Simultaneous Increases of Leptin and Gonadotropin-Releasing Hormone Following Exogenous Estrogen Administration in Women with Normally Menstrual Cycle

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Abstract. The aim of this study was to investigate whether administration of exogenous estrogen affects the changes of leptin and GnRH levels in women with normal menstrual cycle. A total of 18 women received a bolus intravenous injection of 20 mg conjugated estrogen (premarin group) at 0800 during the fifth day of menstrual cycle, while another 18 women were administered 20 mL of normal saline as the control group. Fasting blood samples were collected at 0, 4, 8, 24, 28, 32, 48, 56, 72 and 96 hours after injection for analyses of leptin, GnRH, estrone (E₁), estradiol (E₂), LH and FSH.

Both the mean plasma levels of E₁ and E₂ were significantly increased from 4 hours and significantly sustained elevated levels up to 72 hours after injection of premarin. Simultaneous significant increases of leptin and GnRH levels were observed at 28, 32 and 48 hours after injection, while the controls remained constant. The mean LH and FSH levels were initially suppressed and then significantly increased at 56 and 72 hours after premarin administration. Leptin appears to be involved in the regulation of positive feedback mechanism of estrogen by conveyance of metabolic signal to affect the release of GnRH in hypothalamus, while its participation in the modulation of negative feedback remains unknown.

Key words: Leptin, Gonadotropin-releasing hormone, Estrone, Estradiol

LEPTIN, a novel adipocyte-derived hormone [1], has been recognized as a metabolic signal to the reproductive system because ob/ob mice showed significantly elevated serum levels of luteinizing hormone (LH) and increased ovarian and uterine weight after treatment with leptin [2]. Leptin can prevent the reduced pulsatile LH secretion during fasting by conveying some information about nutrition to the hypothalamus which releases a pulsatile gonadotropin-releasing hormone (GnRH) secretion [3] and can induce a dose-related increase in gonadotropin and GnRH release from the cultures of pituitary and hypothalamic cells in adult rats [4]. In addition, in our previous study of female transgenic skinny mice, sustained overexpression of leptin results in accelerated puberty and late-onset hypothalamic hypogonadism [5]. Therefore, leptin may play a role as a mediating signal of nutrition to affect the gonadotropin release by modulation of pulsatile GnRH secretion in hypothalamus [3–5]. On the other hand, a positive relationship between estrogen and leptin has been suggested. Leptin was found to be increased after addition of estrogen in the cultures of isolated human [6] and rat [7] adipocytes and also was found to be increased in women undergoing controlled ovarian hyperstimulation for assisted reproduction [8, 9]. These women had high levels of estrogen, and the data strongly indicated that estrogen has a stimulatory effect on the production of leptin from adipose tissue.

Exogenous estrogen administration is usually utilized in a clinical testing for examination of positive and negative feedback effect; thus, the differentiation
between a hypothalamic or pituitary lesion for amenorrhea can be made [10]. An impaired positive feedback of estrogen results in the decrease of LH secretion and is responsible for hypothalamic amenorrhea [11]. However, the mechanism by which estrogen triggers the release of GnRH in hypothalamus and whether leptin links to this mechanism remains unknown in humans. The purpose of the study was to determine the sequence of changes in plasma leptin and GnRH concentrations following exogenous administration of conjugated estrogen in order to clarify their interrelationship in positive and negative feedback mechanisms along the hypothalamic-pituitary-ovarian-adipose tissue axis during the follicular phase of normal menstrual cycle.

Materials and Methods

Study subjects

A total of 36 normally menstrual women with cycle lengths of 28–32 days, between the ages of 23–34 years old, volunteered for this study. All subjects had to record their basal body temperature before the study and had a biphasic pattern with normal E$_2$ and progesterone changes during the three previous cycles. None of the women were taking any medication and none had any systemic disease or symptom related polycystic ovarian disease. There was no history of weight loss/gain more than 10% of body weight during the past six months. The average body mass index (BMI) was 21.6 ± 0.4 kg/m$^2$ and did not change during the study period. The study was approved by the hospital’s review board and informed consent was obtained from each subject. Eighteen of these women were given a bolus intravenous injection of 20 mg conjugated estrogen (Premarin, Ayerst Laboratories, Rouses Point, NY) in 20 mL solution at 0800 on the fifth day of their menstrual cycle after fasting (premarin group), while the remaining 18 (control group) received an intravenous injection of 20 mL of normal saline as the control group. Fasting blood samples were collected at 0, 4, 8, 24, 28, 32, 48, 56, 72 and 96 hours after the injection. Plasma was immediately separated and stored at −80°C until the hormonal assay.

