Maternal and Fetal Modulators of Lipid Metabolism Correlate With the Development of Preeclampsia

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The pregnancy syndrome preeclampsia is associated with placental dysfunction, dyslipidemia, and endothelial cell activation, and is a major cause of maternal and fetal morbidity and mortality. In this report, a nested case-control study of matched preeclamptic and normal pregnant women was used to investigate the association of maternal and fetal modulators of lipid metabolism with pregnancy outcome. Maternal body mass index (BMI), triglyceride levels, and nonesterified fatty acid (NEFA) concentrations were all significantly increased in women who developed preeclampsia ($P < .01$). Human placental lactogen (hPL), which is secreted by the syncytiotrophoblast layer of the fetal placenta and reportedly has lipolytic activity, also was found to be elevated in women with preeclampsia ($P < .01$). By contrast, hemoglobin levels were not found to be statistically different between the two groups of women, indicating that the increased plasma lipids and hPL were not a result of hemoconcentration in preeclamptic patients. The results suggest a multihit hypothesis for the pathophysiology of preeclampsia in which maternal obesity and a placental lipolytic hormone (hPL) converge to adversely affect free fatty acid concentrations in the maternal circulation.

THE PREGNANCY-SPECIFIC SYNDROME preeclampsia has a prevalence rate of 4% to 7% of all pregnancies and is a major cause of maternal and perinatal morbidity worldwide.[1] Its etiology is only now becoming better understood. Seven years ago, we first postulated that two interrelated pathogenic factors underlie the development of preeclampsia: (1) inadequate trophoblast invasion of the maternal endometrial stroma and spiral arterioles, and (2) generalized maternal endothelial cell activation or injury, leading to microthrombus formation and vasospasm.[2] A variety of studies support these hypotheses. The histochemistry of the placental beds of preeclamptic women demonstrates impaired trophoblast invasion and failure of vascular remodeling,[3] and Doppler flow studies confirm decreased spiral artery and placental perfusion in this condition.[4] Multiple markers of endothelial cell dysfunction have been reported in preeclampsia, including elevated circulating concentrations of endothelin-1[5] and cellular fibronectin.[6,7] Morphological[8] and functional[9] evidence of endothelial injury also has been documented. Familial studies of preeclampsia suggest that both maternal and fetal genetic factors converge in a complex manner to effect the clinical syndrome of preeclampsia.[10]

Recent studies have demonstrated increased maternal vascular deportation of trophoblast cells in preeclampsia,[11] and syncytiotrophoblast membrane vesicles have been shown to injure endothelial cells in vitro.[12] The association of maternal obesity as an independent risk factor for the development of pregnancy hypertension and preeclampsia[13,14] also has been ascertained. In addition, abnormalities of intracellular lipid metabolism have been associated with the endothelial cell injury and altered prostaglandin metabolism[15-17] observed in preeclampsia. To clarify pathophysiological mechanisms that translate localized placental dysfunction into a systemic maternal vascular disease, we used a nested case-control study of lipid metabolism in normal and preeclamptic pregnancies. We postulated that maternal (adipocyte mass) and fetal (placental syncytiotrophoblast deportation of a lipolytic hormone) factors could influence vascular lipid utilization in pregnancy. We observed that maternal plasma triglyceride, nonesterified fatty acid (NEFA), and human placental lactogen (hPL) concentrations were elevated in women who developed preeclampsia.

SUBJECTS AND METHODS

After provision of written informed consent, pregnant women with singleton gestations presenting to the obstetrical service of the University of California, San Francisco (UCSF) were recruited to participate in a protocol approved by the UCSF Committee on Human Research. Nonfasting serum samples were collected in 5 mmol/L EDTA during the late third trimester of pregnancy before the onset of labor or administration of antihypertensive agents or intravenous fluids. Blood samples were drawn at 36 to 38 weeks' gestation from asymptomatic patients and antedated delivery by an average of 2 to 3 weeks. For women who ultimately developed preeclampsia, blood sampling occurred 1 to 2 weeks before the clinical diagnosis of this syndrome. The samples were coded and frozen at −70°C.

