Inhibin B: A Potential Marker of Gonadal Activity in Patients with Anorexia Nervosa during Weight Recovery

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Inhibin B is a product of the gonads and a marker for ovarian follicular development. This was a cross-sectional study designed to assess awakening of the reproductive function by studying secretion pattern of inhibin B during the weight restoration in patients with anorexia nervosa (AN). Twenty patients with AN participated at low weight [body mass index (BMI) 14.3 ± 0.3 kg/m²], 22 partially weight recovered AN (BMI 17.4 ± 0.1 kg/m²), 16 reached goal weight but did not restore menstrual cycles (BMI 19.5 ± 0.1 kg/m²), and 13 reached goal weight and had at least six consecutive menstrual cycles (BMI 19.3 ± 1.0 kg/m²). Nineteen eumenorrheic females with BMI 19.8 ± 0.4 kg/m² served as controls. At low weight, patients had low basal leptin, inhibin B detectable in all samples, and LH remained low, all significantly lower than in controls (P < 0.01). At weight gain, basal leptin increased, median inhibin B increased (detectable in 66.7% of samples), and LH remained low, all significantly lower than in controls (P < 0.01). Weight-recovered/amenorrheic patients further increased basal leptin, inhibin B was detectable in all samples, and LH remained low, all significantly lower than in controls (P < 0.01). In weight-recovered/cycling patients, basal leptin, median inhibin B, and LH, as expected, were not different from healthy volunteers. Inhibin B values correlated significantly with leptin (P = 0.000) and BMI (P = 0.000). In summary, gonads in patients with AN who gain weight are not entirely quiescent but have a low level of activity. Inhibin B is an early marker of gonadal activity, and with weight gain, awakening of the reproductive function is gradual, whereas factors triggering the onset of menstrual cycles still remain unknown (nutritional fat intake, psychological). (J Clin Endocrinol Metab 89: 1838–1843, 2004)

ANOREXIA NERVOSA (AN) is a syndrome of self-starvation due to disturbances in body image and intense fear of becoming obese. It affects adolescent girls and young women and is associated with multiple endocrine abnormalities, the most prominent being hypothalamic amenorrhea (restrained GnRH pulse generator). One third of patients recover with few sequelae, and one third recover sufficiently to return to a productive level of functioning but continue to struggle with issues of weight and shape. For the remaining one third of patients, the illness is chronic (1, 2). The distortions of body image and fear of becoming fat persist during recovery.

Inhibins are heterodimeric glycoprotein hormones comprised of a common α-subunit linked to either βA (inhibin A) or βB subunit (inhibin B) and are produced by gonads (3, 4). Inhibin B is produced by the granulosa cells in small antral follicles and reaches a peak during the early follicular phase, whereas inhibin A rises during the late follicular phase and reaches peak during the luteal phase (5). Inhibins exhibit unique patterns of secretion across the follicular phase of the menstrual cycle. Both differential secretion of LH and FSH and the stage of follicle development determine the patterns of inhibin A and B secretion in the normal menstrual cycle (6). During puberty both inhibin A and B increase (7), and changes in inhibins during pubertal maturation seem to be a reflection of ovarian maturation (8). Prepubertal girls are found to have detectable levels of inhibin B in contrast to inhibin A. Inhibin B increases slightly from prepuberty and peaks during midpuberty after which no further changes are seen. Serum inhibin B levels correlates positively with age and FSH concentrations several years before the clinical onset of puberty (9). During early adolescence there is a marked increase in inhibin B between ages 10 and 12 yr after which there are no further changes, suggesting that prepubertal gonads are not entirely quiescent but have a low level of activity (10, 11). It has been suggested that inhibin B concentrations might reflect early follicular activity (9).

Ovarian function is related to body fat mass. Obesity as well as weight loss is associated with menstrual irregularities. Leptin is an adipocyte hormone secreted into the circulation (12). Leptin conveys a signal of the amount of energy stores to the brain and mediates its effect through specific receptors in the hypothalamus. There leptin alters the expression of several hypothalamic neuropeptides and thereby regulates energy intake and expenditure (13–15). Serum leptin levels correlate closely with the amount of fat mass, with higher levels observed in obese subjects (16). On the other end, AN is a state of acquired leptin deficiency. Fasting serum leptin levels in AN are low (17) and rise with weight.

Abbreviations: AN, Anorexia nervosa; BMI, body mass index; CV, coefficient of variation.

