Menstrual status and serum leptin levels in anorectic and in menstruating women with low body mass indexes

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Objective: To evaluate serum leptin levels in anorectic women, menstruating women with low body mass indexes (BMI) and normally menstruating women with normal BMI.

Design: Prospective study.

Setting: University clinics.

Patient(s): Fourteen amenorrheic patients with anorexia nervosa (group A), 11 menstruating women with a BMI <18 kg/m² (group B), and 20 normal controls.

Main Outcome Measure(s): Determination of BMI, caloric intake, total fat mass, ovarian volume, and serum leptin, insulin-like growth factor I, FSH, LH, E2, PRL, and TSH levels.

Intervention(s): None.

Result(s): Mean BMI and fat mass were similar in groups A and B and significantly higher in controls. Mean caloric intake was significantly lower in group A than in group B and controls. Median serum leptin levels were significantly lower in group A than in group B and controls, and significantly lower in group B than in controls. Median serum insulin-like growth factor I levels were significantly lower in group A than in group B and controls. Binary segmentation analysis of groups A and B showed that LH was the most relevant variable in differentiating the two groups, followed by leptin.

Conclusion(s): A threshold of leptin levels exist above which, even in the presence of low body mass indexes, the menstrual function is preserved. (Fertil Steril 2002;78:376–82. ©2002 by American Society for Reproductive Medicine.)

Key Words: Amenorrhea, anorexia nervosa, leptin, low body mass index

Leptin is a 167-amino-acid protein mainly produced by adipocytes. It is encoded by the ob gene (1). In the mouse the absence of this gene produces the obese (ob/ob) phenotype, characterized by obesity, hyperphagia, hyperglycemia, hyperinsulinemia, insulin resistance, hyperthermia, impaired immune response, and infertility (2). Thus, it seems that leptin plays a pivotal role in the regulation of energy balance and body composition and it has been proposed that it might exert its action through a central pathway, which may hypothetically involve the inhibition of neuropeptide Y synthesis or release (3, 4).

In humans, plasma levels of leptin have been shown to be strongly correlated with total and percent body fat mass and body mass index (BMI) (weight/height²), being two- to four-fold higher in obese than in normal subjects (5). On the other hand, cerebrospinal fluid leptin levels in obese subjects have been found to be 30% higher in comparison to lean subjects and serum leptin levels 318% higher (6). Therefore, a resistance to leptin may be hypothesized in obese human subjects.

It has been hypothesized that low leptin levels could be a signal of a deficient amount of body fat to the central nervous system and could induce the inhibition of the reproductive function, which is characteristic of the hypogonadotropic hypogonadism of patients with weight loss-related amenorrhea (7). This inhibition is fundamental during starvation, as both menstrual bleeding and pregnancy would in-
duce an increase in energy demands that the body could not satisfy.

Leptin might interfere with the reproductive function through its interaction with neuropeptide Y. This neuropeptide inhibits GnRH and gonadotropin production (8), and is, in turn, inhibited by leptin (4). Low leptin levels may allow higher neuropeptide Y levels and, therefore, interfere with the synthesis and release of GnRH. Other possibilities are the interaction with other central neuroendocrine pathways, or direct actions on GnRH or gonadotropin synthesis/release (9). Even a direct effect of leptin at ovarian levels has been proposed (10).

Anorexia nervosa is a serious psychiatric disorder characterized by alterations in eating habits leading to decreased caloric intake and chronically low weight, increased physical activity, hormonal changes, and amenorrhea due to hypogonadotropic hypogonadism. Recent studies have shown that serum leptin concentrations are significantly lower in anorectic women in comparison to normal controls and are correlated to insulin-like growth factor I (IGF-I) levels (11–17). Low serum IGF-I levels are an index of starvation (18).

In clinical practice it is frequent to observe women with a BMI <18 kg/m², suggested to be pathological, who are regularly menstruated. This subset of women probably have a normal reproductive function and are generally considered to be “constitutionally lean” (19, 20).

It could be hypothesized that, if low serum leptin levels play an important role in the etiology of hypogonadotropic hypogonadism of patients with anorexia nervosa, then constitutionally lean, regularly menstruating subjects should have higher leptin levels than anorectic women, even in the presence of similarly low BMI.

To test this hypothesis we evaluated serum leptin levels in hypogonadotropic hypogonadic women with anorexia nervosa, in constitutionally lean women, and in normal controls. We also evaluated body composition and serum IGF-I levels to verify whether differences in fat mass or in nutritional status could explain the different menstrual status.