Experimental Subjects and Clinical Definitions

The subjects were identified retrospectively from a nested case-control study. Patients were assigned as having preeclampsia or normal pregnancy by previously established strict criteria[7] as recommended by the National Institutes of Health consensus conference for investigations of pregnancy hypertension.[18] Preeclamptic women were nulliparous, with pregnancy-onset hypertension, proteinuria, hyperuricemia, and reversal of hypertension and proteinuria within 12 weeks after delivery. Pregnancy-onset hypertension is defined as an increase of 30 torr systolic or 15 torr diastolic blood pressure compared with values obtained before 20 weeks' gestation, or an absolute blood pressure of at least 140/90 torr. Proteinuria is designated as greater than or equal to 2+ (100 mg/dL) on a voided specimen or greater than or equal to 1+ (30 mg/dL) on a catheterized specimen. Hyperuricemia is defined as at least 5.5 mg/dL (≥1 standard deviation above the normal mean concentration

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at term[19]). None of our patients met criteria for severe preeclampsia. Patients from both diagnostic groups (n = 31 per group) were matched for nulliparity, race, maternal age at delivery (±3 years), and duration of pregnancy (±2 weeks).

Measurements of Blood Pressure and Body Mass Index

Mean arterial blood pressure (MAP) was calculated from the average of blood pressure readings (Korotkoff V, seated position) taken before 20 completed weeks' gestation and again during the late third trimester (≥37 weeks' gestation), before fluid or pharmacologic (including anesthetic) therapy, according to the equation, MAP = (diastolic + [systolic − diastolic]/3). Body mass index (BMI), defined as weight (kg)/height^2 (m^2), was selected as the best practical index of truncal obesity and body composition.[20] The height and weight of each patient were obtained from prenatal records. Placental weight was determined at the time of delivery.

Plasma Triglyceride and NEFA Determinations

Plasma triglycerides were quantified in matched patients using a microtiter-plate modification of the Sigma triglycerides diagnostic kit (St Louis, MO). The colorimetric assay had a sensitivity of 1 mg/dL with interassay and intraassay coefficients of variation of 3% and 1%, respectively. NEFAs were quantified using a microtiter-plate modification of the NEFA C diagnostic kit (Wako Pure Chemicals, Richmond, VA). In our laboratory, this assay had a sensitivity of 0.02 mmol/L with interassay and intraassay coefficients of variation of 12% and 3%, respectively.

Plasma hPL Determinations

Plasma hPL concentrations were quantified in matched patients (n = 31 per group) by a specific radioimmunoassay (DSL, Los Angeles, CA) with a sensitivity of less than 1 μg/mL and interassay and intraassay coefficients of variation of 9% and 8%, respectively.

Statistics

Results are presented as the mean ± SD for each study group. Kolmogorov-Smirnov analyses demonstrated that results from all the analyses were normally distributed and that no significant differences ($P > .05$) existed in data distribution between the two groups of patients. Comparisons between matched preeclamptic and normal pregnant controls were analyzed using unpaired Student's *t* tests. For all analyses, two-tailed tests were accepted as demonstrating significant differences for *P* less than .05.

RESULTS

Nulliparous pregnant women were identified retrospectively from an ongoing prospective cohort study and were matched for race, maternal age, and gestational age at delivery as already described. A summary of demographic, clinical, and biochemical findings for each of the two study groups is presented in Table 1. Maternal age and early-pregnancy (< 20 weeks' gestation) MAP did not differ between the two groups ($P \geq .40$). By definition, late-pregnancy (≥ 37 weeks' gestation) MAP (Fig 1), proteinuria, and serum uric acid levels were significantly greater in the preeclamptic group ($P < .01$). Women in whom the diagnosis of preeclampsia was established were noted to have a higher mean prepregnancy BMI (24.8 ± 5.5 kg/m^2) compared with the normal control group (22.1 ± 6.2 kg/m^2), but this difference did not reach statistical significance ($P = .13$). However, late-pregnancy BMI (Fig 2) was significantly greater in women who developed preeclampsia (33.1 ± 6.7 *v* 28.3 ± 6.3 kg/m^2, $P = .01$; Table 1).

