

Oestradiol plus progesterone treatment increases serum leptin concentrations in normal women

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BACKGROUND: Previous studies have alluded to a role for both oestradiol and progesterone in the secretion of leptin from fat cells in the human, although direct evidence has yet to be obtained. The study aim was to assess serum leptin concentrations in normally cycling women receiving exogenous oestradiol and progesterone. **METHODS:** Normally cycling women were investigated in an untreated spontaneous cycle (control, $n = 10$), a cycle treated with oestradiol (oestradiol cycle, $n = 10$) and a cycle treated with oestradiol plus progesterone (oestradiol + progesterone cycle, $n = 6$). Oestradiol was given to the women through skin patches on cycle days 2, 3 and 4, and progesterone intravaginally on cycle days 3, 4 and 5. Serum concentrations of leptin, oestradiol, progesterone, FSH and LH were measured in daily blood samples. **RESULTS:** During the treatment, serum oestradiol and progesterone concentrations increased significantly. In the oestradiol cycles, leptin concentrations were not affected by treatment and did not differ from those in controls. In the oestradiol + progesterone cycles, leptin concentrations (mean \pm SEM) increased in all women from cycle day 3 (8.6 ± 1.1 ng/ml) to days 5 (12.2 ± 1.8 ng/ml, $P < 0.01$) and 6 (11.9 ± 2.0 , $P < 0.05$), and were at these points significantly higher than in the control cycles ($P < 0.05$). The mean percentage increase from day 3 to the peak concentration on days 5 or 6 was $62.6 \pm 6.8\%$. Leptin concentrations returned to the pretreatment value on day 7, together with the concentrations of oestradiol and progesterone. In the oestradiol + progesterone cycles, leptin concentrations correlated significantly with oestradiol and progesterone concentrations, but not with FSH and LH concentrations. **CONCLUSIONS:** These results show, for the first time, that leptin secretion can be stimulated in women by the administration of oestradiol plus progesterone. This may explain the increased concentrations of leptin during the luteal phase of the normal menstrual cycle.

Key words: human/leptin/oestradiol/progesterone

Introduction

Leptin, a product of the *ob* gene, is a protein that is almost exclusively secreted in fat cells (Zhang *et al.*, 1994). It was thought initially that leptin is the antiobesity hormone, but soon it was realized that this protein expresses various activities both at central and peripheral sites (Mantzoros and Moschos, 1998; Messinis and Milingos, 1999). Animal studies have shown that oestradiol may play a role in the control of leptin secretion, since treatment of rats with this steroid can prevent the ovariectomy-induced decrease of serum leptin concentrations, and of the expression of the *ob* gene in adipose tissue (Shimizu *et al.*, 1997; Yoneda *et al.*, 1998; Chu *et al.*, 1999). However, the role of ovarian steroids in the control of leptin secretion in humans is not clear.

Normal women have significantly higher serum leptin concentrations than normal men (Rosenbaum *et al.*, 1996; Shimizu *et al.*, 1997). Also, in the luteal phase of the normal menstrual

cycle, serum leptin concentrations are significantly higher than in the follicular phase (Hardie *et al.*, 1997; Mannucci *et al.*, 1998; Messinis *et al.*, 1998; Quinton *et al.*, 1999; Ludwig *et al.*, 2000), while significant positive correlations have been found between leptin and oestradiol or progesterone concentrations (Hardie *et al.*, 1997; Paolisso *et al.*, 1998; Messinis *et al.*, 1999). Although these studies suggest that ovarian steroids may play a role in leptin secretion in humans, no direct evidence has been provided as yet. Recently, a significant decrease in serum leptin concentrations was found following bilateral ovariectomy in normal women (Messinis *et al.*, 1999) and, although treatment with oestradiol was without any effect, the addition of progesterone prevented this decrease, suggesting that progesterone plays a role in the control of leptin secretion (Messinis *et al.*, 2000). A hypothesis was developed from these results that the increase in leptin concentrations during the second half of the menstrual cycle may be related to changes in the steroidal milieu during the

periovulatory period and the luteal phase. The present study was undertaken to test this hypothesis further by examining the effect of treatment with oestradiol and progesterone on leptin concentrations in normal premenopausal women.

