Leptin concentrations in the follicular phase of spontaneous cycles and cycles superovulated with follicle stimulating hormone

I.E. Messinis1,4, S. Milingos1, K. Zikopoulos2, G. Kollios3, K. Seferiadis3 and D. Lolis2

1 Department of Obstetrics and Gynaecology, University of Thessalia, Larissa and Departments of 2 Obstetrics and Gynaecology and 3 Biological Chemistry, University of Ioannina, Greece
4 To whom correspondence should be addressed

It has been reported that oestradiol may play a role in the production of leptin from adipocytes. To investigate this relationship further, nine normally ovulating women were studied during two menstrual cycles, i.e. an untreated spontaneous cycle and a cycle treated with follicle stimulating hormone (FSH) from cycle day 2 until the day of human chorionic gonadotrophin (HCG) injection. Serum leptin values on cycle day 2 did not differ significantly between the spontaneous and the FSH cycles. In the spontaneous cycles, leptin values declined gradually and significantly up to day 7 and then increased progressively up to the day of luteinizing hormone (LH) surge onset, at which point they achieved the highest values. In the FSH cycles, serum leptin values increased gradually and significantly up to day 6, remaining stable thereafter, and were in the midfollicular phase significantly higher than in the spontaneous cycles. Significant positive correlations were found between mean values of leptin and mean values of oestradiol during the second half of the follicular phase in the spontaneous cycles and during the first half in the FSH cycles. A significant negative correlation was found between these two parameters in the spontaneous cycles during the first half of the follicular phase. Serum leptin levels were significantly higher in the midluteal than in the follicular phase in both cycles. These results demonstrate for the first time significant changes in leptin values during the follicular phase of the human menstrual cycle and a significant increase during superovulation induction with FSH. It is suggested that oestradiol may be involved in the regulation of leptin production in women.

Key words: leptin/FSH/oestradiol/ovary

Introduction

Leptin is a protein of 16 kDa molecular mass that is produced by adipocytes under the regulation by the ob gene (Zhang et al., 1994). This protein seems to regulate fat stores and body weight through an effect on appetite and fat metabolism (Halaas et al., 1995; Caro et al., 1996). It has been suggested that leptin is important for fertility in mice since animals that lack this protein become very obese and infertile, while treatment with leptin reduces weight and restores fertility (Barash et al., 1996; Chehab et al., 1996). The production of leptin can be affected by various hormonal factors including glucocorticoids and insulin (De Vos et al., 1995; Saladin et al., 1995; Kolaczynski et al., 1996; Papaspyrou-Rao et al., 1997).

Recent studies have investigated serum leptin values in women with polycystic ovary syndrome but the results have been controversial (Brzechffa et al., 1996; Chapman et al., 1997; Laughlin et al., 1997; Mantzoros et al., 1997; Rouru et al., 1997). Also, a possible action of leptin on the ovary has been postulated both from the specific binding of this protein in granulosa cells (Spicer and Francisco, 1997) and the fact that insulin-like growth factor-I-mediated enhancement of follicle stimulating hormone (FSH)-stimulated oestriadiol synthesis by rat and human granulosa cells in vitro can be inhibited by leptin (Agarwal et al., 1997; Zachow and Magoffin, 1997). Furthermore, a relationship between oestrogen and leptin has been recently suggested. In particular, data in rodents have shown a reduction in serum leptin concentrations and decrease in the expression of ob gene in adipose tissue of ovariectomized animals, changes which are reversed by oestradiol administration (Shimizu et al., 1997). In the same study, higher concentrations of leptin were found in women than in men as well as in premenopausal compared to postmenopausal women (Shimizu et al., 1997). Also, recent data in normal women have shown higher values of leptin in the luteal than in the follicular phase of the cycle (Hardie et al., 1997; Shimizu et al., 1997) as well as in the periovulatory period than in the follicular phase (Hardie et al., 1997). However, fluctuations in leptin values during the cycle based on daily blood samples have not been investigated.

The present study was undertaken to investigate serum values of leptin during the follicular phase of natural cycles and cycles superovulated with FSH in order to obtain more insight into the relationship between this protein and gonadal steroids.

Materials and methods

Patients

Nine normally cycling women attending the infertility clinics volunteered for the study and gave written informed consent. They were 24–35 years old (mean 29.2 years) with 5–8 years duration of unexplained infertility.

