

Serum Leptin Concentrations during the Menstrual Cycle in Iranian Healthy Women

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Abstract- Leptin, a circulating 16-kd polypeptide consisting of 167 amino acids, appears to be involved in the body weight homeostasis. Moreover leptin plays an important role for the reproductive system, early embryogenesis, and fat metabolism during pregnancy and puberty. Significant correlations have been found between leptin and sexual hormones, which is a cytokine and has hormonal properties. The aim of this study was to determine serum leptin levels during the menstrual cycle, and the association between serum leptin and reproductive hormones in young, healthy Iranian women. 42 healthy women volunteered for the study. They all had regular menstrual cycles, with cycle length varying between 26 and 32 days. None of them used oral contraceptives. All were of normal weight, with body mass index (BMI) < 25 Kg/m². Fasting blood samples were collected during the follicular phase, mid cycle and luteal phase of the menstrual cycle. FSH and LH were measured with coated tube immunoradiometric assay. Estrogen and progesterone were measured using antibody-coated tubes. Serum Leptin concentration were measured by Leptin (sandwich) ELISA. In menstruating women, serum leptin increased from 13.15±1.60 ng/ml in the early follicular phase to 16.57±1.68 ng/ml ($P<0.01$) at the luteal phase. Serum leptin concentration negatively correlated with LH and progesterone ($P<0.05$). Mean serum leptin levels correlated with body mass index (BMI) ($r =0.78$, $P<0.001$).

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Key words: Leptin, Menstrual cycle, Iranian women

Introduction

Leptin is a circulating 16 KD polypeptide consisting of 167 amino acids, encoded by obese gene and is located on chromosome 7 in humans (1). It is a hormone produced by adipocytes which provides information on the availability of fat stores to the hypothalamus and acts as a signal regulating appetite and energy expenditure to both rodents and humans (2). Leptin concentration is related to the mass of adipose tissue, is an adipocyte secreted hormone and appears to be involved in the regulation of body weight homeostasis (3). Moreover, leptin plays an important role for the reproductive system, in the gonadal function (4,5). It has also been shown that leptin is involved in early embryogenesis (6), fat metabolism during pregnancy (7), and puberty (8).

Several authors found circulating leptin in a pulsatile (3) but also in a circadian and circa cyclic pattern (9,10).

The exact mechanism by which leptin controls fertility is unknown. There are data controversies

regarding variations of leptin during menstrual cycle and its relation to the sex hormones (11). Moreover no study dealing with leptin levels and sex hormones in Iranian women has been performed. The aim of this study was to determine serum leptin levels and sex hormones during menstrual cycle in healthy Iranian women.

Patients and Methods

This study was performed on 42 Iranian women. They were recruited through advertisements at the Tehran University Campus and most of them were students. Written consent was provided by all. Their ages were between 18-35 years and reported regular menstrual cycles. Other inclusion criteria were normal BMI (18-25 Kg/m²) and good health without any problem and endocrine disorders. They all had regular menstrual cycles, with cycle length varying between 26 and 32 days. None of them used oral contraceptives. All were of normal weight, with body mass index (BMI) < 25

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Kg/m². Three separate blood samples were taken 1) during days 3-5 from onset of menstrual cycle 2) during days 13-17 (according to basal body temperature of volunteers) 3) 3-5 days before onset of next menstrual cycle.

All blood samples were drawn after an overnight fast between 8.00 and 10.00 A.M from the cubital vein. All blood samples were allowed to clot and centrifuged within 30 min after vein puncture. The obtained serum was divided into two aliquots and frozen at -80°C until the measurements were performed.

Measurements of leptin, FSH, LH, estradiol and progesterone

Serum leptin levels were determined by commercially available highly sensitive ELISA kit (Diagnostics Biochem Canada Inc) having the following intra- and inter-assay variation (AV): <0.05 and 0.<09 .

Commercially available immunoassay kits were used for FSH (FSH IRMA Kavoshyar, Iran), LH (LH IRMA Kavoshyar, Iran). Estradiol and progesterone levels were determined using ELISA kits DRG, Germany).

Statistical Analysis was performed with the SPSS software version 11.0. The changes of the studied hormones and leptin throughout the menstrual cycle were examined with Pearson correlation test. All tests were two-tailed and statistical significance was considered for $P < 0.05$. Levels of hormones and leptin were expressed as mean \pm SEM.

