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# Leptin, Soluble Leptin Receptor and Leptin Gene Polymorphism in Relation to Preeclampsia Risk

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## Summary

Few investigators have simultaneously evaluated leptin, soluble leptin receptor (SLR) and leptin gene polymorphisms in preeclampsia cases and controls. We examined these three biomolecular markers in 40 preeclampsia cases and 39 controls. Plasma leptin and SLR concentrations were determined using immunoassays. Genotype for the tetranucleotide repeat $(TTTC)_n$, polymorphism in the 3′-flanking region of the leptin gene was determined using PCR. Alleles of the polymorphism were characterized by size distributions [short repeats (class I); and long repeats (class II)]. Logistic regression was used to calculate odds ratios (OR) and 95 % confidence intervals (CI). Leptin concentrations were higher in our cases than in the controls (53.1±4.7 vs. 17.7±2.4 ng/ml, $p<0.05$). SLR concentrations were slightly lower in our patients than in the controls (25.7±1.9 vs. 29.1±1.1 ng/ml, $p>0.05$). Elevated leptin (≥ 14.5 ng/ml) was associated with a 3.8-fold (CI 1.0-14.4) increased risk; whereas low SLR (< 28.5 ng/ml) was associated with a 6.3-fold (CI 1.7-23.2) increased risk of preeclampsia. The I/II genotype was associated with a 3.8-fold increased risk of preeclampsia (OR=3.8; 95 % CI 0.8-18.0); and the II/II genotype was not observed among our cases (0 % vs. 33 % $p<0.001$). Larger studies would be needed to confirm and further clarify the relations between functional variants in the leptin gene and preeclampsia risk.

**Key words**

Leptin • Leptin receptor • Leptin gene-polymorphism • Obesity • Pregnancy • Preeclampsia

## Introduction

Leptin, an adipocyte-derived peptide that binds to receptors to initiate a cascade of biological processes involved in regulating food intake and energy expenditure, has been implicated in the pathogenesis of preeclampsia (Williams *et al.* 1999a, Poston 2002). Preeclampsia, a hypertensive disorder of pregnancy, is known to be associated with placental hypoxemia secondary to shallow endovascular cytotrophoblast invasion in spiral arteries (Redman 1997), insulin resistance and hyperinsulinemia (Kaaja *et al.* 1995), chronic systemic inflammation (Williams *et al.* 1999b), maternal pregestational adiposity (Mahomed *et al.* 1998) and sympathetic nervous system over-reactivity (Schobel *et al.* 1996).



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Leptin circulates in free form or bound to its receptors (Houseknecht *et al.* 1996, Sinha *et al.* 1996, Diamond *et al.* 1997). Hormonal factors, age as well as the concentrations of soluble leptin receptors are known to influence total and free leptin concentrations in humans (Friedman and Halaas 1998, Mantzoros 1999, Mann *et al.* 2003). Multiple leptin receptor isoforms have been identified in human placentas and other tissues (Henson *et al.* 1998). Additionally, leptin is known to be positively correlated with indices of adiposity such as body fat mass and body mass index (BMI) while concentrations of soluble leptin receptors (SLR) are known to be inversely correlated with such indices (Yannakoulia *et al.* 2003).

Emerging evidence suggests that the leptin gene may be a candidate gene for hypertension through its direct effect on blood pressure, through its effects on adipose tissue metabolism, or indirectly through its effect on obesity. Investigators recently reported that carriers of the shorter class I allele of the highly variable tetranucleotide repeat $(TTTC)_n$, polymorphism in the 3′-flanking region of the gene are more likely to be hypertensive than non-carriers of the allele (Moffett *et al.* 2002, Shintani *et al.* 2002). Few investigators have simultaneously evaluated leptin, SLR and leptin gene polymorphisms in preeclampsia cases and controls. We, therefore, examined these three biomolecular markers in 40 preeclampsia cases and 39 controls.

## Methods

Subjects for this analysis were recruited between April 1998 and January 2000 as part of a case control study designed primarily to study the epidemiology of preeclampsia. Details regarding data collection methods have been previously described (Sorensen *et al.* 2003). During the study period, women with preeclampsia and normotensive women were recruited from Swedish Medical Center, Seattle, Washington, USA, and Tacoma General Hospital, Tacoma, Washington, USA. Institutional Review Committees at both institutions reviewed and approved the research described herein. All participants provided written informed consent.

