Retinol-binding protein 4 and insulin resistance in preeclampsia

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Abstract. Preeclampsia is characterized by the onset of high blood pressure and proteinuria during pregnancy, which results in substantial maternal and neonatal morbidity and mortality. Insulin resistance has been observed before the onset of preeclampsia, and has implicated in its pathophysiology. Recently, retinol-binding protein 4 (RBP4), which carries retinol in circulation, has been shown to be a potential regulator of insulin resistance originating from adipose tissue. Here we measured insulin resistance and RBP-4 levels in patients with preeclampsia and in women with normal pregnancies matched for gestational age and body mass index at Okayama University Hospital. Our aim was to examine the potential role of RBP4 in the pathophysiology of this disorder. There were no significant differences in RBP4 levels between all patients with preeclampsia and controls. However, the RBP4 level and homeostasis model assessment as an index of insulin resistance (HOMA-IR) in overweight patients with late-onset preeclampsia were significantly higher than in overweight controls carrying normal pregnancies and in normal weight women with late-onset preeclampsia. In contrast, there were no significant differences between the overweight and normal weight groups among patients with early-onset preeclampsia and in healthy pregnant women. These data suggest that RBP4 might act in the pathophysiology of late-onset preeclampsia via increased insulin resistance in obese women.

Key words: Preeclampsia, Insulin resistance, Retinol binding protein 4

PREECLAMPSIA is a pregnancy-specific and multi-system disorder characterized by the onset of high blood pressure and proteinuria, which develop after 20 weeks of gestation in previously normotensive women or which are superimposed on preexisting hypertension. In severe cases, several other symptoms, such as renal and/or liver failure and eclampsia, complicate the clinical features. In addition, it can also complicate pregnancies with fetal growth restriction, with nonassuring fetal status or even those with fetal death. It occurs in about 5% of all pregnancies and results in substantial maternal and neonatal morbidity and mortality [1, 2].

It is widely accepted that physiological insulin resistance persists in pregnant women, so glucose tolerance might be lower than in the nonpregnant state. Maternal characteristics such as obesity, diabetes and insulin resistance increase the risk for preeclampsia [3–5]. Thus, insulin resistance has been observed before the onset of preeclampsia and has been implicated in its pathophysiology [6–8]. Adipocytokines such as tumor necrosis factor-α, leptin and adiponectin have been demonstrated to play a role in the regulation of glucose metabolism and insulin resistance in nonpregnant and pregnant women [9–11]. Recently, retinol-binding protein 4 (RBP4), which carries retinol in circulation, was shown to be correlated with insulin resistance [12]. Moreover, it was reported that circulating RBP4 was elevated in a mouse model for insulin resistance and in patients with severe obesity and type II diabetes [13]. Several mechanisms link RBP4 to insulin resistance. RBP4-associated effects include an increase in hepatic gluconeogenesis by enhancing the expression of phosphoenolpyruvate carboxykinase in the liver, and attenuated insulin signaling in skeletal muscle [12]. Thus, RBP4 could be a novel regulator of insulin resistance originating from adipose tissue, but
there are few reports about the potential roles of RBP4 in the pathophysiology of preeclampsia. Here we analyzed insulin resistance and RBP4 levels in patients with preeclampsia and compared them with normal pregnancies at similar stages to examine their potential roles in the pathophysiology of preeclampsia.

