Disturbances in gonadal axis in women with anorexia nervosa

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ABSTRACT. Anorexia nervosa negatively affects multiple body systems including the reproductive system. **Aim**: To assess the disturbances in the hypothalamic-pituitary-gonadal axis (HPG) and the relationship between the gonadotropins and body weight, duration of the disease and amenorrhea. We studied 40 female anorectic patients (aged 14-31 years) with a body mass index (BMI) 13.14±1.80 kg/m² and a degree of weight loss 28.67±8.74%. Fifteen healthy, age-matched women with normal weight served as controls. **Methods**: We investigated the disturbances in the gonadotropin levels before and after stimulation with gonadotropin-releasing hormone (GnRH) 100 µg i.v. One week later 100 mg of clomiphene citrate (CC) was administered orally for 5 days. **Results**: Basal levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were significantly lower in the patients. The responses of LH to GnRH were diminished, but those of FSH were exaggerated. However, after clomiphene citrate administration, LH increased 5.4 times whereas FSH increased 1.7 times. The basal levels of LH were significantly correlated with body weight (r=+0.373, p<0.05), BMI (r=+0.385, p<0.01) and percentage of the weight loss (r=-0.356, p<0.05). FSH levels were positively correlated with the duration of the disease (r=+0.481, p<0.01) and amenorrhea (r=+0.540, p<0.01). **Conclusions**: Our study demonstrates dissociation in the secretion of gonadotropins after hypothalamic stimulation in anorectic patients. It also reveals the relationship between alterations in the hormones of the HPG axis, not only with the changes in body weight, but also with the duration of the disease.


INTRODUCTION

It is a well known fact that there exists a close relationship between body weight and the human reproductive system. Anorexia nervosa (AN) is a typical example of this association in which refusal to eat leads to severe weight loss, which in turn interrupts the menstrual cycle, ultimately resulting in anovulation and infertility. AN and bulimia nervosa (BN) affect up to 5% of women in the reproductive age group and is prevalent among adolescent girls and young women (1).

Many endocrine and metabolic disturbances have been described in patients with AN. However, the exact mechanism and affected brain structures are not clear and need to be investigated further. A number of genetic, neurological, hormonal, psychological, and social factors are implicated in the pathophysiology (2). In the last several decades, many investigators have attempted to uncover the mechanism responsible for the disturbances typically seen in the hypothalamic-pituitary-gonadal (HPG) axis in patients with AN under basal conditions as well as after stimulation with GnRH or clomiphene citrate (CC).

Several studies have shown that patients with AN experience hormonal disturbances, but no clinical correlation was determined (3-6). Moreover, uniform data regarding the nature of hormonal disturbances in patients with AN and their relationship with clinical manifestations are lacking. Basal levels of luteinizing hormone (LH) have been described as lower than normal (7-9) or within the normal range (10), and those of follicle-stimulating hormone (FSH) as higher than normal (11, 12) or within the normal range (10, 13, 14). The levels of gonadotropins did not correlate with the duration of amenorrhea (15). Some studies reported a significant correlation between basal levels of LH and weight loss (12, 14, 15) whereas others found no such correlation (8). Some authors have reported diminished or absent response of LH after gonadotropin-releasing hormone (GnRH)
stimulation, but there is no consensus on this issue (8, 12). GnRH stimulation of pituitary gonadotrophs yielded a normal (8, 9) or blunted (13, 14) FSH response.

Unfortunately, the results of these studies lack uniformity, which probably can be attributed to the small number of patients involved in these studies. Therefore, we performed this study in an attempt to achieve clarification on the level of disturbances in the gonadal axis in patients with AN and to attain a better insight into their relationship with body weight, duration of the disease, and amenorrhea. For this purpose, we evaluated the responsiveness of the pituitary gland by measuring the basal level of gonadotropins as well as their levels after separately stimulating the pituitary with GnRH - a hypothalamic hormone that stimulates only LH and FSH from the pituitary, and CC, which triggers the hypothalamus. In this way, we believe that the disturbances in the HPG axis of anorexic patients under different settings can be observed.

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**PATIENTS AND METHODS**

The study was approved by the Institutional Review Board, Clinical Center of Endocrinology, Medical University, Sofia, Bulgaria. The study involved 40 female patients with diagnosis of AN having median age of 18.5 years (range, 14-31 years), with an average body mass index (BMI) of 15.1±1.80 kg/m² (x ± SD) and an average degree of weight loss of 28.6±8.74%. Fifteen healthy, age-matched women with normal weight (BMI 21.8±1.33 kg/m²) and regular menstrual cycles served as controls.

