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Treatment of normal women with oestradiol plus progesterone prevents the decrease of leptin concentrations induced by ovariectomy

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To study the role of oestradiol and progesterone in the secretion of leptin, 21 normally ovulating women were recruited from those scheduled for ovariectomy plus hysterectomy performed in mid-follicular phase of the cycle. Seven of the women were used as controls and received no hormonal treatment post-operatively. Another seven women received oestradiol (oestradiol group) and the remaining seven women received oestradiol plus progesterone (oestradiol plus progesterone group). Serum leptin values showed a temporal but significant increase 24 h after the operation and were significantly correlated with the cortisol and progesterone values, which increased temporarily at 12 h. At that time a marked decline in oestradiol concentrations was seen. After the temporal increase, leptin values in the controls and the oestradiol group decreased significantly up to day 4 ($P < 0.05$), while in the oestradiol plus progesterone group they increased ($P < 0.01$) and were significantly higher than in the other two groups ($P < 0.05$). Body mass index (BMI) was the most important variable accounting for the changes in leptin values post-operatively, but in the oestradiol plus progesterone group progesterone correlated significantly with leptin independently of BMI. These results suggest that progesterone and cortisol can stimulate leptin secretion in women regardless of oestradiol concentrations.

Key words: leptin/oestradiol/ovariectomy/ovary/progesterone

Introduction

Leptin, a product of the *ob* gene, is a protein that is secreted by the adipocytes (Zhang *et al*., 1994). Evidence has been provided that this substance may participate in various metabolic and endocrine processes including reproductive function (Mantzoros and Moschos, 1998; Messinis and Milingos, 1999). It has recently been demonstrated that in women, leptin and oestradiol values show significant positive correlations both in the follicular phase of the menstrual cycle and during treatment with follicle-stimulating hormone (FSH) (Messinis *et al*., 1998). Also in rats, the significant decrease in serum leptin values that follows ovariectomy can be prevented by treatment with oestradiol (Shimizu *et al*., 1997; Chu *et al*., 1999). A significant decline in leptin concentrations after bilateral ovariectomy, which was significantly correlated with progesterone values, has also been seen in normal women (Messinis *et al*., 1999).

Although these results suggest that ovarian steroids may play a role in leptin secretion, treatment of normal women with oestrogen plus progesterone in the form of the oral contraceptive pill or hormone replacement therapy did not have any effect on serum leptin concentrations (Kohrt *et al*., 1996; Castracane *et al*., 1998; Cella *et al*., 2000). It may be that the doses of the steroids used in these preparations were not sufficient to stimulate leptin secretion. It remains, therefore, to investigate whether induction of 'physiological' concentrations of oestradiol and progesterone in blood, similar to those seen in the normal menstrual cycle, can affect leptin secretion. Furthermore, previous data in women have shown a temporal increase in leptin values within the first 24 h after ovariectomy (Messinis *et al*., 1999), but the reason for this phenomenon is not known. The present study was undertaken to investigate the mechanisms of changes in leptin values following ovariectomy in normal women and to study further the role of oestrogen and progesterone in the secretion of this protein.

Materials and methods

Patients

The study included 21 normally ovulating women who volunteered for the study and gave written informed consent. Approval for the study was obtained from the local ethical committee. Clinical and endocrine characteristics of the women are shown in Table I. There were no significant differences in the age, body mass index (BMI), FSH, luteinizing hormone (LH), leptin and cortisol values between the three groups of women before the onset of the study. All women were studied during the first week following bilateral ovariectomy plus total abdominal hysterectomy performed for benign lesions of the genitalia, such as fibroids and menorrhagia. The operation was performed in the mid-follicular phase of the cycle (follicle size 14–16 mm in diameter by ultrasound). The women were divided into three groups based on whether they received hormonal treatment during the post-operative period. In the control group ($n = 7$), the women received no treatment. In the oestradiol group ($n = 7$), the women received oestradiol through three skin patches (Estraderm TTS®; Ciba-Geigy, Athens, Greece) releasing 100 μg/24 h. The first patch was applied immediately after the operation and the other two on post-operative days 3 and 6. All patches were removed on the day of discharge (day 7). In the oestradiol plus progesterone group ($n = 7$), the women received oestradiol, as in the oestradiol group, plus progesterone intravaginally from days 2–6 (Utrogestan® capsules,

