Leptin During and After Preeclamptic or Normal Pregnancy: Its Relation to Serum Insulin and Insulin Sensitivity

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Hyperleptinemia may be part of the insulin resistance syndrome. We studied serum leptin in preeclampsia, which is an insulin-resistant state, and sought associations between leptin and insulin or insulin sensitivity during and after pregnancy. Twenty-two proteinuric preeclamptic women and 16 normotensive controls were studied during the third trimester. Leptin was higher in preeclampsia (mean ± SE, 34.6 ± 3.9 *v* 20.0 ± 3.3 μg/L, $P = .002$) and correlated directly with the level of proteinuria ($r = .47$, $P = .03$) and normal pregnancy ($r = .52$, $P = .04$), whereas insulin sensitivity as assessed by an intravenous glucose tolerance test showed no relationship to leptin. Leptin was 19.0 ± 3.6 μg/L in 14 preeclamptic women and 10.1 ± 2.0 μg/L ($P = .11$) in 11 controls 3 months after delivery. Leptin correlated directly with insulin both in preeclamptic puerperal women ($r = .63$, $P = .02$) and in controls ($r = .81$, $P = .003$). Leptin and insulin sensitivity correlated only in preeclamptic puerperal women ($r = -.59$, $P = .02$). In conclusion, (1) serum leptin is elevated in preeclampsia, (2) insulin is an important determinant of serum leptin in preeclamptic and normotensive women both during pregnancy and in the puerperium, and (3) hyperleptinemia may be part of the insulin resistance syndrome also in women with prior preeclampsia.

LEPTIN is a novel hormone produced almost exclusively by adipocytes,[1] and it plays an important role in physiology in experimental animals and in humans.[2] Briefly, leptin may be the hormonal signal to the brain of the adequacy of energy stores, and leptin appears to activate the hypothalamic centers regulating energy intake and expenditure. The regulation of leptin synthesis and release in humans is not fully understood, but several pieces of evidence imply that insulin regulates leptin secretion.[3-7] Insulin resistance, independent of adiposity, is associated with an elevated plasma leptin concentration,[8,9] and hyperleptinemia has been suggested to be part of the insulin resistance syndrome.[10]

There are profound changes in body fat stores and energy metabolism during human pregnancy. Circulating leptin increases by 100% to 200% during normal pregnancy, reaching peak levels between 20 and 30 weeks of gestation,[11,12] which may be part of the physiological adaptation in pregnancy. Such pregnancy-induced elevations in serum leptin may be derived from both adipocytes and placental trophoblasts,[13,14] but the relative contribution of these cells to the circulating leptin pool is presently unknown. Placental leptin production may explain why there is a rapid decrease in serum leptin after delivery.[12]

Preeclampsia is associated with metabolic characteristics similar to those of the insulin resistance syndrome, such as changes in lipids and lipoproteins, hyperinsulinemia, and hyperuricemia.[15-19] Hyperleptinemia has been suggested to be part of the insulin resistance syndrome.[10] However, little is known about leptin metabolism and its relation to glucose homeostasis in preeclampsia. Previous data on leptin in preeclampsia are controversial: circulating levels of leptin have been reported to be either normal[11] or elevated[14] in severe proteinuric preeclampsia. In addition, no data have been previously reported on the relationship between the serum leptin level and insulin sensitivity during preeclampsia or normal pregnancy. The present study was thus undertaken to examine the serum leptin level and its relation to the insulin level and insulin sensitivity during and after preeclampsia and normal pregnancy.