Materials and Methods

Pregnant Japanese women who visited the Okayama University Hospital, Department of Obstetrics and Gynecology (Okayama, Japan), were included in this study. Twenty normal pregnant and nonpregnant women with no complications were enrolled as controls for measuring normal RBP4 levels during pregnancy, while 64 healthy pregnant women with no complications at first trimester were examined for the correlation between RBP4 level and insulin resistance. In addition, 60 pregnant women with severe preeclampsia were also analyzed to investigate the relationships of preeclampsia with RBP4 levels and insulin resistance. Of these women with severe preeclampsia, 19 women had severe early-onset preeclampsia diagnosed before gestational week 32, and 19 age-, gestational week-, parity- and body mass index (BMI)-matched healthy women with normotensive pregnancies who delivered early because of preterm ruptured membranes or preterm labor were chosen as ‘early control’ patients. Forty-one patients had severe late-onset preeclampsia diagnosed in gestational week 32 or later and 41 age-, gestational week-, parity- and BMI-matched healthy women with normotensive pregnancies were chosen as ‘late control’ patients. Severe preeclampsia was defined as either severe hypertension (systolic blood pressure > 160 mmHg or diastolic blood pressure > 110 mmHg) or severe proteinuria (>2.0 g of protein collected in a 24 h urine sample). None of the patients with preeclampsia and controls had any history of renal disorders, diabetes or essential hypertension, or multiple gestations. Control women and patients with preeclampsia were subdivided into two groups: overweight or obese (‘overweight group’; BMI ≥ 25 kg/m²) and normal weight women (‘normal weight group’; 18.5 kg/m² < BMI < 25 kg/m²). Fetuses were defined as ‘small for gestational age’ if they were below the 10th percentile for gestational age in Japanese standards. Clinical records were reviewed carefully and some patients were interviewed further at the time of sample collection. Those who did not meet the above criteria were eliminated from the study. The Institutional Ethics Review Board of Okayama University Hospital approved this study and all subjects gave informed consent.

Blood samples were collected from patients with preeclampsia soon after the diagnosis of the disease and none of the subjects received any medication before sampling. Samples from healthy pregnant women in their second and third trimester were matched with patients with preeclampsia by age, gestational week, parity and BMI to avoid possible biases. All samples were collected between 2006 and 2009. Immediately after sample collection, serum was separated by centrifugation and stored at –80 °C until use.

Serum levels of RBP4 were determined using a specific enzyme-linked immunosorbent assay (ELISA) following the manufacturer’s instructions (AdipoGen Inc., Incheon, Korea). Fasting insulin and glucose levels were determined by fluorescence enzyme immunoassay (Tosoh Corp., Tokyo, Japan) and the glucose oxidase method (Shino-Test Corp. Tokyo, Japan), respectively. The homeostasis model assessment as an index of insulin resistance (HOMA-IR) was calculated from the fasting insulin concentration (µU/mL) × fasting glucose concentration (mg/dL) / 405 [14]. All samples were examined in duplicate and mean values of individual serum concentrations were used for statistical analysis. Samples for measurement of RBP4 were diluted 1/100 prior to the assay. The minimum detectable concentration in this assay was 1.0 ng/mL. The intra- and interassay coefficients of variation for the RBP4 ELISA were <3.8% and 6.9%, respectively.

Statistical analysis

All values are expressed as the mean ± SD. The Kruskal–Wallis test and Scheffe’s test were used for statistical analysis using StatView software (Abacus Concepts, Berkeley, CA, USA) and P < 0.05 was taken as statistically significant.

Results

Patient characteristics

There were no significant differences between patients with early or late onset preeclampsia and their respective controls with regard to maternal age, smoking habit, BMI before pregnancy, and the gestational week of delivery and at recruitment. As expected, systolic and diastolic blood pressures were higher, birth weights were lower and the percentage of concep-
RBP4 concentrations in preeclampsia

We first examined the serum levels of RBP4 in patients with early- and late-onset preeclampsia and controls. The concentration of serum RBP4 in patients with late-onset preeclampsia was significantly higher than in healthy pregnant women, but there was no significant difference in RBP4 levels between women with early-onset preeclampsia and controls (Fig. 2A). Next, to evaluate the association between RBP4 and obesity, we examined RBP4 in overweight (n = 8) and normal weight (n = 11) groups among patients with early-onset preeclampsia and early control patients, and in overweight (n = 18) and normal weight (n = 23) groups among patients with late-onset preeclampsia and in late pregnancy controls. RBP4 levels in overweight patients with late-onset preeclampsia were significantly higher than in normal late pregnancy over-

Maternal RBP4 concentrations in healthy pregnant women and patients with preeclampsia