100 mg/capsule; Faran, Athens, Greece). The dose of progesterone was 200 mg on day 2 (100 mg 12 h apart) and 300 mg/day from day 3 onwards (100 mg every 8 h). The intention was to induce concentrations of oestradiol and progesterone that are normally found in the mid- to late follicular and mid-luteal phases of the menstrual cycle respectively. Information regarding the dosages was obtained from pilot experiments performed in two volunteers before the onset of the study. In particular, two dosages of oestradiol (50 μg and 100 μg) and two dosages of progesterone (100 mg and 300 mg) were given to each of the two women during the follicular phase of two spontaneous cycles as follows: oestradiol on cycle days 2, 5 and 8 and progesterone on days 3, 4, 5, 6 and 7. The 50 μg dose of oestradiol and the 100 mg dose of progesterone induced serum oestradiol and progesterone concentrations that in peaks did not exceed 500 pmol/l and 10 nmol/l respectively, while with the higher doses concentrations approaching 1000 pmol/l and 20 nmol/l respectively were achieved. All operations were performed in the morning (0800 h) and lasted less than 90 min. No complications occurred during the operation in any of the women and the blood loss did not exceed 200 ml. The post-operative period was uneventful in all cases. A blood sample was taken from all women on the day of the operation (day 0), i.e. before the administration of the anaesthetic drugs. Another blood sample was taken 12 h after the operation. Further blood samples were obtained from each woman daily (0900 h) on days 3–7. In all blood samples oestradiol, progesterone, leptin and cortisol were measured.

Hormone assays

Oestradiol was measured in serum using a microparticle enzyme immunoassay (AxSYM Estradiol assay). Kits were purchased from Abbott Laboratories (Abbott Park, IL, USA). The results are expressed as pmol/l. For progesterone measurement in serum a solid-phase, chemiluminescent enzyme immunoassay was used (IMMULITE Progesterone). Kits were purchased from DPC (Los Angeles, CA, USA). The results are expressed as nmol/l. Leptin was measured in all serum samples in duplicate using a radioimmunoassay method and all samples were assayed in one batch. Kits were purchased from Linco Research (RIA, Linco Research, St Charles, MO, USA) and contained human leptin antibody prepared in rabbit and raised against highly purified human leptin and standards and tracer prepared with human leptin. The results are expressed as ng/ml. Cortisol was measured in serum using a fluorescence polarization immunoassay (TDx/TDxFLx Cortisol assay). Kits were purchased from Abbott Laboratories. The results are expressed as μg/dl. The lower limits of detection for oestradiol, progesterone, leptin and cortisol were 73 pmol/l, 0.6 nmol/l, 0.5 ng/ml and 0.64 μg/dl respectively, while interassay and intra-assay coefficients of variation were 8.4 and 7.1%, 7.2 and 5.8%, 6.3 and 7.0% and 6.4 and 7.8% respectively.

Table I. Clinical and hormonal parameters in the three groups of women before the operation (day 0)

	Control group	Oestradiol group	Oestradiol plus progesterone group
Women (*n*)	7	7	7
Age (years)	44.4 ± 0.5 (42–46)	44.8 ± 0.5 (42–46)	43.5 ± 0.6 (41–46)
BMI (kg/m^2)	25.7 ± 1.2 (21–29)	26.3 ± 0.8 (23–29)	25.3 ± 1.5 (20–28)
FSH (IU/I)	7.6 ± 0.7 (6.2–10.5)	7.4 ± 1.1 (6.0–10.7)	6.4 ± 0.6 (5.5–9.3)
LH (IU/I)	4.9 ± 0.5 (4.5–6.0)	5.0 ± 0.5 (4.6–6.0)	5.4 ± 0.5 (4.9–6.3)
Leptin (ng/ml)	13.3 ± 3.4 (4.2–24.9)	15.6 ± 2.0 (6.0–21.7)	16.0 ± 3.9 (2.8–24.3)
Cortisol (μg/dl)	24.4 ± 1.8 (21.5–34.7)	21.4 ± 4.1 (13.2–32.8)	18.2 ± 1.0 (14.3–31.1)

Values are mean ± SEM. Ranges are in parentheses.
BMI= body mass index.

