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Pre-eclampsia disrupts the normal relationship between serum leptin concentrations and adiposity in pregnant women

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Summary. The adipocyte hormone, leptin, is secreted in proportion to adipose mass and is implicated in the regulation of energy balance via its central actions on food intake and sympathetic nervous system activity. The placenta was also shown recently to be a possible source of leptin in pregnant women, raising the possibility that the normal relationship between leptin and adiposity may be altered in pre-eclampsia. We therefore sought to assess the extent to which maternal second trimester serum leptin concentrations differed for women who would subsequently develop pre-eclampsia and those who would remain normotensive. This nested case–control study population comprised 38 women with pregnancy-induced hypertension and proteinuria (pre-eclampsia) and 192 normotensive women. Multiple least-squares regression procedures were used to assess the independent relationship between leptin concentrations and risk of pre-eclampsia. Serum leptin concentrations, measured by radioimmunoassay, were highly correlated with maternal pre-pregnancy and second trimester body mass index ($r = 0.71$ and $r = 0.74$ respectively; $P < 0.001$ for both) among normotensive women,

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and to a lesser extent among women who developed pre-eclampsia ($r = 0.29$ and $r = 0.42$; $P = 0.09$ and 0.02 respectively). Among women with a pre-pregnancy body mass index of $\leqslant 25$ kg/m^2, pre-eclampsia cases compared with controls had higher mean second trimester leptin concentrations after adjustment for confounding factors. In contrast, pre-eclampsia cases had lower mean leptin concentrations than controls for those women with a pre-pregnancy body mass index above 25 kg/m^2. Other factors in addition to the level of adiposity may therefore influence serum leptin concentrations in pre-eclamptic pregnant women. Our results suggest the possibility that leptin, like several other placentally derived substances (e.g. steroid hormones, eicosanoids and cytokines), may be involved in the pathogenesis of pre-eclampsia. Further work is needed to confirm our findings and to assess the metabolic importance and determinants of leptin concentrations in uncomplicated and pre-eclamptic pregnancies.

Introduction

Pre-eclampsia continues to be an important cause of maternal mortality as well as perinatal morbidity and mortality worldwide.[1] Although there is increasing evidence that many of the symptoms of pre-eclampsia may be attributed to diffuse endothelial dysfunction,[2] the cause of this complex multisystem maternal disorder remains unknown. Clinically, pre-eclampsia is characterised by maternal high blood pressure, proteinuria and oedema. Women with the disorder also experience profound, albeit transient, endocrine disturbances including hyperlipidaemia, particularly hypertriglyceridaemia,[3,4] excessive lipid peroxidation or oxidative stress,[5,6] insulin resistance,[3] sympathetic nervous system over-reactivity;[7] plasma elevations of pro-inflammatory cytokines,[8,9] elevations in the placental hormone, human chorionic gonadotropin,[10] and an imbalance in the placental production and circulation of the eicosanoids, thromboxane and prostacyclin.[11,12] Poor utero-placental perfusion, secondary to incomplete endovascular invasion by cytotrophoblasts is considered the primary aetiological lesion of pre-eclampsia.[2,13] Putative epidemiological risk factors for pre-eclampsia include primiparity, young and advanced maternal age, and multiple pregnancies.[14,15] Increased maternal adiposity, as measured by high pre-pregnancy body mass index, is also a strong and consistently identified risk factor for the disorder,[14–16] although little is known about the biological mechanisms underlying this epidemiological association.

Leptin, the product of the *ob* gene, is produced primarily by adipose tissue and has been implicated in the regulation of energy balance via its central actions on food intake and energy expenditure.[17] In humans, circulating leptin concentrations correlate with adiposity,[18,19] and decrease after weight loss,[19,20] fasting[20] or caloric

restriction.[20] Recent studies, however, suggest that during pregnancy leptin is not derived solely from the adipocyte. Maternal serum leptin concentrations have been shown to increase during pregnancy to values greater than those predicted for the degree of adiposity.[21–23] Emerging evidence from *in vivo* and *in vitro* studies indicate that the placenta, an important endocrine organ, is capable of synthesising and secreting leptin into maternal and fetal circulation and may contribute to pregnancy-associated elevations in circulating leptin concentrations. Placentally derived leptin is also thought to be a source of leptin detected in amniotic fluid, and arterial and venous cord blood.[24]

Given that pre-eclampsia is known to be associated with poor placental endovascular invasion, diverse endocrine disturbances, and increased maternal pre-pregnancy adiposity, and that leptin has been shown, by some,[23] although not all investigators,[24] to be correlated with adiposity during pregnancy, we sought to (1) assess the relationship between maternal second trimester serum leptin concentrations in relation to maternal adiposity; and (2) assess maternal second trimester serum leptin concentrations in women who remained normotensive throughout pregnancy compared with women who developed pre-eclampsia.