Materials and methods

Patients

The study included 10 healthy normally ovulating women aged between 23 and 36 years (median age 26.5 years) with a mean (\pm SEM) body mass index (BMI) of $20.2 \pm 0.3 \text{ kg/m}^2$. The women had not been receiving any medication or hormonal treatment for at least the previous 6 months. Only women with a cycle length of between 26 and 32 days were included (mean \pm SEM, 28.6 ± 0.9 days). Ovulation was confirmed in all women by ultrasound scans of the ovaries and serum progesterone measurement ($>30 \text{ nmol/l}$; mean $47.2 \pm 6.0 \text{ nmol/l}$) before admission to the study. The women volunteered for the study and provided their written informed consent. The study was approved by the local Ethical Committee.

The women were investigated during the early follicular phase of three different cycles. The first two cycles were consecutive, while between the second and the third cycle there was a break of at least 1 month. However, due to inconvenience caused by the blood sampling, four of the 10 women withdrew from the study after they had completed the first and second cycles. During the first cycle (control), no treatment was given to the women ($n = 10$). In the second cycle (oestradiol cycle), the women ($n = 10$) were given oestradiol through skin patches (Estraderm TTS; Ciba-Geigy, Athens, Greece) at a dose of $100 \mu\text{g}/24 \text{ h}$ on cycle day 2 (09:00 h) and $150 \mu\text{g}/24 \text{ h}$ on days 3 and 4. All patches were removed on cycle day 6 (09:00 h). In the third cycle (oestradiol+progesterone cycle), the women ($n = 6$) received oestradiol as in the oestradiol cycle plus progesterone (Utrogestan caps; Faran, Athens, Greece) on cycle days 3, 4 and 5 at the daily dose of 400 mg i.v. (two doses of 200 mg , 12 h apart). The intention was to induce serum concentrations of oestradiol and progesterone approaching respectively those seen during the preovulatory period and the luteal phase of the normal menstrual cycle (Messinis and Templeton, 1988). Information regarding the doses of these two steroids was obtained from pilot experiments performed in two volunteers prior to the onset of the study. In each of the three cycles, blood samples were taken from days 2 to 10 (09:00 h) after an overnight fast. In the treated cycles, the samples were drawn each time before the application of treatment. Further blood samples were taken in the control cycles on day 21, and in the two treatment cycles on days 25 and 31. Concentrations of oestradiol, progesterone, leptin, FSH and LH were measured in all blood samples.

Hormone assays

Leptin was measured in all samples in duplicate using a radioimmunoassay kit (RIA; Linco Research, St Charles, MO, USA) which contained human leptin antibody prepared in rabbits and raised against highly purified human leptin, together with standards and tracer prepared with human leptin. Leptin concentrations were expressed as ng/ml, the lower limit of detection being 0.5 ng/ml . For the measurement of oestradiol, a microparticle enzyme immunoassay (MEIA) was used (AxSYM Estradiol; Abbott Laboratories, Abbott Park, IL, USA); the lower limit of detection for oestradiol was 73 pmol/l . Progesterone was measured in serum using a solid-phase, chemiluminescent enzyme immunoassay (Immulite progesterone; DPC, Los Angeles, CA, USA); the lower limit of detection for progesterone was 0.6 nmol/l . FSH and LH were measured in serum using MEIA (AxSYM FSH and AxSYM LH respectively; Abbott Laboratories);

the lower limits of detection for FSH and LH were 0.37 IU/l and 0.5 IU/l respectively. The inter- and intra-assay coefficients of variation for leptin, oestradiol, progesterone, FSH and LH were 6.2 and 7.1%, 2.1 and 5.7%, 9.2 and 8.1%, 3.1 and 4.3%, and 2.4 and 4.2% respectively.

Statistical analysis

The results were analysed using a one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. Statistical calculations for the hormone data values were carried out following log transformations, though the arithmetic means of values were presented. Correlations between various parameters were calculated using Pearson's product moment correlation coefficient analysis.