To evaluate the gestational pattern of RBP4 secretion, we examined serum levels of RBP4 in healthy pregnant women and nonpregnant controls. There were no significant changes in the RBP4 levels throughout the course of the pregnancies and no significant differences between pregnant and nonpregnant women (Fig. 1A). There were also no significant differences in serum RBP4 levels between all patients with preeclampsia and controls (Fig. 1B). The RBP4 levels were significantly correlated with HOMA-IR at first trimester in healthy pregnant women (Fig. 1C). In addition, there were significant correlations between RBP4 levels and BMI (r=0.813, P<0.001).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of Patients with Early and Late Onset Preeclampsia and of Controls</th>
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<tbody>
<tr>
<td></td>
<td>Early control</td>
</tr>
<tr>
<td>n</td>
<td>19</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.3 ± 4.8</td>
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<tr>
<td>Primigravida number (%)</td>
<td>14 (74%)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>2 (10%)</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>102 ± 18</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>64 ± 13</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.5 ± 4.2</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>29.1 ± 2.0</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1201 ± 144</td>
</tr>
<tr>
<td>Small for gestational age (%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
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NS: not significant

RBP4 concentrations in patients with early- and late-onset preeclampsia and controls

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HOMA-IR in patients with early- and late-onset preeclampsia and controls

To evaluate insulin resistance, we examined the HOMA-IR in patients with early- and late-onset preeclampsia and controls. HOMA-IR in patients with late-onset preeclampsia was significantly higher than that in healthy pregnant women, but there was no significant difference in HOMA-IR between patients with early-onset preeclampsia and controls (Fig. 3A). Next, to evaluate the association between HOMA-IR and obesity, we examined HOMA-IR in overweight (n = 8) and normal weight (n = 11) groups among patients with early-onset preeclampsia and controls and in normal weight women with late-onset preeclampsia, but there were no significant differences between the overweight and normal weight groups among patients with early-onset preeclampsia and healthy pregnant women (Fig. 2B).

**HOMA-IR in patients with early- and late-onset preeclampsia and controls**

Fig. 2  Serum RBP4 concentrations in patients with early- and late-onset preeclampsia and controls. (A) Serum concentrations of RBP 4 among patients with early- and late-onset preeclampsia and controls. (B) Comparison of RBP 4 level between obese and normal weight women among those with early- and late-onset preeclampsia and controls. Values are shown as the mean ± SD; *P < 0.01.

Fig. 3  HOMA-IR estimates in patients with early- and late-onset preeclampsia and controls. (A) HOMA-IR estimates among patients with early- and late-onset preeclampsia and controls. (B) Comparison of HOMA-IR results between obese and normal weight women in the early- and late-onset preeclampsia groups and controls. Values are shown as the mean ± SD; *P < 0.01.
ly-onset preeclampsia and early control patients, and in overweight (n = 18) and normal weight (n = 23) groups among patients with late-onset preeclampsia and late pregnancy controls. The HOMA-IR was significantly higher in the overweight group compared with the normal weight group in patients with late-onset preeclampsia and in the normal late overweight controls, but there was no significant difference between overweight and normal weight groups in patients with early-onset preeclampsia and healthy pregnant women (Fig. 3B).

Discussion

An increased understanding of the molecular mechanisms underlying preeclampsia has led to several potential areas of investigation, including angiogenic factors [15, 16]. We and others have demonstrated that even mild endothelial dysfunction under a minor imbalance of angiogenic factors might cause overweight patients to suffer from late-onset preeclampsia, and that obesity might not play a dominant role in the pathophysiology of early-onset preeclampsia [17–19], suggesting different profiles of early- and late-onset preeclampsia. In this study, there were no significant differences in RBP4 levels between all patients with preeclampsia and normal controls. However, the RBP4 concentration of women with late-onset preeclampsia was significantly higher than in normal late pregnancy controls. In contrast, there were no significant differences in RBP4 levels between patients with early-onset preeclampsia and normal early pregnancy controls. In addition, a significant difference in serum RBP4 levels between obese and normal weight women was observed only among those with late-onset preeclampsia. So far, we have found only a few reports about the relationship between RBP4 and preeclampsia. Two studies demonstrated that the RBP4 level was increased in obese patients with preeclampsia compared with normal controls [20, 21], and our previous report demonstrated a significant increase of RBP4 in late onset patients with preeclampsia compared with normal late pregnancy controls [22]. In contrast, Stephan et al. [23] observed no significant difference in RBP4 levels between patients with preeclampsia and normal control subjects, but all the patients in their study were lean and showed early-onset preeclampsia. Taken together, RBP4 levels appear to be increased significantly among obese patients with late-onset preeclampsia.

