Alterations of serum resistin in normal pregnancy and pre-eclampsia

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ABSTRACT
Resistin is expressed in human placenta and has been postulated to play a role in regulating energy metabolism in pregnancy. However, changes in serum resistin levels in normal pregnancy and in the setting of pre-eclampsia are far from understood. The purpose of the present study was to clarify the alterations in serum resistin level in normal pregnancy and pre-eclampsia. Blood samples were taken from 28 healthy non-pregnant women, 27 women in the first, 26 in the second and 26 in the third trimesters of normal pregnancy and 25 women with pre-eclampsia. Serum resistin concentrations were determined by using an ELISA, and mean serum resistin levels were compared with one-way ANOVA. Serum resistin levels were not significantly different among non-pregnant women and women in the first and second trimesters of pregnancy (P > 0.05 for all). Serum resistin was significantly elevated in the third trimester of normal pregnancy compared with non-pregnant women (P < 0.001) and women in the first (P < 0.001) and second (P < 0.001) trimesters of pregnancy. Serum resistin level was significantly lower in women with pre-eclampsia than women in the third trimester of normal pregnancy (P < 0.001), but was comparable with those of non-pregnant women and women in the first and second trimesters of pregnancy (P > 0.05 for all). In conclusion, we found an increase in serum resistin in the third trimester of normal pregnancy, but this increase was not present in pre-eclampsia. We postulate that these associations may offer insight into the mechanisms of maternal adaptation to pregnancy and the pathogenesis of pre-eclampsia.

INTRODUCTION
Dramatic changes in metabolism occur during pregnancy to meet the demands of fetal growth and to prepare energy reserves for delivery and breastfeeding. Altered insulin sensitivity is among these metabolic changes [1,2]. Insulin resistance is observed in normal pregnancy and is maximal in the third trimester [2–7]. Insulin resistance has been proposed to be exaggerated in pre-eclampsia compared with normal pregnancy and to play a role in the pathogenesis of the disease [2–7], although different opinions exist [2,4,8,9]. Factors, including human placental lactogen, prolactin, insulin-like growth hormones, steroid hormones and leptin, have been proposed to be involved in the maternal adaptation to pregnancy and make contributions to insulin resistance in pregnancy and pre-eclampsia [1,2,4,10,11].

Resistin is a novel hormone that is secreted by human adipocytes and mononuclear cells and is probably associated with insulin resistance. Resistin impairs glucose intake by adipocytes, increases plasma glucose concentration and thus decreases insulin sensitivity. In the setting of non-pregnancy, the serum resistin level is significantly higher in obese subjects than in lean ones and, thus, resistin has been proposed to link obesity to insulin resistance. Resistin is expressed in the human placenta and has been postulated to play a role in regulating energy metabolism in pregnancy [10,12];
Table 1 Maternal age, gestational age and BMI in the study subjects

Values are means (range) or means ± S.E.M. No significant difference was found among groups for maternal age. The gestational age and BMI were not significantly different (P > 0.05) between the third trimester of normal pregnancy and pre-eclampsia. *P < 0.05 compared with non-pregnant women; †P < 0.05 compared with women in the first trimester; ‡P < 0.05 compared with women in the second trimester.

<table>
<thead>
<tr>
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<th>Non-pregnant women</th>
<th>First trimester</th>
<th>Second trimester</th>
<th>Third trimester</th>
<th>Pre-eclampsia</th>
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<td>27</td>
<td>26</td>
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<tr>
<td>Gestational age (weeks)</td>
<td>—</td>
<td>9.6 (6–12)</td>
<td>19.2 (13–26)</td>
<td>36.8 (31–41)</td>
<td>35.9 (29–41)</td>
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<td>BMI (kg/m²)</td>
<td>20.0 ± 0.10</td>
<td>21.9 ± 0.11</td>
<td>23.4 ± 0.13</td>
<td>25.9 ± 0.12‡</td>
<td>27.4 ± 0.16‡‡</td>
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however, the changes in serum resistin levels in normal pregnancy and in the setting of pre-eclampsia are far from understood.

