Leptin concentrations in normal women following bilateral ovariectomy

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Materials and methods

Patients

The study included 20 normally cycling women who volunteered for the study and gave written informed consent. Approval for the study was obtained from the local ethical committee. In all women ovulation was confirmed by ultrasound and serum progesterone measurement before admission to the study. Clinical and endocrine characteristics...
of the women are shown in Table I. All women were studied during the postoperative period following a laparotomy performed under general anaesthesia. Fourteen of them underwent bilateral ovariectomy plus total hysterectomy because of benign conditions of the genitalia, such as fibroids and menorrhagia, while the ovaries were normal. In seven of the 14 women (group 1), the operation was performed during the mid- to late follicular phases of the cycle, i.e. when the size of the dominant follicle was 15–16 mm in diameter as assessed by ultrasound, and in the remaining seven (group 2) in the early to midluteal phase, i.e. 5 days after the spontaneous luteinizing hormone (LH) surge. The latter was detected by daily urine evaluation using kits for LH measurements (Organon LH color; Organon Hellas, Greece) and was confirmed by two or three blood samples on the day on which the test was positive. The remaining six of the 20 women were used as controls (group 3). These women underwent cholecystectomy for benign conditions of the gallbladder, such as cholecystitis, and the operation was performed in early to midfollicular phases of the menstrual cycle (days 4–7). All operations were performed in the morning (0800 h) and lasted less than 90 min. During the operation there were no complications and the loss of blood did not exceed 200 ml. The postoperative period was uneventful in all cases and the women were discharged home on postoperative days 7 (group 3) or 8 (groups 1 and 2). During the operation the presence of a dominant follicle in group 1 or a corpus luteum in group 2 was confirmed. Blood samples were taken from all women starting on the day of the operation, i.e. before the administration of the anaesthetic drugs and continuing initially every 12 h and then every 24 h up to the day of discharge. In all blood samples FSH, LH, oestradiol, progesterone and leptin concentrations were measured.

### Hormone assays

For FSH and LH measurements, immunoassay-based on enhanced luminescence were used (Amerithite FSH and Amerithite LH assay respectively; Amersham International plc, Amersham, UK). The results are expressed as IU/I of standards calibrated against the WHO 2nd IRP of human FSH (58/549) and the 1st IRP of human LH (68/40). Oestradiol was measured using a competitive immunoassay based on enhanced luminescence (Amerithite Estradiol-60 assay; Amersham). The results are expressed as pmol/l. For progesterone measurement a competitive immunoassay was used (Kodak Amerithite Progesterone assay; Amersham). 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. The lower limits of detection for FSH, LH, oestradiol and progesterone were 0.5 IU/I, 0.12 IU/I, 50 pmol/l and 0.35 nmol/l respectively, while interassay and intra-assay coefficients of variation were 7.5 and 6.0%, 9.0 and 6.8%, 9.1 and 8.0%, and 7.0 and 6.6% respectively. The lower limit of detection for leptin was 0.5 ng/ml, while inter- and intra-assay coefficients of variation were 6.2 and 7.1% respectively.

### Statistical analysis

For the purpose of the statistical analysis, hormone results were transformed into logarithms in order to achieve an approximately normal distribution of the data. However, in the results the arithmetic means are presented. For comparisons within the same group, statistical analysis was performed using one-way analysis of variance (ANOVA) and paired t-test as appropriate, while for comparisons between groups two-way ANOVA was used (effects of time and of women). Correlations between various parameters were calculated by using simple and multiple linear regression.

