Serum levels of the adipokine lipocalin-2 are increased in preeclampsia

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ABSTRACT. Background: Preeclampsia (PE) is a serious complication in pregnancy which increases the future risk for vascular and metabolic disease in both mother and newborn. Recently, lipocalin-2 has been introduced as a novel adipokine which contributes to obesity, insulin resistance, and vascular disease. Aim: In the current study, we investigated lipocalin-2 serum levels in PE patients as compared to healthy gestational age-matched controls. Subjects and methods: Lipocalin-2 serum concentrations were quantified by enzyme-linked immunosorbent assay in control (no.=22) and PE (no.=22) patients. Furthermore, lipocalin-2 levels were correlated to clinical and biochemical measures of renal function, glucose, and lipid metabolism, as well as inflammation. Results: Median maternal lipocalin-2 concentrations were significantly increased in PE (121.3 μg/l) as compared to control subjects (99.8 μg/l) (p<0.05). Furthermore, circulating lipocalin 2 correlated positively with diastolic blood pressure, creatinine, and C reactive protein. In multivariate analyses, creatinine and C reactive protein remained independently associated with lipocalin-2 levels. Conclusions: We demonstrate that maternal lipocalin-2 concentrations are significantly increased in PE. Furthermore, markers of renal function and inflammation independently predict circulating lipocalin-2.

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INTRODUCTION

Preeclampsia (PE) which is characterized by hypertension, proteinuria, and endothelial dysfunction is a serious cardiovascular complication in pregnancy (1). It shares risk factors with the metabolic syndrome including insulin resistance and obesity (1). Both mother and newborn have a significantly increased future risk for metabolic and vascular diseases as a consequence of a preeclamptic pregnancy (1). The pathogenesis of PE has been characterized in more detail in recent years. Thus, angiogenic and anti-angiogenic factors including soluble fms-like tyrosine kinase 1 and endoglin are dysregulated in PE (2-5). Furthermore, various adipocyte-secreted factors – the so-called adipokines – play an important role in the pathogenesis of the disease. Thus, concentrations of the appetite-suppressive adipokine leptin are increased in PE and upregulation of leptin precedes the clinical onset of the disease (6). This hyperleptinemia might be a compensatory response to increase nutrient delivery to the underperfused placenta (7). Furthermore, the proinflammatory adipokine tumor necrosis factor α (TNFα) was increased about 2-fold in women with PE (8). Studies in pregnant rats suggested that this increase in TNFα concentrations is sufficient to increase mean arterial pressure by 27 mmHg (9). Moreover, we have recently demonstrated significant upregulation of the adipokines visfatin (10), adipocyte fatty acid binding protein (11), and adiponectin (12) in PE patients. Recently, lipocalin-2 has been introduced as a novel adipokine which contributes to obesity, insulin resistance, and associated vascular disease (13-15). Lipocalin-2 is a member of the large family of lipocalins which exhibit high affinity for small hydrophobic ligands such as steroids and pheromones (16, 17). It is interesting to note in this context that another member of the lipocalin superfamily, retinol-binding protein-4, has recently been characterized as an adipocyte-secreted protein that impairs glucose metabolism and insulin sensitivity (18). Lipocalin-2 plasma concentrations and mRNA expression in adipose tissue and liver are upregulated in obese mice as compared to lean controls (13). In accordance with these findings, upregulation of lipocalin-2 was shown in multiple rodent models of obesity (14). Circulating lipocalin-2 concentrations in humans significantly and positively correlated with adiposity, hypertriglyceridemia, hyperglycemia, and insulin resistance, whereas a negative correlation was found with HDL cholesterol (13). Interestingly, lipocalin-2 levels were downregulated by thiazolidinediones in both human subjects in vivo (13) and fat cells in vitro (14). In addition, convincing evidence has been presented that circulating lipocalin-2 is elevated in patients with coronary heart disease as compared to controls (19).

In the current study, we sought to investigate whether maternal lipocalin-2 levels are altered in PE and might potentially contribute to the present and future metabolic and vascular risk of the disease. We determined lipocalin-2 levels in 22 control and 22 PE patients and correlated serum concentrations of this adipocyte-secreted factor to clinical and biochemical measures of renal function, glucose, and lipid metabolism, as well as inflammation.
MATERIALS AND METHODS

Subjects

The study design has been described recently (20). In brief, 22 pregnant women with PE and 22 gestational age-matched controls were recruited from the Department of Obstetrics, University of Leipzig. PE was defined as gestational blood pressure elevation >140 mmHg systolic (SBP) or >90 mmHg diastolic (DBP) accompanied by proteinuric in women who were normotensive before 20 weeks of gestation as previously described (21). Body mass index (BMI) was calculated as weight before pregnancy divided by squared height. Homeostasis model assessment of insulin resistance (HOMA-IR) was determined as previously described (22). Patients with renal diseases, diabetes mellitus, generalized inflammation, and malignant diseases were excluded from the study. The study was approved by the local Ethics Committee. All subjects gave written informed consent before taking part in the study.

