Leptin concentrations in normal women following bilateral ovariectomy

I.E.Messinis1,5, S.D.Milingos1, E.Alexandris2, I.Kariotis2,3, G.Kollios4 and K.Seferiadis4

1Department of Obstetrics and Gynaecology, University of Thessalia, 22 Papakiriazi Street, 41222 Larissa, State Departments of 2Obstetrics and Gynaecology and 3General Surgery, Larissa and 4Department of Biological Chemistry, University of Ioannina, Ioannina, Greece
5To whom correspondence should be addressed

To study the relationships between gonadal steroids and leptin, 20 women with normal cycles were investigated during the postoperative period following a laparotomy. Fourteen women underwent bilateral ovariectomy plus total hysterectomy either in the mid- to late follicular phase (n = 7, group 1) or in the early to midluteal phase (n = 7, group 2). The remaining six of the 20 women underwent cholecystectomy in the early to midfollicular phase of the cycle and were used as controls (group 3). In all three groups, serum leptin values decreased rapidly up to postoperative day 4. Then, leptin values increased significantly only in group 3 (P < 0.05). Leptin values before and after the operation showed significant positive correlations with body mass index (BMI), oestradiol and progesterone. However, with multiple regression analysis, BMI was the only parameter significantly correlated with leptin in group 3 (days 0 and 4–7), whereas in groups 1 and 2 progesterone and BMI showed independent significant correlations with leptin (days 0 and 8, r = 0.601 and r = 0.602 respectively). These results demonstrate for the first time a significant reduction in leptin concentrations in normal women following bilateral ovariectomy. Although BMI seems to be the predominant factor, it is also suggested that oestradiol and progesterone may participate in the control of leptin production during the human menstrual cycle.

Key words: body mass index/leptin/oestradiol/ovariectomy/ovary

Introduction

Leptin, a 16 kDa protein, is a product of the ob gene secreted by adipose tissue (Zhang et al., 1994). In humans, the secretion of leptin is performed in a pulsatile fashion (Licinio et al., 1998). This protein appears to regulate fat stores and body weight by decreasing appetite and increasing thermogenesis (Halaas et al., 1995; Caro et al., 1996a). The ob/ob mice that lack leptin become very obese and infertile and develop insulin resistance. However, fertility is restored in these animals after treatment with recombinant leptin (Barash et al., 1996; Chehab et al., 1996). In humans, the relationship between leptin and obesity is rather obscure. An association between obesity and leptin resistance has been proposed (Caro et al., 1996b; Schwartz et al., 1996), but the mechanism is not clear. Recently, a leptin binding factor has been identified in human serum which may influence the physiological response to leptin (Diamond et al., 1997). On the other hand, although human obesity has not been linked to mutations of the leptin gene, recently a mutation of the gene for leptin was detected in two severely obese children with very low serum leptin concentrations (Montague et al., 1997).

Since obesity is one of the symptoms in a significant proportion of women with polycystic ovary syndrome (PCOS), several studies have investigated changes in leptin concentrations in this syndrome (Brzechffa et al., 1996; Chapman et al., 1997; Laughlin et al., 1997; Mantzoros et al., 1997; Rouru et al., 1997). Although the results are conflicting, it has become evident that leptin may play a role in certain cases of PCOS and may act as a link between fat and reproduction. Recent studies have detected leptin receptor mRNA in the human ovary and specific binding of leptin in ovine granulosa cells (Cioffi et al., 1996; Spicer and Francisco, 1997), whereas experiments in vitro have shown that leptin may directly affect follicle stimulating hormone (FSH)-induced production of oestradiol by rat granulosa cells (Zachow and Magoffin, 1997). A possible relationship between oestrogen and leptin has been recently postulated. In rats, ovariectomy reduced significantly serum leptin values and the expression of ob gene in certain sites of white adipose tissue, changes which were reversed by oestradiol supplement (Shimizu et al., 1997; Yoneda et al., 1998). In humans, lower concentrations of leptin have been found in postmenopausal compared to premenopausal women and in men compared to pre- or postmenopausal women, while during the normal menstrual cycle the concentrations of leptin are higher in the luteal than in the follicular phase (Hardie et al., 1997; Shimizu et al., 1997; Messinis et al., 1998).