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gain both in plasma and cerebrospinal fluid (18). Weight gain is associated with highly variable leptin responses that may be due to differences in the acute nutritional state before the blood sampling. Although dynamic changes in serum and cerebrospinal fluid leptin have been shown with changes in nutrition or body weight, leptin values show a delay in recovery in comparison with fat mass restoration (18). It is well known that the reproductive system is highly sensitive to states of energy deficit. A number of studies have been performed that provide evidence that leptin may influence gonadotropin release at the level of the hypothalamus. Leptin (116-130) (an active fragment of the native molecule) intracerebroventricular administered to fasted adult male rats increased LH pulse frequency, amplitude, mean levels, and net secretion (19). Similar results were reported by other investigators (20, 21).

Leptin appears to play a key role in proper functioning of the reproductive system. Thus, reproductive function is restored in leptin-deficient mice by administration of recombinant leptin (13). Long-term recombinant leptin treatment resulted in initiation of puberty with pulsatile gonadotropin secretion in an obese child with congenital leptin deficiency (22). Patients with congenital lipoatrophy, another leptin-deficient state, resume menstrual cycles during chronic treatment with recombinant leptin (23). Leptin can regulate gonadotropin levels during starvation at a central level (24). In a recent study, leptin administration prevented fasting-induced decrease in LH pulsatility in humans mainly at the level of the hypothalamus (25). All of these data are consistent with a role for leptin as a gate for normal reproductive function when adequate energy stores are achieved and suggest that leptin may have a role for treating reproductive dysfunction seen in low leptin states such as chronic undernutrition due to self-induced starvation (AN).

The aim of this cross-sectional study was to examine the relationship between degree of weight recovery and endocrine signals in patients with anorexia nervosa.

**Subjects and Methods**

**Subjects**

After providing informed consent, we investigated patients with AN in a cross-sectional study, divided into four groups. The first group (Low Wt.) consisted of 20 AN patients at low weight [age 19.1 ± 0.8 yr, body mass index (BMI) 14.3 ± 0.3 kg/m²]. The second group (Gain Wt.) consisted of 22 AN patients during weight recovery period (age 21.5 ± 0.9 yr, BMI 17.4 ± 0.1 kg/m²). The third group (Goal weight without menstrual cycles, Goal Wt. without m.c.) consisted of 16 weight-recovered AN patients without menstrual cycles (age 22.4 ± 0.8 yr, BMI 19.5 ± 0.1 kg/m²). The fourth group (Goal Wt. with m.c.) consisted of 13 weight-recovered AN patients with at least six regular cycles (age 21.5 ± 0.7 yr, BMI 19.3 ± 1.0 kg/m²). We compared these AN patients with 19 healthy eumenorrheic females aged 25.0 ± 0.7 yr with BMI 19.8 ± 0.5 kg/m². The study had been approved by the hospital ethics committee. An informed consent for participating in the study was obtained from all subjects.

**Study protocol**

Studies were conducted in the morning after overnight bed rest and overnight fast. Blood samples were obtained by venepuncture for hormonal analysis, and a standard LH-RH test was performed with ΔLH calculated (peak LH minus basal LH value). Patients who restored menstrual cycles and healthy subjects were studied in the early follicular phase of the cycle.

BMI was defined as weight (kilograms) divided by the square of height (square meters). In a subgroup of 12 AN patients body, composition (body fat and lean body mass) as well as bone mineral density was assessed using dual-energy x-ray absorptiometry DPX-L scanner (Lunar, Madison, WI).

**Hormone assays**

Leptin was measured in pooled serum from three samples taken at 15-min intervals at 0800 h in the morning after an overnight fast by commercial RIA (Linco, St. Charles, MO) with detection limit of 0.5 ng/ml, intraassay coefficient of variation (CV) of 6%, and interassay CV of 8%.

Serum inhibin B was measured by immunassay kit ELSA (Oxford Bio-Innovation, Oxford, UK) for the specific measurement of dimeric inhibin B with detection limit of 10 pg/ml, intraassay CV of 8%, and interassay CV of 13%.

FSH was measured by immunoradiometric assay (INEP, Zemun, Yugoslavia) with detection limit of 1.15 U/liter, intraassay CV of 6.3%, and interassay CV of 10.7%.

LH was measured in pooled serum from three samples taken at 15-min intervals by immunoradiometric assay (INEP) with detection limit of 0.3 IU/liter and intraassay CV of 5.8% and interassay CV of 9.86%.

Estradiol was measured by RIA (INEP) with detection limit of 50 pmol/liter, intraassay CV of 9%, and interassay CV of 10%.

