Increased plasma levels of adipokines in preeclampsia: relationship to placenta and adipose tissue gene expression


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PREECLAMPSIA IS CHARACTERIZED BY HYPERTENSION, increased vascular resistance, proteinuria, edema, and coagulopathy and has been linked to maternal systemic endothelial cell dysfunction (43). Delivery of the placenta results in clinical resolution, and placenta is viewed as the essential organ in the development of preeclampsia (42). Increased risk of preeclampsia has been reported with increased obesity (37). Development of insulin resistance in the third trimester of pregnancy (21), together with adipose tissue accumulation (11), is a possible adaptation of the maternal metabolism to optimize fetal nutrition. Insulin resistance has a potential role in pregnancy-induced hypertension, and extensive insulin resistance is often observed during preeclampsia (45). Furthermore, placenta secretes a variety of hormones that may play a role in both gestational insulin resistance and preeclampsia.

Adipose tissue has an endocrine function, secreting several metabolically active proteins, such as leptin, resistin, adiponectin, TNF-α, and IL-6, termed adipokines (50). During pregnancy, the placenta is an additional source of adipokines like leptin and resistin (17, 58). The anti-diabetic and anti-atherogenic properties of adiponectin are well documented (6), and in contrast to other adipokines, plasma adiponectin levels are decreased in obesity, insulin-resistant diabetes, and coronary vascular disease (2, 18, 19, 25, 54, 60). Resistin circulates at high concentrations in diet-induced and genetic forms of obesity in mice, modulating insulin action on hepatic glucose production (4, 35, 38, 44, 49). From several human studies it can be concluded that circulating resistin levels are proportional to the degree of adiposity (3, 10, 52, 57), but the role of resistin in type 2 diabetes is unclear. Leptin is an important secretory protein regulating satiety and fatty acid oxidation (15), and it is positively related to body fat mass (27). Leptin may play an important role during pregnancy (17).

The aim of our present study was to compare plasma concentrations of the adipokines adiponectin, resistin, and leptin in preeclamptic (PE) and healthy pregnant (HP) women undergoing cesarean delivery and relate these data to the mRNA expression levels in adipose tissue and placenta. We concluded that circulating levels of adipokines were elevated in preeclampsia, whereas the mRNA levels in adipose tissue were similar. In addition, placental levels of resistin mRNA were not significantly different between the study groups, whereas elevated levels of leptin mRNA in placenta were associated with preeclampsia.

MATERIALS AND METHODS

Patient selection. The pregnant women included in this study were previously healthy, with uncomplicated pregnancies. In particular, no women with chronic hypertension, renal disease, or diabetes were included. All pregnancies were singletons. Women in both the PE (n = 15) and HP (n = 23) control groups delivered by caesarean section at Ulleval University Hospital in Oslo, Norway, and none of the women were in active labor at the time of caesarean delivery. Caesarean delivery was performed in PE because vaginal delivery was not considered appropriate to disease progression and/or unfavorable cervical ripening. HP women were normotensive, undergoing cesarean delivery due to breech presentation or psychological reasons.

Preeclampsia was defined as the rise in blood pressure after 20 wk of gestation to >140/90 mmHg on at least two occasions 6 h apart in
a previously normotensive woman, combined with proteinuria. Krotkoff phase I (first beat heard) and phase V (disappearance of sound) were used to determine systolic (SBP) and diastolic blood pressure (DBP), respectively. Proteinuria was defined as a protein dipstick reading of at least two midstream urine samples 6 h apart, in the absence of urinary tract infection. Informed written consent was obtained from all participants. The Regional Committee of Medical Ethics in Norway approved the study protocol.

**Clinical data.** Blood was sampled from the antecubital vein (in an arm without intravenous infusion ongoing) after ≥6 h of fasting just before the application of spinal anesthesia in EDTA-containing vials or in serum glasses. The EDTA-blood was kept on ice for ≤30 min until centrifugation at 2,000 g for 10 min at 4°C. Blood for serum samples was left at room temperature for 30–60 min until centrifugation at 2,000 g for 10 min at room temperature. Plasma and serum samples were stored at −80°C until analyses. Pregnancy duration was based on routine ultrasonographic screening between gestational weeks 17 and 20. Information regarding parity, gravidity, maternal height, pre-pregnancy weight and weight at delivery, and infant birth weight was gathered from the medical records and by interviewing the patients.

