Changes in plasma leptin during the menstrual cycle
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Abstract
Objective: To measure the plasma concentration of leptin, which is expressed in ovarian follicles and may have a reproductive function, in healthy women during the menstrual cycle.

Design: This study included nine women with regular menstrual cycles (mean ± S.E.M. age 28 ± 2 years: body mass index 23.9 ± 1.8 kg/m²). From the onset of menses, fasting blood samples were collected every 1–2 days throughout the menstrual cycle. As a control, plasma leptin was measured in six postmenopausal women and six men every other day for 28 days.

Results: In menstruating women, plasma leptin increased from 14.9 ± 2.9 ng/ml in the early follicular phase to 20.4 ± 4.2 ng/ml (P < 0.01) at the midluteal phase and returned to the baseline by the subsequent menses. In contrast, leptin concentrations did not change significantly in postmenopausal women or men. The changes in plasma leptin during the menstrual cycle were not related to changes in sex hormones.

Conclusions: The cause of the increase in plasma leptin during the late follicular and luteal phases of the menstrual cycle is not clear. It may be attributed to augmented adipocyte production of leptin in response to increased caloric intake or hypothalamic release of neuropeptide Y, or to release of leptin from mature ovarian follicles. Leptin may have a role in regulating the menstrual cycle and preparing the body for the metabolic demands of pregnancy.

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Introduction
Leptin, the ob gene product (1), appears to contribute to the regulation of reproduction. Leptin deficiency in the ob/ob mouse is associated with hypogonadism and sterility that can be corrected by leptin replacement (2, 3). Moreover, leptin administration has been shown to accelerate puberty in normal rodents (4–6). Ahima et al. (7) found leptin prevented the starvation-induced delay in ovulation in female mice and the decrease in testosterone concentrations in males. These effects were associated with increased luteinizing hormone (LH) concentration, suggesting that leptin acts at the level of the hypothalamic–pituitary axis. Leptin has also been shown to exert direct effects on hypothalamic–pituitary release of gonadotropin (8) and ovarian steroidogenesis (9) in vitro. More recently, Cioffi et al. (10) showed that pre-ovulatory human follicles synthesize leptin. To gain further insight into the interrelationships among leptin, gonadotropins, and female sex steroids, we determined plasma leptin concentrations during the normal menstrual cycle.

Subjects and methods

Subjects
This study included nine healthy women aged 28 ± 2 (mean ± S.E.M.) years with a body mass index (BMI) of 23.9 ± 1.8 kg/m². All women had regular menstrual cycles and none was taking any medications. From the onset of menses, fasting blood samples were collected every other day for the first 9 days, every day for the next 7 days, and then every other day until the next menses. Blood samples were collected between 0700 and 0900 h, at the same time ± 30 min for every woman, for measurement of plasma concentrations of glucose, insulin, leptin, estradiol, progesterone, LH, and follicle-stimulating hormone (FSH). The day of the midcycle gonadotropin surge was identified by the coincident occurrence of three of the following: day of the LH peak, day of the FSH peak, day of or day after the estradiol peak, and first day of the progesterone concentration doubling or exceeding 2.0 nmol/l (11).

In addition, two control groups were included. The first comprised six postmenopausal women aged 60 ± 4 years with a BMI of 29.8 ± 2.3 kg/m² and the second, six men aged 44 ± 5 years with a BMI of 25.3 ± 0.6 kg/m². All controls were healthy and not taking any medications. Fasting blood samples were collected every other day for 29 days between 0700 and 0900 h, at the same time ± 30 min for each subject, for measurement of plasma glucose, insulin, and leptin.

The study was approved by the Institutional Review Board and all participants gave informed consent.
Biochemical analyses

Glucose was measured by the glucose oxidase method. All hormones were determined by radioimmunoassay. Insulin was measured with a double-antibody technique using a specific antibody for human insulin, raised in guinea pigs and having <0.2% cross-reactivity with proinsulin (Linco Research, St Louis, MO, USA); the interassay coefficient of variation was 6–8% and the sensitivity was 12 pmol/l. Leptin was determined with a double-antibody technique using a polyclonal antibody, raised in rabbits, against recombinant human leptin (Linco Research). This assay is highly specific for human leptin, with no cross-reactivity with other human peptides; the interassay coefficient of variation was 5–7% and the sensitivity was 0.5 ng/ml. LH was measured with a double-antibody assay using monoclonal antibodies with <0.01% cross-reactivity with FSH, human chorionic gonadotropin (HCG), and thyroid-stimulating hormone (TSH) (Nichols Institute, San Juan Capistrano, CA, USA); the interassay coefficient of variation was 2–5% and the sensitivity was 0.1 mIU/ml. FSH was determined with a double-antibody assay using monoclonal antibodies with <0.01% cross-reactivity with LH, HCG, and TSH (Nichols Institute); the interassay coefficient of variation was 3–8% and the sensitivity was 0.2 mIU/ml. Estradiol was measured using antibody-coated tubes (Diagnostic Products Corporation, Los Angeles, CA, USA). This assay had very low cross-reactivity with other steroids, a sensitivity of 0.03 nmol/l, and an interassay coefficient of variation of 4–9%. Progesterone was measured using antibody-coated tubes (Diagnostic Products Corporation); the assay had a very low cross-reactivity with other steroids, a sensitivity of 0.06 nmol/l, and an interassay coefficient of variation of 6–10%.