**Statistical analysis**

Statistical analyses were performed using SPSS statistical software (SPSS for Windows, release 10.0, SPSS, Chicago, IL). Descriptive statistics are presented as mean values ± se for BMI, leptin, and FSH or as medians and interquartile intervals for inhibin B, LH, ΔLH and estradiol. For inhibin B, the percent of samples in which inhibin was below the assay detection limit of 10 pg/ml. Differences in BMI, leptin, and FSH between groups were compared with parametric one-way ANOVA (least significant differences post hoc test for multiple comparisons) because these data had a Gaussian distribution. The data for inhibin B, LH, ΔLH, and estradiol did not have a Gaussian distribution and nonparametric statistics were used. Differences in hormone concentration between groups were tested by Kruskall-Wallis ANOVA or Mann-Whitney U test as appropriate. Because we performed 28 consecutive statistical analyses, we chose a level of significance of 0.05/28 = 0.001 (a-adjustment according to the modified Bonferroni procedure). Correlation between various parameters in patients and healthy subjects was analyzed using Pearson correlation (for data with normal distribution: BMI, leptin, and FSH) or Spearman correlation coefficient (for skewed data: inhibin B, LH, ΔLH, and estradiol).

**Results**

**Healthy controls**

Comparative clinical data in the 19 eumenorrheic controls and patients with AN during weight recovery are summarized in Table 1 and Fig. 1.

**Patients with AN at low weight**

At low weight with BMI 14.3 ± 0.3 kg/m², basal leptin level was 1.1 ± 0.2 ng/ml, inhibin B level was above the assay detection limit in only 15% of samples and LH in only 5% of samples, and estradiol level was 50 ± 0.7 pmol/liter, as expected, significantly lower than in healthy controls (leptin 8.1 ± 0.9 ng/ml, median inhibin B 30.0 with interquartile range of 54 pg/ml, median LH 3.1 with interquartile range 2.1 IU/liter, estradiol 100 ± 0.9 pmol/liter; P < 0.01; Table 1, Fig 1). Basal leptin level, inhibin B, LH, ΔLH, and estradiol were significantly lower than in other groups (P < 0.01).
TABLE 1. Clinical characteristics and hormonal analysis of patients with AN and healthy control subjects

<table>
<thead>
<tr>
<th></th>
<th>AN</th>
<th>Healthy controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Wt. (n = 20)</td>
<td>Wt. gain (n = 22)</td>
<td>Goal Wt. without m.c. (n = 16)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>14.3 ± 0.3,a</td>
<td>17.4 ± 0.1c,b</td>
<td>19.5 ± 0.1b</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.1 ± 0.2,a</td>
<td>3.9 ± 0.5a,b</td>
<td>5.7 ± 0.8a,b</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>Undetected 85.0%</td>
<td>Undetected 33.3%</td>
<td>Undetected 33.3%</td>
</tr>
<tr>
<td>FSH (IU/liter)</td>
<td>3.9 ± 0.9</td>
<td>6.3 ± 0.6b</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>LH (IU/liter)</td>
<td>1.0 (1.0)a</td>
<td>1.0 (1.1)c,b</td>
<td>1.0 (1.8)c,b</td>
</tr>
<tr>
<td>ΔLH (IU/liter)</td>
<td>0 (3.4)c</td>
<td>11.5 (15.6)c,b</td>
<td>17.4 (14.2)c,b</td>
</tr>
<tr>
<td>Estradiol (pmol/liter)</td>
<td>50 (0)a</td>
<td>60 (0.10)</td>
<td>60 (0.07)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE (for BMI, leptin, and FSH) or median with interquartile range (for inhibin B, LH, ΔLH, and estradiol). For inhibin B, the percent of undetected values are given. Wt., Weight; m.c., menstrual cycle.
a Significance of difference from control values, P < 0.001.
b Significance of difference from Low Wt., P < 0.001.

Patients with AN during weight gain
During weight gain at BMI 17.4 ± 0.1 kg/m², basal leptin level was 3.9 ± 0.5 ng/ml, median inhibin B level was 13.4 with interquartile range 23.7 pg/ml, and median LH was 1.0 with interquartile range 1.1 IU/liter. Basal leptin level, inhibin B, LH, and ΔLH were significantly lower than controls (P < 0.01; Table 1, Fig. 1). Only leptin and inhibin B were significantly higher than in low-weight group (P < 0.001, Table 1, Fig. 1).
Patients with AN with goal weight without menstrual cycles

In the patients with AN who reached goal weight with BMI of 19.5 ± 0.1 kg/m², leptin level was 5.7 ± 0.8 ng/ml, median inhibin B level was 18.6 with interquartile range of 22.3 pg/ml, and median LH level was 1.0 with interquartile range of 1.8 IU/liter. Basal leptin level, inhibin B, LH, and estradiol were significantly lower than in healthy controls (P < 0.01; Table 1, Fig. 1). Leptin and inhibin B were significantly higher than in the low-weight group (P < 0.001; Table 1, Fig. 1).

