SHORT COMMUNICATION

Relationship between leptin and oestrogens in healthy women

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Abstract

Objective: Leptin, a protein secreted by white adipocytes, plays a relevant role in the regulation of body weight and food intake. A possible role for sex hormones in the regulation of leptin secretion has been suggested; however, the effect of variations in oestrogen concentration on serum leptin levels has not been described so far.

Methods: In study 1, serum leptin concentrations were measured on days 3, 10, 17 and 24 of the menstrual cycle in 18 healthy, lean, regularly menstruating women, aged 18–35 years. Serum oestradiol, progesterone, testosterone, Δ4-androstenedione, dehydroepiandrosterone sulphate (DHEAS), LH and FSH concentrations were also determined. In study 2, serum leptin and oestradiol levels were measured on the 5th and 7th day of ovarian stimulation with human FSH (225 IU daily) during an in vitro fertilisation programme for infertility in 20 women aged 25–45 years.

Results: The results from study 1 show a physiological fluctuation of leptin levels during the menstrual cycle, which has not been described previously. Leptin levels are significantly lower in the early follicular phase. The results of study 2 show a parallel increase in serum oestrogen and leptin concentrations during FSH administration.

Conclusions: The fluctuation in leptin levels during the menstrual cycle observed in study 1 is compatible with the hypothesis of a stimulatory effect of oestrogen on leptin secretion. The results of study 2 support the hypothesis of a relevant role for oestrogen in the regulation of leptin secretion. Leptin fluctuations during the menstrual cycle are consistent with reported perimenstrual variations in food craving and consumption.

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Introduction

Leptin, the protein encoded by the OB gene, enhances satiety and reduces food intake, participating in the regulation of body weight (1). The main determinant of plasma leptin concentrations in humans appears to be total body fat content (2–4, 6–9). After adjustment for body fat, with females showing higher leptin concentrations than males (10), normally menstruating women show higher leptin levels than postmenopausal women (4). Moreover, significant fluctuations in serum leptin concentrations during the menstrual cycle have been reported in a small sample of healthy volunteers (5). Therefore, the hypothesis of a stimulatory effect of oestrogen and/or progesterone, and of an inhibitory action of androgens, on plasma leptin concentrations has been formulated (4). This hypothesis is supported by the reported increase in leptin levels during pregnancy (5, 11) and the observed inverse correlation between serum leptin and testosterone concentrations in males (12). The aim of the present study was to correlate circulating leptin levels with physiological variations in oestrogen and progesterone secretion.

Objects and methods

Study 1

Eighteen healthy volunteers aged 18–35 years with a body mass index (BMI) of <27 kg/m² were enrolled in the study. All participants were nulliparous, had regular menstrual cycles (28 ± 1 days during the last 6 months), had never been previously treated with oestrogens and/or progestins, and had not taken any medication in the preceding 4 weeks. None of the subjects had a previous or current history of thyroid or other endocrine disease, diabetes mellitus, renal failure or hepatic diseases. All participants gave their informed consent before enrolment. The subjects had a mean (± S.D.) age of 29.0 ± 5.5 years, a height of 163.6 ± 5.6 cm, a weight of 58.5 ± 9.5 kg and a BMI of 21.8 ± 3.2 kg/m². Blood
samples were collected at 0830 h, after a 12 h overnight fast, on day 3 (± 1) from the beginning of menstruation, and then again every 7th day for 3 weeks (on days 10, 17 and 24) for measurement of oestradiol, progesterone, testosterone, Δ4-androstenedione, dehydroepiandrosterone sulphate (DHEAS), follicle-stimulating hormone (FSH) and luteinising hormone (LH).

**Study 2**
A consecutive series of 20 patients taking part in an *in vitro* fertilisation programme at the Department of Obstetrics and Gynaecology of the University of Florence was studied. All patients had been enrolled for tubal infertility and/or because of a male factor of infertility; those enrolled for different reasons were excluded from the study. All participants gave their informed consent. Patients had a mean age of 35.5 ± 5.8 (range 25–45) years, a height of 168.8 ± 6.1 cm, a weight of 65.0 ± 6.5 kg and BMI of 20.9 ± 2.2 kg/m². All the patients had regular menstrual cycles and none was affected by any relevant medical disease. Purified human FSH (Serono, Rome, Italy), 225 IU daily, was administered i.m. starting on the 3rd day from menstruation. Blood samples were withdrawn at 0830 h, after an overnight fast, on the 5th and 7th day of oestradiol stimulation, for the measurement of oestradiol and leptin.

