Longitudinal analysis of maternal plasma leptin concentrations during normal pregnancy and pre-eclampsia

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Leptin concentrations have been found to be elevated in cross-sectional studies of established pre-eclampsia. Circulating concentrations of leptin were measured in a cross-sectional study to confirm these findings (19 women with pre-eclampsia and 13 normal pregnant controls) and in a longitudinal study to establish the timing of the increase in leptin concentrations (samples obtained at 16, 20, 24, 28, 32, 36 and 38 weeks gestation from eight women who went on to develop pre-eclampsia and seven normal pregnant controls). In the cross-sectional study, plasma leptin concentrations were significantly greater in women with pre-eclampsia than in normal controls (P = 0.001). In the longitudinal study, it was found that circulating leptin concentrations rose gradually to 32 weeks and thereafter declined slightly in normals. The concentrations in women destined to develop pre-eclampsia were consistently higher from 20 weeks gestation (P = 0.04–0.003) and, in contrast to the normal controls, rose markedly from 32 weeks as pre-eclampsia developed. This study confirms that plasma leptin concentrations are increased in established pre-eclampsia and reports for the first time that leptin concentrations are elevated before pre-eclampsia is clinically evident.

Key words: leptin/pre-eclampsia/pregnancy

Introduction

Leptin, a protein product of the obesity (Lep°b) gene, is synthesized and secreted by adipocytes (Halaas et al., 1995; Masuzaki et al., 1997). It is also synthesized by the placenta and may contribute to the circulating leptin concentrations during pregnancy (Masuzaki et al., 1997; Mise et al., 1998). The significant increase in maternal circulating leptin during the first and second trimesters of normal pregnancy is suggested to be in response to the marked changes in maternal weight, energy expenditure and hormonal status. However, recent data show that the increase occurs in early pregnancy, before any changes in body fat or resting metabolic rate, supporting the idea that hormonal factors may be responsible (Highman et al., 1998). Cross-sectional data show plasma concentrations of leptin are much higher in pregnancies complicated by pre-eclampsia compared to normal controls (McCarthy et al., 1999). However, the behaviour of circulating leptin concentrations before pre-eclampsia develops has not been studied. This study was carried out to confirm the reported increase in plasma leptin in pre-eclampsia (McCarthy et al., 1999), to investigate the changes in plasma leptin concentrations with the development of pre-eclampsia and to relate leptin concentrations to other clinical markers of the disease.

Materials and methods

In the cross-sectional study, fasting venous blood samples were obtained from 19 women admitted to hospital with pre-eclampsia in the third trimester and 13 normal pregnant controls. The two groups were chosen to be similar in maternal age, gestational age and prepregnancy body mass index (BMI). All the women were Caucasians, non-smokers and none had received antihypertensive treatment.

In the longitudinal study, samples were obtained from seven nulliparous women with normal uncomplicated pregnancies and eight women who subsequently developed pre-eclampsia. The aim of the longitudinal study was to establish the timing of the increase in circulating leptin concentrations in pregnancies destined to be complicated by pre-eclampsia. In order to recruit the appropriate number of subjects for the study, a power calculation was performed using the cross-sectional data. The mean SD of the data was 4.9 and the difference between the groups 11.6. In order to be able to show a significant difference in leptin concentrations with established pre-eclampsia at P = 0.05 and with a power of 95%, it was calculated that a sample size of seven in each arm would be needed. Pre-eclampsia was defined according to the criteria of hypertension, proteinuria, hyperuricaemia and reversal of hypertension and proteinuria after the pregnancy. Hypertension was defined as an increase in blood pressure from 32 weeks as pre-eclampsia developed. This study was carried out to confirm the reported increase in plasma leptin in pre-eclampsia (McCarthy et al., 1999), to investigate the changes in plasma leptin concentrations with the development of pre-eclampsia and to relate leptin concentrations to other clinical markers of the disease.