### Results

Serum concentrations of FSH and LH (mean ± SEM) before the onset of the operation (day 0) were significantly lower in group 2 (3.5 ± 0.7 and 2.1 ± 0.5 IU/I respectively) than in group 1 (6.8 ± 1.4 and 5.9 ± 1.1 IU/I respectively, \( P < 0.05 \)) and group 3 (6.2 ± 0.6 and 5.4 ± 0.6 IU/I respectively, \( P < 0.05 \), Table I). Both FSH and LH values increased gradually but significantly from the day of the operation up to postoperative day 8 both in group 1 (32.9 ± 2.0 and 12.8 ± 1.7 IU/I respectively, \( P < 0.01 \)) and group 2 (15.4 ± 3.1 and 5.5 ± 1.0 IU/I respectively, \( P < 0.01 \)). Details of gonadotrophin changes in 10 of the 14 patients of groups 1 and 2 are reported elsewhere (Alexandris et al., 1997). In group 3, both FSH and LH showed a temporal but significant increase 12 h from the operation (\( P < 0.05 \), Figure 1). Then, both gonadotrophins decreased on day 2, remaining stable from day 3 to day 6 and increasing on day 7, indicating the onset of an LH surge.

Serum leptin values (mean ± SEM) on day 0 were significantly higher in group 2 (44.7 ± 4.5 ng/ml) than in groups 1 (22.7 ± 3.4 ng/ml, \( P < 0.05 \)) and 3 (20.2 ± 3.8 ng/ml, \( P < 0.05 \), Table I, Figure 1) with no significant difference between groups 1 and 3. During the first 24 h following the operation, leptin values increased significantly in all three groups, peaking on day 1 (\( P < 0.05 \)) and remaining on that day significantly higher in group 2 than in groups 1 and 3 with no significant difference between groups 1 and 3 (Figure 1). Then, leptin values declined significantly from days 1–4, gradually in groups 1 and 3 and more rapidly in group 2 (\( P < 0.01 \)) with no significant difference between the three groups. From day 4 to day 7 or 8 after the operation serum leptin concentrations did not change significantly in groups 1 and 2, but increased significantly in group 3 (\( P < 0.05 \)); however, there were significant differences between the three groups at any point (Figure 1).

Serum oestradiol values (mean ± SEM) on day 0 did not differ significantly between groups 1 (286 ± 57 pmol/l), 2 (270 ± 71 pmol/l) and 3 (227 ± 38 pmol/l), although they were lower in group 3. Oestradiol values decreased significantly in groups 1 and 2 at 12 h from the operation (\( P < 0.05 \) and
Further up to day 8 with no significant difference between the two groups at any point (Figure 1). In group 3, however, serum oestradiol values showed a temporal but significant increase on day 1 ($P < 0.05$), decreasing slightly on day 2 and then increasing gradually up to day 7 ($500 \pm 78 \text{ pmol/l}$, $P < 0.01$, Figure 1). Oestradiol values were significantly higher in group 3 than in groups 1 and 2 during the whole postoperative period (Figure 1). Serum concentrations of progesterone (mean $\pm$ SEM) were on day 0 significantly higher in group 2 ($16.9 \pm 1.2 \text{ nmol/l}$) than in group 1 ($4.5 \pm 0.6 \text{ nmol/l}$, $P < 0.01$) and group 3 ($1.2 \pm 0.2 \text{ nmol/l}$, $P < 0.01$). After the operation, in groups 1 and 2 progesterone values decreased rapidly during the first 24 h, particularly in group 2 and gradually thereafter up to postoperative day 8 with no significant difference between the two groups at any point (Figure 1). In group 3, serum progesterone values showed a temporal but significant increase 12 h from the operation ($P < 0.01$) remaining low throughout the rest of the postoperative period (Figure 1). The described changes in FSH, LH, oestradiol and progesterone concentrations during the postoperative period in group 3 (Figure 1) resemble those seen during the mid- to late follicular phase of the normal menstrual cycle.

A slight but non-significant decrease in body weight (mean $\pm$ SEM) was noted on postoperative day 8 compared with day 0 in group 1 ($0.53 \pm 0.20 \text{ kg}$) and group 2 ($0.71 \pm 0.30 \text{ kg}$) with no significant difference between the two groups. In group 3, the decrease in body weight on day 7 ($3.2 \pm 0.2 \text{ kg}$) was significantly greater than in groups 1 and 2 ($P < 0.01$). BMI values were available in groups 1 and 2 on days 0 and 8 and in group 3 on days 0, 4, 5, 6 and 7. BMI (mean $\pm$ SEM) did not change significantly on postoperative day 8 compared with the value on day 0 both in group 1 ($26.1 \pm 1.1$ and $26.3 \pm 1.1 \text{ kg/m}^2$ respectively) and group 2 ($26.7 \pm 0.7$ and $26.9 \pm 0.8 \text{ kg/m}^2$ respectively), while it decreased significantly in group 3 from day 0 ($28.2 \pm 1.5 \text{ kg/m}^2$) to day 7 ($26.3 \pm 1.5 \text{ kg/m}^2$, $P < 0.001$). A significant decrease in BMI was also seen in group 3 from day 0 to day 4 ($27.3 \pm 1.5 \text{ kg/m}^2$, $P < 0.001$) and from day 4 to day 7 ($P < 0.001$).