MATERIALS AND METHODS

Subjects

Patients with anorexia nervosa, of at least 15 years of age with a BMI <18 kg/m², amenorrheic, who met the Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV) criteria for this condition, were consecutively recruited from the outpatient clinic for eating disorders in the Department of Clinical and Experimental Medicine of our medical school (21). Constitutionally lean women of at least 15 years of age, with normal menstrual cycles and BMI <18 kg/m² were selectively recruited from the same outpatient clinic. These patients had no other complaints apart from low body weight and BMI and the DSM IV evaluation excluded the presence of any eating disorder in this group. Normal weight (BMI >18 and <25 kg/m²), healthy control women were recruited among patients attending the outpatient clinic of the Department of Gynecology and Obstetrics for benign gynecological conditions. Normal ovulatory function was confirmed in lean women and in controls by the detection of ovulatory P levels during the luteal phase.

Fourteen anorectic female subjects (group A), 11 constitutionally lean women (group B), and 20 normal women (regular menses and BMI >18 kg/m²) (controls) were enrolled in the study.

The study was approved by our internal review board. All patients and their parents/guardians gave their informed and written consent for the participation to the study.

Methods

Weight, height, BMI, ovarian volume, total fat mass, serum leptin, IGF-I, gonadotropins, E₂, and PRL levels were determined for all patients. Caloric intake was assessed by a 7-day record, and menstrual function was evaluated by an accurate history of the characteristics of menstrual bleeding.

Blood sampling was performed in the morning (between 9:00 and 11:00 a.m.) after an overnight fasting in the follicular phase for patients in group B and controls and randomly in group A patients (who were all amenorrheic). On the same day of blood sampling, an ultrasound scan was done to determine ovarian volume. All ultrasound scans were performed by one of two operators (G.A.T. and C.D.C.) using a Toshiba SSA-250A with a 5-MHz transabdominal probe (Toshiba Medical Systems, Rome, Italy) and applying the formula of the ellipsoid: $D_1 \times D_2 \times D_3 \times 4/3 \times \pi \times 0.52$.

Blood samples were stored at −70°C until determinations were performed.

Total fat mass was determined by dual energy x-ray absorptiometry using a Lunar DPX (Lunar Corp., Madison, WI) densitometer. The precision of scans for the determination of fat distribution was determined by repeated scans in five subjects for 3 consecutive days. The coefficient of variation (CV) was <1%.

Serum leptin levels were determined in duplicate using a human leptin RIA (Linco Research, St. Charles, MO), with an intra-assay coefficient of variation (CV) of 3.4–8.3%, an interassay CV of 3.6–6.2% and a sensitivity of 0.5 ng/mL.

Serum luteinizing hormone (LH) levels were determined using a RIA (DiaSorin, Saluggia, Italy) with a sensitivity of 0.2 U/L and an intra-assay and interassay CV of 2.7–6.0 and 0.45–3.0, respectively. Serum FSH levels were determined using an RIA (DiaSorin, Saluggia, Italy) with a sensitivity of 0.2 U/L and an intra-assay and interassay CV of 1.4–2.6 and 4.2–6.3, respectively. Serum PRL were determined using a RIA (DiaSorin) with a sensitivity of 0.5 ng/mL and an intra-assay and interassay CV of 1.58–2.80 and 6.2–8.0, respectively. Serum IGF-I were measured using a RIA (CIS, 377
TABLE 1

Characteristics and serum hormonal levels in subjects studied.