Subcutaneous adipose tissue adjacent to the lower abdominal incision and placenta biopsies were obtained from the participants during the caesarean delivery. The biopsies were immediately frozen in liquid nitrogen, stored at −80°C, and homogenized into powder with a pestle and mortar in liquid nitrogen before analyses.

**Blood analyses.** Plasma adiponectin level was determined using the Human Adiponectin RIA Kit [coefficient of variation (CV) ≤6.21%, interassay CV ≤9.25%; Linco Research, St. Charles, MO], and leptin was assayed by Sensitive RIA Human Leptin Kit (intra-assay CV ≤6.21%, interassay CV ≤8.9%; BioVendor, Brno, Czech Republic), IL-6 (intra-assay CV ≤11.1%, interassay CV ≤16.5%; R&D Systems, Oxon, UK), and TNF-α (intra-assay CV ≤8.8%; interassay CV ≤16.7%; R&D Systems) concentrations in plasma were measured by ELISA. Serum concentrations of C-peptide and insulin were determined by Immulite 2000 C-peptide kit (DPC-Bierman, Bad Naunau, Germany) and Human Insulin Specific RIA kit (intra-assay CV ≤4.4%, inter-assay CV ≤6.0%; Linco Research), respectively. The homeostasis model assessment (HOMA) model (31) was employed to estimate β-cell function (HOMA-β) and insulin resistance (HOMA-IR). Estimates were obtained with C-peptide and specific insulin concentrations, respectively, using the HOMA2 Calculator version 2.2, downloaded from www.dtu.ox.ac.uk (Diabetes Trials Unit, Churchill Hospital, Oxford, UK). Serum concentration of total cholesterol, HDL cholesterol, and triglycerides were determined by routine enzymatic methods on a Cobas Integra instrument (Roche, Basel, Switzerland). The atherogenic index was calculated as [(total cholesterol – HDL cholesterol)/HDL cholesterol]. LDL cholesterol concentration was calculated as [total cholesterol – [HDL cholesterol] – 0.45[triglycerides]). Colorimetric/enzymatic assays were used to measure plasma concentrations of glucose (Bayer, Tarrytown, NY) and free fatty acid (Wako Chemicals, Neuss, Germany). Due to limited samples, TNF-α concentration was not measured in four subjects, one in the PE and three in the HP groups. For the same reason, IL-6 concentration was not determined for two HP subjects.

