Potential interaction of brain natriuretic peptide with hyperadiponectinemia in preeclampsia

Hisashi Masuyama, Etsuko Nobumoto, Seiji Inoue, and Yuji Hiramatsu

Department of Obstetrics and Gynecology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Submitted 27 October 2011; accepted in final form 5 January 2012

BNP and adiponectin showed significant positive correlations in both normal-weight and overweight preeclampsia patients. Moreover, significant differences in adiponectin levels were found between normal and overweight women with preeclampsia (23), and the adiponectin level was negatively correlated with the soluble VEGF receptor 1 level in preeclampsia (39). Since hypoadiponectinemia is associated with impaired endothelium-dependent vasodilatation (36, 40) and represents an independent risk factor for hypertension (12), adiponectin may maintain endothelial function, and its deficiency may lead to endothelial dysfunction/hypertension.

On the other hand, plasma brain natriuretic peptide (BNP) levels are increased in patients with congestive heart failure (CHF), and measurements of these levels are widely used to assess the presence, severity, and prognosis of CHF (20). Also, BNP has beneficial effects in patients with heart failure because of its various biological actions (17, 26). Moreover, adiponectin has been identified as a risk factor for cardiovascular disease (16, 29) and CHF (11). Recently, adiponectin has been reported to have cardio-protective effects against ischemia-reperfusion injury (35) and hemodynamic stress (18, 34) in mice. In addition, the BNP level has been detected as alterations in the concentrations of biological markers, including vascular endothelial growth factor and TGFβ (10, 21, 25). Consequently, placenta-derived factors that may be partially responsible for the maternal syndrome of preeclampsia are potentiated. The result is a generalized endothelial dysfunction manifested by hypertension, proteinuria, and thrombotic microangiopathy.

Maternal characteristics such as obesity, diabetes, and insulin resistance increase the risk of preeclampsia (5, 13, 15). Adiponectin is an adipocyte-derived hormone that acts as an anti-diabetic, antiatherogenic, and anti-inflammatory adipocytokine, and decreased circulating adiponectin levels are associated with obesity, insulin resistance, and type 2 diabetes (6, 19, 38). Recent reports have demonstrated that plasma adiponectin concentrations are paradoxically elevated in women with preeclampsia (28, 31, 39), although preeclampsia is characterized by an increase in insulin resistance (33). The elevation of circulating adiponectin may be a physiological response to the endothelial dysfunction caused by antiangiogenic factors derived from the placenta in women with preeclampsia. Moreover, significant differences in adiponectin levels were found between normal and overweight women with preeclampsia (23), and the adiponectin level was negatively correlated with the soluble VEGF receptor 1 level in preeclampsia (39). Since hypoadiponectinemia is associated with impaired endothelium-dependent vasodilatation (36, 40) and represents an independent risk factor for hypertension (12), adiponectin may maintain endothelial function, and its deficiency may lead to endothelial dysfunction/hypertension.

PREECLAMPSIA IS A PREGNANCY-SPECIFIC multisystem disorder characterized by the onset of high blood pressure and proteinuria, which develop after 20 wk of gestation in previously normotensive women or are superimposed on preexisting hypertension. The disorder occurs in about 5% of all pregnancies and results in substantial maternal and neonatal morbidity and mortality (3, 37). Abnormal vascular growth and impaired endothelial function in the placenta are associated with abnormal pregnancy conditions such as preeclampsia and result from inadequate trophoblast invasion of maternal spiral arteries during early gestation (10, 25). Defective placental development may be reflected in the maternal circulation and can be detected as alterations in the concentrations of biological markers, including vascular endothelial growth factor and TGFβ (10, 21, 25). Consequently, placenta-derived factors that may be partially responsible for the maternal syndrome of preeclampsia are potentiated. The result is a generalized endothelial dysfunction manifested by hypertension, proteinuria, and thrombotic microangiopathy.