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 14)</th>
<th>Group B (n = 11)</th>
<th>Controls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD age (yr)</td>
<td>18.6 ± 2.9b</td>
<td>25.7 ± 6.2e</td>
<td>35.5 ± 8.6</td>
</tr>
<tr>
<td>Mean caloric intake (kcal/die)</td>
<td>870 ± 220f,e</td>
<td>2158 ± 378</td>
<td>2546 ± 474</td>
</tr>
<tr>
<td>Mean ± SD total fat mass (kg)</td>
<td>4.4 ± 2.4d</td>
<td>6.9 ± 3.2e</td>
<td>14.2 ± 4.3</td>
</tr>
<tr>
<td>Mean ± SD ovarian volume (cm³)</td>
<td>3.7 ± 1.5bf</td>
<td>5.8 ± 0.2</td>
<td>6.2 ± 1.3</td>
</tr>
<tr>
<td>Median [range] leptin (ng/mL)</td>
<td>1.5 [1.1–3.3]bf</td>
<td>3.3 [1.6–6.1]f</td>
<td>9.0 [3.0–21.0]</td>
</tr>
<tr>
<td>Median [range] IGF-I (ng/mL)</td>
<td>115.8 [65.1–282.4]f,e</td>
<td>208.0 [161.8–354.2]</td>
<td>200 [160.1–300.3]</td>
</tr>
<tr>
<td>Median [range] FSH (IU/L)</td>
<td>3.7 IU/l [0.1–7.7]f</td>
<td>5.0 [2.2–19.2]</td>
<td>8.4 [4.9–14.8]</td>
</tr>
<tr>
<td>Median [range] LH (IU/L)</td>
<td>0.7 [0.6–1.8]f,e</td>
<td>5.0 [3.0–12.3]</td>
<td>7.8 [4.3–10.5]</td>
</tr>
<tr>
<td>Median [range] estradiol (pg/mL)</td>
<td>25 [12–45]f,e</td>
<td>52.5 [21.2–81]</td>
<td>56.5 [40.5–79.1]</td>
</tr>
<tr>
<td>Median [range] PRL (ng/mL)</td>
<td>5.7 [2.3–12.3]f,e</td>
<td>14 [9.3–25.0]</td>
<td>11.5 [6.5–19.7]</td>
</tr>
<tr>
<td>Median [range] TSH (mIU/L)</td>
<td>1.7 [0.1–4.4]</td>
<td>2.2 [1.2–3.8]</td>
<td>3.5 [1.3–3.5]</td>
</tr>
</tbody>
</table>

Note: Group A = patients with anorexia nervosa; group B = lean menstruating subjects.

* P<.001 vs. controls.

** P<.001 vs. controls.

† P<.05 vs. controls.

‡ P<.01 vs. group B.


Gif-sur-Yvette, France) with a sensitivity of 0.45 ng/mL and an intra-assay CV of 1.2–2.1 and an interassay CV of 3.9–6.4.

Serum TSH were determined using a RIA (Diagnostic Products Corporation, Los Angeles, CA) with a sensitivity of 0.03 mIU/L and an intra-assay CV of 1.5–3.6 and an interassay CV of 3.7–9.8.

Statistical Analysis

Statistical analysis of the data was performed on an IBM compatible PC using a statistical software (Statistica for Windows, StatSoft Release 4.5, Inc., 1993, Statsoft, Inc., Tulsa, OK). A Shapiro-Wilk W test was performed to evaluate the distribution of data. Differences among groups for variables with a normal distribution in all groups (age, ovarian volume, caloric intake, and total fat) were evaluated using ANOVA followed by Scheffe’s procedure for post-hoc comparison of means. Results for these variables are reported as mean ± SD. A Mann-Whitney U test was used to compare BMI, leptin, IGF-I, gonadotropins, E₂, PRL, and TSH as these variables showed a non-normal distribution in group A. Results for these variables are reported as median (range). Serum leptin was repressed on BMI, total body fat, ovarian volume, IGF-I, FSH, LH, E₂, PRL, and TSH separately for controls and constitutionally lean women, using the Pearson product–moment correlation. The same correlations were calculated using a linear model and the Spearman R test in anorectic subjects. Significance was set at P<.05.

To determine which variable was more important in differentiating anorectic women from normally menstruating lean women, we performed a binary segmentation analysis (22–24). The aim of this analysis is to classify a series of individuals in homogeneous, most differentiated groups through a series of dichotomic divisions. This technique divides the sample on the basis of the response of the predictive value selected by the split criteria into two groups that have a higher homogeneity in comparison to the initial sample.

RESULTS

Fourteen patients with anorexia nervosa, 11 constitutionally lean subjects, and 20 controls were studied and their characteristics and serum hormonal levels are reported in Table 1.

Mean age of controls was significantly higher in comparison to group A (P<.001) and group B (P<.001). Mean age of subjects from group A was significantly lower than in group B (P=.03). Mean caloric intake was significantly lower in group A (870 ± 220 Kcal/day), in comparison to group B (2,158 ± 378 Kcal/day) and controls (2,546 ± 474 Kcal/day) (P<.01). No significant difference was observed between group B and controls. Median BMI was significantly lower in group A (16.9 kg/m² [range, 13–18.1 kg/m²]) and group B (16.6 kg/m² [range, 13.8–17.9 kg/m²]) in comparison to controls (23.4 kg/m² [range, 19.3 kg/m²–28.7 kg/m²]) (P<.0001). No significant difference was observed between group A and B. Mean total fat mass was significantly higher in controls (14.2 ± 4.3 kg) in comparison to group A (4.4 ± 2.4 kg) and group B (6.9 ± 3.2 kg).
Median serum LH levels were significantly lower in anorectic patients in comparison to subjects in group B and controls (0.7 IU/L [range, 0.6–1.8 IU/L] vs. 5.0 IU/L [range, 2.3–11.3 IU/L] and 7.8 IU/L [range, 4.3–10.5 IU/L], respectively). The LH and leptin levels were not significantly correlated in any group.