From the original studied population, we randomly selected 40 cases with detailed evaluation in the present study. Preeclampsia was defined as persistent (6 or more hours) blood pressure of at least 140/90 mm Hg arising after 20 weeks gestation with concomitant proteinuria. Proteinuria was defined as urine protein concentration ≥ 30 mg/dl or more (or 1+ on a urine dipstick) in at least two random specimens collected at least 4 hours apart. We also randomly selected 40 women with pregnancies uncomplicated by gestational hypertension and proteinuria to serve as controls. Women with pregestational diabetes and women with a medical diagnosis of chronic hypertension were not eligible for this study. All subjects were non-Hispanic Caucasians.

From a structured questionnaire and medical records, we obtained covariate information including maternal age, height, pre-pregnancy weight, last measured weight before delivery, reproductive and medical histories, and medical histories of first-degree family members. Infant weight was also collected from medical records. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. BMI were computed for each woman before their pregnancy, and BMI at delivery.

Maternal non-fasting blood samples, collected in 10 ml Vacutainer tubes during their labor and delivery stay, were frozen at –80 °C until analysis. Maternal plasma leptin and SLR concentrations were measured using enzyme immunoassays (Diagnostic Systems Laboratory, Inc., Webster, Texas, USA). The intra- and inter-assay coefficients of variation for both assays were <8 %. The precise molecular weights of leptin and soluble leptin receptors are unknown. Hence, we are unable to report concentrations in SI units. The free leptin index was defined as the ratio of plasma leptin and SLR concentrations (Yannakoulia *et al.* 2003).

Maternal genotype for the tetranucleotide repeat $(TTTC)_n$, polymorphism in the 3′-flanking region of the leptin gene was determined using polymerase chain reaction (PCR) procedures as decribed previously (Shintani *et al.* 1996, 2002). We used the (5'-6-FAM-AGT TCA AAT AGA GGT CCA AAT CA-3') forward primer sequence, and the (5'-TTC TGA GGT TGT GTC ACT GGC A-3') reverse primer sequence, both from the human leptin gene, in PCR reactions containing 100 ng of genomic DNA template, 0.6 μM of each primer, 25 μl PCR Master Mix 2X (Applied Biosystems, Foster City, CA, USA) and deionized sterile water in a final 50 μl volume. The PCR was performed for 32 cycles of 15 s at 94 °C, 15 s at 55 °C and 20 s at 72 °C with an initial denaturation of 5 min at 95 °C and a final extension of 10 min at 72 °C. PCR products were diluted in DI formamide, run on a 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and analyzed according to the manufacturer's specifications. The

alleles comprised two groups based on different size distributions of the tetranucleotide $(TTTC)_n$ repeats where shorter repeats (< 160 base-pairs) were specified as class I alleles, and longer repeats (≥ 160 base-pairs) were specified as class II alleles (Moffett *et al.* 2002, Shintani *et al.* 2002). All assays were performed without preceding knowledge of case-control status. One control subject with insufficient maternal plasma and genomic DNA was deleted, thus leaving 40 cases and 39 controls available for the present study.

We examined the frequency distributions of maternal sociodemographic characteristics and medical and reproductive histories according to case-control status. Spearman's correlation coefficients were used to estimate the associations between continuous variables. We used logistic regression procedures to estimate odds ratios (OR) and 95 % confidence intervals (CI) (Rothman and Greenland 1998). Final models included maternal age, parity, and covariates that altered unadjusted ORs by 10 %. All analyses were performed using STATA 7.0 statistical software. All continuous variables were presented as mean ± S.E.M. and the reported p-values are two-tailed.

**Table 1**. Sociodemographic and other characteristics of preeclampsia cases and normotensive control subjects (Seattle and Tacoma, Washington, 1996-2000)

| | **Cases (n=40)** | **Controls (n=39)** |
|---|---|---|
| *Maternal age (years)* | 32.5±0.9 | 32.7±0.7 |
| *Married* | n=35 87.5 % | n=35 87.7 % |
| *Nulliparous* | n=32 80.0 % | n=23 59.0 %* |
| *Smoked during pregnancy* | n=6 15.0 % | n=1 2.5 %* |
| *Family history of hypertension* | n=25 62.5 % | n=14 35.9 %* |
| *Pre-pregnancy BMI ($kg/m^2$)* | 26.3±1.0 | 22.3±0.6* |
| *BMI at delivery ($kg/m^2$)* | 38.8±4.9 | 27.8±0.7* |
| *Pregnancy weight gain (kg)* | 16.2±1.2 | 16.5±0.7 |
| *Leptin (ng/ml)* | 53.1±4.7 | 17.7±2.4* |
| *Soluble leptin receptor (SLR) (ng/ml)* | 25.7±1.9 | 29.1±1.1 |
| *Free leptin index*[§] | 2.5 ± 0.3 | 0.7 ± 0.1* |

