Relationships of Ghrelin and Adiponectin Levels to Menstrual Disorders in Female Athletes

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Abstract

Both ghrelin and adiponectin are related to energy balance that regulates food intake.

Objective: The aim of the present study was to explore whether ghrelin and adiponectin would be linked to menstrual disorders in athletic females.

Subjects and Methods: A total of 40 athletic girls, ages 13-21 yr, including 15 with amenorrhea (AA), fifteen eumenorrheic athletes (Eu A) with regular menstruation and 10 non athletic served as controls. Fasting blood was drawn for assay of peripheral hormones including active ghrelin, adiponectin, sex hormone binding globulin (SHBG), estradiol (E2) and testosterone using ELISA.

Results: Amenorrheic girls had significantly lower body mass index than controls (p<0.05), and were strongly positively correlated to gonadal steroid levels (p<0.001). Both ghrelin and adiponectin levels were significantly higher in AA than Eu A and control groups (p<0.05).

Conclusions: Body mass index is a positive predictor to gonadal steroids. Higher ghrelin and adiponectin levels in AA compared with normal menstruating athletic females, and their associations with low gonadal steroids might explain menstrual disorders among athletics.

Key Words: Adiponectin – Amenorrhea – Athletes – Ghrelin.

Introduction

MANY women now complete in strenuous exercise. The sports participation is a wise experience for a teenage athlete. Common gynecologic problems found in female athletes are delayed menarche, menstrual dysfunction and dysmenorrhea [1].

The International Olympic Committee (IOC) has defined amenorrhea as one period or less per year [2]. Amenorrhea is reported in as many as 24% of adolescent athletes, and the prevalence depends on the nature and duration of exercise as well as the athlete's nutritional status [3]. Endurance athletes such as gymnasts, track runners, ballet dancers and swimmers are reported to be at increased risk of amenorrhea. In adults, a negative state of energy balance in amenorrhea has been attributed to cause disruption of gonadotrophin pulsatility [4].

Ghrelin known as orexigenic hormone; is a ligand for the growth hormone secretagogue receptor. It is a peptide produced primarily by endocrine cells in the gastrointestinal tract [5]. Ghrelin levels correlate inversely with adiposity at baseline. Moreover, circulating ghrelin increases in response to weight loss [6]. It has been implicated in the regulation of the hypothalamo-pituitary-gonadal (H-P-G) axis in some, but not all, subjects.

Adiponectin is a hormone expressed in adipose tissue. It is found in high concentrations in circulating plasma and is an important factor for central control of energy homeostasis. One of the reasons for interest in adiponectin and exercise is the relationship that both adiponectin and exercise have to substrate utilization [7].

A greater understanding of the neuroendocrine factors that predispose to hypogonadism in some but not all athletes could lead to development of therapeutic strategies that target menses resumption as a means of regaining fertility [8].

The purpose of this study was to evaluate ghrelin and adiponectin levels and their relations to amenorrhea in athletics to provide the optimal way for management.
Subjects and Methods

Subject selection: Forty adolescent girls, 13-21 years old from ElShams club, were enrolled in this study. Informed consent and assent were obtained from all subjects and their parents preliminary to the study. The design of the study was approved by the Ethical Committee of our Institute (Atomic Energy Authority).

Inclusion criteria:
This included 15 girls who met the criteria for diagnosis of AA, 15 Eu A and 10 nonathletic controls.
• Amenorrhea (for AA): Absence of menses for > three consecutive cycles after initiation of menses or absence of menarche at >16 years [9].
• Eumenorrhea (Eu A): > nine menses (cycle length 21-35 days) in preceding year.
• Non-athlete healthy controls where weight bearing exercise activity is less than two hours a week and or they are not participating in organized team sports. They did not meet endurance criteria and had no history of amenorrhea or menarchal delay.

Girls with AA and Eu A were endurance athletes, and self reported a history of one of the following for at least 6 months (i) at least 4 h of aerobic weight-bearing training of the legs weekly (ii) at least 30 miles of running weekly, or (iii) at least 4 h of specific endurance training weekly (modified from adult criteria. Eumenorrheic and nonathletic controls were examined in the early follicular phase of their cycles. None of our subjects admitted to using performance enhancing drugs, however, we did not screen for this as part of the study. There was no report of use of such agents based on information obtained from primary care physicians of our study subjects.

Potential enrollees were excluded from study participation if they had any organic or psychiatric chronic illness, a history of pregnancy, symptoms of cold intolerance, galactorrhea, hirsutism, an abnormal TSH or elevated FSH (indicative of hypergonadotropic hypogonadism) or a history of recent rapid weight loss or gain within the past year. Enrolled subjects were not taking any medications, birth control, or any other type of hormonal preparation.

Body mass index (BMI) was calculated using the formula: (weight (kg)/height m\(^2\)) [10].

Specimen: Fasting labs were obtained for active ghrelin, adiponectin, E\(_2\), testosterone and SHBG. Ten ml venous blood were collected into dry clean tubes after 12 hours over night fasting. Blood samples were left to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes, carefully the serum layer was removed and stored at -20°C until further analysis. Serum was required per duplicate determination. Repeated freeze-thaw cycle was avoided.