Patients with AN with goal weight and regular cycles

Patients with AN who reached goal weight and recovered regular menstrual cycles had BMI of 19.3 ± 1.0 kg/m², basal leptin level of 8.5 ± 1.3 ng/ml, median inhibin B of 26.0 with interquartile range of 33.7 pg/ml, and median LH level of 3.1 with interquartile range of 2.1 IU/liter, and as expected, this did not differ from healthy controls (P > 0.05; Table 2).

Inhibin B values correlated significantly with leptin (r = 0.446, P = 0.000; Fig. 2A) and BMI (r = 0.465, P = 0.000; Fig. 2B). BMI correlated with leptin during weight gain (r = 0.707, P < 0.01; Fig. 2C).

Body composition in weight-recovered AN

Table 2 summarizes data for body composition and hormones for 12 patients with AN. Six reached goal weight but were amenorrheic, whereas the other six reached goal weight and had six regular menstrual cycles. BMI, percent of body fat, leptin, and FSH levels did not appear as significant determinants of menstrual status in weight-restored patients with AN. As expected, significant differences were found in the duration of amenorrhea, anteroposterior spine bone density, and LH levels (P < 0.05; Table 2).

TABLE 2. Results and data for body composition in 12 weight-recovered patients with AN with and without menstrual cycles (m.c.)

<table>
<thead>
<tr>
<th>Weight-recovered AN (n = 12)</th>
<th>Goal Wt. without m.c. (n = 6)</th>
<th>Goal Wt. with m.c. (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>22.5 ± 1.8</td>
<td>23.2 ± 1.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>51.3 ± 2.0</td>
<td>54.5 ± 2.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.4 ± 0.4</td>
<td>19.3 ± 0.6</td>
</tr>
<tr>
<td>Onset of AN (yr)</td>
<td>16.5 ± 1.0</td>
<td>19.8 ± 1.9</td>
</tr>
<tr>
<td>Duration of amenorrhoea (yr)</td>
<td>5.2 ± 1.2</td>
<td>1.9 ± 0.6*</td>
</tr>
<tr>
<td>Duration of recovery period (yr)</td>
<td>1.8 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>29.6 ± 1.1</td>
<td>30.8 ± 1.8</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>35.2 ± 1.7</td>
<td>37.5 ± 1.8</td>
</tr>
<tr>
<td>AP spine bone density (g/cm²)</td>
<td>0.97 ± 0.03</td>
<td>1.14 ± 0.05*</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>7.3 ± 1.2</td>
<td>7.4 ± 1.1</td>
</tr>
<tr>
<td>FSH (IU/liter)</td>
<td>8.9 ± 1.5</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td>LH (IU/liter)</td>
<td>1.2 ± 0.2</td>
<td>3.8 ± 1.0*</td>
</tr>
</tbody>
</table>

Wt., Weight.

* P < 0.05.

![Fig. 2. Correlations between BMI and leptin (A; r = 0.707, P = 0.001), between BMI and inhibin B (B; r = 0.465, P = 0.000), and between leptin and inhibin B (C; r = 0.446, P = 0.000) in all patients with AN.](image-url)
Discussion

We have demonstrated that in patients with AN, during weight gain but with persisting amenorrhea, ovaries are not entirely quiescent but have a low level of activity as evidenced by inhibin B levels. Inhibin B was detectable in only 15% of samples at low weight but in 66.7% of samples after weight gain. Median levels of inhibin B were low in the low-weight patients with AN and higher during weight gain. Inhibin B and FSH were detectable in patients with AN at weight gain, which may be compatible with sporadic follicular growth under the influence of FSH. It has been demonstrated that inhibin B is a sensitive marker of gonadal function in prepubertal children and can be used as a potential marker of early follicular activity (8, 9). Inhibin B concentrations increase significantly with FSH concentrations in the earlier phases of puberty, but the positive relationship disappears during later phase of puberty, suggesting that, in cycling women, it is not the level of circulating FSH per se that is critical in follicle growth but the duration of elevation above a critical threshold (10). Changes in inhibin B in this study agree in general with those observed during puberty in which inhibin concentrations varied dynamically with pubertal maturation (9).

