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Peptide YY in adolescent athletes with amenorrhea, eumenorrheic athletes and non-athletic controls ☆

Melissa Russell [a,b], Jenna Stark [a], Shriddha Nayak [a], Karen K. Miller [a], David B. Herzog [c], Anne Klibanski [a], Madhusmita Misra [a,b,*]

[a] Neuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
[b] Pediatric Endocrine Unit, Massachusetts General Hospital for Children and Harvard Medical School, Boston, MA, USA
[c] Harris Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

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ABSTRACT

Background: Bone mineral density (BMD) is lower in amenorrheic athletes (AA) compared with eumenorrheic athletes (EA). Decreased energy availability and altered levels of appetite regulating hormones (ghrelin and leptin) in AA contribute to hypogonadism, an important cause of low BMD. The role of other nutritionally regulated hormones such as peptide YY (PYY) and adiponectin in mediating gonadal status and bone metabolism remains to be determined.
Objectives: Our objective was to determine whether PYY and adiponectin are higher in AA compared with EA and contribute to hypogonadism and impaired bone metabolism in AA.
Methods: We determined PYY and adiponectin in 16 AA, 15 EA and 16 non-athletic controls 12–18 years old, and other nutritionally dependent hormones including ghrelin, leptin and IGF-1. We also measured testosterone, estradiol, PINP and NTX (markers of bone formation and resorption) and BMD.
Results: PYY was higher in AA than EA (111 ± 52 vs. 61 ± 29 pg/ml, $p<0.05$), whereas adiponectin did not differ between groups. Although activity scores did not differ, BMI was lower in AA than EA and a larger proportion (62.5% vs. 6.7%) reported disordered eating, indicating lower energy availability. PYY and adiponectin were independent predictors of testosterone in a regression model ($p=0.01$ and 0.04), but did not predict estradiol. PYY, but not adiponectin, was an independent and negative predictor of PINP ($p=0.002$) and lumbar bone mineral apparent density *Z*-scores ($p=0.045$) in this model.
Conclusion: High PYY levels (but not adiponectin) differentiate AA from EA, and may be an important factor contributing to low bone density in athletes.

Introduction

A state of reduced energy availability has been implicated in the hypogonadotropic hypogonadism and subsequent low bone mineral density (BMD) seen in adult exercising women [1] and adolescent athletes [2]. Of concern, amenorrhea affects as many as 24% of adolescent athletes, and endurance athletes in particular are at high risk for developing hypogonadotropic hypogonadism and low BMD. However, not all endurance athletes develop amenorrhea, and alterations in neuroendocrine factors in low energy states (where energy intake cannot keep pace with expenditure) that subsequently impact secretion of gonadotropin releasing hormone (GnRH) and gonadotropins are still being elucidated. We have previously reported that higher ghrelin and lower leptin levels in athletes with amenorrhea (AA) compared with eumenorrheic athletes (EA) predict lower levels of gonadal steroids in AA, and have postulated that alterations in ghrelin and leptin may play a role in differentiating between athletes who will or will not develop hypothalamic amenorrhea [3]. Both ghrelin and leptin reflect the state of energy availability and have important and opposing effects on the hypothalamo–pituitary–gonadal (H–P–G) axis [4,5]. However, there are likely other neuroendocrine factors linking energy status to reproductive function in athletes that are yet to be been identified. Peptide YY (PYY) and adiponectin are important hormones affected by the state of energy availability [6,7] that may impact the H–P–G axis [8,9] and bone [10–12].

PYY is an anorexigenic peptide secreted primarily by endocrine L cells of the distal gut [13] in response to intraluminal nutrients [14]. Its levels increase after food intake and PYY promotes satiety by binding to Y2 receptors of neuropeptide Y (NPY) within the hypothalamus and inhibiting NPY secretion [15,16]. Levels of PYY are low in obesity [17] and high in anorexia nervosa [6], indicating altered PYY secretion at the extremes of energy availability. Importantly, high PYY levels decrease GnRH mediated gonadotropin secretion in rodent models

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* Corresponding author. Neuroendocrine Unit, Massachusetts General Hospital, Boston, MA 02114, USA. Fax: +1 617 726 8528.
E-mail address: mmisra@partners.org (M. Misra).

