Plasma leptin concentrations are increased in women with premenstrual syndrome

N.Anim-Nyame¹, C.Domoney¹, N.Panay¹, J.Jones², J.Alaghband-Zadeh³ and J.W.W.Studd¹,³

¹Academic Department of Obstetrics and Gynaecology, Chelsea & Westminster Hospital, 369 Fulham Road, London SW10 9NH and
²Department of Chemical Pathology, Imperial College School of Medicine, Charing Cross Hospital, Fulham Palace Road, London W6 8RX, UK
³To whom correspondence should be addressed at: Fertility and Endocrinology Unit, The Lister Hospital, Chelsea Bridge Road, London W1H 8RH, UK. E-mail: jsstud@lister.prestel.co.uk

Leptin is a metabolic regulator of the hypothalamic-pituitary-gonadal axis, and plays an important role in human reproduction. Its neuro-endocrine effects are mediated by interactions with receptors in the hypothalamus, where emotional drive is also controlled. We postulated that circulating leptin concentrations are increased in premenstrual syndrome (PMS), and that this may be associated with the psychological symptoms of the disease. We obtained fasting venous samples from 32 women with PMS and 28 women with asymptomatic menstrual cycles, matched for age, body mass index and menstrual cycle length. Leptin concentrations were measured by radioimmunoassay. Leptin concentrations increased significantly during the luteal phases of the menstrual cycles of the control and PMS groups as compared with the follicular phase, having excluded the 11 women with PMS and six controls found to be anovulatory on the basis of mid-luteal plasma progesterone concentrations from the analysis. A greater increase was observed in women with PMS than the controls ($P = 0.00006$ and 0.003 respectively). Although leptin concentrations in the follicular and luteal phases were higher in PMS than the controls, the difference was only statistically significant between the follicular phases ($P = 0.001$). There was no clear relationship between leptin and oestradiol or progesterone in this study. These findings suggest that leptin may play a role in the pathophysiology of the disease, and requires further evaluation.

Key words: leptin/neuro-endocrine/neurosteroids/premenstrual syndrome

Introduction

Leptin, the hormone produced by adipocytes (Zhang et al., 1994), plays an important role in the regulation of food intake, energy expenditure and body mass mediated by its receptors in the hypothalamus (Halaas et al., 1995; Pelleymounter et al., 1995). There is also accumulating evidence that it plays a role in human reproduction. This is supported by observations that leptin acts as a metabolic signal to the hypothalamic-pituitary axis (Barash et al., 1996). It appears to be involved in regulation of the menstrual cycle, as circulating concentrations differ in the different phases of the menstrual cycle. Plasma concentrations are increased in the late follicular and luteal phases of the normal cycles (Raid-Gabriel et al., 1998).

Although leptin receptors are expressed on ovarian granulosa cells (Ciolfi et al., 1997), and leptin treatment of bovine granulosa cell culture inhibits oestadiol production (Spicer and Francisco, 1997, 1998), the major effects of leptin on reproduction appear to be centrally mediated. Evidence from animal studies has shown that the neuro-endocrine effects of leptin are more marked when administered centrally than peripherally, suggesting that some of its effects are mediated by direct action on central receptors (Campfield et al., 1995; Stephens et al., 1995). Leptin appears to have a permissive role in the onset of puberty by reactivation of the hypothalamic-pituitary-gonadal axis (Clarke and Henry, 1999; Quinton et al., 1999). Furthermore, leptin deficiency results in hypogonadotrophic hypogonadism, impaired sexual maturation and infertility, which are corrected by leptin replacement (Ahima et al., 1996, 1997; Chehab et al., 1996).

Premenstrual syndrome (PMS) is a group of menstrually related chronic, cyclical disorders manifested by psychological and physical symptoms in the second half of the menstrual cycle. It has been suggested that the psychological symptoms of the disease may be due to abnormal neuro-endocrine response to normal ovarian function in susceptible women. Since the hypothalamus controls emotional behaviour, and the metabolic and neuro-endocrine effects of leptin are mediated by its receptors in the hypothalamus, we postulated that circulating leptin concentrations were increased in PMS, and that contributes to the disease by stimulation of its hypothalamic receptors. In this study, we have compared circulating leptin concentrations in the follicular and luteal phases of the menstrual cycle in PMS and symptom-free controls.