**Tissue analyses.** Adipose tissue mRNA was isolated with GenoPrep mRNA beads (GenoVision, Oslo, Norway) from abdominal subcutaneous adipose tissue (200 mg) samples, as described by the manufacturer, and quantified by spectrophotometry. The adipose tissue biopsies from 12 PE patients and 12 HP subjects yielded RNA in sufficient amounts and quality and subsequently underwent Northern analysis. From placental tissue (~100 mg), total RNA was first isolated with Tri Reagent (Brunschwig, Amsterdam, The Netherlands), as described by the manufacturer, and quantified by spectrophotometry. Its integrity was evaluated on agarose gel, and from 30 μg of placenta total RNA, mRNA was isolated with GenoPrep. Placental biopsies yielding RNA in sufficient amounts and quality underwent Northern analysis (12 PE patients, 19 HP women). For Northern analysis, mRNA was eluted in 0.05 M 3-(N-morpholino)propanesulfonic acid (pH 7.0) containing 1 mM EDTA, 5.6% formaldehyde, and 40% formamide and loading buffer (2 mM Na-phosphate buffer, 1% Ficoll-400, and 0.025% bromophenol blue). RNA was separated on 1% agarose gels containing 6.7% formaldehyde and blotted onto Hybond N nylon membranes (Amersham, Little Chalfont, UK). The blots were hybridized, as previously described (41), with cDNA probes for the following genes: adiponectin (26), leptin, glyceroldehyde-3-phosphate dehydrogenase (GAPDH) (41), and human ribosomal protein L27 [RPL27; American Type Culture Collection (ATCC)]. The cDNA probes were labeled with [32P]dCTP using a Megaprime DNA labeling kit (Amersham). The blots were analyzed by a PhosphorImager (Molecular Dynamics, Sunnyvale, CA), and the signal intensities were measured. Bands of 4.5 and 3.4 kb were visualized during probing with adiponectin and leptin cDNA, respectively. For each patient, relative levels of target mRNA in tissues were calculated by dividing its signal measurement with the signal measurement of a housekeeping gene (RPL27 in adipose tissue and GAPDH in placenta) to correct for differences in gel loading. Human resistin mRNA (RETN) was measured with the LightCycler instrument (Roche Diagnostics, Mannheim, Germany). Using the Platinum Quantitative RT-PCR Thermoscript One-Step System (Life Technologies), a 137-bp fragment was amplified from the 238- to 374-bp region of the RETN (GI:13435379) with the forward (5'–CCGAGGCTTTGCGGCTAC-3') and reverse (5'–CTCAGGGGCTGACACGAGAC-3') primers. PCR products were detected with a labeled TaqMan probe specifically for the amplified fragment of the RETN: 5'-FAM-TCGTGGGATGTGCGCGGAGA-3' TAMRA (DNA Technology, Aarhus, Denmark). For use as a positive control DNA template in the RT-PCR reactions, a full-length cDNA copy (1–470 bp) of the RETN (GI: 13435379) was cloned with a TA-cloning kit (Invitrogen, Carlsbad, CA) from U-937 (CRL-1593.2, ATCC) mRNA and control sequenced. Prior to RT-PCR, the samples of mRNA were treated with amplification grade DNase I (Invitrogen). Duplicate RT-PCR reactions were performed in narrow glass capillaries with a reaction volume of 25 μl containing 0.1 μg of mRNA, 0.5 μM of each primer, and 0.3 μM probe. The RT step at 55°C for 30 min was followed by a denaturation step of 95°C for 5 min, followed by a three-step PCR (95°C for 15 s, 64°C for 60 s, and 72°C for 13 s). Sizes of PCR products were confirmed on 3% agarose gels after runs. Control reactions were set up to control for amplification from DNA contamination, and resistin amplification was not detected without Thermoscript RT present. As housekeeping genes, human GAPDH and β-actin (ACTB) were amplified in duplicate with a Pre-Developed TaqMan Assay Reagents Control kit according to the instructions (Applied Biosystems, Langden, Germany). Relative resistin and housekeeping mRNA levels were determined by relating to a standard curve (serial dilution of mRNA), using the second derivative maximum method in the LightCycler Software version 3.5. Using the housekeeping gene as an internal control, the relative amount of tissue resistin mRNA was calculated for each sample as (relative RETN mRNA)/(relative ACTB mRNA) in adipose tissue and (relative RETN mRNA)/(relative ACTB mRNA) in placenta. Detectable resistin mRNA signals were quantified in PE patients (adipose tissue, n = 13; placenta, n = 13) and HP women (adipose tissue, n = 19; placenta, n = 21).

**Statistical analyses.** The StatView 4.5 software for Macintosh was used for most analyses, and SPSS 13 for Windows was used for binary logistical regression. Data from the two groups of patients are presented as means ± SE and were log10 transformed if necessary to obtain normal distribution, evaluated by the use of F-tests (P > 0.05) and Z-score histograms. Differences between group means were tested by an unpaired Student’s t-test, and the independence of variables was explored using binary logistical regression tested by Wald statistic.
RESULTS

Clinical data were collected from the PE and HP groups to characterize the study groups (Table 1). Mean SBP and DBP were increased in PE, as was expected from the inclusion criteria. PE women had shorter mean pregnancy duration and lower infant weight in absolute measurements and relative to gestation length (weight percentile), whereas the two groups had similar mean age, parity, gravidity, and BMI (both prepregnancy and at delivery).