Serum leptin concentrations before and after the operation correlated significantly with BMI in groups 1 and 2 combined ($r = 0.632$, $P < 0.001$, $n = 28$, Figure 2b). A significant positive correlation between leptin and BMI was also found in group 3 before and after the operation ($r = 0.892$, $P < 0.001$, $n = 30$) (Figure 2a). Serum leptin values correlated significantly with oestradiol values from postoperative days 4–7 in group 3 ($r = 0.480$, $P < 0.05$, $n = 24$, Figure 3a) and from day 0 to day 8 in group 1 ($r = 0.467$, $P < 0.001$, $n = 91$, Figure 3c). In group 2, the correlations between leptin and oestradiol values were not significant. Significant positive correlations were also found between serum leptin and progesterone concentrations from postoperative day 0 to day 8 in group 1 ($r = 0.239$, $P < 0.05$, $n = 91$, Figure 3d) and group 2 ($r = 0.217$, $P < 0.05$, $n = 91$, Figure 3d) and from day 0 to day 7 in group 3 ($r = 0.323$, $P < 0.05$, $n = 54$, Figure 3b). When multiple regression analysis was performed in group 3, the significant positive correlation between leptin and oestradiol shown in Figure 3a was eliminated and leptin correlated significantly only with BMI. When leptin values on days 0 and 8 of groups 1 and 2 were combined, by simple regression analysis they correlated significantly with BMI ($r = 0.602$, $P < 0.01$, $n = 28$), progesterone ($r = 0.601$, $P < 0.01$, $n = 28$) and oestradiol values ($r = 0.386$, $P < 0.05$, $n = 28$).
A significant reduction in leptin values was seen in both phases of the cycle during the week following the operation which, however, was preceded by a rapid increase during the first 24 h after the operation. This temporal increase in leptin values is difficult to explain. It seems rather unlikely that this is related to the abrupt decrease in oestradiol and progesterone concentrations as a similar temporal increase in leptin values was also seen in women of group 3, in whom serum oestradiol values, instead of declining, increased significantly. So far, oestradiol has been found to exert a stimulatory effect on leptin production in rats in vitro (Murakami et al., 1995). An explanation for this early increase in leptin values might be that during the incision of the abdominal wall manipulation of the s.c. fat tissue took place and as a result leptin was released in high amounts into the circulation, but this needs to be investigated. Finally, one cannot exclude the possibility that the early increase in leptin following the operation was a response to the surgical stress, as happened with gonadotrophins and gonadal steroids in this and previous studies (Messinis et al., 1996; Alexandris et al., 1997).

After the temporal increase, leptin values declined rapidly in all three groups of women up to postoperative day 4 to concentrations that were significantly lower than before the operation. At the same time, changes in oestradiol values varied considerably among groups, indicating that leptin changes during the immediate period following ovariectomy are independent of oestradiol. These results contradict data in rats in which treatment with oestrogen reversed the significant reduction in serum leptin concentrations and in the expression of ob gene in white adipose tissue that was seen 2–8 weeks after ovariectomy (Shimizu et al., 1997; Yoneda et al., 1998). It is evident, therefore, that factors other than oestradiol controlled leptin secretion during the postoperative period in the present study. Such factors could be changes in food intake, reduction in fat stores and body weight and consequently in BMI and decrease in motor activity. These factors, however, are interrelated and, although fat stores were not measured in the present study, only a small reduction in body weight with no significant changes in BMI was seen in the groups of ovariectomized women 1 week after the operation. Since body weight measurements were not available in the ovariectomized women during the greater part of the postoperative period, one cannot exclude the possibility that a significant reduction in body weight occurred in these women during the first 4 days after the operation at a time when great restrictions in food intake were applied. Although dramatic changes in leptin values in response to changes in food intake are not expected in normal or obese subjects (Korbonits et al., 1997), a recent study has shown that even a 4% reduction in body weight over a period of 7 days resulted in a 61% decrease in leptin values in men and women (Dubuc et al., 1998). These data, together with the significant positive correlations between leptin values and BMI that were seen in our patients during the postoperative period, indicate that changes in leptin values following the operation were predominantly determined by changes in this parameter.