SUBJECTS AND METHODS

With the permission of our ethics committee and after receiving the informed consent of the subjects, we studied 38 nulliparous women between 29 and 39 weeks of gestation.[20] The enrollment period was January 1, 1996 to April 30, 1997. All subjects were healthy before pregnancy, but during pregnancy, 22 developed preeclampsia (blood pressure >140/90 mm Hg at least 2 times 6 hours apart and proteinuria ≥0.3 g in a 24-hour urine collection), whereas 16 remained normotensive and nonproteinuric. Otherwise, the groups were comparable, and factors such as the body mass index (BMI), edema, or gestational week did not differ. All subjects had normal glucose tolerance as determined by a standard 75-g 2-hour oral glucose tolerance test (OGTT) (Table 1). One to 7 days after the OGTT, the whole-body insulin sensitivity of the subjects was measured using the minimal model technique after an overnight fast and in the absence of any medication as previously described.[20] Briefly, a bolus of glucose (0.3 g/kg body weight) was injected intravenously (IV) at 9 AM, followed by a dose of insulin (0.03 IU/kg IV Velosulin Human; Novo Nordisk Pharmaceuticals, Bagsvaerd, Denmark) 20 minutes later. Blood samples were collected as follows: 2 at baseline and then 1 at 4, 6, 8, 10, 19, 22, 29, 37, 67, 90, and 180 minutes (13 samples altogether) after administration of the glucose bolus. Using the simultaneous changes in glucose and insulin, the insulin sensitivity of the subjects was calculated.[20,21] Serum samples collected before glucose injection were assayed for leptin. The subjects were invited to a similar IV test 12 weeks after delivery, and 14 women from the preeclamptic group and 11 from the control group participated in the second study. At the follow-up examination, all signs of preeclampsia were absent. All subjects were still in amenorrhea, and a total of 10 women from each group lactated.

Serum leptin levels were assessed by specific radioimmunoassay ([RIA] Human leptin RIA kit; Linco Research, St Louis, MO), and all samples were tested in the same assay to eliminate the effect of interassay variation. The intraassay coefficient of variation at low

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Table 1. Clinical Characteristics of the Study Population

Characteristic	Women With Preeclampsia (n = 22)	Normotensive Pregnant Women (n = 16)	P
Age (yr)	30.4 ± 1.0	31.8 ± 1.1	NS
Weeks of gestation at study	36.3 ± 0.5	35.2 ± 0.7	NS
BMI pre-pregnancy (kg/m²)	22.6 ± 0.5	21.9 ± 0.8	NS
BMI at study (kg/m²)	27.0 ± 0.7	26.5 ± 0.8	NS
Proteinuria (g/24 h)	2.0 ± 0.6		
Uric acid (mmol/L)	0.37 ± 0.02	0.27 ± 0.01	.0001
2-h glucose in OGTT (mmol/L)	6.4 ± 0.2	6.1 ± 0.3	NS
2-h insulin in OGTT (μU/mL)	93.8 ± 6.5	50.6 ± 6.4	.0001
Fasting insulin in IVGTT (μU/mL)	7.4 ± 0.6	6.1 ± 0.6	NS
Systolic blood pressure (mm Hg)	142 ± 2	120 ± 3	.0001
Diastolic blood pressure (mm Hg)	96 ± 2	73 ± 2	.0001
Weeks of gestation at delivery	38.3 ± 0.4	40.4 ± 0.3	.0003
Infant birthweight (g)	2,764 ± 120	3,546 ± 89	.0001
Placental weight (g)	552 ± 30	648 ± 25	.03
Women reexamined after delivery (n)	14	11	
Weeks after delivery at reexamination	12.8 ± 1.0	13.6 ± 1.0	NS
BMI at reexamination (kg/m²)	23.9 ± 0.8	23.5 ± 1.0	NS
Fasting insulin in IVGTT (μU/mL)	5.4 ± 0.6	3.5 ± 0.3	.02

NOTE. Data are the mean ± SE.
Abbreviations: NS, nonsignificant; IVGTT, IV glucose tolerance test.

concentration (2.8 μg/L) was 4.7%, and at medium concentration (15.6 μg/L) 3.8%. The measurement of plasma insulin and insulin sensitivity have been described previously.[20]

Data analyses were performed with the Statview II program (Abacus Concepts, Berkeley, CA). Continuous variables are presented as the mean ± SEM. Leptin values were logarithmically transformed to normalize the distribution. Paired and unpaired Student's *t* tests were used in comparisons between paired and unpaired items, respectively. Univariate regression analysis was used to evaluate relations between the serum leptin concentration and the variables.