The cross-sectional data in this study further suggest that for similar circulating leptin levels and percent of body fat, some patients with AN restore their reproductive function, whereas others do not. In those who restore menstrual cycles, circulating inhibin B levels do not differ from healthy subjects in the early follicular phase. In those who remain amenorrheic despite weight recovery, inhibin B levels are lower than in healthy subjects yet higher than in low-weight patients with AN. LH remained low in these patients, illustrating its insensitivity as a marker of gonadal activity. As for the estradiol, because the estradiol values in the weight gain and goal-weight groups were very close to assay detectability levels, the use of a more sensitive assay would likely improve the usefulness of its measurement.

Leptin may serve as a permissive signal for the onset of puberty and the maintenance of reproductive function thereafter (26). This is illustrated by showing that pubertal pattern of gonadotrophin release was evident in a 10-yr-old girl with congenital leptin deficiency after 12 months of replacement with recombinant leptin (22) and by restoring menstrual cycles in females with congenital lipodystrophy after several months of treatment with recombinant leptin (23). Interestingly, despite a continuous increase of serum leptin concentrations during weight gain, one group of patients with normal BMI did not reestablish cycles and both leptin and inhibin B were lower, compared with normal women and those AN patients who did resume cycling, suggesting that inhibin B may be a marker for resumption of ovarian activity and that weight gain itself is not a good marker. There are several possible explanations for the differences in leptin and body weight restoration during weight gain. First, nutritional factors influence leptin concentrations in humans and the impact of dietary fat composition on serum leptin concentrations has been proposed (27–29). It has been proposed that hypoleptinemia in normal-weight women with hypothalamic amenorrhea may reflect inadequate fat intake and/or other subclinical nutritional disturbances (30). Most of our weight-recovered patients with anorexia nervosa still restricted dietary fat intake (i.e., substituted dietary carbohydrates for fat). Our patients were not energy restricted but, due to persisting fear of obesity, consumed low-fat diets, and there are data that isocaloric substitution of dietary carbohydrate for fat enhances leptin sensitivity in the central nervous system failing to trigger an increase in appetite (31). Recently much interest has been focused on the proliferator-activated receptors, which are transcription factors involved in the differentiation of adipocytes and may play a role in energy homeostasis. This receptor can be modified by diet (32).

Second, minor weight gains in patients with AN are associated with highly variable leptin responses (33). There could be individual differences in leptin and body fat restoration during weight gain. Furthermore, healthy individuals with similar degree of adiposity exhibit great variations in plasma leptin concentrations (16). The presence of polymorphisms in the promoter region of the human leptin gene has recently been associated with variations in circulating leptin levels, and the potential importance of the common −2548G/A promoter variant has been reported (34). Although many studies describe body composition cross-sectionally in women with AN, little research has systematically investigated the changes in body composition and body fat distribution that occur with normalization of body weight (33). Some data suggest a predilection for the accumulation of truncal (visceral) fat relative to the extremity fat (35, 36). In a selected subgroup of weight-recovered patients with AN in our study, BMI, leptin, percent fat, or percent truncal fat did not appear as significant determinants of the menstrual status.

Third, the distortions of body image and fear of becoming fat persist in patients with AN during recovery, and they continue to struggle with issues of weight and shape. It is presumed that in AN neurotransmitters and different neuropeptides are involved in the pronounced distorted psychology, which might affect neuropeptidergic signaling involved in weight control. Thus, the possibility that central mechanisms regulating energy homeostasis during recovery still overcome the effect of peripheral signals. It has been shown that particular stressors might reduce leptin secretion, mainly via adrenergic pathways (37). Insufficient leptin may then be further involved in a set of behaviors that are often disturbed in psychiatric patients (e.g., sexuality, sleep, and motor activity), and thus, a vicious cycle is set up. All together this may encourage leptin administration after sufficient weight gain in patients with AN. By encouraging leptin administration after achieving goal weight, one might hasten restoration of gonadal function, given the positive association between leptin and inhibin B found in this cross-sectional study.

Taken together, inhibin B may be a marker for resumption of ovarian activity. Weight gain itself is not a good marker because one group of patients with AN with normal BMI did not reestablish cycles, and both inhibin B and leptin were lower, compared with normal women. Ovaries in these patients are not entirely quiescent but display a low level of...
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30. Yannakoulia M, Yannakouris N, Blueher S, Matals A, Klimis-Zacas A, Mantzoros C 2000 Decreased leptin levels in normal weight women with hypothalamic amenorrhea: do they have the same characteristics as patients with anorexia nervosa? Hormones 7:285–290

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