Data are means ± S.E.M. * p <0.05, [§] Ratio of plasma leptin (ng/ml) and SLR (ng/ml)

## Results

Characteristics of preeclampsia cases and controls are summarized in Table 1. Maternal plasma leptin concentrations were significantly higher among cases than in the controls (53.1±4.7 vs. 17.7±2.4 ng/ml, p<0.05). Maternal plasma SLR concentrations were lower in cases as compared with the controls, although this difference did not reach statistical significance (25.7±1.9 vs. 29.1±1.1 ng/ml, p>0.05). Free leptin index values were significantly higher between our cases as compared with the controls (2.5±0.3 vs. 0.7±0.1, p<0.05). Figure 1 summarizes the correlation coefficients for leptin, SLR and the free leptin index, respectively, with maternal pre-pregnancy BMI, BMI at delivery, pregnancy weight gain and infant birth weight.

We next evaluated the risk of preeclampsia according to whether mothers had elevated leptin (≥ 14.5 ng/ml), reduced SLR (< 28.5 ng/ml) or elevated free leptin index (≥ 0.48). Cut-points for each analyte were determined on the basis of median values observed among control subjects. Relative to women with lower maternal leptin concentrations (< 14.50 ng/ml), women with elevated concentrations (≥ 14.50 ng/ml) had a 5.4-fold increased risk of preeclampsia (OR=5.4; 95 % CI 1.8-15.7) (Table 2). The association remained significant, though it was slightly attenuated after the adjustment for confounders (adjusted OR=3.8; 95 % CI 1.0-14.4). Low maternal SLR concentrations were associated with a 6.3-fold increased risk of preeclampsia (adjusted OR=6.3; 95 % CI 1.7-23.2). Finally, women with elevated values of the free leptin index (i.e. values ≥ 0.48) had an almost 5-fold increased risk of preeclampsia (adjusted OR=4.9; 95 % CI 1.2-19.8) as compared with those with lower values.

**Table 2.** Odds ratios (OR) and 95 % confidence interval (95 % CI) of preeclampsia risk in relation to maternal plasma concentrations of leptin, soluble leptin receptor (SLR), and free leptin index (Seattle and Tacoma, Washington, 1996-2000)

| | Cases n | Controls n | Crude OR (95 % CI) | Adjusted OR* (95 % CI) |
|---|---|---|---|---|
| *Plasma leptin (ng/ml)* | | | | |
| < 14.5 | 6 | 19 | 1.0 reference | 1.0 reference |
| ≥ 14.5 | 34 | 20 | 5.4 (1.8-15.7) | 3.8 (1.0-14.4) |
| *Plasma SLR (ng/ml)* | | | | |
| < 28.5 | 31 | 19 | 3.6 (1.4-9.6) | 6.3 (1.7-23.2) |
| ≥ 28.5 | 9 | 20 | 1.0 reference | 1.0 reference |
| *Free leptin index (Leptin/SLR ratio)* | | | | |
| < 0.48 | 4 | 19 | 1.0 reference | 1.0 reference |
| ≥ 0.48 | 16 | 20 | 8.6 (2.6-28.6) | 4.9 (1.2-19.8) |

*Adjusted for maternal age, pre-pregnancy BMI, parity and physical activity, first degree family history of hypertension.

**Table 3.** Adjusted and unadjusted odds ratios (OR) and 95% confidence interval (95 % CI) of preeclampsia risk in relation to maternal leptin genotype (Seattle and Tacoma, Washington, 1996-2000).

| Genotype | Cases n | Controls n | Crude OR (95 % CI) | Adjusted OR (95 % CI) |
|---|---|---|---|---|
| *Genotype* | | | | |
| I/I | 9 | 9 | 1.0 reference | 1.0 reference |
| I/II | 31 | 17 | 1.8 (0.6-5.5) | 3.8 (0.8-18.0) |
| II/II | 0 | 13 | --- | --- |

*Adjusted for maternal age, pre-pregnancy BMI, parity and first degree family history of hypertension.