RESULTS

Leptin levels were significantly higher in preeclampsia than in normotensive pregnancy (34.6 ± 3.9 *v* 20.0 ± 3.3 μg/L, $P = .002$). The difference in leptin remained essentially similar even if 1 preeclamptic woman with high leptin (93.2 μg/L) was excluded from the statistical analysis (31.7 ± 3.0 *v* 20.0 ± 3.3 μg/L, $P = .004$). Also, the ratio of leptin to the BMI was higher in preeclamptic women than in normotensive women (Fig 1). A positive relation between leptin and fasting insulin emerged during both preeclampsia ($r = .47$, $P = .03$) and normal pregnancy ($r = .52$, $P = .04$; Fig 2).

The range of insulin sensitivity was 0.26×10^{-4} to $2.52 \times 10^{-4} \cdot min^{-1} \cdot \mu U/mL$ in preeclamptic women ($1.11 \pm 0.15 \times 10^{-4} \cdot min^{-1} \cdot \mu U/mL$) and 0.45×10^{-4} to $3.62 \times 10^{-4} \cdot min^{-1} \cdot \mu U/mL$ in controls ($1.77 \pm 0.19 \times 10^{-4} \cdot min^{-1} \cdot \mu U/mL$, $P = .009$ *v* preeclamptic women),[22] but insulin sensitivity was not related to leptin levels in either group (Fig 2).

There was a direct correlation between the serum leptin concentration and pre-pregnancy BMI in preeclamptic women ($r = .49$, $P = .02$) and in controls ($r = .70$, $P = .003$). The relation between the serum leptin and BMI at study emerged only in controls ($r = .67$, $P = .004$). Serum leptin correlated with the degree of proteinuria ($r = .46$, $P = .03$), but not with blood pressure or serum uric acid. Furthermore, no relations emerged between serum leptin and the weight of the infant or placenta or the weeks of gestation in either group.

Serum leptin levels measured 3 months after delivery were decreased significantly in the preeclamptic group and the control group (Fig 1). Leptin tended to be higher in puerperal women with prior preeclampsia (19.0 ± 3.6 μg/L) than in normal pregnancy (10.1 ± 2.0 μg/L, $P = .11$). This tendency was also present when leptin was related to the BMI (0.76 ± 0.13 *v* 0.41 ± 0.06 μg/L per kg/m², $P = .07$). Serum leptin correlated with fasting insulin in the puerperal preeclamptic group ($r = .63$, $P = .02$) and the control group ($r = .81$, $P = .003$), and also with insulin sensitivity in the puerperal preeclamptic group ($r = -.59$, $P = .02$) but not in the control group (Table 1 and Fig 3). Serum leptin also correlated with the puerperal BMI in the preeclamptic women ($r = .74$, $P = .002$) and the control group ($r = .81$, $P = .005$).

DISCUSSION

During pregnancy, in addition to leptin production, adipocyte leptin is also produced by the trophoblasts.[13,14] These data suggest a role for leptin in the physiology of pregnancy. In view

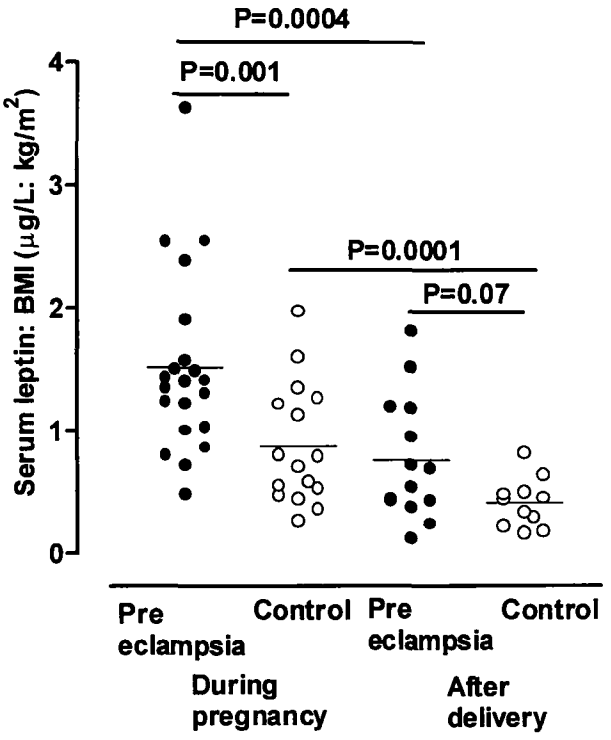

Fig 1. Ratio for the serum leptin level to the current BMI in 22 preeclamptic women (●) and 16 controls (○) during pregnancy and at reexamination 3 months after delivery (14 women with prior preeclampsia, ●); 11 women with prior normotensive pregnancy, ○). Means are indicated by horizontal lines.