Statistical analyses

Data are expressed as means ± S.E.M. Statistical analyses were performed with the programs of SPSS, Inc. (12). Menstrual cycle profiles for all women were aligned relative to the day of the midcycle gonadotropin surge (day 0). Within-group comparisons were performed with repeated measures ANOVA. The Tukey test was used for multiple comparisons. Linear regression or Pearson correlations, or both, were used to evaluate the between- and within-subject relations of different variables, using Bland and Altman’s approach for analysis of repeated data (13, 14). Between-subject correlations were performed on subject means – that is, means of all measurements of each variable during the study (13). The within-subject relation between the change in leptin and those in other variables was evaluated with analysis of covariance (14).

Results

In menstruating women, all cycles were ovulatory. LH, FSH, estradiol, and progesterone showed the expected changes (Fig. 1). Body weight did not vary significantly during the menstrual cycle. Plasma leptin was 14.9 ± 2.9 ng/ml at the onset of menses and fluctuated minimally around that value during the early follicular phase (days −13 to −9). Leptin concentrations started to increase in the late follicular phase and reached a plateau by the time of the midcycle gonadotropin surge ($P < 0.01$). Subsequently, leptin concentrations...
remained approximately the same throughout the midluteal phase (days +5 to +9), declining in the late luteal phase (days +10 to +14), and returned to baseline by the start of the next menses (Fig. 1). Thus plasma leptin increased from 14.9 ± 2.9 in the early follicular phase to 20.4 ± 4.2 ng/ml at the midluteal phase – an increase of 51 ± 14% (P < 0.01). In contrast, neither postmenopausal women (P = 0.59) nor men (P = 0.34) showed a significant change in plasma leptin over a period of 29 days.

Fasting insulin concentration was 62 ± 10 pmol/l at the beginning of the menstrual cycle. It fluctuated around the baseline during the follicular phase increasing to 77 ± 11 by the time of the midcycle gonadotropin surge. Plasma insulin concentration remained the same through most of the luteal phase, to decrease to baseline by the onset of the subsequent menses (Fig. 1). Overall, the changes in insulin were significant (P = 0.03), but the differences among early follicular, midcycle, and midluteal concentrations did not reach statistical significance. Plasma insulin did not change significantly in postmenopausal women or men.

In menstruating women, plasma leptin concentration correlated with BMI and with insulin concentration (r = 0.79, 0.74 respectively; P < 0.01 for each), but not with glucose, LH, FSH, estradiol, or progesterone concentrations. The relation between insulin and leptin became weaker, however, after adjustment for BMI (r = 0.62; P = 0.08). The changes in leptin concentrations correlated with those in insulin, estradiol, progesterone, and LH (r = 0.48, 0.42, 0.29, 0.26 respectively; P < 0.01 for each), but not with those in FSH or glucose. In a multiple regression model that included all variables, only the changes in insulin and progesterone were significantly associated with those in leptin, explaining 16 and 5% of its variance respectively.

**Discussion**

This work shows a distinctive pattern of change in plasma leptin during the menstrual cycle in women of reproductive age, whereas no significant changes were observed in postmenopausal women or men over a period of 29 days. Leptin concentrations started to increase in the late follicular phase, reaching a plateau at the midcycle gonadotropin surge and the early luteal phase. These changes occurred in absence of a significant change in fat mass and do not appear to result from stimulation of adipose tissue production of leptin by sex steroids. Multiple regression showed that estradiol was not significantly related to, and progesterone accounted for only 5% of, the change in leptin. This finding is consistent with studies showing that hormone replacement therapy in postmenopausal women (15, 16) and oral contraceptives (17) had no effect on plasma leptin.

The cause of the changes in leptin during the menstrual cycle is, thus, unclear. It could be attributed to variations in caloric intake, because plasma leptin has been shown to decrease with short-term fasting (18, 19) and increase (20) with acute overfeeding. Although food intake was not measured in this work, several studies have shown that caloric intake is greater in women during the luteal phase of the menstrual cycle (reviewed in reference 21). Alternatively, these changes in plasma leptin concentration could be hypothalamic in origin, mediated by the neuropeptide Y peptidergic system that links neural processes regulating reproduction with those maintaining energy homeostasis (22–24).

A further possibility is that the increase in leptin during the late follicular and luteal phases could be due to its production elsewhere than in adipose tissue. Although leptin was believed to be produced exclusively by adipocytes (1), Cioffi et al. (10) have recently shown that it is expressed at the mRNA and protein levels in human granulosa and cumulus cell of ovarian follicles. In addition, they found that ovarian hyperstimulation with HCG increased plasma leptin in women undergoing treatment for infertility. It is plausible, therefore, that the increase in leptin around the time of ovulation was due to its release from mature ovarian follicles.

The significance of the changes in plasma leptin during the menstrual cycle is unclear. Leptin receptors are present in the reproductive organs (25, 26) and leptin has been shown to exert direct effects on hypothalamic–pituitary gonadotropin release (7, 8) and ovarian steroidogenesis (9). Therefore, leptin may have a role in regulating the menstrual cycle. In this regard, it is notable that amenorrhea, menstrual irregularities, and infertility are common in women with extremes of leptin concentrations, including elite athletes (27) and those with anorexia nervosa (28) and obesity (29). Moreover, increased leptin concentrations in the luteal phase may help prepare the body for the metabolic demands of pregnancy. Indeed, leptin concentrations have been shown to increase two- to threefold in pregnant women (30–32) and rodents (26, 33, 34).

In conclusion, plasma leptin increased by approximately 50% during the late follicular and luteal phases of the menstrual cycle. The mechanism of this increase is not known. It could be explained, however, by increased adipocyte production of leptin in response to increased caloric intake or hypothalamic neuropeptide Y release. Alternatively, this increase may reflect the release of leptin from mature ovarian follicles. Leptin may have a role in regulating the menstrual cycle and preparing the body for the metabolic demands of pregnancy.

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References