Finally, we evaluated the preeclampsia risk in relation to maternal leptin genotype. The class I allele frequency was significantly higher among cases than the controls (p=0.039). Among cases, the class I allele frequency was 61 % (49/80) and the class II allele frequency as 39 % (31/80). The corresponding allele frequencies among controls were 45 % (for class I allele [35/78]) and 55 % (for class II allele [43/78]). As shown in Table 3, women with the I/II genotype, as compared with those with the I/I genotype, had an increased risk of preeclampsia (adjusted OR=3.8; 95 % CI 0.8-18.0). No preeclampsia cases had the II/II genotype, whilst one third of the controls had this genotype (p<0.001).

## Discussion

In this study, elevated maternal leptin concentrations were associated with a 3.8-fold increased risk of preeclampsia. Low maternal plasma SLR concentrations were associated with a 6.3-fold higher risk. When the two analytes were combined to estimate maternal free leptin status, those with higher values for free leptin index (≥ 0.48 above the control population median value) had a 4.9-fold increased risk of preeclampsia. Lastly, we noted that the I/II genotype of tetranucleotide repeat $(TTTC)_n$ polymorphism in the 3′-flanking region of the leptin gene was higher in our cases than in the controls.

Our findings concerning the relation between elevated maternal plasma leptin and preeclampsia risk are consistent with most previous reports (Williams *et al.* 1999a, Laivuori *et al.* 2000, Chappell *et al.* 2002, Gursoy *et al.* 2002, Ning *et al.* 2004). Our findings regarding the relation between maternal SLR concentrations and