Approval for the study was obtained from the local Ethical Committee. All women had menstrual cycles of normal duration (27–34 days) with normal FSH levels and a normal luteal phase, as assessed by serum progesterone measurement and ultrasound scans.
of the ovaries prior to the onset of the study. The body mass index (BMI) varied between 20 and 25 kg/m² (mean 22.1). All women were investigated during two menstrual cycles, i.e. an untreated spontaneous cycle (first cycle) and a cycle treated with FSH (second cycle). Treatment with FSH started on cycle day 2 at the dose of 225 IU (3 ampoules) per day i.m. (75 IU per ampoule, Metrodin HP; Serono, Faran, Greece) and continued up to the day of human chorionic gonadotrophin (HCG) administration. The dose of FSH was chosen, based on our previous experience from women participating in research studies, as producing an appropriate ovarian response (Messinis et al., 1991). In two women, due to the presence of more than three follicles >16 mm, HCG was withheld and the women were advised to avoid intercourse. None of the women during the experimental period underwent in-vitro fertilization treatment. During both cycles, daily blood samples (0900 h) were taken from the women starting on cycle day 2 up to the day of onset of the endogenous luteinizing hormone ( LH) surge in the spontaneous cycles and up to the day of HCG administration in the FSH cycles. The LH surge was detected in all women using a urinary LH measurement (Organon LH colour; Organon Hellas, Athens, Greece) and was confirmed by blood samples. In the spontaneous cycles, the women were not given the HCG injection. Ultrasound scans of the ovaries for follicle measurement started in the spontaneous cycles on day 8 and in the FSH cycles on day 7. In all blood samples FSH, LH, oestradiol and progesterone were measured. A further blood sample for progesterone and leptin measurements was taken from the women 7 days after the detection of the LH surge or the HCG administration. All blood samples were stored at –20°C until assayed. None of the women became pregnant during the period of the study.

Hormone assays

FSH and LH were measured in serum with the use of immunometric assays based on enhanced luminescence (Amerlite FSH and Amerlite LH assay respectively; Amersham International plc, Amersham, UK). The results are expressed as IU/l of standards calibrated against the World Health Organization 2nd International Reference Preparation (IRP) of human FSH (58/549) and the 1st IRP of human LH (68/40). Oestradiol was measured in serum using a competitive immunoassay based on enhanced luminescence. Kits were purchased from Amersham (Amerlite Estradiol-60 assay Amersham International plc Amersham, UK). The results are expressed as pmol/l. For progesterone measurement a competitive immunoassay was used. Kits were purchased from Amersham (Kodak Amerlite Progesterone assay). 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 (St Charles, MO, USA) and contained human leptin antibody prepared in rabbit and raised against highly purified human leptin and standards and tracer 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/l, 0.12 IU/l, 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 for detection of leptin was 0.5 ng/ml, while interassay and intra-assay coefficients of variation were 6.5 and 7.0% respectively.

Statistical analysis

The results were statistically analysed using one-way analysis of variance unless stated otherwise. Variations in leptin values throughout different periods of the follicular phase as well as from the spontaneous to the FSH-treated cycles were assessed by calculating the variance ratio (F). In the statistical calculations, hormone results were log-transformed to obtain approximately normal distributions.

Results

In all women during the spontaneous cycle an LH surge was detected. The cycle day (mean ± SEM) on which the LH surge started was 12.2 ± 0.5. On that day, LH values (mean ± SEM) were 18.8 ± 2.8 IU/l. In none of the women was an LH surge detected in the FSH cycle before the administration of HCG. The mean (± SEM) cycle day on which HCG was given was 10.6 ± 0.3 and the duration of the follicular phase was significantly shorter in the FSH than in the spontaneous cycles (P < 0.05). Multiple follicular development occurred in the women during treatment with FSH. The number of follicles ≥16 mm in diameter on the day of HCG was 3.0 ± 0.6. Ovulation of at least one follicle was confirmed in the women by ultrasound in both cycles.

Figure 1 shows serum concentrations of leptin, FSH, LH and oestradiol during the follicular phase of the spontaneous and the FSH-treated cycles. During the administration of FSH, serum concentrations of FSH and consequently of oestradiol increased and the levels were significantly higher than in the spontaneous cycles (Figure 1). As a result of the increase in oestradiol values serum values of LH decreased and the concentrations were significantly lower than in the spontaneous cycles. Serum leptin values (mean ± SEM) on cycle day 2 did not differ significantly between the spontaneous (10.4 ± 1.3 ng/ml) and the FSH-treated cycles (8.7 ± 1.9 ng/ml). In the spontaneous cycles, leptin levels declined gradually from day 3 to day 7 (6.4 ± 1.3 ng/ml, F3,40 = 3.63, P < 0.05). A declining pattern was seen in seven of nine women (77.8%) and in four of them the decrease was >50% of the initial value. Leptin values then increased gradually up to cycle day 10 (9.4 ± 1.4 ng/ml, F10,49 = 2.96, P < 0.05) at which point they did not differ significantly from those on cycle day 2. In the FSH-treated cycles, serum leptin values increased progressively and significantly from day 2 to day 6 (12.9 ± 1.2 ng/ml, F5,40 = 4.06, P < 0.05). Then, leptin values did not change significantly up to day 10 (11.5 ± 1.4 ng/ml). On days 5, 6 and 7 leptin concentrations were significantly higher in the FSH than in the spontaneous cycles (Figure 1).