Adipokine levels in plasma. Adipokines may affect metabolism during pregnancy, and we examined whether plasma concentrations of the adipokines adiponectin and resistin were altered in preeclampsia. Samples from fasting patients showed that the PE group had higher mean plasma levels of adiponectin (50%) and resistin (22%) than the HP control group (Table 2). Elevated plasma levels of leptin in the third trimester have been associated with preeclampsia, and in the present study concentrations were measured to characterize the study groups. As expected, the PE group had increased mean plasma levels of leptin (52%) compared with HP (Table 2). In addition, we measured plasma levels of proinflammatory adipokines, and the PE group had higher mean levels of IL-6 (126%) and TNF-α (54%) compared with controls (Table 2). Variables of glucose metabolism were measured to reveal their associations with preeclampsia and adipokine levels in plasma. We found no significant differences in the mean fasting levels of insulin, C-peptide, and glucose between the PE and HP groups (Table 2), although mean levels of insulin (55%) and C-peptide (35%) were higher in the PE compared with the HP group. In the HOMA-β, normal insulin secretion is set to 100%. Compared with this, insulin secretion was increased in both PE and HP (Table 2). However, β-cell insulin secretion did not differ significantly between the groups. In addition, the HOMA model was used to estimate insulin resistance (HOMA-IR). The mean HOMA-IR in both PE and HP was elevated compared with an index of 1.0, which is considered normal in this model (Table 2). Although mean HOMA-IR in PE was 56% higher than in controls, it did not reach statistical significance (P = 0.353). Alterations in plasma lipids may be associated with preeclampsia, but we found no significant differences between lipid variables in PE and HP (Table 2).

Because adipose tissue mass and insulin resistance may influence plasma adipokine levels, possible confounding was explored. Binary logistic regression was used to evaluate whether adipokine levels in plasma are independent variables in relation to the diagnosis (PE or HP as dependent variables). Before and after controlling for BMI (both prepregnancy and at delivery), the highest plasma concentrations of adiponectin, resistin, and leptin were associated with individuals in the PE group. Before and after controlling for HOMA-IR, only the elevated plasma concentrations of adiponectin and leptin levels were associated with PE individuals. In contrast, increased plasma resistin levels in PE individuals were not independent of HOMA-IR, indicating that differences in resistin plasma levels between PE and HP may be confounded by insulin resistance (regression data not shown).

Relationships between plasma concentrations of adipokines and other measured variables were assessed by calculating Spearman rank correlation coefficients within the diagnostic subsets (Table 3). By the use of multiple linear regression analysis on the combined patient groups that we controlled for possible confounding variables, we identified variables making a unique contribution to the prediction of adiponectin, resistin, and leptin concentrations in plasma (Fig. 1).

Adipose tissue mass is assumed to be a main determining factor of plasma adipokine levels. We observed that BMI before and during pregnancy and the weight of the newborns were positively correlated with plasma concentrations of leptin in the control group (Table 3), whereas they were not related to leptin levels in PE subjects. BMI did not display any significant correlation with plasma levels of adiponectin or resistin.

Insulin resistance may be linked to alterations in plasma adipokine levels. Plasma leptin concentrations correlated pos-

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Table 1. Clinical variables from the pregnancies of PE and HP women at delivery

<table>
<thead>
<tr>
<th>Variable</th>
<th>PE (n = 15)</th>
<th>HP (n = 23)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>30.9 ± 1.7</td>
<td>31.3 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Parity</td>
<td>0.47 ± 0.17</td>
<td>0.39 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1.87 ± 0.22</td>
<td>2.13 ± 0.25</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.0 ± 1.3</td>
<td>23.8 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg²</td>
<td>29.3 ± 1.2</td>
<td>30.2 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>166 ± 4</td>
<td>120 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>105 ± 2</td>
<td>74 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pregnancy duration, days</td>
<td>231 ± 6</td>
<td>270 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Newborn weight, g</td>
<td>1,977 ± 197</td>
<td>3,483 ± 80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight, percentile</td>
<td>38 ± 4</td>
<td>58 ± 5</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Values are means ± SE. PE, preeclamptic; HP, healthy pregnant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, not significant. *Prepregnancy.