Assays

Venous blood was drawn into serum tubes from each patient after an overnight fast. None of the women was in labour at the time of the blood sampling. Serum was separated immediately by centrifugation at 4000 g for 10 min and frozen at –80 C. Serum insulin was measured with a two-site chemiluminescent enzyme immunometric assay for the Immulite automated analyzer (Diagnostic Products, Los Angeles, CA, USA). Leptin (Mediagnost, Reutlingen, Germany), adiponectin (Mediagnost, Reutlingen, Germany), and lipocalin-2 (Biovendor, Modrice, Czech Republic) serum levels were determined with commercially available enzyme-linked immunosorbent assays (ELISA) according to the manufacturers’ instructions. Creatinine, glucose, cholesterol, triglycerides, and C reactive protein were measured by standard laboratory methods in a certified laboratory.

Statistical analysis

SPSS software version 11.5 (SPSS, Chicago, IL) was used for all statistical analyses. Differences between control and PE patients were assessed by Mann-Whitney-U test. Correlations were performed using the Spearman’s correlation method. To adjust the effects of covariates and identify independent relationships, multivariable linear regression analyses were performed. Distribution was tested for normality using Shapiro-Wilk W test and non-normally distributed parameters were logarithmically transformed. A p-value of <0.05 was considered as statistically significant in all analyses.

RESULTS

Lipocalin-2 serum levels are significantly increased in PE patients as compared to controls

Clinical characteristics of the subgroups studied (Control, PE) are summarized in Table 1 and variables are given as median (25th percentile, 75th percentile). Maternal serum lipocalin-2 concentrations were significantly increased in PE patients (121.3 μg/l) as compared to control subjects (99.8 μg/l) (p<0.05) (Table 1). Age, BMI, and gestational age at blood sampling were not significantly different in the 2 groups (Table 1). SBP and DBP, serum creatinine, triglycerides, leptin, adiponectin, and C reactive protein were significantly elevated in PE patients as compared to healthy pregnant controls (Table 1). In contrast, fasting glucose, fasting insulin, HOMA-IR, and cholesterol were not different between the 2 groups (Table 1).

Univariate correlations

Serum lipocalin-2 concentrations significantly and positively correlated with DBP, creatinine, and C reactive protein (Table 2). In contrast, lipocalin-2 was not associated with markers of glucose (fasting glucose, fasting insulin, HOMA-IR, adiponectin) and lipid (triglycerides, cholesterol) metabolism.

Multivariate correlations

Multiple regression analysis revealed that PE predicted circulating lipocalin-2 levels independent of age (Table 3, Control PE No. 22 22 Lipocalin-2 (μg/l) 99.8 (85.1, 116.0) 121.3 (96.8, 131.7) Age (yr) 29.0 (24.8, 34.3) 32.5 (29.8, 35.3) BMI (kg/m²) 20.9 (19.4, 22.5) 22.4 (20.3, 25.4) SBP (mmHg) 100 (98, 115) 160 (145, 180) DBP (mmHg) 65 (60, 73) 65 (60, 73) Heart rate (beats/min) 72 (68, 80) 72 (68, 80) Gestational age at blood sampling (days) 212 (196, 221) 206 (189, 237) Creatinine (mg/dl) 0.61 (0.53, 0.69) 0.75 (0.68, 0.90) Fasting glucose (mg/dl) 64.42 (56.63, 68.75) 64.60 (61.40, 73.43) Fasting insulin (mU/l) 7.84 (5.15, 9.88) 5.89 (3.44, 10.58) HOMA-IR 1.23 (0.76, 1.56) 1.24 (0.52, 2.47) Cholesterol (mg/dl) 264.50 (230.09, 299.88) 250.39 (230.27, 301.82) Triglycerides (mg/dl) 212.19 (153.34, 283.72) 297.06 (231.44, 380.84) Leptin (μg/l) 24.45 (15.78, 37.28) 53.45 (37.95, 89.85) Adiponectin (mg/l) 6.73 (4.88, 8.35) 11.93 (8.55, 16.03) C reactive protein (mg/l) 1.65 (1.00, 3.42) 8.22 (3.63, 37.21) PE: preeclampsia; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment of insulin resistance.