Hormonal assays

Hormones were measured by commercially available immunoassays. Plasma concentrations of leptin were measured by RIA (Linco Diagnostics, St. Louis, MO, USA) with inter-assay and intra-assay coefficients of variation of 8.5% and 5.3%, respectively, with a sensitivity of 0.5 ng/mL. Plasma GnRH concentrations were measured by enzyme-linked immunoassay kits (ELISA, Peninsula Laboratories, Inc., San Carlos, CA, USA). Briefly, 0.4 mL plasma was passed through Sep-Pak C$_{18}$ Cartridge (Waters Corp., Associates) and eluted with 3 mL 60% acetonitrile (Nacalai Tesque, Inc., Japan) in 0.1% trifluoroacetic acid. The eluate was lyophilized and reconstituted for ELISA. The inter-assay and intra-assay coefficients of variation were 9.5% and 6.5%, respectively, with a sensitivity of 16 pg/mL. Plasma LH and FSH concentrations were assayed using immunoradiometric assay (Daiichi Radioisotope Laboratories, Tokyo, Japan). The inter-assay and intra-assay coefficients of variation for LH and FSH were 8.3% and 5.2%, 9.2% and 5.3%, respectively. Plasma E$_2$ and E$_1$ levels were determined by RIA (Diagnostic Products Corp., Los Angeles, CA, USA). The inter-assay coefficients of variation (CVs) were E$_2$<9.3% and E$_1$<9.1%, respectively. The intra-assay of CVs were E$_2$<6.7% and E$_1$<6.4%, respectively. Samples from each individual were measured in duplicate and run in the same assay.

Statistical analysis

Statistical analysis of data was performed using Statistical Package for the Social Science (SPSS, Inc., Chicago, IL, USA) for Windows (version 10.0). One-way ANOVA, paired and unpaired t-tests and Wilcoxon tests were applied, when indicated. A $P$ value of <0.05 was considered statistically significant.

Results

Mean (±SEM) plasma levels of E$_1$ and E$_2$ following intravenous injection of premarin are shown in Fig. 1. Mean preinjection levels of plasma E$_1$ and E$_2$ (26.8 ± 3.5 pg/mL and 40.5 ± 5.2 pg/mL, respectively) in the women who received premarin were similar to those in the control group (25.7 ± 3.8 pg/mL and 38.5 ± 5.2 pg/mL, respectively). Circulating levels of E$_1$ and E$_2$
were significantly \((P<0.0001)\) increased at 4 hours \((2080 \pm 157 \text{ pg/mL} \text{ and } 2845 \pm 165 \text{ pg/mL})\), respectively) from preinjection levels and \(E_2\) levels \((75.6 \pm 5.2 \text{ pg/mL})\) were still significantly elevated until 96 hours after premarin administration, while those in the control group revealed significant increases than preinjection levels from 72 to 96 hours after saline injection. The corresponding values of \(E_1\) and \(E_2\) at 4 to 48 hours in the premarin group were significantly \((P<0.0001 \text{ to } P<0.05)\) higher than those in the control group (Fig. 1).

As demonstrated in Fig. 2, the changes of leptin and GnRH levels from preinjection levels \((7.5 \pm 0.5 \text{ ng/mL} \text{ and } 40.3 \pm 3.5 \text{ pg/mL})\), respectively) showed significant increases from 28 hours \((9.6 \pm 0.4 \text{ ng/mL} \text{ and } 61.8 \pm 5.4 \text{ pg/mL})\), sustained to 32 hours \((11.9 \pm 0.6 \text{ ng/mL} \text{ and } 53.8 \pm 4.6 \text{ pg/mL})\) and remained elevated until 48 hours. Leptin levels were significantly elevated until 56 hours \((9.5 \pm 0.5 \text{ ng/mL})\) and then slowly decreased between 72 and 96 hours after injection of conjugated estrogen (Fig. 2). The corresponding values of
leptin and GnRH which increased at 24 to 48 hours in the premarin group were significantly higher than those in control group.

The changes in FSH and LH levels showed a bi-phasic pattern following the loading of conjugated estrogen (Fig. 3). Plasma FSH levels were significantly ($P<0.001$ to $P<0.05$) suppressed for 48 hours, and then showed a significant ($P<0.001$) rebounding increase to 135% of the preinjection level (7.5 ± 0.3 mIU/mL) at 72 hours (9.6 ± 0.3 mIU/mL) after the injection. Plasma LH levels were significantly ($P<0.005$) suppressed at 4 hours after the injection of conjugated estrogen and remained at low levels until 32 hours and then significantly ($P<0.001$) increased to 170% of the preinjection levels (4.7 ± 0.5 mIU/mL) at 72 hours (8.0 ± 0.5 mIU/mL) after the injection. Changes of plasma FSH and LH levels in the control group, however, revealed no significant changes during the study.