Figure 2 shows serum values of leptin and oestradiol during the 4 days before the LH surge onset in the spontaneous cycles or the injection of HCG in the FSH cycles (day 0). Leptin values on day 0 were similar in the two cycles. In the spontaneous cycles, serum leptin values (mean ± SEM) increased significantly from day –4 (7.9 ± 1.5 ng/ml) to day 0 (11.6 ± 1.3 ng/ml, P < 0.05) in parallel with the increase in serum oestradiol concentrations. In contrast, in the FSH cycles serum leptin values did not change significantly from days –4 to day 0. During the same period of time, serum oestradiol concentrations in these cycles increased and were significantly higher than in the spontaneous cycles (Figure 2).

Figure 3 shows significant correlations between serum leptin and oestradiol values. In particular, a positive correlation was seen in the spontaneous cycles between leptin and oestradiol.
values from day –4 to day 0, (Figure 3a). A positive correlation was also found in the FSH cycles between leptin and oestradiol values from day 2 to day 6, (Figure 3b). Finally, a weak negative correlation was found in the spontaneous cycle between leptin and oestradiol values from day 2 to day 7

Figure 1. Serum leptin, follicle stimulating hormone (FSH), luteinizing hormone (LH) and oestradiol values (mean ± SEM) during the follicular phase of (○) spontaneous and (●) FSH-treated cycles in nine normally ovulating women. *P < 0.05, **P < 0.01, ***P < 0.001 (compared with the spontaneous cycles).

The BMI (mean ± SEM) before the onset of the study in the spontaneous cycles was 22.1 ± 0.4 kg/m². BMI value did not change significantly throughout the whole period of the study. Significant positive correlations were found between serum leptin values and BMI in the spontaneous (r = 0.807, P < 0.01) and the FSH cycles (r = 0.701, P < 0.05). Serum concentrations of leptin (mean ± SEM) in the luteal phase did not differ significantly between the spontaneous (15.8 ± 3.2 ng/ml) and the FSH cycles (18.1 ± 4.7 ng/ml, n = 7). In both cycles, leptin values were significantly higher in the luteal than in the follicular phase (P < 0.05). Also, serum progesterone concentrations were similar in the two cycles (30.3 ± 2.6 and 31.2 ± 2.1 nmol/l respectively). However, the duration of the luteal phase was significantly shorter in the FSH (11.7 ± 0.5 days, n = 7) than in the spontaneous cycles (13.6 ± 0.2 days, P < 0.05). None of the women developed ovarian hyperstimulation syndrome during treatment with FSH.

Discussion
The present study is the first in which changes in leptin concentrations were investigated during the follicular phase of spontaneous cycles and cycles stimulated with FSH. There are only two previous studies in which leptin concentrations were measured in spontaneous cycles. In one of them (Shimizu et al., 1997), leptin was measured only in two blood samples during the cycle and the values were significantly lower in the

Figure 2. Serum leptin values (mean ± SEM) during the late follicular phase of (○) spontaneous and (●) FSH-treated cycles in nine normally ovulating women. The data were normalized to the day of onset of the endogenous luteinizing hormone surge in the spontaneous cycles or the day of human chorionic gonadotrophin administration in the FSH-treated cycles (Day 0). *P < 0.05, ***P < 0.001 (compared with the spontaneous cycles).

Figure 3. Serum leptin, follicle stimulating hormone (FSH), luteinizing hormone (LH) and oestradiol values (mean ± SEM) during the late follicular phase of (○) spontaneous and (●) FSH-treated cycles in nine normally ovulating women. **P < 0.01 (compared with the spontaneous cycles).