Adipose tissues express various secretory proteins such as leptin, tumor necrosis factor-α and adiponectin, which regulate energy expenditure, lipid metabolism and insulin resistance [24]. Recent reports have demonstrated that plasma adiponectin and leptin concentrations are not elevated in normal human pregnancies, but are elevated in women with preeclampsia [19, 25–29]. Specially, adiponectin was increased only in women with late-onset preeclampsia [17, 30], and significant differences in adiponectin levels were found between normal and overweight women only among those with late-onset preeclampsia [17]. These data show that there is dysregulation of adipocytokines, including adiponectin and RBP4, among obese women with late-onset preeclampsia. In addition, we found here that the HOMA-IR was significantly increased only in women with late-onset preeclampsia, which is consistent with the report by D’Anna et al. [30]. We also observed that there was a significant difference in the HOMA-IR between obese and normal weight women with late-onset preeclampsia in this study. Moreover, we demonstrated that RBP4 levels were significantly correlated with insulin resistance during pregnancy, as previously reported for nonpregnant women [12, 13]. In addition, we previously demonstrated that even mild endothelial dysfunction caused by a minor imbalance of angiogenic factors might cause overweight patients to suffer from late-onset preeclampsia [17]. Because hyperinsulinemia may directly predispose to hypertension by increased renal sodium reabsorption and stimulation of the sympathetic nervous system and insulin resistance may impair endothelial function [6], the dysregulation of RBP4 might play a role in the pathophysiology of late-onset preeclampsia via insulin resistance, which leads to endothelial dysfunction/hypertension with mild imbalance of placental angiogenic factors, in women with preexisting obesity. However, the RBP4 level in obese women with late-onset preeclampsia was significantly increased compared with that in late obese control, although there was no significant difference of BMI between the two groups (preeclampsia 27.4 ± 1.7 vs. control 27.5 ± 1.5) and we observed a significant correlation between RBP4 and BMI at first trimester in normal pregnancy. Moreover, recent preliminary study have suggested that one possibility for this correlation between RBP4 and insulin resistance may be due to renal insufficiency, which is common in type 2 diabetes [31]. Although we demonstrated that there was no significant difference of RBP4 level between early-onset preeclampsia patients and control, it might be nec-
necessary to examine how RBP4 levels in severe preeclampsia change during the course of the pregnancy to exclude an effect on RBP4 secretion by other causes such as placental angiogenic factors or renal function. In addition, there were no significant changes in the RBP4 levels throughout the course of normal pregnancies, while we haven’t found any reports about the RBP4 expression in placenta of preeclampsia so far. The comparison of placental RBP4 expression between late-onset preeclampsia and control might be required to distinguish the origin of increased RBP4 in obese women with late-onset preeclampsia.

In this study, we demonstrated that the RBP4 concentrations and HOMA-IR were significantly higher in women with late-onset preeclampsia than in normal late pregnancy controls. In contrast, there were no significant differences between patients with early-onset preeclampsia and normal early pregnancy controls. Moreover, we observed that the RBP4 levels and HOMA-IR were significantly higher in the overweight group compared with the normal weight group, but only in women with late-onset preeclampsia. These data suggest that upregulation of RBP4 production might play some role in the pathophysiology of obese women with late-onset preeclampsia via increased insulin resistance. However, further analysis will be required to examine the actual role of RBP4 and the relationships with other important factors, such as placental angiogenic factors, other adipocytokines and renal function in the pathogenesis of preeclampsia.

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**Disclosure of Conflict of Interest**

The authors have nothing to disclose.

**References**