

## —Report on Experiments and Clinical Cases—

## Serum Leptin Concentration in Young Adult Women with Ovulatory Dysfunction

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### Abstract

To investigate the functional role of leptin in human ovulation, we measured serum leptin, LH, FSH and estradiol in 16 young adult women suffering from ovulatory dysfunction with BMI ranging from 17.5 to 24.5 (group A). The control subjects included 12 women with regular ovulation and matched age and BMI (group B). We found that serum leptin concentration in group A subjects was significantly lower than that in group B subjects ( $4.1 \pm 0.5$  vs.  $6.1 \pm 0.4$  ng/ml,  $p < 0.01$ ). The percent body fat, estradiol, LH and FSH concentrations in groups A and B were not significantly different. These results indicate that anovulatory young adult women have lower leptin concentration than women with regular ovulation, thus suggesting a key role for leptin in regular ovulation or ovulatory dysfunction.

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**Key words:** leptin, BMI, percent body fat, ovulation, ovulatory dysfunction

### Introduction

In medical practice, it often happens that a simple gain in body weight induces ovulation failure. Likewise, the loss of body weight, as in anorexia nervosa, can also induce ovulatory dysfunction. Therefore, it is reasonable to assume that appropriate body weight is important for ovulatory function. The body mass index (BMI) is clinically used as an indicator of appropriate body weight. On the other hand, leptin is a protein encoded by the *ob* gene that is expressed in adipocytes<sup>1,2</sup>. It is well known that serum leptin level has a close relationship with BMI or percent body fat<sup>3</sup>. If appropriate BMI is truly important for ovulation,

leptin may also be expected to have a relationship with ovulation function. We previously reported that fluctuations in serum gonadotropin and ovarian steroids levels during the menstrual cycle correlated with fluctuations in serum leptin levels<sup>4,5</sup>. In the present study, we measured and compared the serum leptin, estradiol ( $E_2$ ), LH and FSH levels between two groups of women with matched age, BMI, and percent body fat, one group with ovulatory dysfunction and the other with normal ovulatory function.

### Materials and Methods

#### Subjects

Sixteen women who had ovulatory dysfunction

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with secondary amenorrhea and BMI ranging from 17.5 to 24.5 (group A) were randomly selected from the Outpatient's Clinic in Nippon Medical School Second Hospital. Twelve women with matched age and BMI and regular ovulation (group B) served as control. The mean age and BMI in Groups A and B are shown in **Table 1**. The ovulatory dysfunction in group A subjects was confirmed with basal body temperature (BBT) recording for 3 months, during which they had continuous anovulatory amenorrhea. Their ovaries were observed with trans-vaginal ultrasound as morphologically normal. Cases of premature menopause were excluded from group A subjects. Group B subjects had regular menstrual cycles (28~35 days between onsets of menstruation) and their ovulations were also confirmed with BBT and trans-vaginal ultrasound. All subjects had maintained a stable body weight ( $\pm 2.0$  kg) for more than one year. None of the subjects had prior history of anorexia nervosa, metabolic diseases or was taking any medication. All participants provided written informed consent to participate in this study, and the Declaration of Helsinki (Revised 1975) was strictly observed.

**Methods**

All subjects were recruited for serial venous blood sampling at the Outpatient Clinic of Nippon Medical School Second Hospital. The control subjects were studied in the early follicular phase (3rd day after the onset of menstruation). Every subject was instructed to eat their last meal or snack before 22:00 on the day preceding outpatient visit and to avoid eating or drinking before the visit. The subjects had

at least 1 hour's rest in the sitting position before the samples were taken from a large forearm vein. Blood samples were collected between 9:00 and 11:00. Serum leptin and estradiol were measured by radioimmunoassay (RIA) using the previously described methods<sup>5</sup>. LH and FSH were measured by a commercial chemiluminescent enzyme immunoassay using the previously described methods<sup>4</sup>.

Body weight was measured to the nearest 0.1 kg on a digital scale (Body Composition Analyzer, Tanita TBF-541, Japan). The BMI was estimated by dividing the body weight (Kg) by the square of the height (in meters). Percent body fat was measured by bioelectrical impedance analysis, which is an easy and non-invasive technique, but has limitations in underweight and overweight subjects.

**Statistics**

The data were expressed as means  $\pm$  SE. Statistical analyses were performed with SPSS software (SPSS, Chicago). The age, BMI, percent body fat and serum concentrations of E<sub>2</sub>, LH, FSH and leptin were compared between the groups by Student's *t*-test.