Median serum E_2 levels were lower in anorectic women in comparison to lean women and controls (25 pg/mL [range, 17.3–35.7 pg/mL] vs. 52.5 pg/mL [range, 35.6–66.2 pg/mL] and 56.5 [range, 40.5–79.1 pg/mL], respectively; P = .004). No correlation between serum leptin and E_2 levels was observed in any group.

Median PRL levels were significantly higher in group B and controls in comparison to group A (14 ng/mL [range, 11.5–17.5 ng/mL] and 11.5 ng/mL [range, 11.5–17.5 ng/mL] vs. 5.7 ng/mL [range, 3.9–7.4 ng/mL]; P < .05). No correlation between serum PRL and leptin levels was observed in any group.

No significant difference was observed in TSH levels between groups A and B and controls (group A: 1.7 IU/L [range, 0.1–4.4 IU/L]; group B: 2.2 IU/L [range, 1.2–3.8 IU/L]; controls: 3.5 IU/L [range, 1.3–3.5 IU/L]) and no correlation between leptin and TSH levels was observed in any group.

Binary segmentation best differentiated the two groups using the variable LH, with a rate of 91.8% and a threshold level of 2.4 IU/L (only two women in group B had less than this value). If LH was withdrawn from the analysis, leptin was the next best variable in discriminating between groups A and B, with a rate of 83.8% and a threshold of 2.6 ng/mL.

**DISCUSSION**

Leptin could have an impact on reproductive function. The ob/ob mouse is infertile and shows gonadotropin levels and secretion pattern similar to prepubertal animals (25). In these animals, intraperitoneal administration of leptin corrects the reproductive defect (26). Barash et al. (27) evaluated gonadotropin levels, reproductive organ weights, and histology of ob/ob mice either supplemented or not with intraperitoneal recombinant leptin and found that treated female animals had higher LH levels, uterine and ovarian weights, and number of primary and Graafian follicles. Furthermore, it has been shown that starvation induces a decrease in leptin concentrations in mice, and that the prevention of this decrease with exogenous leptin blunts the changes in gonadal, adrenal, and thyroid axes in male mice and prevents the delay in ovulation in female mice (12), suggesting that one of the main physiological roles of leptin may be the regulation of the neuroendocrine system during starvation.

Leptin stimulates FSH and LH release by the hypothalamic-pituitary axis in vitro and of GnRH from median emi-
nence arcuate nuclear explants (9). Furthermore, inoculation of leptin into the third ventricle of animals primed with estrogen in vivo causes a significant increase in LH plasma levels (9).

Evidence that leptin may have a role in the reproductive function in humans are few but convincing. First, many investigators found a gender difference in serum leptin levels (28, 29). Second, although there is no direct evidence of an influence of leptin on the reproductive function, amenorrheic anorectic women have serum leptin levels significantly lower than normal subjects, and it has been hypothesized that low leptin levels could be a signal of a deficient amount of body fat to the central nervous system and could induce the inhibition of the reproductive function (11–17). Moreover, very recently, lower leptin levels were found in normal weight female patients with stress-related hypothalamic amenorrhea (30, 31).

In this study we confirm that anorectic women have significantly lower serum leptin levels in comparison to normal weight women. Our results are in accordance with previous studies (11–17). Our main finding is the demonstration that anorectic women not only have significantly lower leptin levels in comparison to controls, but also in comparison to lean menstruating women with similar BMI and fat mass. The presence of a dispersion of two values of leptin toward the upper level in group B might in part decrease the statistical significance of this finding. However, the lack of correlation between serum leptin and BMI in anorectic subjects, but not in lean women, strongly suggests that in these patients leptin secretion is profoundly deranged. Our findings could support the hypothesis that leptin plays an important role in the maintenance of reproductive function. Indeed, it could be hypothesized that a threshold value of leptin has to be present to allow normal reproductive function. Beyond this value, hypogonadotropic hypogonadism is induced, and the correlation with BMI is lost. Ballauff et al. (17) recently demonstrated that during the refeeding of anorectic patients, leptin increments parallel those of gonadotropins during the first weeks of treatment and that serum leptin levels of 1.85 μg/L are associated with gonadotropin levels at or above the minimal level observed during the menstrual cycle in healthy women, and hypothesized the presence of a threshold of leptin levels to be maintained to have normal menstrual function.