Methods

A cohort of 1383 women providing a second trimester serum sample to a central laboratory between December 1993 and August 1994, and who later delivered at the Swedish Medical Center, constitute the base cohort study population wherein the present case–control study is nested. Each blood sample, collected at 15–22 weeks' gestation, was centrifuged and serum was separated and stored at –20 °C after being subjected to routine biochemical analyses as part of a second trimester screening protocol for detecting pregnancies complicated by neural tube defects and Down's syndrome. Serum remaining after routine testing was transferred to the Swedish Medical Center and stored at –70°C until analysis.

From this base cohort population of 1383 pregnant women, we identified 39 patients who subsequently developed pre-eclampsia, the diagnosis of which was made in accordance with the guidelines of the American College of Obstetricians and Gynecologists.[25] Using serial maternal blood pressure readings and maternal urinary protein results abstracted from medical records, pre-eclampsia was defined as a sustained (6 or more hours) 15-mmHg diastolic rise or a 30-mmHg rise in systolic blood pressure with proteinuria; if first trimester blood pressures were unknown, a sustained (6 or more hours) blood pressure $\geqslant 140/90$ mmHg was used. Proteinuria was defined as urine protein concentration of $\geqslant 30$ mg/dL for two random specimens collected at least 4 h apart. Primiparity was not a criterion for diagnosis for this investigation. In this base study population of 1383 primiparous and multiparous gravidas, the incidence of pre-eclampsia is estimated at 2.8% (39/1383). Of the 39 pre-eclampsia cases, one subject had an

inadequate amount of serum for leptin determination. Hence, 38 cases were available for analysis.

Eligible controls were subjects who remained normotensive throughout pregnancy. Owing to practical constraints, it was not possible to include all normotensive pregnant women as controls. Therefore, using a stratified random sampling algorithm, with stratification based on 3-month intervals within which serum samples were collected, we identified 192 women to serve as controls. Women with missing information for blood pressure and urinary protein test results ($n = 4$) were not eligible for inclusion in this investigation, as a diagnosis of pre-eclampsia could not be ruled out with certainty. Twenty-seven women with pregnancy-induced hypertension without proteinuria (i.e. gestational hypertensives) were not included in this study. Women with chronic hypertension preceding the index pregnancy were not eligible for inclusion in this research. The large number of controls provides approximately a 1:5 case to control ratio, thus maximising statistical power.

Serum leptin concentrations were determined by radioimmunoassay[26] with reagents supplied by Linco Research, St Charles, MO, USA. The range of the standard curve is 0.5–100 ng/mL. The intra- and interassay coefficients of variation are $< 8\%$. The antibody used in the assay does not cross-react with human insulin, proinsulin, glucagon, pancreatic polypeptide or somatostatin.

Information on maternal socio-demographic characteristics, reproductive and medical histories, and labour and delivery characteristics, as well as anthropometric measurements (maternal height, pre-pregnancy weight and weight at 18–22 weeks' gestation) were abstracted from maternal medical records. Gestational age was based on the last menstrual period and (when possible) confirmed by ultrasound examination, conducted before 20 weeks' gestation. Body mass index, used as a measure of maternal overall adiposity, was calculated as weight (in kilograms) divided by height (in metres) squared. Maternal pregnancy weight gain (up to 18–22 weeks' gestation) was calculated by subtracting maternal pre-pregnancy weight from maternal weight measured at 18–22 weeks' gestation.