(Figure 3c). During the rest of the follicular phase in the spontaneous and the FSH cycles the correlations between mean leptin and oestradiol values were not significant.
The role of oestradiol in leptin production

Figure 3. Correlations between leptin and oestradiol values in serum during (a) the late follicular phase of spontaneous cycles (days –4 to 0 of Figure 2), (b) the early follicular phase of follicle stimulating hormone-treated cycles (cycle days 2 to 6 of Figure 1) and (c) the early follicular phase of spontaneous cycles (cycle days 2 to 7 of Figure 1).

midfollicular than in the midluteal phase, while in the other study (Hardie et al., 1997), leptin was measured in blood samples taken every third day in the two phases of the cycle and every day at midcycle. The latter study, however, found considerable variability in leptin values throughout the menstrual cycle, but when the results were grouped according to the functional stage of the cycle, values were significantly higher in the periovulatory period than in the rest of the follicular phase. In the present study leptin values, measured in blood samples taken every day in the spontaneous cycles, showed a steady decline during the first half of the follicular phase and a gradual recovery during the second half with highest values achieved at midcycle. It should be emphasized, however, that although a declining pattern was apparent in the majority of the women, only on day 7 of the cycle were leptin concentrations significantly lower than on cycle day 2, and this may be related to the individual variability in leptin values ranging for example on day 2 from 3.2 to 16.7 ng/ml.

This decrease in leptin values during the first half of the follicular phase is difficult to explain. During the same period of time, serum oestradiol concentrations increased only slightly, but the pattern of increase correlated negatively with the pattern of leptin decrease. Although there is some evidence that oestradiol stimulates the production of leptin in vivo (Shimizu et al., 1997), the possibility that slowly rising concentrations of oestradiol may exert suppressing effects cannot be excluded. During the second half of the follicular phase in the spontaneous cycles, leptin values increased significantly in parallel with the increase in oestradiol values, suggesting that at that stage of the cycle oestradiol may stimulate the production of leptin from the adipocytes. That oestradiol may be involved in the control of leptin production in women is also supported by the fact that premenopausal women have higher levels of leptin than postmenopausal women and in general women have higher values than men (Shimizu et al., 1997). Moreover, ovariectomy in rats resulted in a significant reduction in the expression of the ob gene in various sites of white adipose tissue as well as in a reduction in serum leptin concentrations and these changes were reversed by the administration of oestradiol to these animals (Shimizu et al., 1997). The fact that leptin levels in this study, as in previous studies (Hardie et al., 1997; Shimizu et al., 1997), were higher in the luteal than in the follicular phase is a further support to the involvement of gonadal steroids in the mechanism of leptin production during the normal menstrual cycle. One could assume that progesterone in addition to oestradiol stimulated leptin production during the luteal phase, but this remains to be clarified.

In the present study, a significant rise in leptin values was found during the first half of FSH treatment after which leptin values remained stable despite the continuous administration of FSH. Since FSH exerts direct effects on the ovary, one would assume that leptin is an ovarian product. This assumption is further supported by recent data demonstrating the expression of leptin at the level of mRNA and protein by granulosa and cumulus oophorous cells (Antczak et al., 1997; Cioffi et al., 1997) as well as by the specific binding of this protein in granulosa cells (Spicer and Francisco, 1997). Nevertheless, another recent study has failed to detect ob gene expression in human granulosa cells (Billig et al., 1997). Therefore, although the production of leptin by the ovary is not excluded, it is also possible that the production of this protein by the adipocytes is indirectly affected by FSH through an effect of various ovarian substances. During the first half of the follicular phase of the FSH-treated cycles, the pattern of leptin increase correlated significantly with that of oestradiol. It is tempting, therefore, to speculate that the early rise in oestradiol values during the FSH treatment prevented leptin values from declining and stimulated further the production of this protein. The fact that leptin concentrations failed to increase further in the FSH cycles, despite the continuous rise of oestradiol values to supraphysiological levels, suggests that there is a limit to the extent to which oestradiol can stimulate leptin production in women. In fact, no significant correlation was found between
leptin and supraphysiological oestradiol values during the second half of the follicular phase in the FSH cycles, while in both cycles leptin values correlated with oestradiol values only when the latter were within the physiological range. It is evident from these results that serum leptin concentrations do not reflect the degree of ovarian hyperstimulation induced by FSH. Finally, the possibility that the described changes in leptin values in the present study are related to changes in the amount of fat tissue is not likely, since although fat stores were not measured, BMI did not change significantly throughout the period of the study.

The physiological importance of the present findings is not clear. It is not known whether the changes described during the spontaneous cycles reflect changes in the metabolism. In certain conditions, e.g. polycystic ovary syndrome which may be associated with obesity and metabolic dysfunction, changes in leptin values may represent a link between metabolism and reproduction (Conway and Jacobs, 1997). It should be taken into account that the women who participated in this study were infertile, and therefore the present results may not directly apply to the normal population. Certainly, the role of leptin during the normal menstrual cycle requires further investigation.

In conclusion, the present study demonstrates for the first time that leptin concentrations in the normal menstrual cycle decline during the first half and increase during the second half of the follicular phase, with higher values at midcycle and highest values in the luteal phase. During treatment with FSH, the decline in leptin values is prevented and a further increase is stimulated. It is suggested that oestradiol may be an important regulator of leptin production in women.

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References


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