To obtain insight into the relationship of resistin to normal pregnancy and pre-eclampsia, we conducted the present investigation to measure serum levels of resistin in healthy non-pregnant women, women in the first, second and third trimesters of normal pregnancy and women with pre-eclampsia.

MATERIALS AND METHODS

Subjects
A cross-sectional study was conducted in the Women’s Hospital of Zhejiang University, according to the protocol approved by the Hospital Ethics Committee. Informed consent was obtained from all the participants. In the present study, 28 healthy non-pregnant women, 27 women in the first trimester, 26 in the second trimester and 26 in the third trimester of normal pregnancy, and 25 women with pre-eclampsia were recruited. All subjects were nulliparous Chinese women. Both gestational age and BMI (body mass index; weight/height²) were matched for the third trimester group and pre-eclampsia group at the time of sampling. Inclusion criteria were healthy non-pregnant women of reproductive age, women in the first, second and third trimesters of normal pregnancy and women with pre-eclampsia. Pregnancy was diagnosed based on missed menstruation and positive hCG (human chorionic gonadotropin) test. Gestational age was calculated according to the last menstrual period and was confirmed by ultrasound in early pregnancy. Pre-eclampsia was diagnosed according to strict criteria: a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg on two occasions at least 6 h apart occurring after 20 weeks of gestation in a pregnant woman with previously normal blood pressure and detectable urinary protein (≥1 by dipstick or ≥0.3 g/24 h). Exclusion criteria were multiple gestation, diabetes mellitus, chronic hypertension, infectious diseases recognized in pregnancy, premature rupture of membrane, active labour, polyhydramnios and signs of other concurrent medical complications.

Sample collection and resistin analysis
One fasting blood sample was taken from each subject. All samples were kept at room temperature for at least 30 min to allow the blood to clot and were then centrifuged at 2000 g for 15 min. Serum was collected and stored at −80 °C until assayed. Resistin was determined by ELISA (BioVendor Laboratory Medicine; Brno, Czech Republic), and assays were conducted according to the manufacturer’s instructions. The sensitivity for the determination was 0.2 ng/ml. When a serum pool was used as a control, intra- and inter-assay coefficients of variation were <4.0 % and 7.2 % respectively.

Data analysis
Serum resistin concentrations were normally distributed, as determined using the Kolmogorov–Smirnov test (Z = 0.752 for non-pregnancy, 0.761 for the first trimester, 0.677 for the second trimester, 0.636 for the third trimester of pregnancy and 1.204 for pre-eclampsia; P > 0.05 for all), and are presented as means ± S.E.M. One-way ANOVA and Bonferroni’s multiple comparison test were used to compare means. Maternal age and gestational age are presented as means and range. Prism 3.0 (GraphPad Software, San Diego, CA, U.S.A.) was used for data analysis. P < 0.05 was considered significant.

RESULTS
The maternal age, gestational age and BMI at the time of sampling are summarized in Table 1. Mean ages were not significantly different among the five groups, and the gestational ages at sampling were similar between women in the third trimester of normal pregnancy and those with pre-eclampsia (P > 0.05). BMI was significantly different in the three trimesters of normal pregnancy (P < 0.001); however, BMI was not significantly different between pre-eclampsia and the third trimester of normal pregnancy.
normal pregnancy ($P > 0.05$). As expected, women with pre-eclampsia delivered at a significantly ($P < 0.001$) earlier gestational age [37.0 (33–41) weeks] than normal pregnant women in the third trimester [40.0 (38–41) weeks], and birth weights were significantly ($P < 0.001$) lower in the pre-eclampsia group (2197 ± 136 g) than in the third trimester group (3495 ± 97 g).

Serum resistin levels were not significantly different among non-pregnant women (5.450 ± 0.418 ng/ml) and women in the first (6.660 ± 0.546 ng/ml) and second (5.085 ± 0.461 ng/ml) trimesters of pregnancy ($P > 0.05$ for all). However, serum resistin levels were significantly increased in women in the third trimester of pregnancy (8.659 ± 0.698 ng/ml) compared with non-pregnant women ($P < 0.01$) and women in the first and second trimesters of pregnancy ($P < 0.001$ for both). Women with pre-eclampsia had significantly ($P < 0.001$) lower levels of serum resistin (5.162 ± 0.484 ng/ml) compared with those in the third trimester of normal pregnancy. Serum resistin levels in pre-eclampsia were not significantly different when compared with levels in non-pregnant women and women in the first and second trimesters of normal pregnancy ($P > 0.05$ for all).