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Table 2. Blood variables in PE and HP women at delivery

<table>
<thead>
<tr>
<th>Variable</th>
<th>PE (n = 15)</th>
<th>HP (n = 23)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, μg/ml</td>
<td>18.3 ± 2.2</td>
<td>12.2 ± 1.1</td>
<td>0.011</td>
</tr>
<tr>
<td>Resistin, ng/ml</td>
<td>5.68 ± 0.41</td>
<td>4.65 ± 0.32</td>
<td>0.028*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>34.4 ± 3.2</td>
<td>22.7 ± 2.1</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>6.34 ± 1.02</td>
<td>2.80 ± 0.31</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>1.89 ± 0.18</td>
<td>1.23 ± 0.10</td>
<td>0.002</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>110 ± 34</td>
<td>71 ± 26</td>
<td>NS</td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>1059 ± 136</td>
<td>783 ± 71</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.3 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>β-Cell function (HOMA-β)</td>
<td>157 ± 12</td>
<td>165 ± 35</td>
<td>NS*</td>
</tr>
<tr>
<td>Insulin resistance index (HOMA-IR)</td>
<td>2.5 ± 0.8</td>
<td>1.4 ± 0.1</td>
<td>NS*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>6.6 ± 0.5</td>
<td>6.8 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>3.3 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Atherogenic index, mmol/l</td>
<td>3.6 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>3.2 ± 0.2</td>
<td>2.8 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.44 ± 0.05</td>
<td>0.42 ± 0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. HOMA-β, homeostasis model assessment for β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance. *Data were log10 transformed.
Elevated blood pressure is a feature of preeclampsia, and SBP was positively correlated with plasma adiponectin concentration in the PE group (Table 3). Analysis of data from both PE and HP, combined with linear regression, confirmed a correlation between plasma adiponectin concentration (dependent variable) and plasma HDL cholesterol concentrations in the PE group (Fig. 2B). This correlation was still significant after controlling for BMI, pregnancy duration, and maternal age ($b = 0.484; P = 0.009$), but not HOMA-IR.

Preeclampsia has been associated with altered blood lipid profiles, and we found that the atherogenic index, as well as plasma concentrations of HDL cholesterol (Table 3), correlated with resistin plasma concentrations in the PE group. Applying linear regression analysis on both groups combined confirmed a correlation between plasma resistin concentrations (dependent variable) and plasma HDL cholesterol concentrations (Fig. 1B). This correlation was still significant after controlling for BMI, pregnancy duration, and maternal age ($b = 0.484; P = 0.009$), but not HOMA-IR.

Adipokine mRNA levels in adipose tissue. We investigated whether the differences in plasma levels of adiponectin, resistin, and leptin between the PE and HP groups could be associated with differences in adipose tissue expression of the adipokines. Analysis of the relative mRNA level in abdominal subcutaneous adipose tissue by Northern blotting (Fig. 2A) did not demonstrate differences in mean expression levels of the adiponectin and leptin genes between women in the PE and HP groups (Fig. 2B). Resistin mRNA was not detectable by Northern blotting in the adipose tissue (data not shown), but relative levels could be measured using real-time RT-PCR (MATERIALS AND METHODS). Mean resistin mRNA levels in the adipose tissue were 42% higher in PE than in HP, but this was not statistically significant when data were log$_{10}$ transformed ($P = 0.574; 95\% CI = 0.345 - 0.810$; Fig. 2C). (One outlying data point from the PE group was omitted because it was nearly 16-fold higher than the PE mean).

In the PE group, adiponectin concentrations in plasma showed a positive correlation with adiponectin mRNA levels in adipose tissue (Table 4), whereas no significant correlation was observed in the control group. Resistin and leptin concentrations in plasma were unrelated to the respective mRNA level in adipose tissue (Table 4).

Levels of resistin mRNA (dependent variable) in adipose tissue correlated with HOMA-IR index in linear regression analysis of the combined PE and HP groups (Fig. 2C). This correlation was also significant after controlling for BMI and pregnancy duration ($b = 0.450; P = 0.028$) but not maternal age. Moreover, there was an inverse relationship between resistin and adiponectin mRNA levels in adipose tissue (Fig. 2D), and this was also significant after controlling for BMI, pregnancy duration, and maternal age ($b = -0.747; P = 0.001$).

Adipokine mRNA levels in placenta. Placenta is a potential source of adipokines during pregnancy; hence, we examined whether the increased plasma concentrations of adiponectin, resistin, and leptin in PE could be associated with placental overexpression of the respective genes. However, by Northern blot analysis and real-time RT-PCR we could not detect any adiponectin gene expression in placenta biopsies from the subjects (data not shown). Leptin expression was detected by Northern blotting in the placenta (Fig. 3A), and the mean level of leptin mRNA was threefold higher in the PE than in the HP group ($P = 0.003$; Fig. 3B). Resistin mRNA was detected not on Northern blots, but by real-time RT-PCR. The analysis of patient placenta biopsies did not show any statistically significant difference in the respective mRNA levels.