We confirmed the existence of this threshold using two different populations with similar BMI but different menstrual status. The mild discrepancy between our level of leptin and that of Ballauff et al. may be due to differences in the populations, nutritional status, and the main parameter considered (menstrual status in our study, gonadotropin concentrations in Ballauff’s).

The question arises: why women with similar BMI and fat mass have different serum leptin levels? We believe that this difference may be due to the nutritional status of the two groups. Indeed, the caloric intake is higher in lean women than in anorectic patients and disordered eating has been associated to hypoleptinemia in women with functional hypothalamic amenorrhea (31). Moreover, serum IGF-I levels were significantly lower in anorectic women. Because IGF-I is considered to be an index of nutritional status, this finding supports the view that anorectic patients and lean women are different in this regard.

An alternative hypothesis is that lower E2 levels in anorectic patients could induce lower leptin levels in comparison to lean and control subjects who have normal E2 levels. In vitro, estrogens have been shown to stimulate leptin production from human adipocytes (32). However, other in vivo studies showed that E2 does not directly affect leptin secretion in mice (33).

Moreover, our group has recently found higher leptin levels in postmenopausal untreated women in comparison to premenopausal women and postmenopausal women taking hormone replacement therapy (34). The difference in age between controls and patients of groups A and B is unlikely to be an important factor in determining the observed differences in leptin levels. Leptin has been reported to be inversely related to age (35). However, this was true only when a very wide range of ages (up to 80 years) was considered. Other studies, with average ages similar to our subjects did not find an independent relation between age and plasma leptin level (5).

Leptin may exert its effects on the reproductive function through a central modulation of neuropeptide Y production, as reported by Stephens et al. (4). Neuropeptide Y is known to inhibit GnRH production, therefore it is possible that lower leptin levels could induce an increased release of neuropeptide Y and a suppression of GnRH and consequently, of the reproductive axis. Also a direct action of leptin on the hypothalamic production of GnRH may be hypothesized, as it was found that leptin stimulates the production of GnRH from rat hypothalamus in vitro (9). Finally, because the leptin receptor was found on the ovary (10), a direct action of leptin in the female gonads may be hypothesized.

On the other hand, the serious neuroendocrine derangement caused by anorexia nervosa could be the cause and not the result of low leptin levels in anorectic subjects. However, leptin levels significantly lower than controls are present also in regularly menstruating lean women.

The LH levels were significantly lower in anorectic subjects in comparison to lean women, but no difference was observed in FSH concentrations. This could be explained by the fact that LH is much more sensitive to GnRH deficiency than FSH (36). Because anorexia nervosa induces a severe hypogonadotropic hypogonadism characterized by a deep depression in GnRH secretion, it can be hypothesized that LH is the first gonadotropin whose secretion is significantly decreased. Furthermore, a direct stimulating effect of leptin
on LH, but not on FSH, secretion has been demonstrated, therefore the significantly higher leptin levels observed in lean subjects could have stimulated the hypothalamus in secreting more LH than in anorectic women (9).

The binary segmentation analysis performed on anorectic and normally menstruating lean women showed that the major determinant in differentiating the two groups is LH. This indicates that the amenorrheic state in anorectic women is determined by an hypothalamic dysfunction. Nevertheless, this analysis cannot rule out a role for leptin in determining this reproductive defect, as an effect on this hormone at the hypothalamic level determines an alteration in GnRH secretion as well. This indicates that the amenorrheic state in anorectic women with lean menstruating women was leptin.

This finding supports the hypothesis of a threshold of circulating leptin level that is needed for the maintenance of normal menstrual function and that the role of this hormone in regulating reproductive function is probably exerted at the hypothalamic level. Further studies are needed to verify this hypothesis.

The presence of lower PRL levels in anorectic women than in the other two groups is in agreement with previous studies on hypothalamic amenorrhea (36). An interesting, although speculative, hypothesis could be that leptin acts on PRL production, either by an inhibition of hypothalamic production of dopamine or through a direct action. In fact, it has been demonstrated that leptin stimulates PRL secretion from the rat pituitary in vitro (9).

In conclusion, our study confirms that anorectic women have lower serum leptin levels than normal women but also of women with low BMI but preserved menstrual function (constitutionally lean, normally eating women). This is consistent with the hypothesis that leptin levels are fundamental for reproductive function, independent of BMI, and that there is a threshold in leptin levels beyond which reproductive function is lost. Furthermore, below this threshold, a correlation between BMI and leptin is lost. In subjects with similarly low BMI and fat amount, the nutritional status is probably the most important factor in determining leptin levels.

References