**Results**

The mean percent body fat, serum leptin, E<sub>2</sub>, LH and FSH levels were  $23.2 \pm 1.4$ ,  $4.1 \pm 0.5$ ,  $50.2 \pm 11.6$ ,  $6.3 \pm 1.1$ , and  $8.6 \pm 0.8$  respectively in group A subjects and  $27.7 \pm 0.8$ ,  $6.1 \pm 0.4$ ,  $61.6 \pm 8.7$ ,  $3.7 \pm 0.9$ , and  $8.4 \pm 0.8$  respectively in group B subjects. Serum leptin concentration in group A subjects was significantly lower than that in group B subjects

Table 1 Mean age, BMI, percent body fat, Serum leptin, estradiol, LH and FSH concentrations in Group A and B subjects

	Group A (n = 16)	Group B (n = 12)
Mean age (yr)	28 $\pm$ 1	32 $\pm$ 2
BMI	20.3 $\pm$ 0.5	20.8 $\pm$ 0.5
Percent body fat (%)	23.2 $\pm$ 1.4	27.7 $\pm$ 0.8
Leptin (ng/ml)	4.1 $\pm$ 0.5	6.1 $\pm$ 0.4*
E <sub>2</sub> (pg/ml)	50.2 $\pm$ 11.6	61.6 $\pm$ 8.7
LH ( $\mu$ IU/ml)	6.3 $\pm$ 1.1	3.7 $\pm$ 0.9
FSH ( $\mu$ IU/ml)	8.6 $\pm$ 0.8	8.4 $\pm$ 0.8

Values are the means  $\pm$  SE. \*p < 0.01 vs. control subjects

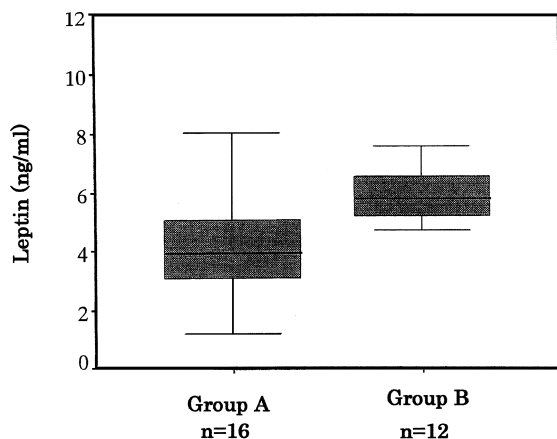


Fig. 1 Boxplot of serum leptin levels in group A and group B subjects. The median (thick line through the boxes), quartile, and extreme values within each group are shown. Serum leptin level in ovulatory dysfunction subjects (group A) was significantly lower than in normal subjects (group B) ( $p < 0.01$ ).

( $4.1 \pm 0.5$  vs.  $6.1 \pm 0.4$  ng/ml,  $p < 0.01$ ; **Table 1 and Fig. 1**). Percent body fat, E<sub>2</sub>, LH and FSH concentrations in group A and group B subjects were not significantly different.

### Discussion

Serum leptin levels were low in underweight women with chronic starvation as seen in anorexia nervosa or in sports women with ovulatory dysfunction<sup>6,7</sup>. Furthermore, serum leptin concentration correlated positively with BMI or percent body fat<sup>3</sup>.

Being underweight condition may lead to ovulatory dysfunction. Subsequently, the cause of ovulatory dysfunction is primarily hypothalamic pituitary dysfunction. Therefore, a long-term underweight condition results in low BMI and percent body fat, low levels of leptin, LH, FSH, E<sub>2</sub>, and finally ovulatory dysfunction. Here, leptin shows an indirect relation with ovulation. In animal studies, administration of leptin prevented starvation-induced anovulation<sup>8</sup>.

Our results suggest a relationship between leptin and human ovulation. Not only age, BMI, and percent body fat but also serum E<sub>2</sub>, LH, and FSH concentrations were matched between the regular ovulatory group and the anovulatory group. Only

the serum leptin concentration in the anovulatory group was significantly lower than that in the regular ovulatory group. This finding suggests that leptin plays an important role in human ovulation.

Several factors involved in the ovulation process, such as serum insulin, thyroid hormone or prolactin level, may affect the leptin level. Serum leptin has been shown to correlate positively with insulin level<sup>11,12</sup>. In addition, the capacity of adipocytes excreting leptin in normal subjects versus women with ovulatory dysfunction should be considered. If leptin excretion from adipocytes in patients with ovulatory dysfunction is lower than in normal subjects, serum leptin level should be low in the former group. However, no reports have been published about adipocytes leptin excretion in patients with ovulatory dysfunction. Further, women with ovulatory dysfunction and normal BMI are clinically considered to have a mentally stressed condition. Therefore, differences in nutritional habits and life style need to be investigated between women with regular ovulation and women with ovulatory dysfunction.

In conclusion, the results of our present study indicate that serum leptin level plays an important role in human ovulatory function.

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