Circulating levels of adiponectin and leptin at 23–25 weeks of pregnancy in women with impaired placentation and in those with established fetal growth restriction

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Abstract
Adiponectin and leptin, two adipose-tissue-derived proteins, have been reported to be elevated in women with established PE (pre-eclampsia). The aim of the present study was to investigate whether alterations in adiponectin and leptin levels predate the development of PE and FGR (fetal growth restriction) in women at increased risk of these complications, as assessed by Doppler examination of the uterine arteries during the second trimester of pregnancy. We also sought to investigate the circulating levels of adiponectin and leptin in women with established severe early-onset FGR. The study included three groups of pregnant women at 23–25 weeks: Group A (n = 44) with normal uterine artery Doppler waveforms, Group B (n = 49) with abnormal Doppler waveforms and normal fetal growth at the time of the examination, and Group C (n = 15) with established severe FGR and abnormal Doppler waveforms. All women had plasma adiponectin and leptin measured by sensitive immunoassays. In Group B, 19 women had a normal outcome, 17 delivered infants with FGR and 13 developed PE. The women who developed PE delivered smaller babies earlier than women with a normal outcome (P < 0.001). There were no significant differences in adiponectin levels between any of the groups (overall P = 0.3). Leptin concentrations, expressed as MoM (multiples of the median) of Group A, were higher in women in Group C, i.e. established severe FGR at 2.5 (1.2–2.7) MoMs (overall P < 0.001), compared with all of the other groups and subgroups. In conclusion, we found that, in pregnancies complicated by severe early-onset FGR, the maternal plasma concentration of leptin is twice as high as in normal pregnancies. However, the second trimester levels of maternal plasma adiponectin and leptin in pregnancies that subsequently develop PE and/or FGR are not significantly different from normal and, consequently, it is unlikely that these markers will be useful as predictors of these pregnancy complications.

Introduction
PE (pre-eclampsia) is a major cause of maternal and perinatal mortality and morbidity [1]. Although its exact pathophysiology remains elusive, the widely accepted model is that of a hypoxic placenta, which releases factor(s) in the maternal circulation that cause endothelial dysfunction, resulting in hypertension and proteinuria [2]. Inflammatory up-regulation and adipocyte lipolysis are also probably involved [3–7]. FGR (fetal growth...
restriction), another major cause of perinatal morbidity and mortality, appears to share the same pathophysiology with PE. In both conditions there is histological evidence of impaired trophoblastic invasion of the uterine spiral arteries and Doppler evidence of increased impedance to flow in the uterine arteries [8,9].

Adiponectin and leptin are two adipose-tissue-derived proteins with important metabolic effects. The plasma concentration of adiponectin, which is derived mainly from adipose tissue and possibly from placenta, is inversely correlated with insulin resistance and is consequently reduced in obesity and Type 2 diabetes, but it is paradoxically increased in PE [10–16].

Leptin, a protein regulating body weight, is produced in large amounts by adipocytes and human placental trophoblasts [17]. Leptin gene expression in the placenta is augmented in severe PE and it has been suggested that placental hypoxia may play a role in this augmentation [18]. Maternal leptin levels have also been reported to be elevated in women with established severe PE and in women destined to develop the condition, but not in women who subsequently delivered infants with FGR [6,19].

The aim of the present study was to investigate whether alterations in adiponectin and leptin levels predate the development of PE and FGR in women at increased risk of these complications, as assessed by Doppler examination of the uterine arteries during the second trimester of pregnancy. We also sought to investigate the circulating levels of adiponectin and leptin in women with established severe early-onset FGR.

MATERIALS AND METHODS

Study participants
This was a retrospective study that included three groups of healthy women with singleton pregnancies at 23–25 weeks of gestation. They were all recruited consecutively from the Ultrasound Department of King’s College Hospital, London, U.K., where colour Doppler examination of the uterine arteries is routinely performed, as described previously [9]. Group A included 44 women with normal uterine artery Doppler waveforms and normally grown fetuses; Group B included 49 women with abnormal uterine artery Doppler waveforms (presence of early diastolic notch bilaterally) and normally grown fetuses at the time of the examination; and Group C included 15 women with estimated fetal weight <5th centile for gestation at the time of the examination (all of them had abnormal uterine artery Doppler waveforms; 11 of these fetuses had absent or reversed end-diastolic flow in the umbilical artery and eight of these fetuses had reduced amniotic fluid). The fetal weight was estimated from the sonographic measurements of head circumference, abdominal circumference and femur length [20].