The distribution of maternal socio-demographic characteristics, medical and reproductive histories according to case–control status was examined. The distribution of continuous variables (e.g. serum leptin concentrations, body mass index and maternal age) were checked and found to be approximately normal, hence parametric statistical analytic procedures were used. Continuous variables are presented as means $\pm$ standard deviation (SD). Unadjusted mean differences in maternal serum leptin concentrations were assessed using Student's *t*-test statistics. Comparisons of categorical variables were made between case and control subjects using chi-squared or Fisher's Exact tests. In an effort to examine the independent relationship between maternal serum leptin and pre-eclampsia risk, we calculated the leptin:pre-pregnancy body mass index ratio as a means of expressing individual leptin concentration per percentage body fat.[19] A similar

ratio was generated using maternal body mass index measured during the second trimester (18–22 weeks' gestation). The Pearson's product–moment correlation coefficient was used to measure the closeness of a linear relationship between maternal second trimester leptin concentrations and selected characteristics including maternal pre-pregnancy weight and pre-pregnancy body mass index. Multiple linear least-squares regression analyses were conducted in order to examine the independent relationship between maternal serum leptin concentrations while controlling for potential confounding covariates such as maternal pre-pregnancy body mass index, age, race, fetal gender and parity. Any covariate that resulted in at least a 10% change in the coefficient for pre-eclampsia in the regression model was considered a confounder and retained in the multivariate model. For analyses which adjusted for maternal pre-pregnancy body mass index, gestational age at blood collection was found to be a confounder and was therefore included in the final regression model. Maternal race and fetal gender were also found to be important confounders in analyses that considered maternal second trimester body mass index. An interaction term between maternal pre-pregnancy body mass index (and second trimester body mass index) and a yes/no indicator variable, where '1' denotes case status and '0' denotes control status, was also included in the model because univariate analyses indicated that the relationship between leptin and maternal pre-pregnancy body mass index varied according to pre-eclampsia outcome. The adjusted R^2 value was provided as a measure of the total variability in maternal serum leptin concentrations explained by each model. Predicted leptin values were calculated from final regression models along with 95% confidence intervals for the predicted values.

Results

Socio-demographic, medical and reproductive characteristics of study subjects are presented in Table 1. Women who developed pre-eclampsia compared with control subjects tended to be heavier, primiparous and more likely to have a positive family history of diabetes. Of note, cases and controls gained a similar amount of weight by the second trimester. The average gestational age of blood collection was similar for the two study groups (16.9 ± 1.8 and 16.5 ± 1.2 weeks' gestation for cases and controls respectively). Among cases, maternal second trimester serum leptin concentration ranged from 5.7 to 46.6 ng/mL, and was 21% higher on average than for control subjects (20.2 ± 1.5 vs. 16.7 ± 0.7 ng/mL; $P = 0.040$) (Table 1). Women who developed pre-eclampsia also had higher leptin concentrations per unit pre-pregnancy body mass index than those women who remained normotensive (0.85 ± 0.06 vs. 0.71 ± 0.03; $P = 0.044$). A similar case–control difference was seen for leptin concentrations corrected for second trimester body mass index.

Table 1. Socio-demographic and medical characteristics of pre-eclampsia cases and normotensive control subjects