Statistical analysis

The results were statistically analysed using one-way analysis of variance. To achieve an approximate normal distribution of the data, before the statistical evaluation hormonal values were transformed into logarithms. However, in the results the arithmetic values are presented unless stated otherwise. Correlations between various parameters were calculated by using simple and multiple linear regressions.

Results

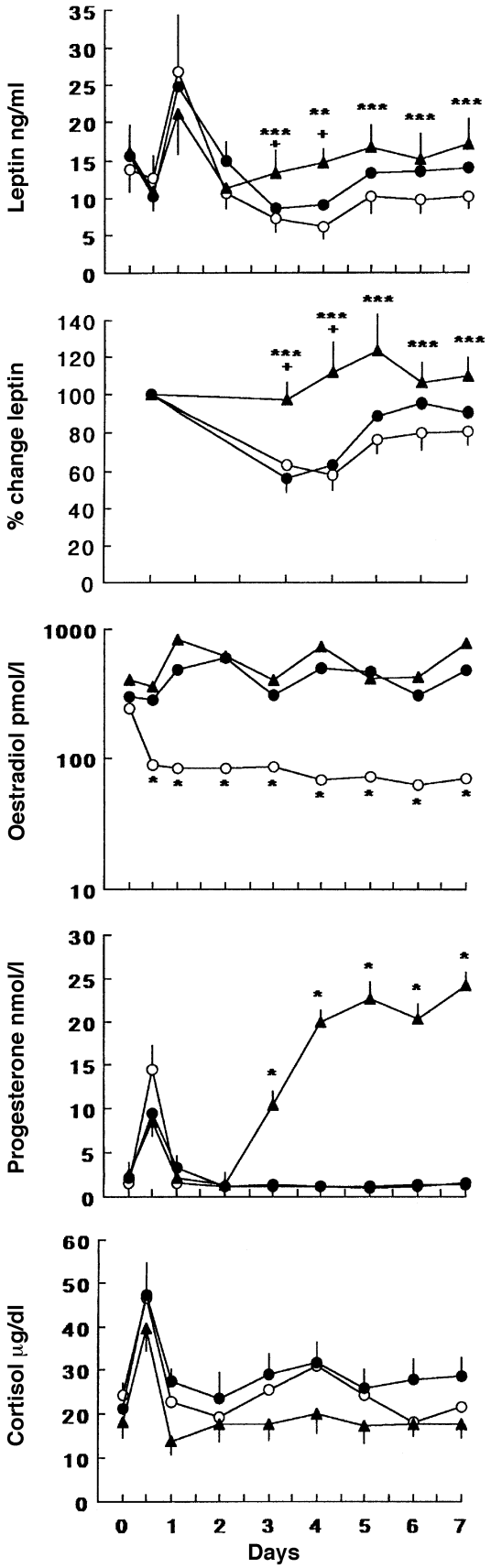
Figure 1 shows serum concentrations of leptin, oestradiol, progesterone and cortisol before and after the operation in the three groups of women. Basal values of these hormones before the onset of the operation (day 0) did not differ between the three groups. Serum leptin values (mean ± SEM) declined significantly 12 h from the operation ($P < 0.05$), before showing a temporal but significant increase at 24 h (post-operative day 1) in all three groups (26.9 ± 7.7, 24.8 ± 2.0 and 21.2 ± 5.7 ng/ml respectively, $P < 0.05$). Leptin values then declined on day 2 in all three groups ($P < 0.01$) and further on days 3 and 4 in the control group (7.2 ± 1.4 and 6.2 ± 1.0 ng/ml respectively, $P < 0.05$) and the oestradiol group (8.5 ± 1.1 and 9.0 ± 1.4 ng/ml respectively, $P < 0.05$), while in the oestradiol plus progesterone group leptin values increased gradually from day 2 to 5 ($P < 0.01$) and 7 ($P < 0.01$). A slight but significant increase in leptin values was seen from day 4 to 5 in the controls and the oestradiol group ($P < 0.05$) with no change thereafter. Leptin values in the oestradiol plus progesterone group were significantly higher than in the control group from day 2 to 7 and the oestradiol group on days 3 and 4 (Figure 1).