**Laboratory methods**
Serum leptin was measured by a competitive RIA for human leptin (Linco, St Charles, MO, USA). Oestradiol, progesterone, LH and FSH were assayed by ELISA (Enzymun-Test; Boehringer Mannheim Immunodiagnostics, Tutzing, Germany). Testosterone was determined using an Automated Chemiluminescence System (Chiron Diagnostics, East Walpole, MA, USA). Δ4-Androstenedione levels were measured by Direct Androstenedione Radioimmunoassay (Diagnostic Product Corporation, Los Angeles, CA, USA), and DHEAS by Chemiluminescent Enzyme-Immuonassay (Immulite; Diagnostic Product Corporation).

**Statistical analysis**
Statistical analysis was performed using SPSS 5·0 for Windows. One-way ANOVA, paired and unpaired Student’s t and Wilcoxon tests were applied whenever appropriate. Correlations were studied by Pearson’s method. Multivariate analysis was performed using the multiple linear regression model.

**Results**

**Study 1**
Serum leptin levels on day 3 of the menstrual cycle were 11.2 ± 0.4 ng/ml (mean ± S.E.M.; n = 18). They correlated significantly with BMI (r = 0.62; P < 0.01), but not with age (r = 0.32). The correlation of leptin levels with BMI was confirmed by multiple regression analysis (P < 0.01), when age was considered as a covariate. No correlation was observed with concentrations of LH, FSH, oestradiol, progesterone, testosterone, Δ4-androstenedione or DHEAS (data not shown). Serum leptin concentrations during the menstrual cycle are summarised in Fig. 1: they show significant variations throughout (P < 0.01; ANOVA). A significant increase was observed on day 10 (P < 0.005; Wilcoxon and Student’s t-tests), 17 (P < 0.001) and 24 (P < 0.05), with respect to day 3. Mean (± S.E.M., n = 18) increases in leptin with respect to day 3 were 23 ± 7% on day 10, 31 ± 7% on day 17 and 35 ± 7% on day 24. Correlation of leptin with BMI was confirmed on day 10, 17 and 24 (r = 0.71, 0.63 and 0.61 respectively; P < 0.01; simple and multiple linear regression). Fractional variations in leptin concentrations from day 3 showed a significant inverse correlation with age on day 10 (r = −0.59; P < 0.05, P < 0.01: multiple regression) but not on day 17 or 24 (r = −0.03 and −0.27 respectively). Concentrations of LH, FSH, oestradiol, progesterone, testosterone, Δ4-androstenedione and DHEAS during the menstrual cycle are reported in Table 1. All subjects showed serum progesterone concentrations above 8 nmol/l on day 17 or 24 or both. Testosterone, Δ4-androstenedione and DHEAS concentrations did not show any significant variation during the menstrual cycle. Fractional variations in leptin concentration from day 3 did not show any significant correlation with fractional increases in oestradiol (r = 0.13, 0.03 and 0.05 on days 10, 17 and 24 respectively; simple and multiple linear regression considering age, BMI, progesterone and androgen levels as covariates).

**Study 2**
On the 5th day of ovarian stimulation, mean ± S.E.M. (n = 20) serum leptin was 11.7 ± 0.4 ng/ml and oestradiol 0.15 ± 0.01 nmol/l. Serum leptin levels correlated significantly with BMI by simple (r = 0.60, P < 0.01) and multiple (P < 0.05) linear regression, while no significant correlation was observed with age (r = 0.23) or serum oestradiol (r = 0.18). After two more days of treatment with FSH, mean ± S.E.M. (n = 20) oestradiol concentrations had risen to 0.63 ± 0.01 nmol/l (P < 0.01). A similar increase in leptin concentrations (Fig. 2) was observed to 14.8 ± 0.4 ng/ml (P < 0.01; two-tailed paired Student’s t-test). The fractional increase in leptin did not show any significant correlation with age, BMI or fractional increase in oestradiol (data not shown).