	Normotensive controls ($n = 192$)		Pre-eclampsia cases ($n = 38$)	
Covariate	Mean ± SD	Range	Mean ± SD	Range
Maternal age (years)	29.3 ± 4.8	(15.0–39.0)	31.0 ± 6.1	(17.0–41.0)
Gestational age at blood collection (weeks)	16.5 ± 1.2	(15.0–20.0)	16.9 ± 1.8	(15.0–22.0)
Maternal height (m)	1.7 ± 0.07	(1.4–1.9)	1.6 ± 0.08	(1.4–1.8)
Pre-pregnant weight (kg)	62.2 ± 12.9	(39.5–120.2)	66.0 ± 10.7	(44.0–106.6)
Pre-pregnancy BMI (kg/m^2)[a]	22.8 ± 4.1	(16.5–39.2)	25.4 ± 4.0	(18.6–39.1)
BMI at 18–22 weeks gestation (kg/m^2)[a]	25.2 ± 4.3	(17.9–47.0)	27.8 ± 3.7	(21.3–37.8)
Weight gain by 18–22 weeks (kg)	6.3 ± 4.2	(–6.8–24.0)	6.3 ± 4.3	(–3.6–17.2)
Birthweight (g)[a]	3402.6 ± 572.1	(397.0–4990.0)	2823.3 ± 974.4	(535.0–4366.0)
Gestational age (weeks)[a]	38.5 ± 2.1	(21.0–42.00)	36.7 ± 3.3	(28.0–42.0)
Serum leptin (ng/mL)[a]	16.7 ± 10.1	(1.1–53.4)	20.2 ± 9.4	(5.7–46.3)
Leptin:pre-pregnancy BMI ($ng/mL:kg/m^2$)[a]	0.71 ± 0.35	(0.07–1.84)	0.85 ± 0.36	(0.22–1.92)
Leptin:18–20 week BMI ($ng/mL:kg/m^2$)[a]	0.64 ± 0.32	(0.06–1.82)	0.76 ± 0.31	(0.22–1.62)
Covariate	*n*	(%)	*n*	(%)
Maternal race				
Black	11	(5.7)	3	(7.9)
Non-black	175	(94.3)	35	(92.1)
Unmarried	30	(15.6)	8	(21.1)
Medicaid recipient	36	(18.8)	8	(21.1)
Primigravid	53	(27.6)	16	(42.1)
Primiparous[a]	93	(48.4)	27	(71.1)
Prior miscarriage	49	(25.5)	8	(21.1)
Prior induced abortion	42	(21.9)	7	(18.4)
Prior stillbirth	3	(1.6)	0	(0)
Cigarette smoker	25	(13.0)	3	(7.9)
Family history of diabetes	60	(32.6)	18	(48.7)
Family history of hypertension	77	(41.4)	14	(38.9)
Male infant	101	(52.6)	18	(47.4)
Large-for-gestational-age	27	(14.1)	3	(7.9)
Small-for-gestational-age[a]	2	(1.0)	8	(21.1)

[a] $P < 0.05$. Fisher's Exact test used if frequencies for categorical covariates were below 5. Chi-squared tests used for all other categorical covariates. Student's *t*-tests were used for continuous covariates.

We next examined the association between maternal serum leptin concentration, pre-pregnancy and second trimester body mass index for women who developed pre-eclampsia (cases). Analyses were repeated for those women who remained normotensive throughout pregnancy (controls). Among control subjects, second trimester leptin concentrations were positively correlated with maternal pre-pregnancy weight ($r = 0.64$, $P < 0.0001$), pre-pregnancy body mass index ($r = 0.71$, $P < 0.0001$) and second trimester body mass index ($r = 0.74$, $P < 0.0001$). Notably, correlation coefficients between second trimester serum leptin and indices of maternal adiposity among pre-eclampsia cases were considerably lower than those estimated for control subjects. Among pre-eclampsia cases, second trimester leptin concentrations were weakly correlated with maternal pre-pregnancy weight ($r = 0.47$, $P < 0.003$), pre-pregnancy body mass index ($r = 0.42$, $P = 0.02$) and second trimester body mass index ($r = 0.29$, $P = 0.09$). Maternal serum leptin concentrations were not correlated with maternal weight gain during pregnancy, gestational age at delivery or infant birthweight. Visual inspection of regression lines estimated for pre-eclampsia cases and controls indicated that the lines crossed at ≈ 25 kg/m^2.

Results from analyses stratified according to maternal adiposity indicated that the relationship between plasma leptin concentrations and pre-eclampsia differed for lean and obese women. Among women with a pre-pregnancy body mass index $\leqslant 25$ kg/m^2, serum leptin concentrations were 33% higher for cases than controls (20.5 ± 10.91 vs. 13.6 ± 6.8 ng/mL; $P = 0.005$). For women with a pre-pregnancy body mass index above 25 kg/m^2, cases had leptin concentrations that were 20% lower than those of controls (22.3 ± 7.5 vs. 27.8 ± 12.1 ng/mL; $P = 0.084$). The results were similar when leptin was expressed per unit pre-pregnancy body mass index. Among women with a pre-pregnancy body mass index $\leqslant 25$ kg/m^2, the leptin:pre-pregnancy body mass ratio was 43% higher in pre-eclampsia cases than in controls (0.91 ± 0.45 vs. 0.63 ± 0.30 ng/mL/kg/m^2; $P = 0.0013$). In contrast, the leptin per pre-pregnancy body mass index value was 18% lower for pre-eclampsia cases than control subjects whose pre-pregnancy body mass index exceeded 25 kg/m^2 (0.79 ± 0.26 vs. 0.96 ± 0.39 ng/mL/kg/m^2; $P = 0.104$).

