies similarly identified increased insulin resistance in women with a history of preeclampsia (21, 22). Therefore, insulin resistance appears to be a potential mechanism linking preeclampsia and future CVD. Although the molecular pathways by which insulin resistance might link preeclampsia with future CVD are unclear, oxidative stress and inflammation, which are features of both diseases, may be involved. For example, the vascular superoxide-producing nicotinamide adenine dinucleotide phosphate reduced oxidase, an enzyme that can be activated by hyperinsulinemia or associated excess free fatty acids, may be particularly important in oxidative stress and inflammation (23–26). Interestingly, we observed significant linear correlation between $HOMA_{IR}$ and VEGF. Insulin induces the expression of VEGF mRNA (27) and reduced expression of VEGF mRNA in insulin-resistant states can be reversed with insulin (9, 28). Therefore, it is tempting to speculate that increased insulin resistance at baseline along with alterations in angiogenesis might act synergistically to predispose certain women to preeclampsia and perhaps future CVD. Indeed, a recent prospective study (29) during pregnancy identified such an interaction: whereas insulin-resistant women and women with disordered angiogenesis were at increased risk of preeclampsia (odds ratio 4.1 and 8.7, respectively), women who demonstrated both alterations were at markedly increased risk (odds ratio 15.1). In this study, there was a suggestion that women with the highest levels of both sFlt-1 and $HOMA_{IR}$ had the greatest odds of prior preeclampsia; however, our small sample size did not permit formal testing of interaction between the two factors.

We acknowledge limitations of this pilot study. First, a small number of subjects were examined once after pregnancy and the difference between the groups' sFlt-1 levels were statistically significant, although relatively small. This is the first study, however, to examine markers of angiogenesis in postpartum women with a history of preeclampsia. Second, the basal levels of sFlt-1 appear to be too low to influence circulating VEGF concentrations, raising the possibility that although sFlt-1 levels are elevated in women with a history of preeclampsia, they may not play a clinically significant role in the postpartum state. Alternatively, it is possible that in disease states such as atherosclerosis, hypoxia could induce sFlt-1 to higher, potentially pathogenic levels in especially those patients with higher baseline levels, such as women with a history of preeclampsia, and thereby contribute to acceleration of atherosclerosis. Finally, it is possible that the circulating levels of the angiogenesis factors we measured may not fully reflect their levels at the endothelial surface. Third, the small number of subjects precluded stratification according to the severity of preeclampsia. We hypothesize that women with severe preeclampsia who are at the highest risk for future CVD (2, 30–32) and who demonstrate the highest levels of sFlt-1 during pregnancy (6, 7) might also demonstrate the highest levels postpartum. Certainly larger studies in the future should investigate whether such a dose-response relationship exists among the severity of preeclampsia, its risk of recurrence, and the magnitude of increase in postpartum sFlt-1 levels. Fourth, we compared women with previous preeclampsia with women with uncomplicated pregnancies. Whether other adverse pregnancy outcomes such as preterm labor or gestational diabetes might also be associated with postpartum alterations in angiogenesis factors is still unknown.

Finally, it is important to acknowledge that we cannot exclude the possibility that preeclampsia was the cause of our postpartum observations rather than a consequence of alterations that antedated pregnancy. This distinction may be less important if further studies confirm that altered angiogenesis either contributes directly to future CVD or at least helps identify high-risk women who might benefit most from early and aggressive cardiovascular risk factor modification. Indeed, a point of this study is to convey to the general medical community the possibility that perhaps closer attention to pregnancy outcome could help identify women at higher risk for future CVD, even when certain evidence of that risk, such as obesity, might have been present before pregnancy, as it was in this study. Whereas obesity is indeed a powerful risk factor for preeclampsia (33), most obese women still do not develop preeclampsia. Conversely, lean women who are insulin resistant are at increased risk of preeclampsia, suggesting an independent association between insulin resistance and preeclampsia (10). Furthermore, separating the independent effects of obesity from its downstream consequences, such as insulin resistance, is difficult and perhaps statistically inappropriate given that they likely

share a common causal pathway (34, 35). Importantly, increased sFlt-1 levels were independent of BMI in this study. Therefore, our results suggest that the phenotype of postpartum women with prior preeclampsia appears to include not only the obesity-insulin resistance syndrome but also alterations in angiogenesis factors.

Acknowledgments

Received March 24, 2004. Accepted September 15, 2004.

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This work was supported by Grants M01RR01066 (Massachusetts Institute of Technology General Clinical Research Center), MO1RR00056 (Magee-Womens Hospital Satellite General Clinical Research Center), RR017376 (to M.W.), HL64144 (to C.A.H.), HD30367 (to J.M.R.), DK02825 (to S.A.K.), DK64255 (to S.A.K.), and HD39223 (to R.T.) from the National Institutes of Health; grants from the American Heart Association (to M.W.), the American Diabetes Association (to J.L.E.), the McGuirk Family Research Foundation; and the Carl Gottschalk Award from the American Society of Nephrology (to S.A.K.).

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