Biochemical measurements:
Ghrelin levels were measured with a commercial enzyme linked immunosorbent assay (ELISA) kit (Phoenix Pharmaceuticals Inc., Belmond, CA, USA). A minimum detectable concentration of 0.08 ng/ml, an intra-assay variation of 5% and an inter-assay variation of 14% are reported in the accompanying information sheet of the kit according to Nakazato, et al. [11].

Adiponectin levels were measured using adiponectin ELISA kit (Linco Research, Inc., St. Charles, Missouri 63304 USA) [12].

Testosterone was assessed using testosterone ELISA kit (RE52151) (IBL-Hamburg GmbH Flughafenstr. 52A, D-22335 Hamburg, Germany) [13].

Sex hormone binding globulin was determined using SHBG ELISA kit (IBL Gesellschaft Für Immunchemie und Immunbiologie mBH Flughafensstrasse 52a - D-22335 Hamburg - Germany) [14].

Estradiol (E\(_2\)) was estimated by using Estradiol EIA Kit (BioVendor Laboratorní medicína, a. s. Cat. No.: RCAN-E-430R, Im Neuenheimer Feld 583, D-69120 Heidelberg-Germany) [15].

Free androgen index (FAI) was calculated using the following formula: total testosterone (ng/ml) x100/SHBG (nmol/l). Samples were stored at -80°C until analysis, and were run in duplicate [16].

Statistical analysis:
Data are presented as mean ± SD, and were analyzed using the Statistica program (version 6). We used ANOVA to determine differences between the 3 groups. On comparing 2 groups simple student t test was used. Ap-value of < 0.05 was considered significant. Regression analyses were performed to determine the correlations between different hormones. Histograms presentations were expressed using Microsoft Office Excel 2007. While Statistica program was used for correlation coefficient.
Results

Baseline characteristics are summarized in Table (1) where AA girls did not differ from Eu A girls and controls with respect to age. Significantly lower BMI was observed in AA than in Eu A and controls (Fig. 1). Age of menarche trended to be higher in AA than in Eu A and controls.

Active ghrelin and adiponectin levels were significantly higher in AA than in the other two groups (Table 2 and Figs. 2,3). In addition, estradiol levels were lower in AA than in Eu A. Total testosterone levels were lower in AA than in Eu A, and FAI was significantly lower in AA than in controls.

Body Mass Index was a positive predictor of gonadal steroids. While both ghrelin and adiponectin correlated inversely with testosterone, free androgen index and E2.

![Fig. (1): Significant lower BMI among AA group compared to both Eu A and controls.](image1)

![Fig. (2): Significant higher ghrelin levels among AA group compared to both Eu A and controls.](image2)

![Fig. (3): Significant higher adiponectin levels among AA group compared to both Eu A and controls.](image3)

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Table (1): Baseline characteristics in athletes with amenorrhea, eumenorrheic athletes and controls.

<table>
<thead>
<tr>
<th></th>
<th>AA (n=15)</th>
<th>Eu A (n=15)</th>
<th>Controls (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16.4±2.6</td>
<td>16.2±1.8</td>
<td>15.9±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.2±3.8*</td>
<td>54.5±2.7</td>
<td>54.9±2.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Height (m)</td>
<td>164±2.4</td>
<td>164±4.9</td>
<td>162.1±3.6</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.1±1.3*</td>
<td>20.1±0.9</td>
<td>20.3±1.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>12.7±1.3</td>
<td>12.7±1.2</td>
<td>12.5±1.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

AA: Athletes with amenorrhea.  
Eu A: Eumenorrheic Athletes.  
Mean ± SD.  
NS: not significant.  
*: p<0.05 compared with controls.  
#: p<0.05 compared with Eu A (student t test).  
BMI: Body mass index.

Table (2): Hormonal parameters in amenorrheic athletes, eumenorrheic athletes and controls.

<table>
<thead>
<tr>
<th></th>
<th>AA (n=15)</th>
<th>Eu A (n=15)</th>
<th>Controls (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active ghrelin (pmol/l)</td>
<td>899±183*#</td>
<td>669±118</td>
<td>680±121</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>26.72±3.8*#</td>
<td>20.88±9.8</td>
<td>19.1±8.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total testosterone (ng/ml)</td>
<td>30.21±7.54*#</td>
<td>49.08±17.13</td>
<td>41.09±10.81</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FAI</td>
<td>1.5±59*</td>
<td>1.9±0.59</td>
<td>2.75±0.91</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E₂ (pg/ml)</td>
<td>87±28.1</td>
<td>87±25.9</td>
<td>83.9±20.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

* p<0.05: Compared with controls.  
# p<0.05: Compared with Eu A.  
FAI: Free androgen index.
Table (3): Correlation coefficients of gonadal steroids with body mass index, ghrelin and adiponectin levels in all subjects (n=40).