Figure 1 also shows the percentage change in serum leptin values in relation to the pre-operative value on day 0. In the controls and the oestradiol group, leptin values (mean ± SEM) decreased respectively on day 3 to 63.2 ± 6.7% ($P < 0.05$) and 55.9 ± 4.5% ($P < 0.001$) and on day 4 to 57.5 ± 7.5% ($P < 0.05$) and 63.1 ± 11.7% ($P < 0.05$), while in the oestradiol plus progesterone group there was no significant change on either day (97.1 ± 12.8% and 112.0 ± 18.2% respectively). The decrease in the control group was also significant on days 5–7 ($P < 0.05$). The difference was significant between the oestradiol plus progesterone group and the controls at all points from days 3–7 and between the oestradiol plus progesterone group and the oestradiol group on days 3 and 4 (Figure 1).

Serum oestradiol values declined significantly as early as 12 h after the operation in the control group and remained below the value of 100 pmol/l up to day 7 (Figure 1). In contrast, in the oestradiol group and the oestradiol plus progesterone group serum oestradiol values did not decline, but remained at or above the pre-operative value throughout the whole experimental period. At all points, serum oestradiol

concentrations were significantly higher in these two groups than in the control group (Figure 1).

Serum values of progesterone showed a temporal but significant increase at 12 h after the operation ($P < 0.001$), returning to the pre-treatment value on day 1 (Figure 1). A significant decrease was seen from days 1–2 ($P < 0.05$). From days 2–7, progesterone values remained very low in the control and the oestradiol groups, while in the oestradiol plus progesterone group, due to the exogenous administration of this hormone, the values increased rapidly from days 2–7 ($P < 0.001$) and remained unchanged at this increased value (Figure 1). A temporal but significant increase ($P < 0.001$), coincident with that of progesterone, was also seen in serum cortisol values 12 h after the operation in all three groups (Figure 1). Then, cortisol concentrations declined on day 1 and did not change significantly thereafter, although there was a trend for lower values in the oestradiol plus progesterone group (Figure 1).



A significant decrease in body weight (mean ± SEM) from days 0 to 7 was seen in the controls (2.7 ± 0.1 kg) and in the oestradiol group (3.6 ± 0.2 kg) ($P < 0.001$), but not in the oestradiol plus progesterone group (1.0 ± 0.5 kg). Consequently, BMI was decreased in the controls and the oestradiol group but not in the oestradiol plus progesterone group. Serum leptin concentrations before and after the operation were significantly correlated with BMI in all three groups combined (day 0: $r = 0.555$, $P < 0.01$, $n = 21$ and day 7: $r = 0.496$, $P < 0.05$, $n = 21$). Significant positive correlations were found from days 2–7 between leptin and progesterone values ($r = 0.409$, $P < 0.01$, $n = 42$) and between leptin and oestradiol values ($r = 0.363$, $P < 0.05$, $n = 42$) only in the oestradiol plus progesterone group. In multiple regression analysis, changes in BMI from days 2–7 was the most important determinant that was significantly correlated with leptin values in all three groups (controls $r = 0.419$, $P < 0.01$; oestradiol group $r = 0.400$, $P < 0.01$; oestradiol plus progesterone group $r = 0.728$, $P < 0.001$, $n = 42$); however, in the oestradiol plus progesterone group progesterone was significantly correlated with leptin independently of BMI. When all three groups were combined, the log-transformed values of leptin increment from 12–24 h following the operation (changes in leptin) were significantly correlated with those of cortisol ($r = 0.687$, $P < 0.001$, $n = 21$) and progesterone ($r = 0.637$, $P < 0.01$, $n = 21$) at 12 h (Figure 2). Between cortisol and progesterone values on day 0 (0 and 12 h) and day 1 there was a significant positive correlation ($r = 0.641$, $P < 0.001$, $n = 63$).

Figure 1. Serum values (mean ± SEM) of leptin, oestradiol, progesterone and cortisol before and after bilateral ovariectomy plus total abdominal hysterectomy performed in mid-follicular phase (day 0) in 21 normally cycling women. (○) Control group ($n = 7$), no hormonal treatment post-operatively. (●) Oestradiol group ($n = 7$), treatment with oestradiol through skin patches (post-operative daysn 0, 3 and 6). (▲) Oestradiol plus progesterone group ($n = 7$), treatment with oestradiol, as in the oestradiol group, plus progesterone intravaginally on days 2–7. $^{*}P < 0.001$, $^{**}P < 0.01$, $^{***}P < 0.05$ (differences in leptin values between the oestradiol plus progesterone group and the controls, differences in oestradiol values between the controls and the two treatment groups and differences in progesterone values between the oestradiol plus progesterone group and the other two groups). $^{\dagger}P < 0.05$ (differences in leptin values between the two treatment groups).