Maternal age, ethnic group, smoking status, parity, BMI (body mass index) and BP (blood pressure) were recorded. BP was measured in the right arm with the subject seated using an ambulatory BP monitor (SpaceLabs Medical 90207). Three measurements were taken and averaged.

The study was approved by the Local Research Ethics Committee, and all women gave written informed consent.

Analysis of adiponectin and leptin
All women had a venous blood sample taken and plasma was separated by centrifugation, frozen and stored at –80 °C. Plasma adiponectin concentration was determined by ELISA (R&D Systems). The intra- and inter-assay coefficients of variability were both 7.5 %. The minimum detectable concentration was 0.01 μg/ml. Leptin was measured using an in-house RIA that was validated thoroughly against the commercially available Linco assay [21]. In this case, the intra- and inter-assay coefficients of variation were < 7 and < 10 % respectively, over the sample concentration range, and the detection limit of the assay was 0.5 ng/ml.

Definition of clinical outcome
Information on pregnancy outcome, including gestation at delivery, birth weight and gender, were obtained from examination of individual patient hospital records. The diagnosis of PE was made according to the criteria of the International Society for the Study of Hypertension in Pregnancy. Under this classification, PE is defined as a DBP (diastolic BP) of at least 110 mmHg on one occasion or a DBP of at least 90 mmHg on two consecutive occasions more than 4 h apart in combination with proteinuria (≥300 mg of total protein in a 24-h urine collection or, if this was not available, ≥+2 proteinuria by dipstick on two consecutive occasions at least 4 h apart), developing after 20 weeks of gestation in previously normotensive women [22]. The diagnosis of FGR was based on the delivery of an infant with a body weight below the 5th percentile for gestation and gender [23].

Statistical analysis
Normality of the distribution of continuous data was examined with the Shapiro–Wilk test. Log-transformation was performed for non-normally distributed data. Descriptive data are expressed as means ± S.D. or as medians (interquartile range) for normally and non-normally distributed data respectively. Comparisons between groups were performed using ANOVA, followed by the Bonferroni post-hoc test, Kruskal–Wallis test or Mann–Whitney test as appropriate. The χ² test was used to compare categorical variables among groups. In order to compare the levels of adiponectin and leptin in the different groups of women, adjusting for BMI, smoking
and ethnic group, variables that are known determinants of both adiponectin and leptin [10,21,24–26], the following steps were taken: (i) patients were subdivided into five groups according to the outcome of pregnancy (Group A: normal Doppler/normal outcome; Group B: abnormal Doppler/normal outcome, abnormal Doppler/development of FGR and abnormal Doppler/development of PE; and Group C: abnormal Doppler/established FGR); (ii) linear regression analysis was used to determine which factors among the maternal demographic and clinical characteristics were significant predictors of the adiponectin and leptin levels in Group A; (iii) the distribution of adiponectin and leptin levels, expressed as MoM (multiples of the median) of Group A, were determined for all of the subgroups in Group B and Group C; and (iv) owing to the small number of patients, case even after exclusion of the three women in Group C who developed PE (results not shown).

Levels of adiponectin and leptin
The multiple regression equations for adiponectin and leptin levels in Group A are: adiponectin = 17.008 – 0.264 × BMI (kg/m²) + (3.434 if smokers, 0 if non-smokers) + (1.599 if Caucasian, – 0.910 if Afro-Caribbean, 0 if any other ethnic group) (R² = 0.305, P = 0.006); and leptin = –27.915 + 2.744 × BMI (kg/m²) – (4.626 if smokers, 0 if non-smokers) – (15.699 if Caucasian, 18.472 if Afro-Caribbean, 0 if any other ethnic group) (R² = 0.665, P < 0.001).

For each patient and using the formulas above, the expected adiponectin and leptin levels and the ratio of the observed to expected value were calculated. The concentrations of adiponectin and leptin (expressed as raw values and MoMs of the unaffected Group A) in the different groups of women are shown in Table 2. All of the values and MoMs of the unaffected Group A) in the different groups of women are shown in Table 2. All of the