Results

All 42 volunteers had ovulatory cycle as judged by luteal progesterone levels above 5 ng/ml. General characteristics of the women included in this study are shown in table 1. In menstruating women the mean serum concentrations of FSH, LH, estradiol, progesterone and leptin of subjects during three phases of a full menstrual cycle are shown in table 2. The serum leptin levels showed a large variation during menstruation cycle in healthy women. Regarding the three phases of menstrual cycle, the mean leptin concentrations during the follicular phase (13.15 \pm 1.6 ng/ml) were significantly lower compared to the luteal phase (16.57 \pm 1.68 ng/ml, $P=0.015$). No significant difference was observed between the midcycle and the luteal phase ($P=0.17$). Also no significant difference was observed between the leptin levels in follicular and midcycle phase ($P=0.16$). The serum leptin concentrations were analyzed in relation to the sex hormones. Significant correlations were found in the follicular phase between leptin and LH levels ($r=-0.314$). Also there is a significant correlation between leptin and progesterone concentrations ($r=-0.360$). No correlation between leptin levels and FSH ($r = 0.03$), or leptin and estradiol ($r = -0.18$) was found, during different phases of menstrual cycle. Also there was a strong positive correlation between leptin concentrations and BMI independent of the cycle stage ($r=0.78$, $p<0.001$) (Figure 1).

Table 1. Basic characteristics of subjects studied

Parameter	No.	Mean \pm SEM
Age	42	27.28 \pm 1.24
Weight	42	58.37 \pm 1.35
Height	42	1.62 \pm 0.08
BMI	42	22.23 \pm 0.47

Table 2. Concentrations of Leptin, FSH, LH, estradiol and progesterone of 42 healthy women during three phases of a full menstrual cycle. Mean values \pm SEM are presented.

Parameter	Follicular phase	Midcycle	Luteal phase
Leptin (ng/ml)	13.15 \pm 1.6	16.04 \pm 2.05	16.57 \pm 1.68
FSH (μ IU/ml)	5.12 \pm 0.37	5.09 \pm 0.41	3.21 \pm 0.32
LH (μ IU/ml)	3.47 \pm 0.25	13.33 \pm 2.24	3.67 \pm 0.46
Estradiol (pg/ml)	26.81 \pm 1.60	104 \pm 11.5	84.0 \pm 6.63
Progesterone (ng/ml)	0.79 \pm 0.19	2.16 \pm 0.48	7.16 \pm 0.76

Serum leptin concentrations during the menstrual cycle

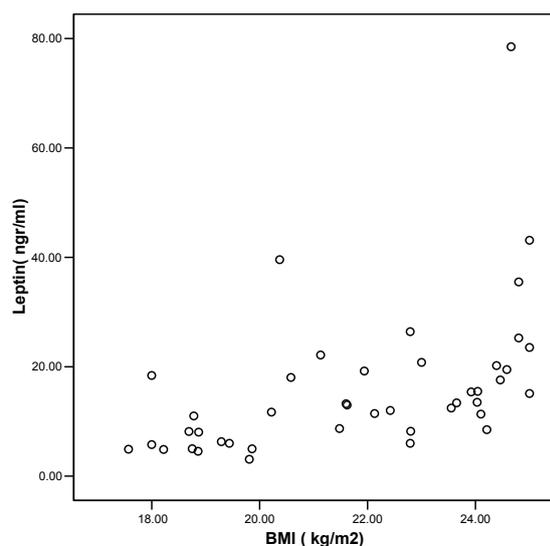


Figure 1. Leptin concentrations in healthy women (n=42) in correlation to body mass index (BMI). Leptin in serum correlate with BMI: $r=0.78, p<0.001$ levels

Discussion

In this study we demonstrated that blood samples from healthy Iranian women show significantly higher serum leptin concentration during the luteal, compared to the follicular phases. We also found correlation between leptin and LH and progesterone levels, but any correlation between leptin levels and FSH or leptin levels and estradiol, was not found.

These results are in accordance with a number of other studies which have shown considerable variation in leptin levels throughout the menstrual cycles with higher levels in the luteal rather than the follicular phase (12-20). A few studies demonstrate that leptin is a mediator of cell proliferation and with respect to the presence of leptin receptors in the endometrium, it is plausible that the luteal phase of the menstrual cycle is accompanied by increased leptin levels. Also the preovulatory surge of estrogen might increase the circulating leptin, which in turn could stimulate the gonads to produce more leptin together with increasing levels of progesterone (21-23).

However other studies also have shown either small but insignificant trends at the end of cycle (24), or no fluctuation at all (25,26). Regarding to the correlations between sexual hormones and leptin, although several studies have shown a correlation between leptin levels with changes in serum estradiol, progesterone and/or LH but results are not uniform and is unclear (15,17-19,24,26,27).

Finally regarding to the correlation of serum leptin level with body mass index we found a strong correlation between serum leptin concentration and BMI, where it is similar to the other researchers have reported in several studies (2,27,28).

Our study has several limitations. Our sample size is small, and there was a large amount of variability in both leptin levels and sex hormones. Although, this suggests a greater deal of variability within the normal population. However in summary, our findings are in accordance to several other researchers in this area and this is the first report of leptin fluctuations during menstrual cycle in Iranian healthy women.

Thus it is hoped that the ongoing research efforts will not only fully elucidate the relation between leptin and sex hormones, but will also result in better understanding of the role of leptin in Iranian women health.

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