Values are Spearman rank correlation coefficients; n = 15 PE and 23 HP. Variables tested were: BMI, SBP, DBP (mmHg), pregnancy duration, newborn weight, adiponectin, resistin, leptin, IL-6, TNF-α (pg/ml), insulin resistance (HOMA-IR); total cholesterol, HDL cholesterol, LDL cholesterol, atherogenic index (mmol/l) = HDL cholesterol/(total cholesterol – HDL cholesterol); triglycerides, free fatty acid (mmol/l). Only statistically significant correlation coefficients are shown. §Pregnancy; *P ≤ 0.05; †P ≤ 0.01.
not reveal any significant difference in resistin mRNA levels (log10-transformed data) between the PE and HP groups ($P = 0.280$), although HP levels tended to be higher (Fig. 3C).

In the PE and HP groups combined, there was only a borderline correlation between leptin concentrations in plasma and mRNA levels in placenta ($r = 0.301; P = 0.105$). Placenta expression of resistin did not correlate with plasma resistin concentrations.

DISCUSSION

Our main finding is that maternal plasma concentrations of several adipokines, including adiponectin, resistin, and leptin, were higher in the third trimester in a group of PE women compared with HP women. Recently, increased plasma concentrations of adiponectin and reduced resistin concentrations have been reported (7, 39) in the plasma of PE women. Maternal plasma leptin concentrations in the third trimester may be increased in preeclampsia (1), whereas midterm leptin concentrations are reduced among women who will develop preeclampsia (8). In agreement with other investigators, we found that plasma concentrations of the proinflammatory adipokines TNF-α (51) and IL-6 (14) were higher in the PE compared with HP group.

We did not detect differences in HOMA-IR and BMI, possibly due to the relatively small number of patients in this study. All participants included in this study underwent caesarian section, thus allowing us to obtain biopsies from adipose tissue as well as placenta. Mean pregnancy duration was inherently shorter in PE compared with HP, although all samples analyzed were taken in the third trimester.

Adiponectin. As opposed to other adipose-derived proteins, plasma levels of adiponectin have been found to be decreased in most metabolic diseases, including obesity (2), dyslipidemia (29), type 2 diabetes (18, 19, 25), gestational diabetes (40), and coronary artery disease (60). Therefore, it was unexpected that adiponectin plasma levels were increased in the pathological state of preeclampsia.

Table 4. Correlation coefficients: adipose tissue adiponectin, resistin, and leptin mRNA levels in PE and HP women at delivery

<table>
<thead>
<tr>
<th>Adiponectin mRNA*</th>
<th>Resistin mRNA*</th>
<th>Leptin mRNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, µg/ml</td>
<td>Resistin, ng/ml</td>
<td>Leptin, ng/ml</td>
</tr>
<tr>
<td>PE ($n = 12$)</td>
<td>HP ($n = 12$)</td>
<td>PE ($n = 13$)</td>
</tr>
<tr>
<td>0.60†</td>
<td>−0.39</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are Spearman rank correlation coefficients. *Relative levels. †$P <= 0.05$.

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Blood pressure is a major clinical manifestation of preeclampsia, and we found that adiponectin concentrations in plasma were correlated with SBP after adjusting for BMI and HOMA-IR, but not after adjusting for gestational length. However, SBP and gestational length in serious preeclampsia may not be truly independent variables. Because the mean gestational length of the HP control group was 39 days longer than the mean length in the PE group, gestational length as a major determinant of adiponectin levels in the patients cannot be ruled out. However, in another study (9), similar plasma adiponectin levels were observed in the second and third trimesters in nondiabetic women. A report of elevated plasma adiponectin in hypertension (28) gives further support to the view that adiponectin levels are elevated in preeclampsia per se, and not only because of shorter gestation. A high concentration of adiponectin in circulation has been suggested to represent a counter-regulatory response aimed at moderating endothelial damage and cardiovascular risk associated with high arterial pressure. Furthermore, adiponectin was increased in patients with renal disease (60) and proteinuria (59) and was inversely correlated to creatinine clearance (28). Plasma creatinine levels were within the reference range for the PE group (data not shown). However, it is possible that modest alterations of renal function could affect adiponectin clearance.