We next sought to assess the association between second trimester leptin concentrations and risk of pre-eclampsia after controlling for maternal pre-pregnancy body mass index and other potential confounding factors. Estimated coefficients and standard errors (SEs) for the covariates included in the final regression model are presented in Table 2. Table 2 shows that the presence or absence of pre-eclampsia is a major determinant of serum leptin concentrations during the second trimester, even after adjusting for body mass index and other confounding factors. The heterogeneity in association between maternal second trimester leptin concentration and risk of pre-eclampsia according to maternal adiposity is evident by the statistically significant interaction term in the model (Table 2). Figure 1 shows the predicted values and 95% confidence intervals of

Table 2. Relationship between maternal serum leptin concentration in the second trimester and pre-eclampsia (adjusted for pre-pregnancy body mass index)

Parameter	Coefficient ± SE	*P*
Pre-eclampsia	31.22 ± 11.51	0.007
Pre-pregnancy body mass index	1.77 ± 0.14	0.000
Pre-eclampsia × body mass index	–1.27 ± 0.46	0.007
Gestational age at blood collection	0.27 ± 0.45	0.543

Leptin expressed in ng/mL; body mass index in kg/m^2.
Of the total variation in maternal second trimester leptin concentration, 44.9% (adjusted $R^2 = 44.9\%$) is explained by this linear regression model.

serum leptin concentrations in relation to maternal pre-pregnancy body mass index (i.e. predicted values from the model described in Table 2). The predicted values of leptin are based on a model which allows for different slopes and intercepts for cases and controls. Briefly, among women with a pre-pregnancy body mass index of $\leqslant 25\ kg/m^2$, pre-eclampsia cases had higher predicted second trimester leptin concentrations than controls. For those women with a pre-pregnancy body mass index above 25 kg/m^2, pre-eclampsia cases had lower predicted leptin concentrations than controls.

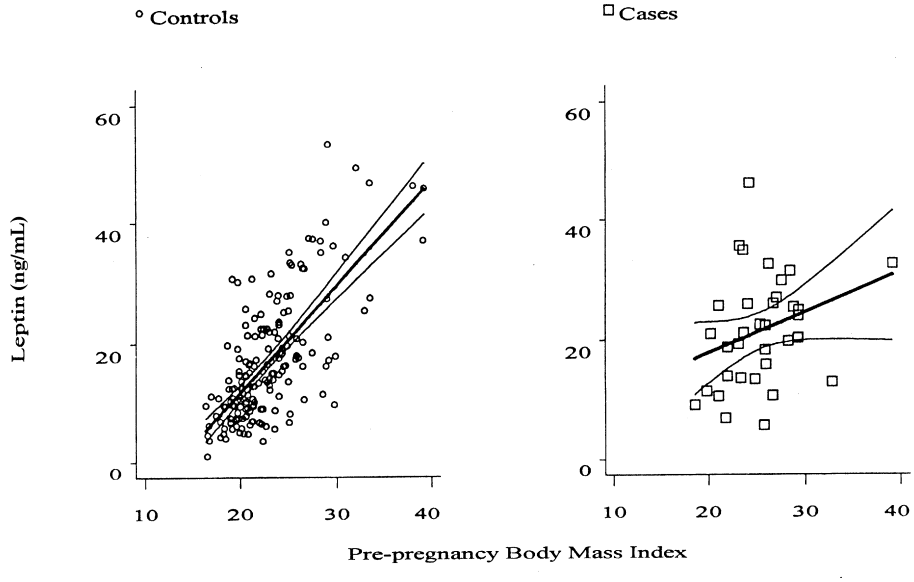

Figure 1. Relationship of maternal second trimester serum leptin concentrations to pre-pregnancy body mass index for pre-eclampsia cases (□) and normotensive control subjects (○). The heavier line represents predicted serum leptin concentrations; the lighter line represents pointwise 95% confidence intervals for predicted points Fitted values and confidence intervals are from the least-squares regression model described in Table 2.