Materials and methods

Fasting plasma leptin concentrations were measured during the mid-follicular (days 5–9) and mid-luteal phases (days 19–23) of the menstrual cycles in 32 women with PMS and 28 with symptom-free cycles (controls). Both groups were matched for age, body mass index (BMI), and duration of menstrual cycle. All the women were presumed ovulatory on the basis of regular menstrual cycle, but this was confirmed retrospectively by mid-luteal plasma progesterone concentrations. The controls were health workers with no medical or psychiatric history. The women with PMS were recruited from the Psychoendocrine Clinic at the Chelsea and Westminster Hospital.
The criteria for entry into the PMS group were: (i) absence of any medical, or psychiatric history; (ii) presence of severe symptoms for more than 12 months, confirmed prospectively by daily symptom rating for two menstrual cycles; (iii) significant positive (worsening in the 14 days prior to menstruation) and negative (improvement after the onset of menstruation) trends in at least three premenstrual distress questionnaire symptoms (PDQ) (Magos 1988), at least one of which is psychological, as determined by Triggs trend analysis (Magos and Studd, 1986); (iv) disruption of normal activities or interpersonal relationships (Reid, 1991). They were instructed to score 15 common PMS symptoms on a scale of 0–3 (no symptoms to severe symptoms) on their symptom chart. The symptoms included loss of efficiency, irritability, weight gain, difficulty concentrating, tiredness, mood swings, tension, restlessness, depression, bloating, breast tenderness, headaches, food cravings, acne, pelvic pain. Heavyness of bleeding was also scored on the same scale.

None of the women used hormonal contraception or had history of hypertension, metabolic or endocrine disease, or used any drugs that may have effects on circulating leptin concentrations. Venous blood was obtained from the antecubital vein, spun immediately and plasma stored at –70°C until assayed for leptin, oestradiol and progesterone. Total circulating leptin concentrations (expressed as ng/l) were measured in duplicates by radioimmunoassay (Linco Research, Inc., St Louis, MO, USA). The assay employed a polyclonal (rabbit) antibody raised against recombinant human leptin. Standards and 125I tracer were also made from recombinant human leptin (Ma et al., 1996). All samples were run in duplicate and the average intra- and inter-assay coefficients of variation were 3 and 8% respectively. The local ethics committee approved the study, and informed consent was obtained from all subjects.

Statistical analysis
Data for the clinical characteristics and plasma progesterone values were normally distributed and the values are expressed as mean ± SD. Plasma leptin and oestradiol values showed a non-gaussian distribution and were expressed as median (interquartile range). Statistical significance between the variables was analysed using paired t-test or Wilcoxon signed-rank paired test for the appropriate data. The strength of association between plasma leptin, oestradiol and progesterone was tested using Spearman’s correlation coefficient. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 4. P < 0.05 was considered statistically significant.

Results
The two groups were similar in age, BMI, blood pressure and length of menstrual cycles (Table I). Eleven of the PMS group and six of the controls were excluded from the analysis because of low progesterone concentrations in the presumed mid-luteal phase. The clinical characteristics of this group were not different from the women included in the analysis. There were no significant differences in plasma progesterone, and oestradiol concentrations between the control group and women with PMS during the equivalent menstrual phases and ovulatory status (Table I). In the control group, plasma leptin concentrations increased significantly from 7.8 (6.5–12.8) ng/l, median (interquartile range), during the follicular phase to 11.3 (8.2–19.2) ng/l (P = 0.003) in the luteal phase. A more significant increase in leptin during the luteal phase was observed in the PMS group from 14.7 (11.4–17.7) ng/l in the follicular phase to 18.2 (12.4–23.3) ng/l in the luteal phase (P = 0.00006) (Figure 1).