The diagnosis of AN was based on the American Psychiatric Association diagnostic criteria - DSM IV (16) and was confirmed by an experienced psychiatrist. All patients were diagnosed with the restricting type of AN (no signs of binge-eating and purging). The duration of the disease ranged from 3-120 months. All participants (patients and healthy women) in the study were of the Caucasian race and were post-menarchal. Individuals were excluded if they had other diseases that could lead to malnutrition such as malabsorption (abnormal excessive systemic loss of nutrients due to diarrhea, hemorrhage, renal failure, or excessive sweating), infection, or addiction to drugs (17). Four of the patients were on medications (oral contraceptives), which were discontinued 2 to 6 months prior to the start of this study to avoid any confounding bias.

We assessed serum levels of LH, FSH, sex hormone binding globulin (SHBG), estradiol, and testosterone in all the participants of the study. The basal levels of gonadotropins and their responses to 100 μg GnRH i.v. (intravenous) at 20, 30, 60 and 90 minutes were studied. One week after the GnRH test, 100 mg of CC (orally) was administered to the patients for 5 days only. Levels of gonadotropins were measured both before and on the 6th day after stimulation with CC.

Serum hormone levels were assessed in blood samples taken at 8 a.m. after an overnight fast. The blood was centrifuged, and the sera were frozen at -21°C till the time of assay. The determination of the hormone levels was performed using commercially available kits. Serum levels of pituitary hormones were determined by immuno radioimmunoassay (IRMA) (Radim, Italy) for the LH (intra-assay 3.87%, inter-assay 4.64%, sensitivity 0.18 IU/l) and FSH (intra-assay 5.1%, inter-assay 4.11%, sensitivity 0.18 IU/l) and by Delfia (time resolved fluorimunoassay) (Pharmacia, Turku, Finland) for SHBG (intra-assay 6.7%, inter-assay 5.1%, sensitivity 0.8 nmol/l), testosterone (intra-assay 4.9%, inter-assay 3.4%, sensitivity 0.2 nmol/l) and estradiol (intra-assay 4.6%, inter-assay 4.9%, sensitivity 0.05 nmol/l).

**Statistical methods**

Statistical evaluation of the data was carried out using the software SPSS 11 for Windows. Student’s t test as well as Wilcoxon signed ranks test was used to analyze the changes in hormonal levels between the patients and the controls. Areas under the curve (AUC) were calculated with the trapezoidal rule. The differences between the highest levels of gonadotropins during the tests and the basal level (Δ), and percentage increase in plasma gonadotropins above the baseline level (Δ%), were calculated and were used as indices of response. After the Kolmogorov-Smirnov test for normality of distribution was done, two-tailed Pearson correlation test was performed to determine the relationship between different parameters. The results were expressed as the mean ± SD. The level of significance was preset at p<0.05.

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**RESULTS**

Basal gonadotropin levels were significantly lower in the patients than in controls (LH: 3.22±3.35 IU/l vs. 7.19±2.34 IU/l, p<0.001; FSH: 3.58±2.5 IU/l vs. 4.96±1.43 IU/l, p<0.05). The responses of LH to GnRH stimulation were diminished in the patients with AN while those
of FSH were exaggerated (Fig. 1). In the anorexic patients, the AUC for FSH (11.13±
7.42 IU/l/min) was higher compared to the healthy women (6.98±1.94 IU/l/min, p<0.05). The AUC for LH was lower in the anorexic patients than in the controls but the difference was not significant. We did not find a statistically significant difference between ΔLH in the patients and ΔLH in the healthy individuals, but ΔFSH, %ΔFSH, and %ΔLH in the patients were significantly higher than those of the healthy women (Table 1). However, after CC stimulation, LH increased 5.4 times from the basal levels whereas FSH increased only 1.7 times the basal levels (LH increased from 2.16±2.46 IU/l to 11.7±13.78 IU/l, p<0.001, and FSH from 3.12 ±2.5 IU/l to 5.17±4.51 IU/l, p>0.05).