The present study was undertaken to examine further the relationships between gonadal steroids and leptin in normal women by investigating changes in leptin concentrations following bilateral ovariectomy.

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
Leptin was measured in all serum samples in duplicate using a competitive immunoassay was used (Kodak Amerlite Estradiol-60 assay; Amersham). The results are expressed as pmol/l. For progesterone assay based on enhanced luminescence (Amerlite Estradiol-60 assay; Amersham). The results are expressed as pmol/l. For progesterone assay based on enhanced luminescence were used (Amerlite FSH and Amerlite LH assay; Amersham). The results are expressed as 0.05 IU/l respectively, and group 2 (15.4 ± 3.1 and 5.5 ± 1.0 IU/l 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) 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
Leptin concentrations in women after ovariectomy

Figure 1. Serum concentrations (mean ± SEM) of leptin, oestradiol, progesterone and gonadotrophins in normally cycling women before and after bilateral ovariectomy plus total abdominal hysterectomy performed (day 0) (○) in the mid- to late follicular phase of the cycle (seven women, group 1) or (●) in the early to midluteal phase of the cycle (seven women, group 2). Another six women underwent cholecystectomy (▲) in the early to midfollicular phase of the cycle. *P < 0.05, **P < 0.01, ***P < 0.001 (differences from the other two groups). FSH = follicle stimulating hormone; LH = luteinizing hormone.

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 ± 78 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 ± SEM) were on day 0 significantly higher in group 2 (16.9 ± 1.2 nmol/l) than in group 1 (4.5 ± 0.6 nmol/l, P < 0.01) and group 3 (1.2 ± 0.2 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 ± SEM) was noted on postoperative day 8 compared with day 0 in group 1 (0.53 ± 0.20 kg) and group 2 (0.71 ± 0.30 kg) with no significant difference between the two groups. In group 3, the decrease in body weight on day 7 (3.2 ± 0.2 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 ± SEM) did not change significantly on postoperative day 8 compared with the value on day 0 both in group 1 (26.1 ± 1.1 and 26.3 ± 1.1 kg/m² respectively) and group 2 (26.7 ± 0.7 and 26.9 ± 0.8 kg/m² respectively), while it decreased significantly in group 3 from day 0 (28.2 ± 1.5 kg/m²) to day 7 (26.3 ± 1.5 kg/m², P < 0.001). A significant decrease in BMI was also seen in group 3 from day 0 to day 4 (27.3 ± 1.5 kg/m², 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 and 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).
When multiple regression analysis was applied, the significant correlation of leptin with oestradiol was eliminated, while the correlation of leptin with progesterone and BMI were preserved. Oestradiol values correlated significantly with those of progesterone on days 0 and 8 in groups 1 and 2 ($r = 0.520$, $P < 0.01$, $n = 28$) and in group 3 from days 4–7 ($r = 0.405$, $P < 0.05$, $n = 24$). No significant correlations were found between BMI and oestradiol or progesterone values in all three groups of women.

Discussion

The present study is the first in which changes in leptin concentrations were investigated in women following bilateral ovariectomy. 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 unrelated 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 cholecystectomized 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 concentrations in women after ovariectomy

Figure 3. Correlations between leptin and oestradiol or progesterone values in normally cycling women after bilateral ovariectomy plus hysterectomy performed in mid- to late follicular phases (seven women, group 1) or in early to midluteal phases (seven women, group 2) and in six women after cholecystectomy (group 3). Operations were performed on day 0. (a) Group 3, postoperative days 4–7 (r = 0.480, P < 0.05, n = 24), (b) group 3, days 0–7 (r = 0.323, P < 0.05, n = 54), (c) group 1, days 0–8 (r = 0.467, P < 0.01, n = 91) and (d) (○) group 1, days 0–8 (r = 0.239, P < 0.05, n = 91) and (●) group 2, days 0–8 (r = 0.217, P < 0.05, n = 91).

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 concentrations similar to those of the follicular phase 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 ovariectomized 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 cholecystectomized 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.

Acknowledgements

We wish to thank Professor O.Tsolas, Director of the Department of Biological Chemistry, University of Ioannina for providing the laboratory facilities for the hormone assays.
References