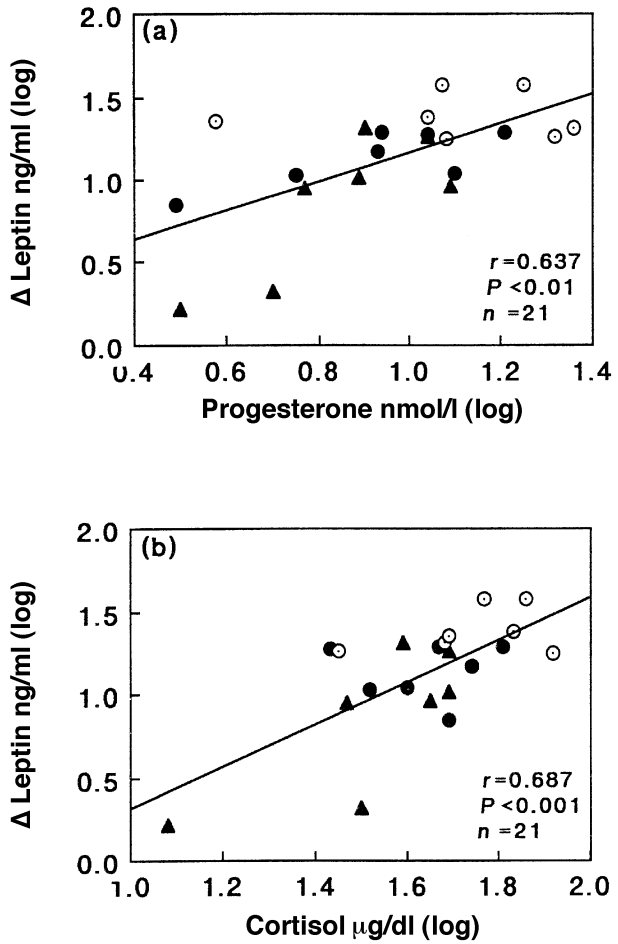


Figure 2. Correlations between the log transformed values of leptin increment (Δleptin) from 12–24 h following bilateral ovariectomy and those of (**a**) progesterone and (**b**) cortisol at 12 h after the operation. Ovariectomy plus total abdominal hysterectomy was performed in mid-follicular phase in 21 normally cycling women. (⊙) Control group (n = 7), no hormonal treatment post-operatively. (●) Oestradiol group (n = 7), treatment with oestradiol through skin patches (post-operative days 0, 3 and 6). (▲) Oestradiol plus progesterone group (n = 7), treatment with oestradiol, as in oestradiol group, plus progesterone intravaginally from days 2–7.

Discussion

The present study confirms previous data that leptin concentrations decline significantly in normal women after bilateral ovariectomy in parallel with the concentrations of oestradiol and progesterone (Messinis *et al.*, 1999). The reason for this decline is not known. Although BMI seems to be an important determinant, the present study shows for the first time that treatment of the women with oestradiol plus progesterone prevented the ovariectomy-induced decrease in leptin values, while oestradiol alone did not have any effect. Since with this treatment, concentrations of oestradiol and progesterone in blood similar to those normally seen in the menstrual cycle were achieved, it is suggested that oestradiol at 'physiological' concentrations is not a principal regulator of leptin secretion, while the role of progesterone is critical.

The present results, in terms of the effect of oestrogen on leptin secretion, do not confirm earlier studies on rats according to which treatment of the animals with oestradiol prevented the ovariectomy-induced decline in leptin values (Shimizu *et al.*, 1997; Chu *et al.*, 1999). The reason for this difference is not known. However, in the studies on rats treatment with oestradiol was applied for a few weeks, while in the present study it was applied for only a few days. It is also possible that species differences are important, since administration of oestradiol to female rats significantly elevated within 12 h amounts of leptin mRNA in adipose tissue and plasma leptin concentrations (Brann *et al.*, 1999), while in the present study in women oestradiol was without any effect. Also, the results of the current study are different from those in previous studies in which treatment of normal women with combinations of oestrogen plus progesterone, in the form of either the oral contraceptive pill or hormone replacement therapy, did not affect leptin values (Kohrt *et al.*, 1996; Castracane *et al.*, 1998; Cella *et al.*, 2000). There are, however, great differences between these and the present study in terms of the design, the aim and the dosages used, which in those preparations may have been insufficient to stimulate leptin secretion.