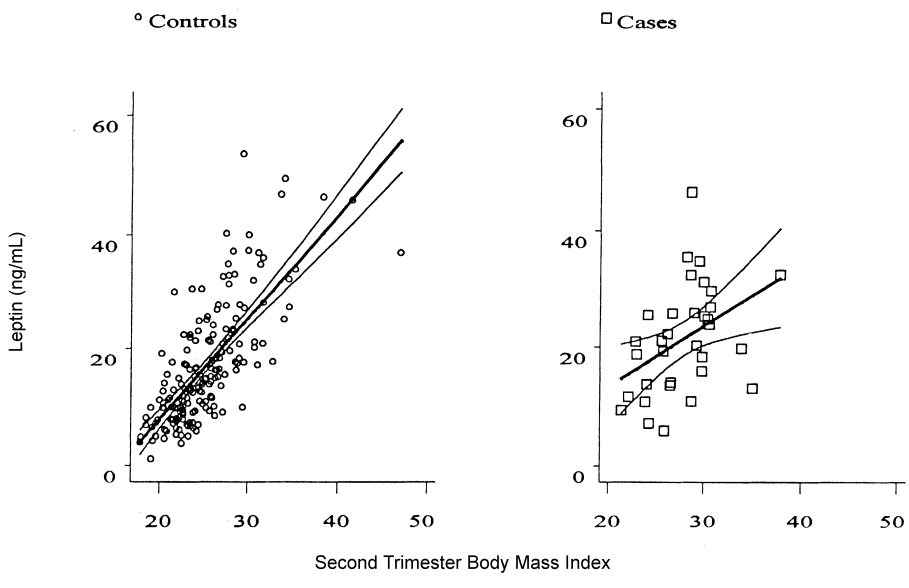

Figure 2. Relationship of maternal second trimester serum leptin concentrations to second trimester body mass index for pre-eclampsia cases (□) and normotensive control subjects (○). The heavier line represents predicted serum leptin concentrations; the lighter line represents pointwise 95% confidence intervals for predicted points. Fitted values and confidence intervals are from the least-squares regression model described in Table 3.

Figure 2 shows the relationship between maternal second trimester body mass index and leptin concentrations with pre-eclampsia, and Figure 3 shows the relationship between pre-eclampsia status and maternal serum leptin per unit pre-pregnancy body mass index. The corresponding results for the last two multivariate models (Tables 3 and 4) are consistent with the observation that the relationship between leptin and pre-eclampsia varies according to maternal adiposity. Furthermore, as seen in Figure 3, the amount of leptin per unit body mass index increases with maternal adiposity for women who remained normotensive throughout pregnancy (circles). In contrast, among women who developed pre-eclampsia (squares), the relationship between serum leptin concentrations and maternal adiposity appears to be disrupted, as the amount of leptin per unit body mass index is similar for lean and obese women. Results from analyses which excluded two subjects with leptin values that were thought to be outliers were not materially different from those reported here.

Discussion

We found that among both pre-eclamptic cases and normotensive controls, maternal second trimester serum leptin concentrations were correlated with maternal pre-pregnancy and second-trimester adiposity. Correlation coefficients