Potential interaction of brain natriuretic peptide with hyperadiponectinemia in preeclampsia. Am J Physiol Endocrinol Metab 302: E687–E693, 2012. First published January 10, 2012; doi:10.1152/ajpendo.00548.2011.— Adiponectin was reported recently to have roles in the pathophysiology of preeclampsia. Moreover, elevation of adiponectin and brain natriuretic peptide (BNP) has been observed in preeclampsia. We examined the possible links between adiponectin and BNP in the pathophysiology of preeclampsia. We performed a cross-sectional study in 56 preeclampsia patients and 56 controls matched for gestational age and body mass index. The BNP, leptin, and adiponectin levels were measured by ELISA, and their mRNA expressions were evaluated in omental adipose tissue by real-time PCR. The effects of BNP on adiponectin and leptin mRNA expression and secretion were investigated in primary cultures of adipocytes from obese and normal-weight women. The BNP, adiponectin, and leptin levels were significantly higher in preeclampsia patients compared with controls. The adiponectin level was increased significantly in normal-weight preeclampsia patients compared with overweight preeclampsia patients. Adiponectin mRNA expression was increased significantly in adipose tissues of preeclampsia patients compared with controls and was also increased significantly in normal-weight preeclampsia patients compared with overweight preeclampsia patients, whereas leptin was not.

BNP and adiponectin showed significant positive correlations in both normal-weight and overweight preeclampsia patients. BNP had a significantly weaker effect on adiponectin in overweight compared with normal-weight preeclampsia patients. Moreover, BNP had a weaker effect on adiponectin production in adipocytes from overweight women compared with adipocytes from normal-weight women using primary culture of human adipocytes. These data suggested that BNP may play a role in hyperadiponectinemia of preeclampsia patients. The weaker effect of BNP on adiponectin production may participate in the pathophysiology of overweight preeclampsia patients.

BNP and adiponectin; obesity; adipocyte; pregnancy
adiponectin levels in patients with preeclampsia and compared them with the levels in women with normal pregnancies to examine the relationship between BNP and adiponectin in the pathophysiology of preeclampsia. We also examined whether the effects of BNP on adiponectin expression and secretion differed in adipocytes derived from obese and normal-weight women to examine the mechanism for the different adiponectin levels between obese and normal-weight patients with preeclampsia.

**MATERIALS AND METHODS**

*Patients.* One-hundred twelve pregnant Japanese women who visited the Department of Obstetrics and Gynecology, Okayama University Hospital, Japan, were included in the present study. Of these 112 women, 56 had severe preeclampsia and 56 were controls with normotensive pregnancies matched for age, gestational age, parity, and body mass index (BMI). Twenty age-matched nonpregnant women and 20 healthy pregnant women in the first, second, and third trimesters were also included as volunteers. According to the definition of the Japan Society of Obstetrics and Gynecology, preeclampsia was defined as persistent elevation of systolic blood pressure to 140 mmHg and diastolic blood pressure to 90 mmHg on two occasions several hours apart, with proteinuria of >300 mg in a 24-h urine collection. 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 in a 24-h urine collection). The healthy pregnant women and preeclampsia patients were subdivided into two groups of overweight or obese (overweight group: BMI ≥25 kg/m²; n = 26) and normal-weight women (normal-weight group: BMI 18.5–25 kg/m²; n = 30), using BMI before pregnancy. Small-for-gestational age was defined as below the 10th percentile for gestational age in the Japanese standards. None of the patients with preeclampsia had any prior history of renal disorder or essential hypertension. The healthy pregnant women had no history of illness and no form of hypertension or renal disorder. The clinical records were reviewed carefully. Some patients were interviewed further at the time of sample collection, and those that did not meet the above criteria were excluded from the study. The study was approved by the Institutional Ethics Review Board of Okayama University Hospital, and all of the subjects provided informed consent. Blood samples were collected from the preeclampsia patients soon after disease onset. All of the preeclampsia patients underwent termination within 1 wk from the time of sample collection, owing to severe preeclampsia. None of the subjects received any medication before blood sampling. The samples from the healthy pregnant women were matched with those from the preeclampsia patients according to age, gestational week, parity, and BMI to avoid possible bias. All of the patients and the healthy controls visited Okayama University, and all blood samples (5 ml from each patient) were collected in the fasting state between 2007 and 2010. Immediately after sample collection, the serum and plasma were separated by centrifugation and stored at −80°C until analysis. The average time of freezer storage for the preeclampsia patients (2.0 ± 1.4 yr) did not differ significantly from that of the controls (2.1 ± 1.6 yr).

*Measurements of BNP, adipocytokines, and homeostasis model assessment as an index of insulin resistance.* The plasma concentration of BNP and serum levels of adiponectin and leptin were determined by ELISA (for BNP, Phoenix Pharmaceuticals, Burlingame, CA; for adipocytokines, R & D Systems, Minneapolis, MN) according to the corresponding manufacturer’s instructions. The ELISA kit for adiponectin was designed to measure total adiponectin, including low and high molecular weight. Fasting insulin and glucose levels were determined by fluorescence enzyme immunoassay (Tosoh, Tokyo, Japan) and the glucose oxidase method (Shino-Test Tokyo, 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 (24).