The basal concentration of SHBG in the patients (165.27±63.5 nmol/l) was significantly higher than those in the controls (96.21±38.04 nmol/l, p <0.01), whereas the levels of estradiol (0.10±0.09 nmol/l) and testosterone (1.49±0.96 nmol/l) in the anorexic patients were significantly lower (p<0.02) than those in the healthy women (0.19±0.10 nmol/l and 2.37±1.05 nmol/l, respectively).

We established a significant correlation of the basal levels of LH with body weight (r=+0.373, p<0.05), with BMI (r=+0.385, p<0.01), and with the percentage of the weight loss (r=-0.356, p<0.05). A similar trend was observed with ΔLH and %ΔLH after the CC stimulation (Table 2). Likewise, basal levels of SHBG were significantly correlated with body weight (r=-0.761, p<0.01), BMI (r=-0.513, p <0.05) and percentage of the weight loss (r= +0.654, p<0.05). In addition, the alterations of FSH were correlated significantly to the duration of the disease (r=+0.481, p<0.01) and the duration of amenorrhea (r=+0.540, p<0.01).

**DISCUSSION**

AN is a disease, which is characterized by various alterations in the endocrine system including the HPG axis. Nutritional deprivation has been implicated as a common cause of the reproductive compromise observed in AN. Amenorrhea is one of the earliest signs of AN. However, the precise mechanism of these disturbances is still unknown. It is considered that it might be a consequence of severe weight loss. On the other hand, an anorexic’s persistent self-starvation could be the result of malfunction of the neurotransmitter systems known to modulate feeding behavior.

In the present study, basal levels of gonadotropins were significantly lower in the 40 anorexic patients than in the 15 healthy controls. These results are in agreement with previous studies (18, 19), but contradicted with others who did not find any significant difference in basal gonadotropins levels between the cases and controls (7, 8, 13, 14).

We established that AN patients have a low

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td><strong>Gonadotropin levels after stimulation with 100 μg gonadotropin releasing hormone [GnRH] (x±SD)</strong></td>
</tr>
<tr>
<td><strong>Indexes</strong></td>
</tr>
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<tr>
<td><strong>Basal level</strong></td>
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<tr>
<td>Patients n=40</td>
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<td>Controls n=15</td>
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<td>p</td>
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AUC=area under the curve, Δ=difference between the highest value during the test and the basal value, %Δ=percentage increase in gonadotropins above the baseline level.
TABLE 2
Relationship between LH and FSH and some clinical indexes.

<table>
<thead>
<tr>
<th>Indexes</th>
<th>After 100 µg GnRH</th>
<th>After clomiphene citrate 100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH basal</td>
<td>LH AUC</td>
</tr>
<tr>
<td>% of weight loss</td>
<td>r =+0.356*</td>
<td>r =-0.374*</td>
</tr>
<tr>
<td>BMI</td>
<td>r =+0.385**</td>
<td>r =+0.341*</td>
</tr>
<tr>
<td>Weight</td>
<td>r =+0.373*</td>
<td>r =+0.382*</td>
</tr>
<tr>
<td>Duration of the disease</td>
<td>r =+0.212</td>
<td>r =-0.008</td>
</tr>
<tr>
<td>Duration of the amenorrhea</td>
<td>r =+0.171</td>
<td>r =-0.155</td>
</tr>
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*p<0.05; **p<0.01

basal level of LH but a good response to stimulation with GnRH; approximately equal values of ΔLH and AUC LH in comparison with the controls were found. Low levels of FSH followed by an exaggerated response to GnRH indicate that the pituitary reserves of FSH are adequate and that the function of gonadotrophs is undisturbed. Also, the increased values of %Δ LH and %Δ FSH in the patients after stimulation show that the capacity of the pituitary was preserved. Therefore, the increase of gonadotropin levels after the administration of GnRH is suggestive of an intact pituitary and a partially disturbed hypothalamus.

In healthy women the levels of LH following CC administration increase by 100%, while the increase in FSH levels are by 50% (20). Some authors found a diminished or failing response of LH to CC in AN patients (18, 21). On the contrary in our study, after CC stimulation, the levels of gonadotropins increased, especially of LH (542%). CC acts as a selective estrogen receptor blocker by binding to the estrogen receptors in the hypothalamus, and thereby decreases the negative - feedback effect of estrogen on the hypothalamus resulting in release of endogenous GnRH from the hypothalamus. This, in turn, increases secretion of FSH and LH.