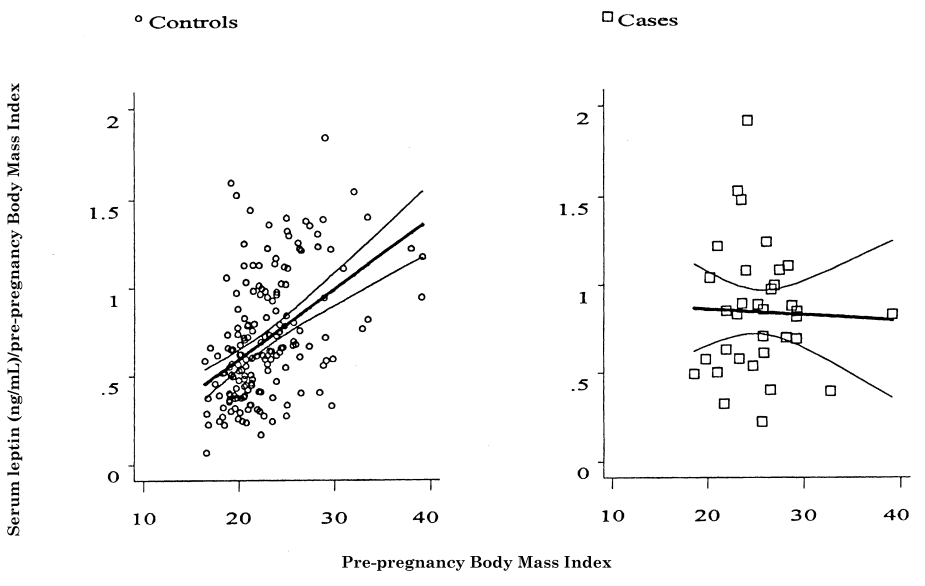

Figure 3. Relationship of maternal second trimester serum leptin concentrations (expressed as per unit body mass index) to pre-pregnancy body mass index for pre-eclampsia cases (□) and normotensive control subjects (○). The heavier line represents predicted serum leptin concentrations; the lighter line represents pointwise 95% confidence intervals for predicted points. Fitted values and confidence intervals are from the least-squares regression model described in Table 4.

between maternal adiposity and leptin concentrations among pre-eclampsia cases, however, were considerably lower than those seen for control subjects. Since the risk of pre-eclampsia and serum leptin concentrations both increase with increasing adiposity, it was necessary to determine whether adiposity-adjusted leptin concentrations were elevated in pre-eclampsia. After adjusting for possible confounding by maternal body mass index, we noted that case–control differences in leptin concentrations varied according to maternal adiposity. Specifically, lean

Table 3. Relationship between maternal serum leptin concentration in the second trimester and pre-eclampsia (adjusted for second trimester body mass index)

Parameter	Coefficient ± SE	*P*
Pre-eclampsia	15.28 ± 10.27	0.139
Second trimester body mass index	1.78 ± 0.13	0.000
Pre-eclampsia × body mass index	–0.56 ± 0.37	0.133
Maternal race (black/non-black)	4.26 ± 2.18	0.052
Infant gender (male/female)	1.81 ± 1.01	0.075

Leptin expressed in ng/mL; Body mass index in kg/m^2.
Of the total variation in maternal second trimester leptin concentration, 52.5% (adjusted $R^2 = 52.5\%$) is explained by this linear regression model.

Table 4. Relationship between maternal serum leptin concentration (expressed as per unit pre-pregnancy body mass index) in the second trimester and pre-eclampsia

Parameter	Coefficient ± SE	*P*
Pre-eclampsia	1.13 ± 0.38	0.003
Pre-pregnancy body mass index	0.04 ± 0.01	0.000
Pre-eclampsia × body mass index	–0.04 ± 0.01	0.004

Leptin expressed in ng/mL per body mass index (kg/m^2).
Of the total variation in maternal second trimester leptin:pre-pregnancy body mass index ratio, 18.8% (adjusted $R^2 = 18.8\%$) is explained by this linear regression model.

women who became pre-eclamptic had elevated leptin concentrations, whereas the reverse was true for obese women who developed this condition.

Maternal second trimester serum leptin concentrations among control subjects in our study are considerably lower than the 20–43 ng/mL reported among unselected pregnant women at term by Schubring *et al*.[22] and Butte *et al*.,[23] which may reflect the relatively low pre-pregnancy body mass index of our control group (22.8 ± 4.1 kg/m^2). Our observation of a strong, statistically significant correlation between maternal second trimester serum leptin concentrations and pre-pregnancy body mass index, however, is in general agreement with results reported for non-pregnant women.[19,20] While our results are at variance with those of Schubring *et al*.,[22] who reported that there was no correlation between maternal leptin concentrations (measured at term) with pre-pregnancy body mass index, Hartman *et al*.[27] in a study of 76 women with uncomplicated pregnancies reported that maternal leptin concentrations and body mass index were correlated ($r = 0.47$). These different outcomes may reflect differences in the timing of maternal blood sampling, pre-pregnancy body mass index and presence or absence of complications such as pre-eclampsia, each of which influence the association between leptin concentrations and adiposity in pregnant women.

We are aware of only one published account of maternal leptin concentrations in pre-eclamptic and normotensive pregnant women. In a cross-sectional case–control study of 27 women with pre-eclampsia and an equal number of controls matched for gestational age at delivery and maternal body mass index, McCarthy *et al*.[28] reported that maternal serum leptin levels at delivery were 28% higher among cases than controls (43.23 ± 19.9 vs. 33.68 ± 18.4 ng/mL). These results are in agreement with our findings, although results from our study extend these findings by demonstrating that the relationship between maternal serum concentrations and pre-eclampsia risk may vary by maternal pre-pregnancy adiposity. Moreover, because we used a nested case–control study design where leptin concentrations were determined using serum collected at 17 weeks' gestation on average, we are able to show that the metabolic dysregulation of serum leptin concentrations precedes the clinical manifestation of pre-eclampsia.