Leptin concentrations in similar phases of the cycle were compared for both groups. Leptin concentrations were higher in both phases of the PMS cycles compared with the control group. When similar menstrual phases were compared between the groups, the difference in leptin was statistically significant only for the follicular phases (P = 0.001 and 0.08, for follicular and luteal phases respectively) (Table I). There was no significant difference in leptin concentrations between the follicular and luteal phases of the menstrual cycle in anovulatory PMS and control women excluded from the analysis.

No significant correlation was observed between leptin concentrations and age, BMI, oestradiol, and progesterone concentrations in both menstrual phases, in the control and PMS groups, and the ovulatory subgroups.

Discussion
We provide the first evidence that leptin concentrations may be increased in women with PMS, compared with women with asymptomatic cycles. The data thus support previous evidence that leptin may have a role in human reproduction (Clarke and Henry, 1999). Previous work has suggested ovulation to be a trigger factor for PMS symptomatology (Hammarback et al., 1991), and therefore only women proven to be ovulatory on the basis of mid-luteal progesterone concentrations were included in the analysis. However, the subgroup of women in this study with progesterone concentrations defined as anovulatory are more likely to represent erratic ovulation or mis-timed blood sampling in the index cycle.

The aetiology of PMS is unknown, and the diagnosis is based on the timing and symptom pattern observed in daily symptom records maintained by the patient. The disorder is not universally acknowledged as a disease, and no medical speciality has accepted responsibility for its treatment, although it is recognized by the legal profession as a disease of the mind. Presently there is little evidence that absolute concentrations (deficiency or excess) of hormones cause PMS or that women with PMS have different hormonal patterns from those without. It has been suggested that the disease may result from an abnormal central nervous response to normal gonadal steroids or their metabolites (Berga, 1998). Such central effects may be direct on the brain or indirect by modulating neurotransmitter receptor responses such as γ-aminobutyric acid A (GABA-A) receptor complex. Metabolites of progesterone (allopregnanolone and pregnenolone) can alter neuronal excitability by interaction with the GABA-A receptors (Rupprecht, 1997) in susceptible women. Genetic predisposition may also contribute to the development of the disease (Freeman and Halbreich, 1998).

Although it is not clear from our data whether the raised leptin concentrations are a cause or a consequence of the disease, there are several possible explanations. Leptin may contribute to the pathophysiology of PMS by direct action on receptors in the hypothalamic neurons (Mercer et al., 1996). The hypothalamus is one of the most important centres of the motor output pathways of the limbic system, controlling food
Leptin in premenstrual syndrome

Table I. Clinical characteristics and hormone profile of subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal controls</th>
<th>PMS group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 22)</td>
<td>(n = 21)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>29.1 ± 2.6</td>
<td>30.2 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>23.8 ± 4.0</td>
<td>24.3 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>112.0 ± 7.0</td>
<td>115.0 ± 19.0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>68.0 ± 7.0</td>
<td>70.0 ± 5.0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Ethnic origin</strong></td>
<td>Caucasian</td>
<td>Caucasian</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Duration of menstrual cycles (days)</strong></td>
<td>28.9 ± 2.0</td>
<td>29.2 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Plasma progesterone (nmol/l)</strong></td>
<td>Follicular</td>
<td>5.2 ± 0.3</td>
<td>6.9 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>21.1 ± 4.3</td>
<td>24.3 ± 5.2</td>
</tr>
<tr>
<td><strong>Plasma oestradiol (pmol/l)</strong></td>
<td>Follicular</td>
<td>175.5 (69–223)</td>
<td>202.1 (149 8–267.3)</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>446.1 (184.8–708)</td>
<td>369.5 (286.5–664.3)</td>
</tr>
<tr>
<td><strong>Plasma leptin (ng/l)</strong></td>
<td>Follicular</td>
<td>7.8 (6.6–12.8)</td>
<td>14.7 (11.4–17.7)</td>
</tr>
<tr>
<td></td>
<td>Luteal</td>
<td>11.3 (8.2–19.2)</td>
<td>18.2 (12.4–23.3)</td>
</tr>
</tbody>
</table>

*Values are expressed as median (interquartile range). The rest are expressed as mean ± SD. P < 0.05 is considered statistically significant. Data from women with presumed anovulatory cycles are excluded from the table (Hammarback et al., 1991).