Results

Serum concentrations (mean \pm SEM) of oestradiol, progesterone, leptin, LH and FSH during the administration of oestradiol (oestradiol cycles) and the control cycles in the 10 women are shown in Figure 1. Concentrations of these hormones on cycle day 2 were similar between the oestradiol and the control cycles. A marked increase in oestradiol concentrations was seen in all women from the onset of treatment on cycle day 2 to day 5 ($1279 \pm 149 \text{ pmol/l}$; $P < 0.001$). Subsequently, oestradiol concentrations declined rapidly and returned to the pretreatment level on cycle day 7 ($P < 0.001$), and showed no significant changes thereafter. In the control cycles, serum oestradiol concentrations increased slightly from days 2 to 7, and progressively from days 7 to 10 ($693 \pm 141 \text{ pmol/l}$, $P < 0.01$). From days 3 to 6, oestradiol concentrations were significantly higher ($P < 0.001$), and on days 9 and 10 were significantly lower, in the oestradiol cycles than in the control cycles ($P < 0.05$). Serum progesterone concentrations were low throughout the experimental period in both the oestradiol and control cycles. The concentrations of leptin in the oestradiol cycles did not change significantly during the treatment period. There was no significant difference in leptin concentrations between the oestradiol and the control cycles from days 2 to 10, although in the latter cycles there was a significant increase from days 7 to 10 ($P < 0.05$). Serum LH and FSH concentrations showed a pattern of changes that was compatible initially with a suppressive effect, and then with a positive effect of oestradiol. Both an LH and an FSH surge were induced by the oestradiol treatment.

The changes in serum oestradiol, progesterone, leptin, LH and FSH concentrations (mean \pm SEM) in the six cycles treated with oestradiol plus progesterone (oestradiol+progesterone cycles), and in the corresponding control cycles, are shown in Figure 2. Pretreatment concentrations of these hormones on cycle day 2 did not differ significantly between the two groups of cycles. During treatment with oestradiol and progesterone, serum oestradiol concentrations showed a pattern of increase similar to that in the oestradiol cycles, with no significant differences at the corresponding points. Oestradiol concentrations were significantly higher on days 3, 4 and 5 ($P < 0.001$), and significantly lower on days 9 and 10 ($P < 0.05$) in the oestradiol+progesterone cycles than in the control cycles. Serum progesterone concentrations in the oestradiol+progesterone cycles showed an abrupt increase from days 3

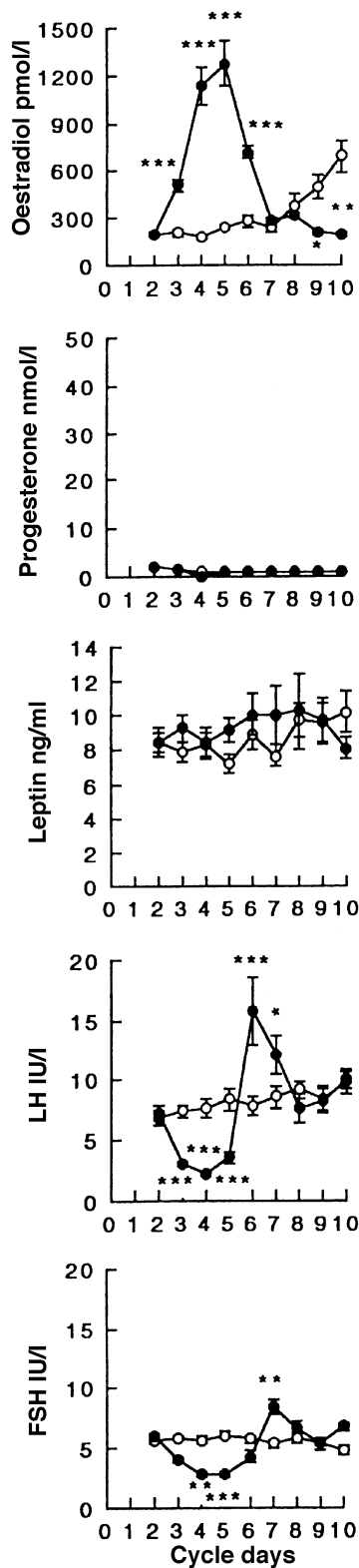


Figure 1. Serum oestradiol, progesterone, leptin, LH and FSH concentrations during the follicular phase of (○) untreated spontaneous (control) cycles and (●) cycles treated with oestradiol through skin patches (from days 2 to 4) in 10 normally cycling women. Values are mean \pm SEM. * P < 0.05, ** P < 0.01, *** P < 0.001 versus control cycles.