Several limitations must be considered when interpreting the results of our study. First, inference from this investigation is hampered, in part, by the relatively small number of cases studied. Without a larger number of pre-eclampsia cases, we cannot thoroughly explore the apparent heterogeneity in the association between leptin concentrations and pre-eclampsia according to maternal adiposity. Second, we cannot completely exclude the possibility that our results are unduly influenced by a few subjects who are not representative of the larger population. Because of this concern, we repeated analyses after excluding two cases with high body mass index and low leptin values and did not observe differences that altered our conclusions.

Although the processes that control the synthesis and secretion of leptin in maternal and fetal circulation during pregnancy are incompletely understood, two independent reports indicate that the placenta is an important source of leptin in human pregnancies. Indirect immunofluorescence analyses of first and third trimester placental tissue revealed strong staining for immunoreactive leptin in first trimester chorionic villi and third trimester syncytiotrophoblasts respectively.[21] These results are in agreement with those of Senaris and colleagues,[24] who demonstrated that leptin is synthesised in syncytiotrophoblast cells harvested from delivered placentas. Studies involving animal models also demonstrate a 2.7-fold increase in uterine leptin receptor mRNA levels concurrent with a 1.8-fold increase in leptin concentration during gestation.[29] Thus, the pregnant uterus probably makes a significant contribution to leptin levels in maternal blood.

This observation may help explain why the correlation coefficient between leptin concentrations and adiposity among women who developed pre-eclampsia was substantially lower than that observed among women who remained normotensive throughout pregnancy ($r = 0.42$ vs. $r = 0.71$). We hypothesise that, in addition to adipose mass, the placenta synthesizes and secretes leptin, and that placentally derived leptin is affected by pre-eclampsia. Thus, pathological placental changes associated with pre-eclampsia are hypothesised to modify the relationship between maternal adiposity and leptin concentrations that is seen among normotensive pregnant women. Although speculative, a scenario in which pre-eclampsia among lean women is associated with increased placental leptin secretion is consistent with our results. Studies to evaluate the expression of leptin mRNA in placental tissue of pre-eclamptic and normotensive pregnant women of variable adiposity are needed to evaluate this hypothesis.

The finding that leptin is associated with generalised sympathoactivation in humans[30] and rats[31] suggests a possible physiological mechanism for our observed association of leptin with pre-eclampsia risk among lean women. In a study of 37 healthy men, basal muscle sympathetic nerve activity, a measure of sympathetic nervous outflow, has been shown to be correlated with plasma leptin concentrations ($r = 0.44$; $P < 0.01$). In a rat model, it was shown that small increases in plasma leptin concentrations, if sustained, produced a two- to

threefold increase in sympathetic nerve activity in several tissues.[31] One may therefore consider the possibility that pregnancy-associated hyperleptinaemia in lean women may increase the risk of sympathoactivation. Heightened sympathetic nervous activity has been shown in pre-eclamptic compared with normotensive pregnant women.[7] In a study of nine pre-eclampsia cases and eight normotensive pregnant controls, Schodel and colleagues[7] reported that sympathetic nerve activity (measured as bursts per 100 heart beats) were over threefold higher in pre-eclamptic women than in controls. Based on these observations, one must consider the possibility that the increased vascular resistance seen in pre-eclampsia is mediated, in part, by factors capable of enhancing sympathoactivation. Results from at least two studies[30,31] raise the possibility that leptin may be one such mediator of sympathoactivation, although leptin has not been shown to cause hypertension in studies to date. Firm conclusions therefore cannot be drawn until direct study of the effect of leptin on sympathetic nervous system activity and cardiovascular regulation is performed in animal models during pregnancy.

In summary, after adjusting for possible confounding factors, we noted that case–control differences in leptin concentrations varied according to maternal adiposity. Further work is needed to provide a better understanding of the metabolic dysregulation of serum leptin concentrations in pre-eclampsia.

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