In healthy persons, administration of GnRH causes a prompt increase in plasma LH and an increase of FSH to a lesser degree. Our data suggest that the FSH response to GnRH in the anorexic patients was higher than that of the LH response: the %ΔFSH increased 6.77 times while %ΔLH increased only 2.5 times. AUC LH and ΔLH in the patients were lower than those of the controls. In contrast, the changes in AUC FSH and ΔFSH in the patients were more pronounced (AUC FSH -1.6 times and ΔFSH -3.3 times) in comparison with the controls. This pattern of response to standard dose of 100 µg GnRH by the way of increased secretion of FSH is typical of prepuberty. Our results suggest that in anorexic patients, the HPG axis returns to the prepubertal state. On the other hand, after oral CC stimulation, the LH response predominates. CC administration has an intrinsic estrogenic effect, especially in the background of the underweight and in those with low estrogen levels, which may partially explain the divergent responses of gonadotropins.

In our study, basal levels of LH were significantly correlated with body weight, BMI and percentage of the weight loss. According to Frisch et al., a critical body mass is essential for maintaining regular menstrual cycles (22). Severe malnutrition and/or body weight loss can cause a reduction in GnRH secretion from the hypothalamus, which in turn leads to suppression of FSH and LH secretion. This effect was observed in experimental animals (23, 24).

To the best of our knowledge, our study is the first one that reports a positive correlation between basal FSH levels and the duration of the disease as well as the duration of amenorrhea.

The basal levels of SHBG in our patients were significantly higher than those of the controls. Basal levels of SHBG in anorexic patients were found to be increased in some studies (25-29) whereas other studies reported reduced SHBG levels (30) or no change in SHBG levels in comparison with controls (10). Most of these investigators could not find a significant correlation between SHBG levels and BMI or degree of weight loss in women with AN (26, 28). Earlier studies demonstrated that patients with AN have lower concentrations of testosterone and insulin, which can explain the variation in the SHBG levels to some degree (29). Furthermore, in these patients, basal levels of SHBG had a strong negative correlation with body weight and BMI and a positive correlation with the percentage of weight loss (29).
Our results show that 1) the changes in the gonadal axis are due to the disturbances in the hypothalamus and 2) all hormonal alterations are in relationship with the weight loss. Severe weight loss is mainly associated with loss of fat tissue. Several other hormones and mediators such as leptin, estrogens, cortisol and other substances are implicated in this process and send signals to the hypothalamus, which influences its activity (31-39). During recuperation, when patients increase their food intake, gonadotropin secretion increases, suggesting that malnutrition could be the most important mechanism involved in reduced gonadotropin secretion (40, 41).

Consequently, body weight is one of the most important factors influencing hormone levels in women with AN. The degree of weight loss is associated with the degree of hormonal disturbances in the gonadal axis.

The pattern of variation in levels of FSH and LH observed after stimulation with GnRH and CC is not fully understood. We found that secretion of LH and FSH followed different modes - GnRH increased FSH to a higher degree in comparison with the controls, whereas CC elevated mainly LH. This dissociation in the response of the gonadotrophs to GnRH and CC may be specific for AN, indicating an alteration in the hypothalamic-pituitary relationship that is unknown at this time. Moreover, some authors have established that the pituitary glands from normal rats and rats suffering from weight loss show different types of responsiveness to GnRH in vitro. A study by Kotsuji et al. found a diminished LH-releasing action of GnRH, an augmented gonadotropin-synthesizing action of GnRH, and an increased FSH release in female rats with weight loss (42), which supports our data.

The question is which factor is responsible for the discrepancy between the changes in serum levels of LH and FSH. One probable cause is the modification of the responsiveness of the pituitary to GnRH owing to weight loss. A question that still remains unanswered is whether the alteration of neuropeptides in anorexic patients is i) a consequence of weight changes or ii) one of the many factors involved in the pathogenesis of AN.

In conclusion, the results of this study demonstrate that the alterations in the HPG axis in anorexic patients are due to disturbances mainly in the hypothalamus and that the activity of the pituitary-gonadal system is preserved. Moreover, it suggests that the disturbances in the HPG axis of anorexic patients correlate directly with the changes in body weight, the duration of the disease, and amenorrhea. Finally, in adolescent and young women with AN, we report a dissociation in the secretion of gonadotropins after stimulation with some form of hypothalamic stimuli.

REFERENCES