Total circulating leptin concentrations (ng/ml) were measured in duplicate by radioimmunoassay as described previously (Ma et al.,...
Table I. Showing the clinical and demographic characteristics of subjects in the cross-sectional study

<table>
<thead>
<tr>
<th></th>
<th>Normal pregnancy (n = 13)</th>
<th>Pre-eclampsia (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.3 ± 3.2</td>
<td>31.7 ± 3.5</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>36.5 ± 1.4</td>
<td>36.0 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.1 ± 1.4</td>
<td>23.7 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>117 ± 8.0</td>
<td>145 ± 10.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70 ± 7.0</td>
<td>98 ± 5</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum urate (mmol/l)</td>
<td>0.21 ± 0.04</td>
<td>0.38 ± 0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Serum creatinine (mmol/l)</td>
<td>65.0 ± 7.2</td>
<td>71.5 ± 10.2</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet count (×10⁹)</td>
<td>236.0 ± 26.6</td>
<td>162 ± 38.0</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Means compared using unpaired test and P < 0.05 considered significant. NS = not significant; BP = blood pressure.

Statistical analysis

The cross-sectional plasma leptin values were not normally distributed and the data are presented as median (range). The data were normalized by log-transformation and comparisons made using an unpaired t-test. The rest of the cross-sectional and all of the longitudinal data were normally distributed and are presented as mean ± SD. The longitudinal data were compared using analysis of variance (ANOVA) for repeated measures. Pearson’s correlation coefficients were calculated and a multiple regression analysis performed to determine which of the variables were independently related to plasma leptin concentrations. For all comparisons, statistical significance was defined as P < 0.05. The Statistical Package for Social Sciences (SPSS), version 8, was used for statistical analysis.

Results

The demographic and clinical characteristics of the subjects taking part in the cross-sectional study are shown in Table I. As expected from the recruitment criteria, the pre-eclampsia group had lower platelet counts (P = 0.002), higher systolic and diastolic blood pressures (both P = 0.001) and higher plasma uric acid concentrations (P = 0.04). There was no significant difference in serum creatinine between the two groups. Plasma leptin was significantly increased in the pre-eclampsia group [27.13, 14.4–49.5 (median, range)] compared to the normal controls [11.7, 7.4–19 (median, range)] (P = 0.001, Figure 1).

The demographic and clinical characteristics of the subjects taking part in the longitudinal study are shown in Table II. In women destined to develop pre-eclampsia, leptin concentrations were consistently higher than normal controls between 20 and 36 weeks gestation. In the normal controls, circulating leptin concentrations rose gradually to 32 weeks and thereafter declined slightly. In contrast, the concentrations in the pre-eclampsia group rose markedly from 32 weeks as the disease developed (Figure 2). Leptin concentrations were significantly higher between 20 and 36 weeks gestation in the women who developed pre-eclampsia compared to normal controls (P = 0.0001, ANOVA for repeated measures). In the longitudinal study, positive correlations were found between the pattern of
change of plasma leptin and both systolic blood pressure ($r = 0.94$, $P = 0.005$ versus $r = 0.69$, not significant, NS) and diastolic blood pressure ($r = 0.88$, $P = 0.02$ versus $r = 0.74$, NS) in pre-eclampsia group.

Leptin concentrations in the cross-sectional group and concentrations at 36 weeks were correlated with clinical and biochemical parameters. In the pre-eclampsia group, significant correlations were observed between plasma leptin and uric acid concentrations ($r = 0.92$, $P = 0.01$ versus $r = 0.56$, NS) and BMI ($r = 0.6$, $P = 0.02$ versus $r = 0.09$, NS) for pre-eclampsia and normal pregnancy respectively. Multiple regression analysis found that only uric acid and BMI were independently related to plasma leptin concentrations in the pre-eclampsia.