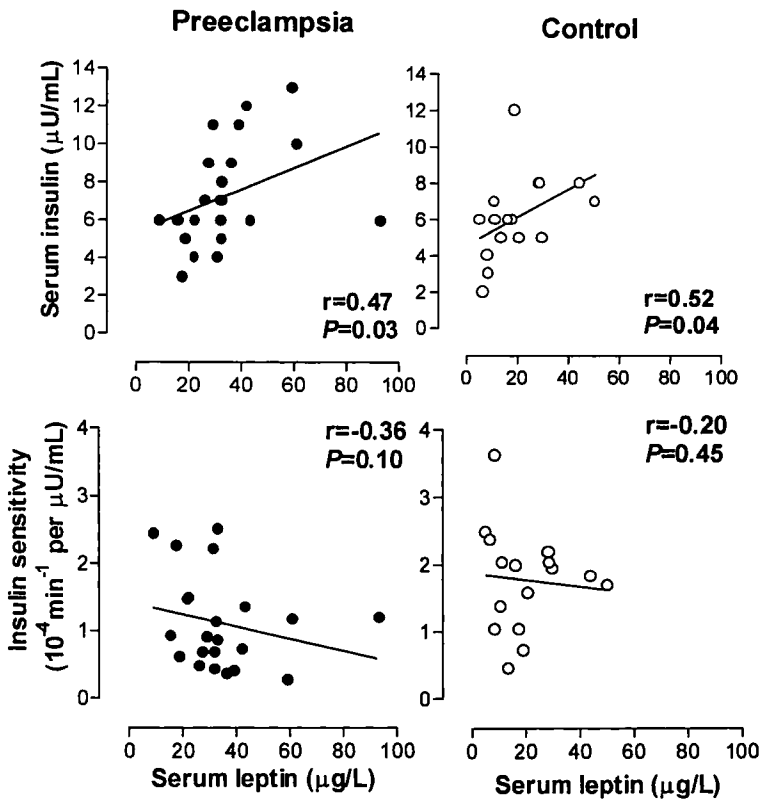

Fig 2. Serum leptin in relation to serum insulin and insulin sensitivity during the third trimester in 22 preeclamptic women (●) and 16 controls (○).

of the previous controversial data on leptin in preeclampsia,[11,14] we compared serum leptin levels in nulliparous women exhibiting well-characterized acute proteinuric preeclampsia and in matched controls who remained entirely normotensive and nonproteinuric throughout pregnancy. Because this study population constitutes part of a larger study on glucose homeostasis in preeclampsia,[20,22] we were able to study in this context whether leptin levels during and after preeclamptic or normal pregnancy are related to insulin sensitivity. Numerous previous data suggest such a relation in men and nonpregnant women[8,9] or in various animal models.[23,24]

Preeclamptic women had higher leptin levels than the control women. Mise et al[14] found elevated leptin levels in 18 women with proteinuric, severe preeclampsia, but not in all women with preeclampsia. Sattar et al[11] reported similar leptin levels in 9 women with proteinuric preeclampsia and 12 controls. The reasons for these discrepant results are unknown; the study populations in different countries or the diagnostic criteria for preeclampsia may have been insufficiently uniform. The prepregnancy BMI and the BMI at study were similar in our preeclamptic and control groups, suggesting that the amount of fat or its distribution showed no major difference between the study groups, although we were unable to assess total body fat or fat distribution in detail. Thus, these factors could hardly explain the higher leptin in preeclampsia. Leptin is eliminated mainly through the kidneys,[25,26] and preeclampsia can be accompanied at least by histological renal changes.[27] The correlation between serum leptin and proteinuria in our patients suggests an association, either direct or indirect, between the elevation in serum leptin and renal changes in preeclampsia, but it should be emphasized that renal function in our patients was normal. It is also possible that elevated leptin levels in preeclampsia are due to a release from placental sources,[13,14] perhaps as a reaction to placental hypoxemia.[14] However, leptin levels were not related to the birthweight, placental weight, or Apgar scores in our study. Hypertension has been associated with leptin elevations in nonpregnant subjects,[28,29] but such a relation was not found in our hypertensive pregnant patients.