PMS = premenstrual syndrome; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; NS = not significant; N/A = not applicable.

The concentrations were also higher during the follicular phase of PMS cycles compared with the controls, suggesting the increase may not be limited to the luteal phase. Although our data cannot explain this observation, high follicular leptin concentrations may have a priming effect on the central nervous system in those women prone to PMS. It may also explain why some women develop symptoms very early during the luteal phase. Further studies will be required to establish precisely when during PMS cycles circulating leptin begins to rise, by daily measurements of plasma leptin concentrations in PMS women.

Although previous observations have suggested that leptin inhibits gonadotrophin-stimulated human luteinized granulosa cell progesterone production (Brannian et al., 1999), there was no clear relationship between leptin and oestradiol or progesterone in this study. The relationship between leptin and insulin-induced oestradiol, LH or FSH were not investigated in this study. This suggests that any possible role played by leptin in the pathophysiology of PMS may be by direct stimulation of the hypothalamus, and not mediated by ovarian steroids.

Another possible mechanism for leptin in PMS is augmentation of central response to neurosteroids and their metabolites, such as allopregnanolone and pregnenolone. Evidence from animal studies suggest that leptin down-regulates neuropeptide-Y (NP-Y) activity via its receptors located on NP-Y neurones in the hypothalamus (Williams et al., 1999). Neuropeptide-Y is a powerful orexetic factor, which plays an important role in feeding (White, 1993), and reproduction (Kalra, 1993). Leptin may play a role in PMS by influencing the NP-Y transmission pathway. Furthermore, it has recently been reported that central serotonin activity is reduced in PMS and plasma concentrations of its metabolites increased during the luteal phase of the PMS cycles. Reduced serotonin activity in PMS may be responsible for the depressive mood experienced by some women with the disease (Freeman et al., 1998). It is also the pharmacological intake and many of the endocrine functions of the body, including emotional behaviour. Although the functional leptin receptors OB-Rb are located in nearly all parts of the brain (Mercer et al., 1996; Guan et al., 1997), they are highly expressed in the hypothalamus. Hypothalamic OB-Rb is expressed in its paraventricular, ventromedial and arcuate nuclei, areas known to be involved in satiety and reproduction (Bray et al., 1981; Menedez et al., 1991).

In this study, although the pattern of change of leptin in PMS was similar to that reported in normal cycles (Raid-Gabriel et al., 1998), the difference between the luteal and follicular leptin was greater in the PMS group. Furthermore,
basis for the use of selective serotonin re-uptake inhibitors for the treatment of PMS (Freeman et al., 1999). Leptin induces brain serotonin metabolism through the L-arginine-nitric oxide pathway (Calapai et al., 1999). Increased serotonin metabolism or uptake will reduce its concentrations and may provide a possible mechanism for leptin in the development of PMS. Leptin concentrations are altered in women with eating disorders (Nakai et al., 1999). Although food craving is a common symptom in PMS, we are unable to explain the relationship between food craving and increased leptin concentrations in PMS, as leptin stimulates the satiety centre. It is not clear whether this phenomenon represents a state of temporary leptin resistance similar to that described in obesity (Lee et al., 1996). However, obese women and those with insulin resistance do not necessarily exhibit PMS. We are currently carrying out studies to investigate whether a relationship exists between insulin resistance and leptin concentrations in women with PMS.

In summary, we have shown that plasma leptin concentrations are higher in PMS compared with asymptomatic menstrual cycles, independent of plasma oestradiol and progesterone concentrations. Although robust conclusions cannot be drawn from these data, as the number of women in the study is small, the probability of such a difference as that observed between the two groups occurring by chance was so small that the findings are likely to represent a true biological difference. The clinical utility of plasma leptin as a tool for the diagnosis of PMS should await independent confirmation of our findings. Further work is required to define the precise role played by leptin in the pathophysiology of PMS, as circulating concentrations may not reflect tissue activity.

References

Received on April 27, 2000; accepted on July 21, 2000