**Discussion**
The present data (study 1) show physiological fluctuations in leptin concentrations during the menstrual
cycle, with the lowest values during the early follicular phase. The observed variations in leptin levels (about 35%) appear to be relevant, and this should be taken into account when studying regularly menstruating women. The results confirm the fluctuation in leptin levels during the menstrual cycle previously described in a smaller sample of healthy volunteers (5). The variation in leptin levels was not related to BMI; however, obese patients were excluded from the study, and therefore the effect of obesity on leptin fluctuation could have been underestimated. An inverse correlation was found between fractional variation in leptin at day 10 and age, meaning that younger females may show smaller increases in leptin levels after menstruation; however, the age range of the subjects studied was narrow, and the sample was not large enough to draw definitive conclusions on this point. The variations in serum leptin concentrations during the menstrual cycle do not appear to be related to fluctuations in androgen levels; in fact testosterone, \( \Delta^1 \)-androstenedione and DHEAS did not show any significant variation throughout the menstrual cycle. Leptin and oestrogen show similar patterns of fluctuation during the menstrual cycle, with a significant increase in serum concentrations on days 10, 17 and 24, suggesting a possible role for oestrogen in the stimulation of leptin secretion. A stimulatory action of progesterone appears to be less likely, as the increase in leptin concentration precedes that of progesterone. A previous study had suggested that a stimulatory effect of progesterone on leptin secretion may be responsible for the fluctuations in leptin levels during the menstrual cycle (5). It should be pointed out that this report, which was based on the observation of a smaller sample of women, did not analyse early and late follicular phase separately, and therefore failed to detect the rise in leptin levels in the late follicular phase. The results of study 2 seem to

Figure 1 Percentage variation in serum leptin concentrations with respect to day 3 in individual subjects throughout the menstrual cycle.

Figure 2 Serum leptin levels in individual patients after 5 and 7 days of therapy with FSH (225 IU/day).
confirm the stimulatory action of oestrogens on leptin secretion, which could be the mechanism underlying fluctuations in serum leptin concentrations during the menstrual cycle, as previously postulated (4). A role for sex hormones in the regulation of leptin secretion is confirmed by the disappearance of the diurnal leptin rhythm in amenorrhoeic women athletes (13), and may be related to the decrease in leptin levels in anorexia nervosa (14, 15) and the increase in leptinaemia during pregnancy (5, 11). A study on postmenopausal women failed to detect any increase in leptin levels after the beginning of hormone replacement therapy (HRT) (16); however, serum concentrations of oestrogens during HRT are far lower than those reached during physiological menstrual cycles. Moreover, serum concentrations of oestrogens in menstruating women, unlike those undergoing HRT, are pulsatile; for this reason, HRT could be an inadequate model for the study of the effect of oestrogen on leptin secretion during the menstrual cycle. However, the possible stimulatory action of oestrogens on leptin secretion should be confirmed with further studies in vitro and in vivo.

Several studies have reported an increase in food consumption and craving (17) during the perimenstrual phase. The reduction in circulating leptin, which enhances satiety, during the same period provides a possible biological explanation for such behavioural modifications. The correlations between serum leptin variations and perimenstrual modifications of eating behaviour need to be further assessed.

References

16 Kohrt WM, Landt M & Birge SJ Jr. Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. Journal of Clinical Endocrinology and Metabolism 1996 81:3890–3895.

Table 1 Concentrations of FSH, LH, oestradiol, progesterone, DHEAS, Δ4-androstenedione and testosterone during the menstrual cycle. Data are expressed as mean values with range in parentheses.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Day 3</th>
<th>Day 10</th>
<th>Day 17</th>
<th>Day 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/l)</td>
<td>8.2 (6.6–12.8)</td>
<td>6.3 (3.4–7.7)</td>
<td>6.1 (4.9–6.4)</td>
<td>4.4 (2.2–9.4)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>3.9 (1.1–4.6)</td>
<td>4.6 (2.3–6.8)</td>
<td>5.5 (2.9–5.8)</td>
<td>4.8 (2.3–9.4)</td>
</tr>
<tr>
<td>Oestrogen (nmol/l)</td>
<td>0.13 (0.05–0.33)</td>
<td>0.24** (0.13–0.76)</td>
<td>0.39** (0.12–1.35)</td>
<td>0.30** (0.18–1.71)</td>
</tr>
<tr>
<td>Progesterone (nmol/l)</td>
<td>1.9 (1.0–18.9)</td>
<td>1.6 (0.7–3.2)</td>
<td>6.8* (1.1–45.7)</td>
<td>27.9** (4.5–64.2)</td>
</tr>
<tr>
<td>DHEAS (μmol/l)</td>
<td>4.9 (1.9–11.5)</td>
<td>5.2 (2.4–10.3)</td>
<td>5.1 (2.1–11.7)</td>
<td>4.2 (2.2–8.0)</td>
</tr>
<tr>
<td>Δ4-Androstenedione (nmol/l)</td>
<td>8.0 (4.0–14.8)</td>
<td>8.4 (5.2–20.1)</td>
<td>10.6 (4.9–20.1)</td>
<td>7.7 (5.1–18.5)</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>2.0 (1.0–2.8)</td>
<td>2.1 (1.2–3.1)</td>
<td>2.1 (1.0–3.4)</td>
<td>1.8 (1.0–2.7)</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 vs day 3 (Wilcoxon test).