*Real-time quantitative PCR.* Total RNA was extracted from adipose tissues of the omentum and primary cultured adipocytes using TRizol reagent (Life Technologies, Carlsbad, CA). Real-time quantitative PCR was performed to measure the mRNA levels of leptin and adiponectin using a StepOne Real-time PCR System and a TaqMan RNA-to-C_{T} Gene Kit (Applied Biosystems, Carlsbad, CA). Specific primers for the human leptin, adiponectin, and β-actin gene sequences were purchased from Applied Biosystems. RNA samples (25 ng) were assayed in triplicate, using 15 pmol of gene-specific primers and 5 pmol of gene-specific probes. Since we observed that there were no significant differences in β-actin expression using another housekeeping gene, GAPDH, as a control (data not shown), human β-actin mRNA levels were measured as an internal control using a predeveloped TaqMan primer and a probe mixture (Applied Biosystems). The mRNA levels of the target genes were normalized by the β-actin mRNA levels.

*Primary culture of human adipocytes.* Two different visceral adipocytes derived from the omental adipose tissues of six obese or normal-weight women were obtained commercially together with culture medium (Zen-Bio, Research Triangle Park, NC). The obese donors were nonsmokers with a mean BMI of 29.0 kg/m² (range: 26.4–31.6 kg/m²) and an average age of 36 yr (range: 29–43 yr), whereas the normal-weight donors were nonsmokers with a mean BMI of 23.4 kg/m² (range: 21.9–24.8 kg/m²) and an average age of 33 yr (range: 27–40 yr). The cells were maintained in adipocyte medium composed of DMEM-Nutrient Mix F-12 medium (1:1, vol/vol), HEPES, FBS, penicillin, and streptomycin in a humidified 37°C incubator with 5% CO₂. The medium was changed every 2 days. Primary cultures of the adipocytes were used to examine the effects of BNP (Sigma-Aldrich, St. Louis, MO) and the functional guanylyl cyclase A type receptor antagonist HS142-1 (Kiyowa Hakko Kogyo, Shizuoka, Japan) on the expression and secretion of adiponectin and leptin. The mRNA expressions of adiponectin and leptin were determined by real-time PCR using specific primers, and the secretions of adiponectin and leptin were examined using the ELISA kits described above.

*Statistical analysis.* All values are expressed as means ± SD. Kruskal-Wallis and Scheffe’s tests were used for intergroup comparisons of the clinical parameters and the levels and expressions of BNP, adiponectin, and leptin. The correlations of BNP with adiponectin or leptin were examined using Spearman’s rank correlation. All statistical analyses were performed with StatView software (Abacus Concepts, Berkeley, CA). P values <0.05 were considered to indicate statistical significance.

**RESULTS**

*Characteristics of the preeclampsia patients and healthy controls.* When we compared variables such as gestational age, maternal age, smoking habit, and BMI before pregnancy between the preeclampsia patients and the controls, we found no significant differences. However, birth weight, small-for-gestational age, and systolic and diastolic blood pressures were significantly higher in the preeclampsia patients than in the healthy pregnant women (Table 1).
Plasma concentration of BNP and serum concentrations of adiponectin and leptin.

To evaluate the gestational pattern of the BNP level, we first examined the plasma BNP concentrations in healthy pregnant and nonpregnant women. There were no significant changes in the BNP levels before or during pregnancy (Fig. 1A). We also examined the plasma BNP level and serum levels of adiponectin and leptin in the preeclampsia patients and normal controls. The plasma concentration of BNP was increased significantly in the preeclampsia patients compared with the healthy pregnant women (Fig. 1B). The serum concentrations of both adiponectin and leptin were also increased significantly in the preeclampsia patients compared with the normal controls (Fig. 1, C and D). In the overall preeclampsia patients, there was a significant correlation between the BNP and adiponectin levels ($r = 0.7756$) but no significant correlation between the BNP and leptin levels ($r = -0.0137$) (Fig. 1, E and F). There were no significant correlations of BNP with the adiponectin and leptin levels in the normal controls (data not shown).