There was no significant difference in adiponectin mRNA expression in adipose tissue from PE and HP women. However, in the PE group, we observed that adipose tissue mRNA levels reflect plasma adiponectin concentrations. Thus adiponectin levels in preeclampsia may be due to ongoing adipose tissue synthesis. Both human adiponectin receptors, ADIPOR1 and ADIPOR2, are abundantly expressed in placenta (56), suggesting that adiponectin may have a physiological function during pregnancy.

Resistin. In the present investigation, we found that plasma resistin levels were increased in the PE women compared with the HP women. In a recent publication (7), the opposite result was reported, with the highest levels of resistin levels in the third trimester of normal pregnancy. Resistin may induce insulin resistance (35, 44) and regulate hepatic glucose homeostasis in rodents (4, 38), as well as glucose uptake in vitro (34), but the role of resistin in humans is unclear. The resistin levels were also significantly different between PE and HP after controlling for BMI but not HOMA-IR. This indicates that elevated resistin levels in preeclampsia are unrelated to differences in fat mass, whereas they may rely on the level of insulin sensitivity. However, we did not observe a direct correlation between plasma resistin levels and the degree of insulin resistance. Most studies (13, 20, 24, 47) fail to show a correlation between plasma resistin and insulin sensitivity in humans.

Obesity may be an important determinant of resistin levels in humans (10, 52, 57). In our study, the subjects were not obese (BMI >30) before pregnancy, and this might explain why BMI did not correlate with plasma resistin levels in pregnancy. Plasma resistin levels were positively correlated with plasma concentration of HDL cholesterol in PE women, which has been reported earlier (46) in subjects at risk of diabetes and in subjects with type 1 and type 2 diabetes.

Resistin mRNA was detected in abdominal subcutaneous adipose tissue and placenta, but mRNA levels did not correlate with plasma resistin concentrations. Heilbronn et al. (16) observed a correlation between serum resistin and resistin mRNA expression from abdominal subcutaneous adipose tissue in obese subjects. In rodents, there is no straightforward relationship between resistin levels in blood and adipose tissue mRNA levels (53). Kielstein et al. (20) reported that resistin concentrations in blood increase with the progressive impairment of renal function and depend on glomerular filtration rates. Thus altered renal function in preeclampsia may be related to elevated plasma resistin levels.

In the present study, the adipose tissue resistin mRNA levels displayed a correlation with the HOMA-IR index, and this may indicate a link between resistin expression and insulin resistance during pregnancy. It is possible that resistin acts locally in adipose tissue to affect insulin sensitivity.

Leptin. We report that third-trimester leptin plasma concentrations are increased in preeclampsia. Studies have shown that leptin levels rise between the first and the last two trimesters of pregnancy and return to prepregnancy levels within the first 30 days postpartum (48). In agreement with our present study, it has previously been shown that circulating leptin is increased in PE women (32, 33). Still, other investigators have concluded that maternal leptin levels are reduced in preeclampsia (23), and in a prospective study (8), the development of preeclampsia has been associated with reduced plasma leptin in the second trimester.

Leptin plasma concentrations and leptin mRNA levels in adipose tissue (data not shown) correlated significantly with BMI and HOMA-IR in the HP group. This relationship was not observed in the PE group, suggesting an extraordinary regulation of leptin levels during preeclampsia. Our present data suggest that placenta expression of leptin may partially explain the high leptin concentrations in PE plasma.

Whereas the cross-sectional design of this study prevents us from drawing conclusions as to what causes preeclampsia, our focus was to monitor adipokine concentrations as they related to tissue expression levels as well as insulin resistance. In summary, we observed increased plasma concentrations of adiponectin, resistin, and leptin in PE vs. HP women. This
incorporate could not be ascribed to differences in mean HOMA-IR, BMI, mRNA levels of adiponectin, resistin, and leptin in adipose tissue, or to resistin mRNA levels in placenta. However, placental levels of leptin mRNA were higher in PE compared with HP women. Elevated plasma levels of adipokines may represent a counterregulatory response, attenuating endothelial dysfunction and metabolic deficiencies in pre-eclampsia.

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