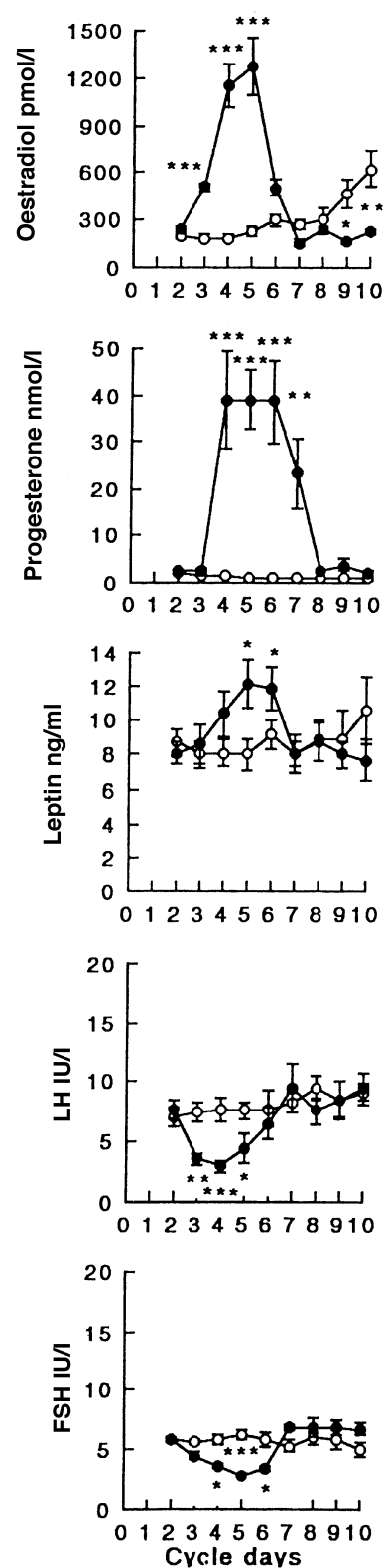


Figure 2. Serum oestradiol, progesterone, leptin, LH and FSH concentrations during the follicular phase of (○) untreated spontaneous (control) cycles and (●) cycles treated with oestradiol through skin patches (from days 2 to 4) plus progesterone intravaginally (from days 3 to 5) in six normally ovulating women. Values are mean \pm SEM. * P < 0.05, ** P < 0.01, *** P < 0.001 versus control cycles.

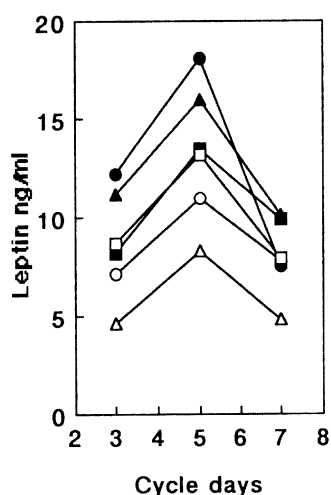


Figure 3. Serum leptin concentrations in individual women treated in the early follicular phase with oestradiol through skin patches (from days 2 to 4) plus progesterone intravaginally (from days 3 to 5).

(2.5 ± 0.4 nmol/l) to 4 (38.9 ± 10.4 nmol/l, $P < 0.001$), remaining high on days 5 and 6 ($P < 0.001$) and decreasing thereafter (Figure 2). In the oestradiol+progesterone cycles, leptin concentrations in serum increased significantly from day 3 (8.6 ± 1.1 ng/ml) to days 5 (12.2 ± 1.8 ng/ml, $P < 0.01$) and 6 (11.9 ± 2.0 ng/ml, $P < 0.05$), and were at these points significantly higher than in the control cycles ($P < 0.05$). Leptin concentrations then declined significantly on day 7 (8.0 ± 0.7 ng/ml, $P < 0.05$) and showed, thereafter, no significant changes up to day 10. This pattern of changes in leptin concentrations occurred in all six women during the oestradiol+progesterone cycles (Figure 3), and the concentrations on days 5 and 6 represented increases over the pretreatment value of day 3 of $46.2 \pm 4.9\%$ and $43.7 \pm 6.0\%$ respectively. When the peak leptin concentration on days 5 or 6 was taken into consideration, the percentage increase over the concentration of day 3 was $62.6 \pm 6.8\%$. In the same cycles, the intra-patient coefficient of variation was 13.8% for blood samples taken on days 2, 3 and 7–10 (low leptin concentrations), and 15.7% for samples taken on days 5 and 6 (high leptin concentrations). In the control cycles, leptin concentrations increased significantly from days 7 to 10. Serum concentrations of LH and FSH showed a pattern of changes that was compatible with a negative effect of oestradiol, while the positive effect was markedly suppressed.