Plasma BNP and serum adiponectin and leptin levels in normal-weight and overweight patients in the preeclampsia and control groups. Next, to evaluate the associations among BNP, adipocytokines, and obesity, we examined angiogenic factors in normal-weight ($n = 30$) and overweight ($n = 24$) preeclampsia patients and controls. We found no significant differences in gestational age, maternal age, smoking habit, or BMI before pregnancy. There were significant differences in birth weight, small-for-gestational age, and systolic and diastolic blood pressures between the preeclampsia patients and controls in the normal-weight and overweight women and no significant differences except for BMI between the normal-weight and overweight preeclampsia patients (Table 2). There were no significant differences in the BNP levels between the normal-weight and overweight groups in both the preeclampsia patients and controls (Fig. 2A). The adiponectin level was significantly lower in the overweight compared with the normal-weight group in the preeclampsia patients but not in the normal controls (Fig. 2B). There were no significant differences in the leptin levels between the overweight and normal-weight groups in the preeclampsia patients and healthy controls (Fig. 2C). HOMA-IR in overweight preeclampsia patients was

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Preeclampsia</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>30.5 ± 4.8</td>
<td>30.7 ± 5.3</td>
<td>NS</td>
</tr>
<tr>
<td>Primigravida no. (%)</td>
<td>39 (69.6%)</td>
<td>39 (69.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>5 (8.9%)</td>
<td>6 (10.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>107 ± 16</td>
<td>169 ± 10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>68 ± 11</td>
<td>112 ± 8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body mass index</td>
<td>24.3 ± 2.7</td>
<td>24.5 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>37.2 ± 1.3</td>
<td>36.7 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at recruitment, wk</td>
<td>37.0 ± 1.3</td>
<td>36.4 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>2,604 ± 214</td>
<td>2,216 ± 341</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Small for gestational age (%)</td>
<td>5 (8.9%)</td>
<td>17 (30.4%)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 56$. NS, not significant.

Fig. 1. Plasma brain natriuretic peptide (BNP) and serum adiponectin and leptin levels in preeclampsia patients, healthy pregnant women, and nonpregnant women. A: plasma BNP levels in nonpregnant and healthy pregnant women. B–D: comparisons of BNP (B), adiponectin (C), and leptin levels (D) between healthy pregnant women and preeclampsia patients. Values are shown as means ± SD. *$P < 0.01$ compared with controls. E and F: correlations of BNP with adiponectin (E) and leptin (F) in preeclampsia patients. ○, Overall preeclampsia patients, including both normal-weight and overweight preeclampsia patients.

AJP-Endocrinol Metab • doi:10.1152/ajpendo.00548.2011 • www.ajpendo.org

Downloaded from http://ajpendo.physiology.org/ by 10.220.32.247 on October 14, 2017
significantly higher than that in normal-weight preeclampsia patients and normal-weight and overweight healthy pregnant women (Fig. 2). We observed no significant correlation of HOMA-IR with adiponectin level in preeclampsia patients (data not shown). There were positive correlations between the BNP and adiponectin levels in both the normal-weight (r = 0.8221) and overweight (r = 0.7855) preeclampsia patients, and the regression coefficient between BNP and adiponectin in overweight preeclampsia patients was lower than that in normal-weight preeclampsia patients (Fig. 2E). However, there were no significant correlations between the BNP and leptin levels in either the normal-weight or overweight preeclampsia patients (Fig. 2F).

### mRNA expressions of leptin and adiponectin in the omental adipose tissue.

The adiponectin gene was significantly upregulated in the omental adipose tissue of preeclampsia patients compared with that in healthy pregnant women (P < 0.01), and there was a significant difference in adiponectin mRNA expression between obese and normal-weight preeclampsia patients (Fig. 3A). There were no significant differences in leptin mRNA expression between the preeclampsia patients and normal controls or between the obese and normal-weight patients in either the preeclampsia patients or normal controls (Fig. 3B).