Discussion

The data confirm that leptin concentrations are higher in established pre-eclampsia (McCarthy et al., 1999) and show for the first time that they are elevated before pre-eclampsia is clinically evident. In addition, it was found that whereas in normal pregnancy leptin concentrations decline late in the third trimester, and were in fact slightly lower than previously reported (Sivan et al., 1998), a rapid increase occurs in the same period in those subjects who developed pre-eclampsia. The higher concentrations of leptin in pregnancies complicated by pre-eclampsia are consistent with observations that placental expression of leptin mRNA is up-regulated in pre-eclamptic placentae (Mise et al., 1998). This group also found that the expression of leptin by the human trophoblast cell line (BeWo cell line) was significantly increased when cultured under hypoxic conditions (5% oxygen) compared with standard conditions (Mise et al., 1998). Placental ischaemia could therefore explain the rapid increase in leptin concentrations during late third trimester in pre-eclampsia. Alternatively, the increase may represent an adaptive response by the feto-placental unit to impaired placental perfusion, mounted in an attempt to meet the energy requirements of the fetus. Finally, there is evidence that inflammatory mediators increase plasma leptin concentrations (Lord et al., 1998), and, in pre-eclampsia, circulating concentrations of the inflammatory cytokines such as tumour necrosis factor-α (TNFα) and interleukin-6 (IL-6) (Vince et al., 1995) are increased. The exact mechanism underlying the increase in circulating leptin concentrations in pre-eclampsia awaits further clarification.

Animal studies have shown that leptin may influence autonomic and cardiovascular function (Haynes et al., 1997; Shek et al., 1998), and its receptors are present in central neural structures that regulate circulatory control (Tartaglia et al., 1995). Chronic infusion of leptin in animal models causes an increase in heart rate and the development of hypertension, and similar findings have been observed in essential hypertension (Narkiewicz et al., 1999). The data in this study show strong associations between the pattern of change of plasma leptin concentrations and blood pressure. It is possible that leptin released from placenta stimulates central receptors that regulate blood pressure and/or heart rate (Tartaglia et al., 1995). Furthermore, a strong association was found between plasma leptin and uric acid concentrations in the pre-eclampsia group. This relationship may have several explanations. Both leptin and urate may be markers of pre-eclampsia and their strong relationship may simply be a reflection of this. In non-pregnant diabetics, leptin is closely related to urate concentrations, independent of BMI and blood pressure (Fruehwald-Schultes et al., 1999), which suggests that one may affect the circulating concentrations of the other. Leptin may contribute to the increased uric acid concentrations as plasma uric acid concentrations are increased by oxidative stress (Davidge, 1998) and leptin has been reported to induce oxidative stress in cultured human endothelial cells (Bouloumie et al., 1999). Thus, there are several possible explanations for the higher leptin concentrations in pregnancies destined to develop pre-eclampsia. Moreover, they may play a role in the development of pre-eclampsia. This possibility requires further evaluation.

Although in this study it was observed that leptin concentrations in pre-eclampsia were independently related to maternal pre-pregnancy BMI in the pre-eclampsia group, other studies have failed to observe such a relationship during normal pregnancy (Highman et al., 1998) and pre-eclampsia (Williams et al., 1999). This may be because weight gain during pregnancy is not solely due to fat deposition (the most important factor for regulating plasma leptin in non-pregnant women). There was no significant difference in haematocrit values and plasma creatinine concentrations between the two groups. Thus, it seems unlikely that haemoconcentration or impaired renal function, which are recognized pathophysiological components of pre-eclampsia, contributed to the high leptin observed in the disease. Although the number of patients in this study is limited, the highly significant statistical difference between the groups favours a true biological finding.

In summary, the data confirm that plasma leptin concentrations are higher in established pre-eclampsia. This study has shown for the first time that this increase pre-dates the development of pre-eclampsia, the occurrence of which is associated with a further marked increase in plasma leptin concentrations. The mechanisms responsible for this increase and the role played by leptin in the development of pre-eclampsia require further study.

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References


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