### Table 1: Demographic, clinical, maternal and neonatal characteristics in each group of women, according to the outcome of pregnancy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normal outcome</td>
<td>Development of FGR</td>
<td>Development of PE</td>
</tr>
<tr>
<td>n</td>
<td>44</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>30 (25–33)</td>
<td>27 (21–31)</td>
<td>28 (21–34)</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>9 (20%)</td>
<td>2 (10.5%)</td>
<td>3 (17.6%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.0 (24.0–30.0)</td>
<td>26.5 (23.5–31.0)</td>
<td>26.0 (22.0–26.5)</td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White (n)</td>
<td>23 (52%)</td>
<td>9 (47.4%)</td>
<td>8 (47.1%)</td>
</tr>
<tr>
<td>Afro-Caribbean (n)</td>
<td>18 (41%)</td>
<td>9 (47.4%)</td>
<td>6 (35.3%)</td>
</tr>
<tr>
<td>Others (n)</td>
<td>3 (7%)</td>
<td>1 (5.3%)</td>
<td>3 (17.6%)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>114 ± 8</td>
<td>114 ± 7</td>
<td>111 ± 7</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>66 ± 6</td>
<td>65 ± 16</td>
<td>64 ± 4.5</td>
</tr>
<tr>
<td>Gestation at delivery (week)</td>
<td>39.0 (38.6–40.4)</td>
<td>41.0 (39.0–41.0)</td>
<td>40.0 (37.0–40.5)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3323 ± 513</td>
<td>3254 ± 407</td>
<td>3200 ± 596</td>
</tr>
</tbody>
</table>

### Results

#### Characteristics of the study participants

In Group A (normal Doppler findings; n = 44), none of the women developed PE and all of them delivered babies of appropriate size. The women in Group B (abnormal Doppler findings and normal fetal growth at presentation; n = 49) were subclassified into three groups according to the outcome of pregnancy: (i) those with no complications (n = 19; 39%), (ii) those who delivered infants with FGR (n = 17; 35%), and (iii) those who developed PE (n = 13; 26%). In Group C (abnormal Doppler findings and FGR at presentation; n = 15), three women had PE and in nine cases there was intra-uterine or early neonatal death. The demographic and clinical characteristics of the groups, obtained at study entry, are shown in Table 1. SBPs (systolic BPs) and DBPs were significantly higher in women who eventually developed PE and in women with established FGR. This was the case even after exclusion of the three women in Group C who developed PE (results not shown).
Table 2  Levels of adiponectin and leptin, expressed as raw values and as MoM of the unaffected Group A, in each group of women according to the outcome of pregnancy

Values are means ± S.D., or medians (interquartile range). Group A, women with normal Doppler examination and normal outcome; Group B, women with abnormal Doppler examination, with subgroups according to the pregnancy outcome; Group C, women with abnormal Doppler examination and established FGR at 23–25 weeks of gestation. *P < 0.01 and †P < 0.001 compared with Group C.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A Normal outcome</th>
<th>Group B Normal outcome</th>
<th>Development of FGR</th>
<th>Development of PE</th>
<th>Group C Established FGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>44</td>
<td>19</td>
<td>17</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Adiponectin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (μg/ml)</td>
<td>10.8 ± 4.8</td>
<td>9 ± 3.9</td>
<td>11.1 ± 4.3</td>
<td>8.8 ± 2.3</td>
<td>12.7 ± 5.8</td>
</tr>
<tr>
<td>MoM of Group A</td>
<td>0.9 (0.7–1.2)</td>
<td>0.8 (0.5–1.0)</td>
<td>0.9 (0.7–1.1)</td>
<td>0.9 (0.6–0.9)</td>
<td>0.9 (0.7–1.2)</td>
</tr>
<tr>
<td>Leptin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (ng/ml)</td>
<td>31.4 ± 18.3 *</td>
<td>28.9 ± 16.2 *</td>
<td>27.2 ± 13.6 *</td>
<td>34.9 ± 14.5</td>
<td>52.2 ± 33.4</td>
</tr>
<tr>
<td>MoM of Group A</td>
<td>0.9 (0.7–1.2)†</td>
<td>1.0 (0.8–1.2)†</td>
<td>1.0 (0.8–1.4)†</td>
<td>1.0 (0.8–1.3)†</td>
<td>2.5 (1.2–2.7)</td>
</tr>
</tbody>
</table>

Figure 1  Box and whisker plot comparing leptin levels, expressed as MoMs of the unaffected Group A, at 23–25 weeks of gestation in the different patient groups

Group A (n = 44), women with normal Doppler and normal outcome; Group B (n = 49), with abnormal Doppler and either normal outcome (n = 19), subsequent development of FGR (n = 17) or subsequent development of PE (n = 13); and Group C (n = 15) with abnormal Doppler and established FGR. Boxes represent the interquartile range and the line represents the median. Whiskers at the top and bottom of the box represent the highest and lowest values. Group C had the highest leptin levels compared with all of the other groups (*P < 0.001).