Table 1. Demographic, Clinical, and Biochemical Findings

Parameter	Preeclampsia (n = 31)	Normal (n = 31)	*P**
Maternal age (yr)	26.6 ± 5.8	28.3 ± 6.1	.40
Early-pregnancy (<20 wk) MAP (torr)	81.7 ± 5.6	80.8 ± 5.0	.61
Late-pregnancy (≥37 wk) MAP (torr)	107.8 ± 9.1	86.2 ± 6.9	<.01
Proteinuria (mg/dL)	>30	<10	<.01
Serum uric acid (mg/dL)	6.4 ± 0.7	3.9 ± 0.7	<.01
Prepregnancy BMI (kg/m^2)	24.8 ± 5.5	22.1 ± 6.2	.13
Late-pregnancy (≥37 wk) BMI (kg/m^2)	33.1 ± 6.7	28.3 ± 6.3	.01
Plasma hPL (μg/mL)	8.4 ± 4.4	5.6 ± 2.9	<.01
Plasma triglycerides (mg/dL)	249 ± 92	192 ± 61	<.01
Plasma NEFA (mmol/L)	0.87 ± 0.40	0.50 ± 0.29	<.01
Hemoglobin (g/dL)	11.9 ± 1.2	12.4 ± 1.3	.09
Placental weight (g)	623 ± 222	706 ± 133	.12

NOTE. Data are presented as the mean ± SD.
*Statistical analysis by unpaired Student's *t* test (2-tailed).

Plasma triglyceride and NEFA concentrations were significantly greater ($P < .01$) in preeclamptic women than in the normal control group (Figs 3 and 4, respectively). Triglyceride levels were higher in preeclamptic women (249 ± 92 mg/dL) than in normal subjects (192 ± 61 mg/dL, $P < .01$). Women with preeclampsia had higher NEFA concentrations (0.87 ± 0.40 mmol/L) compared with the normal control group (0.50 ± 0.29 mmol/L, $P < .01$). As an indicator of hemoconcentration,[21] hemoglobin values were compared and found to be similar in the two study groups (11.9 ± 1.2 *v* 12.4 ± 1.3 g/dL, $P = .09$; Table 1).

Circulating concentrations of hPL, measured by radioimmunoassay at 36 to 38 weeks' gestation (approximately 2 to 3 weeks before delivery), also were found to be significantly higher in women who developed preeclampsia compared with the control group: 8.4 ± 4.4 and 5.6 ± 2.9 μg/mL, respectively

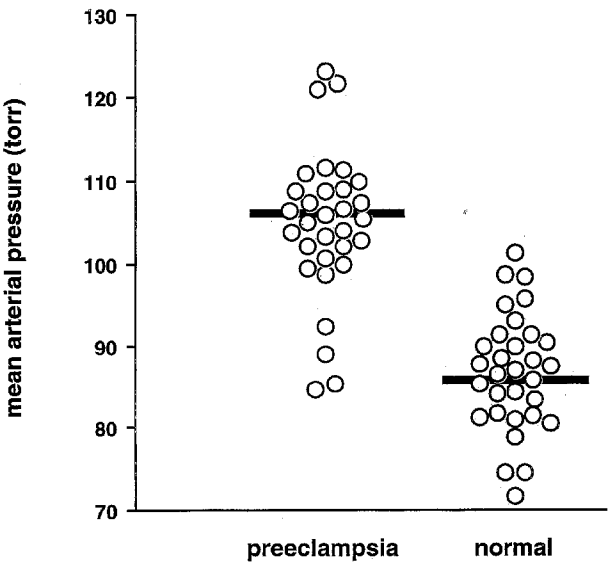

Fig 1. Scattergram of MAP in pregnant women at term (≥ 37 weeks' gestation). Bar indicates group mean. The groups were statistically different by Student's *t* test ($P < .01$).

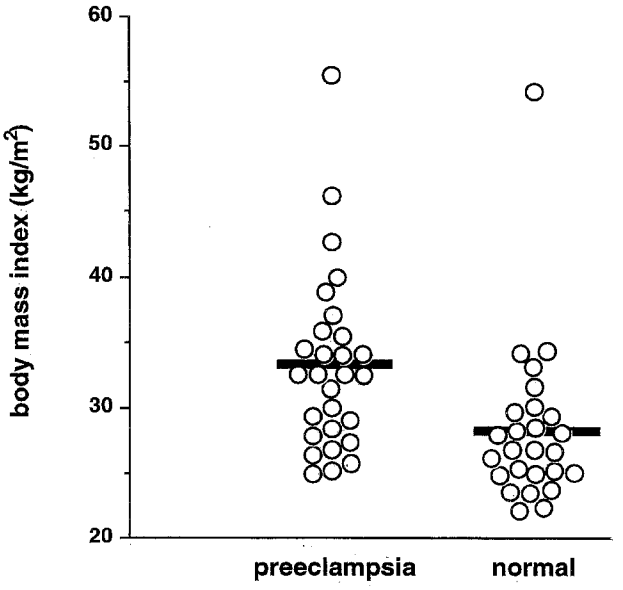

Fig 2. Scattergram of BMI in pregnant women at term. Bar indicates group mean. The groups were statistically different by Student's *t* test ($P = .01$).