Although according to the present results, the role of oestradiol in the control of leptin secretion during the normal menstrual cycle is of minor significance, in-vitro data have shown that oestradiol can stimulate leptin production from human adipocytes in cultures (Casabiell *et al.*, 1998), while oestradiol high affinity binding sites have been detected in fat cells (Wade and Gray, 1978). Additionally, in-vivo data have shown significant positive correlations between oestradiol and leptin values during the follicular phase of the cycle with a significant increase in circulating leptin in the late follicular phase (Messinis *et al.*, 1998; Cella *et al.*, 2000). The possibility, therefore, exists that oestradiol may exert a priming effect on adipocytes thus sensitizing these cells to subsequent stimulants, such as progesterone. However, since in the present study a progesterone alone group was not included, this possibility needs to be further examined. Alternatively, oestradiol may exert a delaying effect on leptin secretion, since a trend for higher leptin values during the second part of the post-operative period was seen in the oestradiol-treated women in this study as compared to controls. However, this requires further investigation.

The finding that leptin values were increased when progesterone was added to the oestradiol regimen could be interpreted as indicating that increased concentrations of oestradiol are a prerequisite for progesterone to exert an effect on leptin secretion in women. However, based on further results of the present study this possibility is not likely. In particular, a temporal increase in leptin values occurred in all women within the first 24 h following ovariectomy. This increase, which has been reported previously, does not seem to be related to the type of the operation, because it has also been seen after cholecystectomy in women (Messinis *et al.*, 1999). Since the temporal increase in leptin values was preceded by a significant increase in cortisol concentrations, which were significantly correlated with leptin values, and since dexamethasone can stimulate leptin production *in vivo* (Larsson and Ahren, 1996; Papaspyrou-Rao *et al.*, 1997), it is possible that a stress-related increase in cortisol was responsible for the subsequent increase in circulating leptin values. Coincident

with the increase of cortisol was an increase in progesterone concentrations and since the ovaries had already been removed, it is suggested that progesterone at that stage was of adrenal origin. The possibility therefore cannot be excluded that the temporal increase in progesterone values contributed to the temporal increase in leptin, because significant positive correlations were also found between progesterone and leptin values. Since at the time of the progesterone increase oestradiol values declined markedly, it is suggested that progesterone may be able to stimulate leptin secretion even in the presence of low concentrations of oestradiol.

From a physiological point of view, the present findings could explain the previously reported higher concentrations of leptin in the luteal than in the follicular phase of the cycle (Hardie *et al*., 1997; Mannucci *et al*., 1998; Messinis *et al*., 1998; Riad-Gabriel *et al*., 1998; Quinton *et al*., 1999; Ludwig *et al*., 2000) as the result of a stimulating effect of progesterone, although there is controversy as to whether leptin and progesterone titres are significantly correlated during the luteal phase (Hardie *et al*., 1997; Quinton *et al*., 1999; Ludwig *et al*., 2000). The disparity between oestradiol plus progesterone group and the other two groups in terms of the decrease in body weight and BMI during the post-operative period is difficult to explain. Nevertheless, it is rather unlikely that this is related to changes in fat stores, since similar dietary restrictions were applied to all three groups of women. It is possible that this is related to water retention, which may happen during the luteal phase of the cycle, although this was not investigated in this study. Finally, the reason for the increase in leptin values in the controls and the oestradiol-treated group during the second half of the experimental period is unclear; however, this may be related to the increased intake of food that was initiated at that stage of the study.

In conclusion, the present study demonstrates for the first time that the temporal increase in leptin values that occurs 24 h after bilateral ovariectomy in pre-menopausal women is preceded by a significant increase in progesterone and cortisol values and a marked decline in oestradiol concentrations. In addition, the subsequent decrease in leptin values below the pre-operative value is prevented by treatment with oestradiol plus progesterone, but not with oestradiol alone. It is suggested that progesterone and cortisol exert stimulating effects on leptin secretion in normal women regardless of the concentrations of oestradiol.

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