### Effects of BNP on the expression and secretion of adiponectin and leptin by primary cultured human adipocytes.

To investigate the effects of BNP on adiponectin and leptin pro-

---

### Table 2. Characteristics of normal-weight and overweight women with preeclampsia and healthy pregnancies

<table>
<thead>
<tr>
<th></th>
<th>Normal Weight (n = 30)</th>
<th>Preeclampsia</th>
<th>P value</th>
<th>Overweight (n = 26)</th>
<th>Preeclampsia</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>30.3 ± 4.1</td>
<td>30.5 ± 4.8</td>
<td>NS</td>
<td>30.7 ± 4.8</td>
<td>30.9 ± 5.4</td>
<td>NS</td>
</tr>
<tr>
<td>Primigravida no. (%)</td>
<td>20 (66.7%)</td>
<td>20 (66.7%)</td>
<td>NS</td>
<td>19 (73.1%)</td>
<td>19 (73.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>3 (10.0%)</td>
<td>3 (10.0%)</td>
<td>NS</td>
<td>2 (7.7%)</td>
<td>3 (11.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>106 ± 14</td>
<td>172 ± 13</td>
<td>&lt;0.01</td>
<td>110 ± 9</td>
<td>165 ± 8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>64 ± 13</td>
<td>113 ± 10</td>
<td>&lt;0.01</td>
<td>67 ± 10</td>
<td>110 ± 14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI before pregnancy, kg/m²</td>
<td>22.5 ± 3.2</td>
<td>22.2 ± 3.7</td>
<td>NS</td>
<td>26.3 ± 2.5</td>
<td>26.5 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>36.8 ± 1.3</td>
<td>36.2 ± 1.6</td>
<td>NS</td>
<td>37.2 ± 1.8</td>
<td>37.0 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>2,568 ± 144</td>
<td>2,145 ± 244</td>
<td>&lt;0.01</td>
<td>2,642 ± 259</td>
<td>2,310 ± 322</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Small for gestational age (%)</td>
<td>3 (10.0%)</td>
<td>11 (36.7%)</td>
<td>&lt;0.01</td>
<td>2 (7.7%)</td>
<td>4 (23.1%)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index.

---

Fig. 2. A–D: plasma BNP (A), serum adiponectin (B) and leptin levels (C), and homeostasis model assessment as an index of insulin resistance (HOMA-IR; D) in normal-weight (N) and overweight (O) women in preeclampsia (PE) patients and healthy pregnant women. Values are shown as means ± SD. **P < 0.01 compared with N PE patients; *P < 0.01 compared with N PE patients and N and O healthy pregnant women. E and F: correlations of BNP with adiponectin (E) and leptin (F) in both N and O PE patients. ○, N PE patients; ●, O PE patients.
duction in adipocytes, we incubated primary cultured human adipocytes with recombinant BNP at concentrations ranging from $10^{-11}$ to $10^{-8}$ mol/l. We found that BNP significantly enhanced adiponectin mRNA expression and adiponectin secretion into the medium in a dose-dependent manner, and these changes were completely inhibited by treatment with HS142-1 (Fig. 4, A and B). Moreover, BNP had significantly weaker effects on adiponectin mRNA expression and adiponectin secretion in adipocytes derived from obese compared with normal-weight women (Fig. 4, A and B). BNP had no significant effects

Fig. 3. mRNA expressions of adiponectin and leptin in adipose tissues of N ($n=6$) and O controls ($n=6$) and N PE ($n=6$) and O PE patients ($n=6$). Values are shown as means ± SD. *$P<0.01$ compared with controls; **$P<0.01$ compared with N controls; ***$P<0.01$ compared with O controls; #$P<0.01$ compared with N PE.

Fig. 4. $A$–$D$: effects of BNP on the mRNA expressions and secretions of adiponectin ($A$ and $B$) and leptin ($C$ and $D$). Values are shown as means ± SD. *$P<0.01$ compared with controls; #$P<0.01$ between adipocytes derived from N and O women.
on leptin mRNA expression or leptin secretion in adipocytes from either obese or normal-weight women (Fig. 4, C and D). No significant changes in leptin mRNA expression or leptin secretion were observed after treatment with HS142-1.

**DISCUSSION**

In this study, we have demonstrated that the BNP, adiponectin, and leptin levels in preeclampsia patients were significantly higher than those in women with normal pregnancies and that there was a significant difference in the adiponectin levels and HOMA-IR between normal-weight and overweight preeclampsia patients but not in the BNP and leptin levels. Adiponectin mRNA expression was increased in omental adipose tissues of preeclampsia patients compared with healthy pregnant women. There was also a significant difference in adiponectin mRNA expression between normal-weight and overweight preeclampsia patients but no difference in leptin expression. Moreover, BNP had significantly different effects on the mRNA expression and secretion of adiponectin in primary cultured adipocytes derived from obese and normal-weight women.