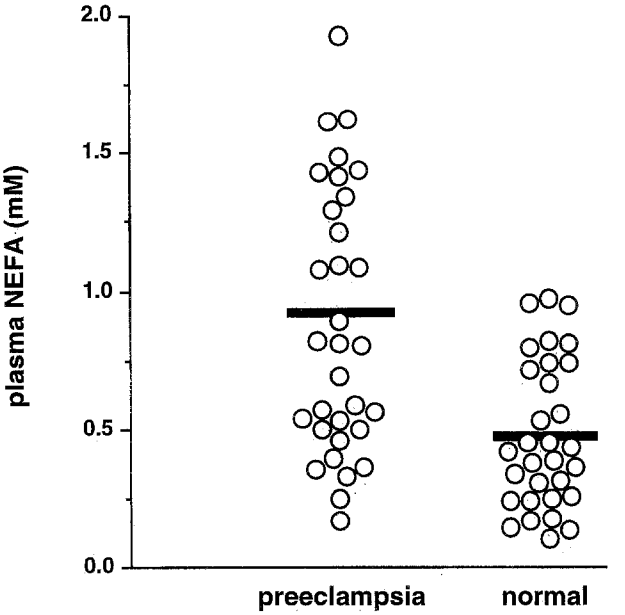

Fig 4. Scattergram of plasma NEFA concentrations. Bar indicates group mean. The groups were statistically different by Student's *t* test ($P < .01$).

($P < .01$; Fig 5). Placental mass, a known determinant of plasma hPL concentration, was smaller in the preeclampsia group, but this difference was not statistically significant (623 ± 222 *v* 706 ± 133 *g*, $P = .12$; Table 1).

DISCUSSION

Over the past decade, three cardinal observations have contributed to our current understanding of the pathogenesis of preeclampsia. First, abnormal placental differentiation, invasion, and vascularization[3,22,23] are consistent with the clinical findings that preeclampsia only occurs with pregnancy or hydatidiform mole and only begins to abate following delivery of the placenta.[24] Second, maternal endothelial cell dysfunction may explain the vascular manifestations of the syndrome, including vasospasm, proteinuria, edema, hypercoagulability, and altered prostaglandin production.[2] Third and most recent are the reported associations of preeclampsia with maternal obesity[13,14] and abnormal lipid metabolism.[15-17] These associations are related to the endothelial injury hypothesis, as triglyceride and lipoprotein toxicity play important roles in the vascular dysfunction of atherosclerosis and preeclampsia.[25,26]

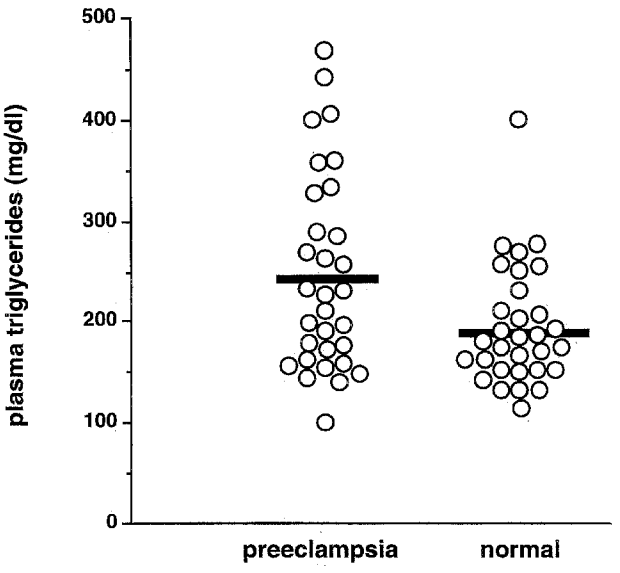

Fig 3. Scattergram of plasma triglyceride concentrations. Bar indicates group mean. The groups were statistically different by Student's *t* test ($P < .01$).