The values of BMI (mean \pm SEM) were similar in the three groups of cycles on day 2 (control, 20.2 ± 0.3 ; oestradiol cycles, 20.3 ± 0.3 ; oestradiol+progesterone cycles, 20.3 ± 0.2 kg/m²). On that day, serum leptin concentrations correlated significantly with BMI in the three groups of cycles combined ($r = 0.504$, $P < 0.01$, $n = 26$). Body weight and BMI did not change significantly during each experimental period. Significant positive correlations were found between leptin and oestradiol concentrations in the group of the control cycles ($r = 0.470$, $P < 0.001$, $n = 84$) and the group of the oestradiol+progesterone cycles ($r = 0.400$, $P < 0.01$, $n = 48$), but not in the group of the oestradiol cycles. In the oestradiol+progesterone cycles, leptin concentrations also

showed a significant positive correlation with progesterone concentrations ($r = 0.320$, $P < 0.05$, $n = 47$). No significant correlations were found between serum leptin and LH or FSH concentrations.

All women in the oestradiol and the oestradiol+progesterone cycles had a menstrual-like bleeding 2–3 days after the end of the treatment. The duration (mean \pm SEM) of the oestradiol cycles (33.3 ± 1.0 days) and the oestradiol+progesterone cycles (36.3 ± 1.6 days) was significantly longer than the duration of the control cycles (28.5 ± 0.8 days) ($P < 0.01$). This was due to the delayed ovulation which was confirmed in all cycles by an increase in progesterone concentration (control cycles day 21, 44.3 ± 4.8 nmol/l; oestradiol cycles day 25, 47.9 ± 3.6 nmol/l; oestradiol+progesterone cycles day 31, 40.7 ± 6.8 nmol/l).

Discussion

The current study is the first to show an increase in serum leptin concentrations in normal women during treatment with exogenous oestradiol and progesterone. It is of interest that when oestradiol alone was given to the women, there was no change in leptin concentration, but the increase was observed when progesterone was added to the oestradiol regimen. This suggests that, in the presence of preovulatory concentrations of oestradiol, progesterone can stimulate leptin secretion. Whether progesterone has the same effect in the presence of low oestradiol concentrations needs to be investigated, however. The aim of the current study was to examine whether a sequence of events, in terms of changes in oestradiol and progesterone concentrations resembling those in the normal menstrual cycle, can affect leptin secretion; the aim was not to investigate the role of the two steroids separately. Although leptin is secreted in a pulsatile manner (Licinio *et al.*, 1997), it is unlikely that in the present study blood sampling interfered with the pulses of this material: first, because the increase in leptin concentration during treatment with oestradiol+progesterone was seen in all women; and second, because the peak concentrations exceeded the pretreatment concentration by more than 60%, which is double the previously reported average pulse height (32%) over the preceding baseline (Licinio *et al.*, 1997). In addition, the within-patient variability of leptin concentrations in the present study was rather small during the entire experimental period.