Discussion

The present study has demonstrated that maternal levels of adiponectin during the second trimester of pregnancy do not appear to predate the development of PE and/or FGR in women at risk of these complications, as assessed by Doppler examination of the uterine arteries. Furthermore, maternal adiponectin levels are not different between women with established early-onset severe FGR and women with uncomplicated pregnancies.

We have shown previously that women who subsequently develop PE have evidence of endothelial dysfunction at 23–25 weeks, as assessed by flow-mediated dilation of their brachial artery [27]. Adiponectin may be considered as a useful marker of endothelial function as it attenuates the excessive inflammatory response in the vascular wall [28] and it also increases NO production by activating and increasing the expression of eNOS (endothelial NO synthase) [29].

In the present study, the lack of a difference in the adiponectin levels between women who subsequently develop PE and/or FGR compared with those with uncomplicated pregnancies may suggest that either adiponectin does not play a fundamental role in the development of PE or that the second trimester of pregnancy is not a good time to use adiponectin as a marker of PE. Studies have demonstrated that the adiponectin concentration decreases throughout normal pregnancy [30] and that its level, in women who subsequently develop PE, is lower in the first and higher in the third trimester compared with women with uncomplicated pregnancies [12,31]. Taken together, these findings indicate that, in women who develop PE later on in pregnancy, the adiponectin levels in mid-pregnancy may overlap with
those of the women with uncomplicated pregnancies. Consequently, despite the fact that adiponectin may play a role in the development of PE, it is unlikely to be a useful biomarker for it in mid-pregnancy. In line with this, we were not able to demonstrate a significant difference in the adiponectin concentration between women with established FGR and women with normal pregnancies. This was the case even after exclusion of the three women in this group who had developed PE.

Another finding of the present study is that maternal leptin concentrations do not appear to predate the development of PE and/or FGR; however, its levels were increased in women with established FGR.

Leptin is a peptide hormone which was introduced as an adipocyte-derived messenger of energy metabolism [32]. The maternal leptin concentration has been reported to be elevated in women with established severe PE and especially in those who delivered infants with FGR as well [18,33]. Longitudinal studies have demonstrated that increases in maternal leptin levels occur weeks prior to the clinical manifestation of PE [6,19]. However, these studies did not comprehensively adjust for potential differences in maternal weight, smoking and ethnic group, factors that are strongly linked to leptin levels [21,25,26]. In the present study, we expressed the levels of adiponectin and leptin as MoMs of the unaffected groups and, consequently, we were able to adjust for all of the potential confounders. We were unable to detect any difference in maternal leptin levels between women who subsequently developed PE and those with uncomplicated pregnancies, and this is in accordance with other studies [34].

Maternal levels of leptin in women with established early-onset FGR were twice as high as in the other groups. Usually FGR is defined as a fetus below a certain weight percentile. This definition includes fetuses with a low genetic growth potential, who do not have a placent al problem, and fetuses that do not reach their growth potential due to placental insufficiency. All of the pregnancies with FGR (Group C) included in our present study were characterized by impaired placentation. However, it has to be mentioned that others have not found elevated maternal levels of leptin in pregnancies with FGR [35,36]. The reason for this inconsistency could be due to the above-mentioned difficulties in defining FGR. Certainly, we cannot exclude that some of the pregnancies with FGR would have developed PE if they did not have their pregnancies terminated. The tendency of elevated maternal BP in pregnancies with FGR when compared with controls, even after exclusion of those women who definitely developed PE, indicate that some of the pregnancies with FGR were possibly close to the threshold of PE and this has to be considered in the interpretation of the data. Therefore it could be stated that, although leptin may play a role in the clinical manifestation of established FGR, it is unlikely that this adipokine plays a fundamental role in the initiation of this condition and, therefore, it is unlikely that leptin alone would be a useful biochemical marker in screening for the development of either PE or FGR.

In summary, maternal levels of adiponectin and leptin during the second trimester of pregnancy do not appear to predate the development of PE and/or FGR and, consequently, it is unlikely that these biochemical markers will be useful in allowing improved prediction of the pregnancy disorders described. However, elevated levels of maternal leptin, but not adiponectin, appear to characterize pregnancies complicated by severe early-onset FGR.

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