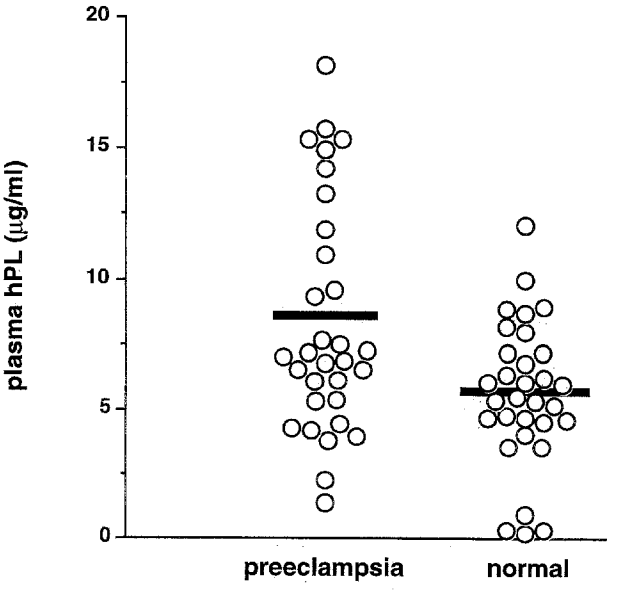

Fig 5. Scattergram of hPL concentrations. Bar indicates group mean. The groups were statistically different by Student's *t* test ($P < .01$).

Our results, which indicate that BMI is increased in preeclamptics at term, cannot distinguish between increased fat accumulation and increased edematous fluid retention. Future studies using more sophisticated methodology to assess body composition will be needed to accurately specify the nature of the increased body weight gain in women developing preeclampsia. Triglyceride and NEFA concentrations also were found to be higher in women with preeclampsia. NEFAs, which are affected less by postprandial lipemia than triglyceride levels, appeared to provide the best discrimination between matched groups of nonfasting pregnant patients.

Elevated concentrations of hPL in primiparous preeclamptic women confirm the findings of Obiekwe et al.[27] hPL is reported to have lipolytic activity on maternal adipose cells[28] and is believed to liberate free fatty acids via growth hormone receptor activation and enhanced sensitivity to endogenous catecholamines.[29] Fat intake and fat-free body mass also have been shown to affect NEFA levels in nonpregnant individuals.[30]

Matching for gestational age at delivery in our nested case-control design necessitated the selection of preeclamptic women delivering at term, and hence is reflective of relatively mild disease. The 30 torr/15 torr blood pressure increase and semiquantitative dipstick proteinuria criteria used in this study are considered by some to overestimate the diagnosis of preeclampsia.[31] Although our inclusion of mild preeclamptics may mitigate its statistical power, the conservatism of our study emphasizes that maternal (BMI and triglycerides) and fetoplacental (hPL) factors contributing to lipid metabolism are elevated significantly in common, mild preeclampsia. More dramatic differences between the two groups might have been expected if cases of severe preeclampsia were included. The criteria used in our study (nulliparity, blood pressure increase over early pregnancy values, proteinuria, hyperuricemia, and postpartum resolution of these signs) are recommended to verify specificity of the preeclampsia diagnosis,[18] not for their clinical utility in patient management.

The molar ratio of free fatty acids to serum albumin is thought to be an important determinant of endothelial cell dysfunction in preeclampsia.[15] Endresen et al[15] reported that this ratio was increased about 1.8-fold in preeclamptic women and demonstrated that high ratios inhibited prostacyclin production by endothelial cells in vitro. Total plasma albumin concentrations were not quantified in this study, but we previously observed similar concentrations in mildly preeclamptic (3.07 ± 0.39 g/dL) and normal (3.21 ± 0.28 g/dL) women ($P > .05$, $n = 19$[32]) in late pregnancy. However, isoelectric focusing indicated that plasma albumin in preeclampsia was qualitatively more acidic than in normal pregnancy,[32] presumably reflecting increased fatty acid binding.

In summary, the data presented herein are consistent with a model of preeclampsia in which poor perfusion causes trophoblastic proliferation[33] and increased maternal vascular deportation of hPL, as described for human chorionic gonadotropin.[34] This, along with maternal obesity, provides a stimulus and a substrate, respectively, for enhanced lipolysis. Delivery of NEFA into the circulation and subsequently into maternal endothelium contributes to cellular lipid accumulation[15] and altered prostaglandin metabolism.[15-17] This combination of maternal and fetal factors is consistent with recent genetic-linkage studies that suggest an interaction between maternal and fetal genotypes in cases of preeclampsia.[10]

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