<table>
<thead>
<tr>
<th></th>
<th>Total Testosterone</th>
<th>FAI</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.6089**</td>
<td>0.7004**</td>
<td>0.6719**</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>-0.2648</td>
<td>-0.2451</td>
<td>-0.1855</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.3889*</td>
<td>-0.4936*</td>
<td>-0.3919*</td>
</tr>
</tbody>
</table>

*: p<0.05 **: p<0.001

Fig. (4: A,B,C): Significant positive correlations between BMI and testosterone, free androgen index and E2.

Discussion

Menstruation is the most important female characteristic; they have a feeling of good health when they have regular menstruation. Sport participation has been reported to delayed menarche when the sport activity began before puberty. Altered menstrual cyclicity can approach 70% in strenuously exercising women. Menstrual dysfunction has been associated with a significant weight loss, decreased body fat, previous history of menstrual dysfunction, stress and intensive training (Piya-Anant, 2008). Amenorrhea occurs in as many as 25 percent of female high school athletes, compared with 2 to 5 percent in the general population. Amenorrhea in athletes is known to cause infertility and early onset of low bone density and may increase the risk of breaking bones. Evidence suggests that intense exercise associated with caloric restriction, and therefore a state of energy deficit, is most responsible for menstrual irregularities among athletes [17].

Female athlete health problems have three components: (1) eating disorders (2) amenorrhea or oligomenorrhea, and (3) osteoporosis or osteopenia. Risk factors include chronic dieting, low self-esteem, family dysfunction, physical abuse, biologic factors, perfectionism, and a lack of nutrition knowledge [18]. Trigger factors include an emphasis on body weight for performance or appearance and pressure to lose weight from parents, coaches, judges, and peers and a drive to win at any cost [19].

Misra, et al. [17] speculated that ghrelin may be an important link between an energy deficit state and the hormones that regulate menstrual function. Adiponectin represents a newly discovered adipose tissue derived hormones; that is associated in health status as well as in glucose and free fatty acid metabolisms [20].

The present study demonstrated higher active ghrelin and adiponectin levels in AA girls compared with Eu A girls and controls, also active ghrelin and adiponectin are independent predictors of gonadal steroids. Our data are consistent with reports of ghrelin administration causing a decrease in gonadotrophin secretion in animal Martini, et al. [21] and Lebrethon, et al. [22] and human studies [23]. These data suggest that higher ghrelin and adiponectin levels may predict which athletes will develop amenorrhea.

Eumenorrheic athletes had somewhat higher weights than sedentary controls, and larger studies are needed to determine whether relatively higher
weights in athletes protect against development of amenorrhea. Warren [24] concluded that energy drain may have an important modulatory effect on the hypothalamic pituitary set point at puberty and, in combination with low body weight, may prolong the prepubertal state and induce amenorrhea. Intensive training and inadequate energy intake may induce delayed menarche and menstrual dysfunction [25].

Objectives neuroendocrine factors may predict which athletes develop amenorrhea and which athletes remain eugonadal. Ghrelin has been implicated in regulation of Gonadotropic releasing hormone secretion having inhibitory effects. Christo, et al. [23] hypothesized that adolescent athletes with amenorrhea (AA) would have higher ghrelin levels than eumenorrheic athletes (Eu A) and would predict levels of gonadal steroids.

Ghrelin was shown to suppress luteinizing hormone (LH) secretion in vitro, and to decrease LH responsiveness to LH-releasing hormone (LHRH) in vivo. Moreover, ghrelin was able to inhibit stimulated testicular testosterone secretion, whereas androgens have been proven independent modulators of circulating ghrelin levels [26]. They added that in the ovary, expression of ghrelin was demonstrated in steroidogenically active luteal cells and interstitial hilus cells. They concluded that ghrelin may operate at different levels of the reproductive system, including the testis and the ovary, which are potential targets for systemic ghrelin actions.

Tanya, et al. [27] reported that adiponectin has a strong relationship with fat mass, plays a role in regulation of glucose and lipid metabolism and is negatively correlated with glucose, insulin, and BMI. It has been well established that adiponectin levels increase in amenorrhea associated with anorexia nervosa. Tanya, et al. [27] found that adiponectin levels are disproportionately high in amenorrheic athletes compared to eumenorrheic athletes with similar body weights. These results are consistent with elevated adiponectin levels observed in association with amenorrhea in AA patients in the present study.

Athletes with amenorrhea are at risk of osteoporosis and stress fracture, much like postmenopausal women. Prevention of this loss of bone mass is one of the main goals of treating amenorrhea. The prevention of female athlete health problems is unknown but improving dietary intake should be done to restore energy and it is not necessary to reduce the intensity of training [28].

Overall, hormonal factors that link energy deficit and the secondary amenorrhea in athletes are not well characterized. These factors are important to be determined in order to develop therapies that will lead to resumption of menstruation and hence improved bone density. It is proposed that ghrelin may cooperate with other regulatory signals, such as adiponectin, in the integrated control of energy balance and hence reverse menstrual disorders.

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