The possibility, however, that oestradiol itself can affect leptin production in women is not excluded. A significant positive correlation between leptin and oestradiol values was seen during the second half of the postoperative period in the cholecystectomy women and this is in accordance with data in mid- to late follicular phase of the normal menstrual cycle (Messinis et al., 1998). At the same time, leptin and oestradiol values increased significantly in this group of women, even though BMI continued to decline. Furthermore, high affinity binding of 17β-oestradiol in the cytoplasmic fraction of various white adipose tissues has been demonstrated in rats (Wade and Gray, 1978).

The finding that in ovariectomized women a significant independent association was found between progesterone and...
leptin values suggests that this steroid may also participate in
the production of leptin by adipocytes. Significant positive
correlations of leptin with progesterone were also found in a
previous study during the normal menstrual cycle (Hardie
et al., 1997). It is possible, therefore, that oestradiol during
the follicular phase of the cycle primes the adipocytes to the
stimulating effect of progesterone. This could explain the
significantly higher values of leptin in the early to midluteal
compared with the mid- to late follicular phase of the cycle
seen in the present and in previous studies (Hardie
et al., 1997; Shimizu et al., 1997; Messinis et al., 1998). The rapid
decline of luteal phase leptin values after ovariectomy to
centres similar to those of the follicular phase of the cycle
seen in the present and previous studies (Hardie et al.,
1997; Shimizu et al., 1997; Messinis et al., 1998). The rapid
decline of luteal phase leptin values after ovariectomy to
centres similar to those of the follicular phase of the cycle
supports this assumption. The possibility that the ovaries may contribute
to the circulating leptin concentrations in women cannot be
excluded despite the fact that in this study leptin values declined
both in the ovarioctomized and the non-ovariectomized women.
Recent data have suggested that the pre-ovulatory follicle itself
may be an important source of leptin (Cioffi et al., 1997).
Oestradiol and progesterone, therefore, may act within the
follicle to increase leptin production at that site. On the other
hand, leptin produced inside the ovary might act as a paracrine
factor to affect steroid synthesis in the follicle and corpus
luteum, since both binding of leptin and a direct effect of this
substance on steroidogenesis have been demonstrated in vitro
(Spicer and Francisco, 1997; Zachow and Magoffin, 1997).
Alternatively, however, changes in leptin concentrations on
days 0–4 in the group of cholocystectomized women could
simply reflect the stage of the cycle in these women, i.e. early
to midfollicular phase, during which a decline in leptin values
has been recently described, although the mechanism is not
clear (Messinis et al., 1998).

From a physiological point of view, these results support
the hypothesis that leptin may be the missing link between
body fat and reproduction (Conway and Jacobs, 1997). Apart
from the relationship with gonadal steroids, this protein may
also affect reproduction through other mechanisms, such as by
controlling early development of embryos before implantation
(Antczak and Van Blerkon, 1997).

In conclusion, the present study confirms previous data that
leptin concentrations are higher in the luteal than the follicular
phase of the cycle. The results demonstrate for the first time
that leptin concentrations following a laparotomy decline
rapidly from the first to the fourth postoperative day both in
ovariectomized and non-ovariectomized women. Although BMI
seems to be the predominant factor responsible for these
changes, it is also possible that oestradiol and progesterone
are involved in the mechanism which controls the production
of leptin during the normal menstrual cycle.

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References


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