**DISCUSSION**

In the present study, we report for the first time serum resistin concentrations in different trimesters of normal pregnancy and in pre-eclampsia. Our findings reveal that the serum resistin level is comparable among non-pregnant women and those in the first and second trimesters of normal pregnancy, but is significantly higher in the third trimester of normal pregnancy. This trend is consistent with the change in BMI during pregnancy. However, in contrast with that found in the third trimester of normal pregnancy, an increase in serum resistin levels in women with pre-eclampsia was not observed.

Our finding of elevated serum resistin level in the third trimester of pregnancy is consistent with the study by Yura et al. [12], who found that resistin expression was greater in term placentas than in chorionic villi of early pregnancy and that blood resistin level was significantly higher in normal term pregnant women than in age-matched healthy non-pregnant women. In a very preliminary observation (M. Dong, D. Chen and H. Wang, unpublished work), we have found that resistin was detected in a trophoblast culture supernatant (approx. 20 ng/ml in Dulbecco’s modified Eagle’s medium after 72 h of culture at a cell concentration of $1 \times 10^6$ cells/ml) by using an ELISA, which confirms the production of resistin by the human placenta. However, the exact mechanisms behind the increase in serum resistin during pregnancy have not been clarified. Based on the findings of Yura et al. [12] and the fact that there is an increase in placenta mass with gestation, it is reasonable to speculate that resistin production by the placenta is the main cause of the increase in serum resistin. Considering that the adipose tissue mass increases during pregnancy and that the expression of resistin in adipose tissue of pregnant women at term does not differ from that of non-pregnant women [10], resistin production by adipose tissue might be one of the causes for the increased serum resistin level in pregnancy. However, we do not know precisely why the serum resistin level was lower in pre-eclampsia. This may be due to the smaller size of the placenta, but we could not exclude the possibility of lower expression of resistin by the placenta in pre-eclampsia. To confirm this possibility, the determination of placental expression of resistin is needed.

The second half of pregnancy is a state of insulin resistance [2,4,6–8,13]. Resistin, along with human placental lactogen, prolactin, steroid hormones and other hormones, decreases insulin sensitivity, whereas leptin increases insulin sensitivity [2,10,12]. The increase in serum resistin in the third trimester of pregnancy is in accordance with insulin resistance in normal pregnancy [4,7,13–15]. This implies that resistin may participate in the regulation of metabolism in pregnancy. Furthermore, pre-eclampsia has been proposed to be an exaggeration of insulin resistance [2], although differing opinions exist [16]. When the role of resistin is linked to the state of insulin resistance in pre-eclampsia, it is paradoxical that the level of serum resistin in pre-eclampsia was significantly lower than that of gestational age- and BMI-matched normal pregnant women. This implies that resistin may not be the main determinant of insulin resistance in pre-eclampsia and that the absence of an increase in serum resistin appears to be a consequence, rather than the cause, of the disease.

However, pre-eclampsia has also been proposed to be a state of leptin resistance [17–19]. Serum levels of leptin [17–19] and adiponectin [20], both of which are adipocyte-derived hormones and increase insulin sensitivity, were reported to be elevated in pre-eclampsia. The alterations in serum resistin, adiponectin [20] and leptin [19] are in accordance with leptin resistance, but contrast with the exaggerated insulin resistance in pre-eclampsia. This highlights the importance of evaluating the roles of these and other associated hormones in the pathogenesis of pre-eclampsia.

In conclusion, we have shown for the first time that the resistin concentration is elevated markedly in the third trimester of normal pregnancy, whereas this increase does not occur in pre-eclampsia, perhaps as a result of a change in placental production of resistin. These data are of major interest given the intense interest in the role of resistin in human metabolism. Therefore elucidating the exact mechanisms for such changes will give further insights into the pathogenesis of pre-eclampsia. Moreover, further studies are required to determine if...
lower serum resistin levels occur before the onset of pre-eclampsia.

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REFERENCES


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