Previous studies have shown that oestradiol can stimulate the secretion of leptin from fat tissue cultures in animals and women (Murakami *et al.*, 1995; Casabiell *et al.*, 1998). A recent study has shown that serum leptin concentrations increased significantly in postmenopausal women after 2 months of treatment with oestradiol at a dose of 2 mg/day (Elbers *et al.*, 1999). That such an effect of oestradiol was not seen in the present study might be related to differences in the protocol used, in which treatment of the women with this steroid was for a much shorter period. Clearly, this finding does not exclude the possibility that oestradiol plays a role in the control of leptin secretion. It is possible that oestradiol exerted a priming effect on fat cells that subsequently responded to progesterone. The effect of combinations of oestradiol and

progesterone on leptin concentrations has also been investigated in previous studies in which normal women were given either the oral contraceptive pill or hormone replacement therapy (HRT), but circulating concentrations of leptin were not affected (Kohrt *et al.*, 1996; Castracane *et al.*, 1998; Cella *et al.*, 2000). There are, however, differences between the present and the previous studies that are mainly related to the type and dosage of the steroids used. In particular, the 'pill' contains synthetic steroids that may act differently from natural steroids, while in HRT formulas the daily dosages used for replacement create circulating concentrations of steroids that may be insufficient to stimulate the fat cells. In the current study, during treatment with oestradiol and progesterone, serum concentrations of oestradiol showed a pattern of increase similar to that seen during the preovulatory period of the normal menstrual cycle and, although progesterone concentrations showed an abrupt increase, the concentrations were similar to those seen in the luteal phase of the cycle (Messinis and Templeton, 1988). It is possible, therefore, that in terms of a stimulating effect of oestradiol and progesterone on leptin secretion, doses of these steroids inducing a rapid increase in blood levels—even for a short period of time—are required rather than the prolonged administration of a low-dose regimen. Whether a threshold mechanism is involved remains to be clarified.

The possibility that in the current study oestradiol plus progesterone treatment stimulated leptin secretion through an effect on pituitary gonadotrophin secretion is not likely. Although in normal women the pulses of leptin are synchronous with the pulses of LH and oestradiol (Licinio *et al.*, 1998), in the current study increments of LH and FSH, in the form of a surge, occurred only during treatment with oestradiol, i.e. when leptin concentrations remained unchanged. In addition, although LH and FSH concentrations declined up to day 5 in both treatment cycles, an increase in serum leptin concentration was seen only during the oestradiol plus progesterone treatment. Furthermore, no significant correlations were found between leptin and LH or FSH concentrations in either treatment group. It is evident, therefore, that the stimulating effect of oestradiol plus progesterone on leptin secretion was not mediated by FSH or LH. The current results are consistent with those of a recent study, according to which treatment with oestradiol plus progesterone prevented the decrease in leptin concentration that was induced by ovariectomy in women (Messinis *et al.*, 2000). From a physiological point of view, the present results support the hypothesis that the increasing concentrations of oestradiol and progesterone during the periovulatory period and the luteal phase of the menstrual cycle are probably responsible for the increased concentrations of leptin during the luteal phase. The fact that in the current study leptin concentrations increased significantly even after a relatively short rise of oestradiol and progesterone concentrations indicates that this process of leptin stimulation by these steroids is rather sensitive. A more sustained elevation of oestradiol and progesterone, therefore, as occurs during the luteal phase of the normal menstrual cycle, would be expected to result in a longer increase in leptin concentrations.

The importance of the increased serum leptin concentrations

during the luteal phase of the cycle is unclear. It has been speculated that leptin at that stage may play a role in embryo implantation, possibly through a mechanism that affects the invasion phase (Gonzalez *et al.*, 2000). Alternatively, increased leptin concentrations may help the body to meet the metabolic demands of a pregnancy (Messinis and Milingos, 1999). In terms of the possible impact that a progesterone-induced increase in leptin concentrations might have in clinical practice, the use of micronized progesterone might be considered in HRT regimens, though this possibility needs to be investigated.

In conclusion, the current study shows for the first time that preovulatory concentrations of oestradiol induced in the early follicular phase of the cycle by the administration of this steroid to normal women did not affect leptin secretion. However, induction of luteal phase concentrations of progesterone, in addition to the increased concentrations of oestradiol, can stimulate leptin secretion. These findings may provide an explanation for the previously reported increased leptin concentrations that occur during the second half of the normal menstrual cycle.

Acknowledgements

The authors thank Professor O.Tsolas, Director of the Department of Biological Chemistry for providing the laboratory facilities for the hormone assays.

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Received on October 10, 2000; accepted on May 4, 2001