We observed no significant changes in the plasma BNP levels among nonpregnant women and pregnant women in the first, second, and third trimesters, although the intravascular volume was increased in late pregnancy and the BNP level was increased significantly in the preeclampsia patients compared with the normal controls, consistent with previous reports (7, 8, 30, 32). In addition, adiponectin was increased significantly in the preeclampsia patients compared with the controls, as reported previously (28, 31, 39). We also demonstrated that BNP was significantly correlated with adiponectin, but not with leptin, in preeclampsia patients, suggesting that some interactions between BNP and adiponectin may exist in preeclampsia patients, similar to the case for patients with CHF (9, 14, 41).

Next, we examined the associations among BNP, adipocytes, and obesity. We observed a significant difference in adiponectin between normal-weight and overweight preeclampsia patients. Although the BNP level was decreased significantly in obese patients with CHF and/or hypertension in a previous study (1), the BNP level in overweight preeclampsia patients was decreased, but not significantly, compared with normal-weight patients in the present study. Interestingly, significant correlations of BNP with adiponectin were also found in both normal-weight and overweight preeclampsia patients, although the correlations had different regression coefficients. These findings suggest that there may be different levels of response to the elevated BNP level for adiponectin production between normal-weight and overweight preeclampsia patients. We also observed that HOMA-IR in overweight preeclampsia patients was significantly higher than that in normal-weight preeclampsia patients, consistent with ours and other previous reports (4, 22). The poor response to BNP in adiponectin production in overweight preeclampsia patients might enhance insulin resistance, which results in endothelial dysfunction.

In our study, different expression levels of adiponectin mRNA in adipose tissues were found between preeclampsia patients and normal controls, and there was a significant difference in adiponectin mRNA expression in adipose tissues between overweight and normal-weight preeclampsia patients. We could not detect the adiponectin gene expression in placenta of either normal or preeclampsia patients by real-time PCR (data not shown), and adiponectin appears to be expressed exclusively in and secreted from adipose tissue (38), suggesting that elevated adiponectin might be derived mainly from adipose tissue during pregnancy. Therefore, we examined the effects of BNP and the different responses to BNP for adiponectin production in adipocytes derived from obese and normal-weight women. As reported previously using subcutaneous adipocytes (42), BNP significantly enhanced the mRNA expression and secretion of adiponectin in omental adipocytes in a ligand-dependent manner, and treatment with HS142-1, a functional guanylyl cyclase A receptor antagonist, abolished the effects of BNP, suggesting that BNP enhanced adiponectin expression in omental adipocytes through a guanylyl cyclase A receptor. In addition, we observed different responses to BNP for the mRNA expression and secretion of adiponectin in omental adipocytes derived from normal-weight and obese women for physiological concentrations of BNP because the physiological level of maternal circulating BNP is between $10^{-10}$ and $10^{-11}$ M. These data suggest that elevation of BNP may enhance adiponectin production in adipose tissues in preeclampsia patients and that different response levels to BNP for adiponectin production may cause the difference in maternal adiponectin levels between normal-weight and overweight preeclampsia patients.

Although BNP was linearly related to the left ventricular functional changes observed in preeclampsia (2), and plasma adiponectin was correlated with the severity of ventricular dysfunction in CHF (27), we did not obtain data about the relationships of left ventricular function with BNP and adiponectin in the present study. Moreover, there was no information about the origin of the elevated BNP. Therefore, further analyses are required to examine the relationships among BNP, adiponectin, and left ventricular function and the mechanism of the BNP elevation, including the origin, in preeclampsia patients.

Taken together, our results suggest that BNP may play a role in the hyperadiponectinemia of preeclampsia patients, and the weak response to BNP for adiponectin production may participate in the pathophysiology of overweight preeclampsia patients. This was only a small-scale cross-sectional study of Japanese patients with preeclampsia, and therefore, examination of larger sample sizes will improve the precision of our findings. And because we have only a small number of patients with gestational hypertension in the same period, it is not enough to investigate and compare the BNP and adiponectin levels in these preeclampsia patients and normal controls. Further analysis will be required to examine whether these factors might also play some roles in gestational hypertension. Moreover, additional experiments will be necessary to examine whether the differences of adiponectin and HOMA-IR and the impaired release of adiponectin in response to BNP really have functional significance in the pathophysiology of preeclampsia.

**GRANTS**

This work was supported in part by a research grant (22591856) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

H.M. did the conception and design of the research; H.M., E.N., and S.I. performed the experiments; H.M., E.N., and S.I. analyzed the data; H.M.
interpreted the results of the experiments; H.M. prepared the figures; H.M. drafted the manuscript; H.M. and Y.H. edited and revised the manuscript; H.M., E.N., S.I., and Y.H. approved the final version of the manuscript.

REFERENCES