**Discussion**

After exogenous estrogen administration, both plasma leptin and GnRH levels were simultaneously and significantly increased in almost the same time frame at 28, 32, 48 hours in the present study. These findings suggested that they might have a close association through endocrine and metabolic interaction in the hypothalamus to affect the release of GnRH during the normal menstrual cycle. In an autoradiographic study of ovariectomized rats [12], after estrogen was injected into the bloodstream, the most heavily distributed area in the brain was the hypothalamus which is considered to be the primary area of estrogen positive feedback and abundant in GnRH neurons [13, 14]. On the other hand, leptin has been recognized to serve as a metabolic signal to reproductive function because leptin administration can increase the LH and FSH levels in ob/ob mice [2]. Another group of investigators, who studied ovariectomized rats, found that fasting-induced suppression of pulsatile LH secretion could be prevented by leptin administration [3], and it was also described that leptin could stimulate the increases of LH and FSH concentrations in the female transgenic skinny mice [5] and cultures of pituitary and hypothalamic cells [4]. In addition, several cell lines of evidence suggest that hypothalamus is the critical target of leptin and GnRH hormones and that they are specifically bound to the hypothalamic neuron cell membrane where both receptors are expressed [13, 14]. Women with inadequate nutritional intake and hypoleptinemia may develop hypothalamic amenorrhea [15, 16], which has been described as a state of impaired positive feedback mechanism in previous studies [11]. Taken together with observations from this study, it seems reasonable to assume that the positive feedback mechanism of estrogen requires the cooperation of leptin to convey metabolic signal to hypothalamus for release and/or synthesis of GnRH in the hypothalamus. However, whether the peripheral increases of leptin from adipocytes after estrogen are directly linked to the simultaneously elevated secretion of hypothalamic GnRH remains to be clarified by further studies.

In regard to the negative feedback effect of estrogen,
we found that the pattern of GnRH releasing was inconsistent with and preceded the gonadotropin secretion. In this study, gonadotropin secretion exhibited a biphasic pattern, i.e., initial decrease in LH and FSH at 4 hours and followed by peak increase in LH and FSH at 72 hours, in response to the injection of exogenous estrogen. The suppression of gonadotropin secretion without a decrease in plasma GnRH levels suggests that the negative feedback effect of estrogen on gonadotropin secretion may not be mediated by GnRH secretion. This finding was comparable to the results of other investigations [17–19], who found estrogen may have different effects on GnRH and gonadotropin secretion; i.e., estrogen may stimulate the increase of plasma GnRH levels, and simultaneously reduce the pituitary responsiveness to GnRH and directly suppress the secretion of gonadotropin from the pituitary.

In an autoradiographic study, the highest concentrations of labeled estrogen injected were found in the pituitary gland of ovariectomized rats [12] and the pituitary cells also expressed the leptin and its receptors [20]. This finding, together with previous reports [17–19], gave further support to the hypothesis that negative feedback of estrogen might be independent of GnRH modulation. However, it is unclear whether the increases of peripheral leptin levels seen in this study are linked to the biphasic pattern of gonadotropin release after estrogen, despite an in vitro study, which showed that the addition of leptin on the cultures of pituitary cells could stimulate the increases of LH and FSH secretion [4].

In this study, E₂ levels abruptly increased, and leptin was simultaneously increased along with the time sequence of estrogen injection. This result provides further evidence for the possibility that supraphysiological levels of estrogen can directly stimulate the production of leptin through the endocrine mechanism. In fact, during controlled ovarian hyperstimulation cycles for assisted reproduction [8, 9], it has been reported that supraphysiological levels of E₂ have stimulatory effect on leptin production from adipocytes. Our present data concur with theirs, and further support the concept that high levels of E₂ may influence the regulation of leptin production during follicular phase.

In conclusion, following exogenous injection of estrogen, circulating leptin and GnRH levels showed simultaneous increases during the normal human menstrual cycle. It is suggested that leptin may be involved in the regulation of positive feedback mechanism of estrogen by conveyance of metabolic signal to affect the release of GnRH in hypothalamus, but whether it participates in the modulation of negative feedback remains unknown.

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References