Preeclampsia appears to be a state of increased insulin resistance.[15,16,18,19] The main site of insulin resistance is muscle tissue. However, adipocytes are also affected, as free fatty acid levels are elevated in preeclampsia, indicating a reduced antilipolytic effect of insulin.[30] Leptin was related to basal insulin in both preeclampsia and normotensive pregnancy, and these data are in keeping with the proposed role of insulin in the regulation of placental leptin production.[31] However, insulin sensitivity was not associated with serum leptin levels in pregnancy, but such an association emerged during the puerperal period, albeit only in women with prior preeclampsia. Thus, it is possible that during pregnancy, placental leptin production may have masked an association between whole-body insulin sensitivity and the serum leptin concentration.

Leptin levels decrease rapidly during the first days after delivery in normal pregnancies,[12] and are apparently normal in the later puerperium in lactating and nonlactating women with a normal pregnancy.[32-34] Because there were no data for leptin levels after preeclamptic pregnancy, we reexamined our patients 3 months after delivery and observed that leptin levels had decreased during the 3 months after delivery also in women with prior preeclampsia. Yet, these women had 2-fold higher

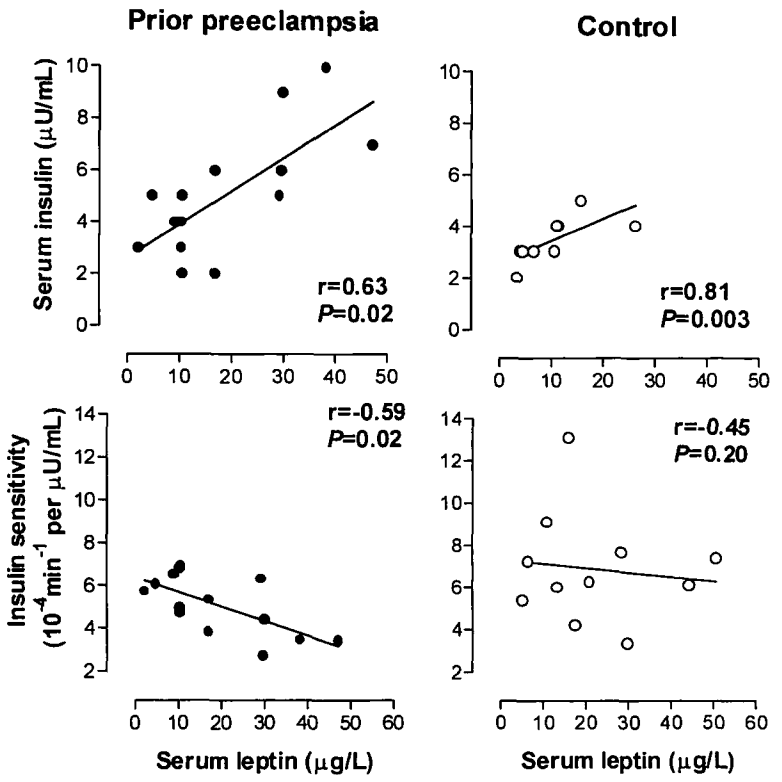

Fig 3. Serum leptin in relation to serum insulin and insulin sensitivity 3 months after delivery in 14 women with prior preeclampsia (●) and 11 women with prior normotensive pregnancy (○).

leptin levels than the controls, although this difference did not reach statistical significance. Moreover, serum leptin was significantly associated with insulin sensitivity in puerperal women who had preeclampsia. These data are in agreement with the proposed role of glucosamine, a factor associated with insulin resistance, as a nutrient-sensing signal to stimulate leptin production.[35] Hyperinsulinemia[36] and hyperandrogenism[37] and frank insulin resistance[20] are characteristic of women with a history of preeclampsia. Our data suggest that hyperleptinemia can be listed among these metabolic and endocrine alterations that are related to the insulin resistance syndrome in women with prior preeclampsia.

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