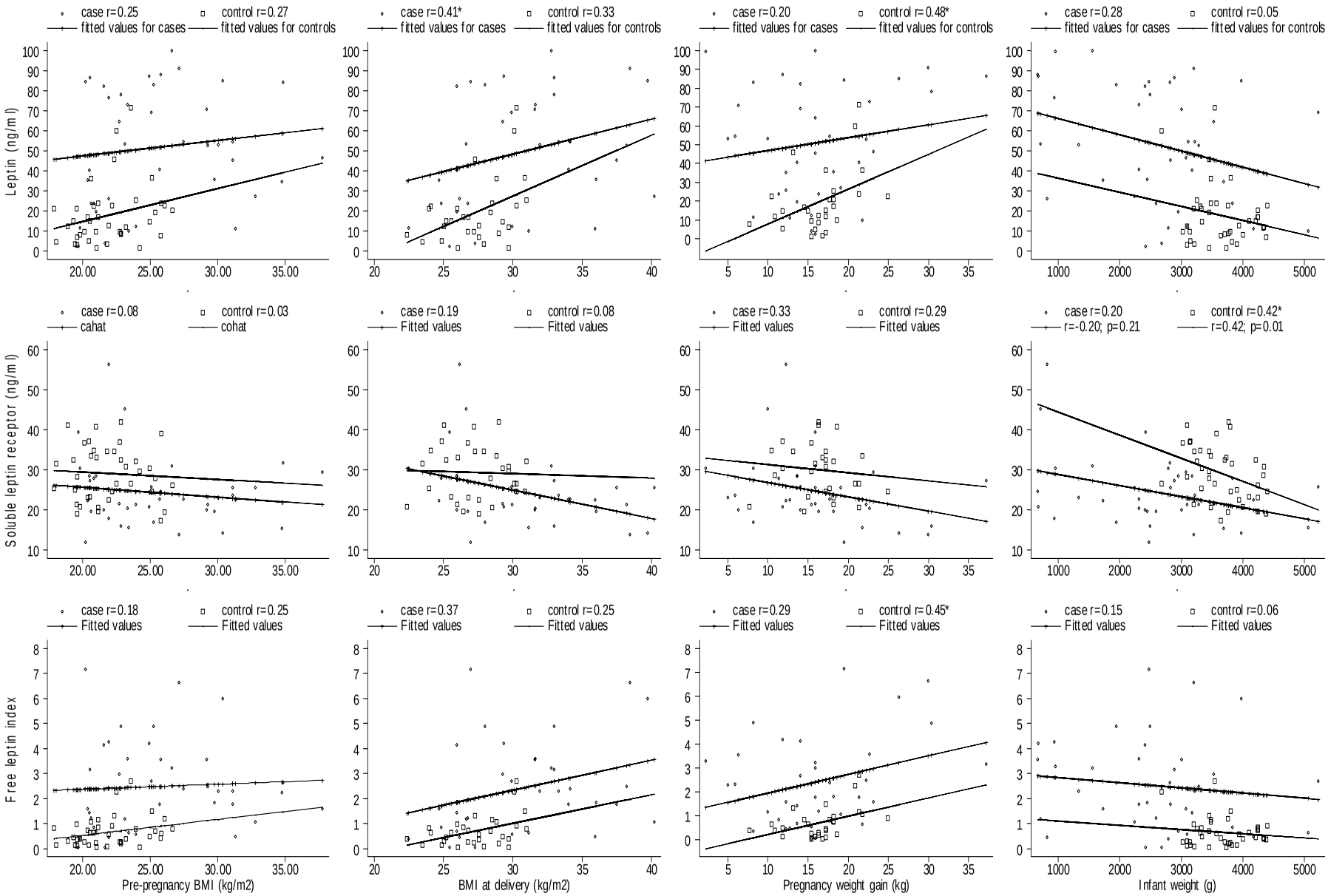


**Fig. 1.** The relationships of leptin (upper row), soluble leptin receptor (middle row) and free leptin index (lower row) with maternal and infant body mass variables pre-pregnancy body mass index among cases (dots) and controls (open squares) with regression lines.

preeclampsia risk are also compatible with reports from Challier *et al.* (2003), the only published paper we were able to locate on the topic. We are unaware of other published studies that have evaluated preeclampsia risk in relation to the maternal genotype for the tetranucleotide repeat $(TTTC)_n$ polymorphism in the 3′-flanking region of the leptin gene (Moffett *et al*. 2002). However, our results are nearly consistent with some, though not all previous studies that have evaluated these genetic variants in non-pregnant hypertensive and normotensive subjects (Onions *et al.* 1998, Shintani *et al.* 2002). In a study of Japanese subjects, Shintani *et al.* (2002) reported that the I/I genotype was associated with an increased frequency of essential hypertension. In the study of Hispanic families, Cheng *et al.* (2001) reported linkage between chromosome 7 (which contains the leptin gene) with lipid homeostasis and blood pressure. However, no such association was supported by results from a study of hypertensive and normotensive African-Americans (Onions *et al.* 1998). Differences in study design, distributions of severe hypertension, as well as ethnic and racial differences across study populations may account for the absence of consistency in these studies.

In our study, the I/II genotype was associated with almost a 4-fold increased risk of preeclampsia; and 33.3 % of controls had the II/II genotype while none of the 40 cases were found to have the genotype II/II. However, chance cannot be excluded as one possible interpretation of this finding. The association of preeclampsia and leptin genotype should be confirmed in greater studies.

Several important limitations must be considered when interpreting the results from our study. First, it is important to note that maternal blood samples were collected in late pregnancy (after diagnosis and during hospitalization for labor and delivery). Our findings are thus subject to potential variations of analyte concentrations during this period. The concordance of our results with many other studies (Williams *et al.* 1999a, Laivuori *et al.* 2000, Chappell *et al.* 2002, Gursoy *et al.* 2002, Ning *et al.* 2003, Challier *et al.* 2003) suggests that observed dysregulation of leptin and SLR concentrations in preeclampsia may be robust and observable despite variations in study design and populations. Nevertheless, longitudinal studies, with serial measurements of maternal leptin and SLR concentrations, are needed to further elucidate temporal changes in the bioavailability of leptin during normal and pathological pregnancies. Second, BMI during pregnancy is an indirect and relatively imprecise measure of maternal adiposity or fat mass. Studies that employ more precise means of determining maternal pre-pregnancy adiposity and changes in fat mass during pregnancy will overcome this important limitation. Lastly, inferences from some of our analyses, particularly the genetic analyses, were hindered by our relatively small number of preeclampsia cases and control subjects. Inferences concerning the relation between leptin gene polymorphism and preeclampsia risk will be extended in a future study.

Although the precise mechanisms for the observed associations remain unknown, several investigators have proposed biologically plausible hypotheses (Williams *et al.* 1999a, Poston 2002, Ning *et al.* 2003). For instance, some investigators have suggested that hyperleptinemia resulting from insulin resistance (Laivuori *et al.* 2000), tissue hypoxemia (Grosfeld *et al.* 2002) or chronic systemic inflammation (Grunfeld *et al.* 1996) may contribute to the etiopathogenesis of preeclampsia. Regardless of the mechanisms, available evidence suggests that leptin and SLR are potentially important biological markers of preeclampsia risk. Future studies that aim to identify modifiable factors that influence the bioavailability of leptin in maternal circulation may yield new strategies for preeclampsia prevention.

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