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[8,18], effects mediated through the Y2 receptor of NPY. Furthermore, PYY has a direct role in the regulation of bone metabolism. Rodents with selective deletion of the Y2 receptor have increased osteoblastic activity [10], suggesting that activation of the Y2 receptor by high levels of PYY may inhibit bone formation. Consistent with this postulate, we have reported an inverse association between PYY and levels of bone turnover markers in girls with anorexia nervosa, an extreme state of low energy availability [6]. In addition, adiponectin is an adipocytokine also affected by the state of energy availability. Adiponectin levels are low in obesity [7] and variable in conditions of low weight [19,20]. *In vitro* studies indicate that adiponectin may reduce GnRH mediated gonadotropin secretion [9] and activate both osteoblast and osteoclast activity [11,12]. However, clinical studies suggest a net negative effect of high adiponectin levels on bone density [19,21]. PYY and adiponectin levels and their association with bone metabolism and gonadal steroids have not been investigated in adolescent athletes.

We hypothesized that PYY and adiponectin levels would be higher in adolescent AA compared with EA and eumenorrheic non-athletic controls consistent with presumed lower energy availability in AA, and would predict hypogonadism and impaired bone metabolism.

Subjects and methods

Subject selection

Forty-seven adolescent girls, ages 12–18, were enrolled in this study. Among the group, 16 girls met the criteria for diagnosis of AA, 15 were EA and 16 were non-athletic controls. Girls with AA or EA were endurance athletes with a self-reported history of one of the following for at least six months: (i) ≥4 h of aerobic weight-bearing training of the legs weekly, (ii) ≥30 mi of running weekly or (iii) ≥4 h of specific endurance training weekly [22]. Girls were considered amenorrheic if (i) they had no menses for at least three consecutive cycles immediately preceding study participation, and had either attained menarche and menstruated regularly for at least six months after menarche and before the period of amenorrhea [22], or (ii) had not attained menarche at 15.3 years of age (mean age at menarche + 2 SDs for girls in the United States) [23]. Athletes with eumenorrhea met criteria for endurance athletes but did not have menarchal delay or amenorrhea. Non-athletic controls did not meet endurance criteria and had no history of menarchal delay or amenorrhea. Eumenorrheic athletes and non-athletic controls had a cycle length between 21 and 35 days. Although none of the subjects met DSM-IV criteria for diagnosis of anorexia nervosa or bulimia nervosa, (based on self-report, reported history from their care providers, and interviews with our study psychiatrist D.H.), some form of disordered eating was reported in eleven athletes (ten AA and one EA). Subjects were recruited through advertisements in area newspapers and mailings to physician offices in the New England area. The Institutional Review Board of Partners Health Care approved the study. Informed consent and assent were obtained from subjects and their parents.

Experimental protocol

Research subjects were evaluated at the General Clinical Research Center (GCRC) of Massachusetts General Hospital (MGH) during an outpatient visit. Subjects were referred to our study only after other causes of amenorrhea (both primary and secondary) had been ruled out, and after their providers had determined that the cause of amenorrhea was energy deficit. Subjects who had an abnormal TSH level, an elevated FSH (indicative of hypergonadotropic hypogonadism), and subjects who were taking hormonal medications were excluded from study participation. A complete history and physical exam were conducted for each subject. Information regarding menstrual history, exercise and eating behavior was confirmed with parents and primary physicians. Weight was measured on a single electronic scale to the nearest 0.1 kg, and height on a single stadiometer to the nearest 0.1 cm in triplicate and averaged. We calculated the body mass index (BMI) for our subjects using the formula: [weight (in kg) / [height (in meters)^2]. Both absolute BMI values and BMI-SDS (BMI-standard deviation scores, based on data compiled by the Centers for Disease Control [24]) are reported. An exercise and physical activity evaluation was obtained for each subject via the Modifiable Activity Questionnaire which has been validated for use in adolescents [25]. A score was calculated (hours of exercise per week) to quantify activity levels of our subjects. A bone age X-ray was obtained and read by a single pediatric endocrinologist employing the methods of Greulich and Pyle [26]. Fasting blood samples were tested for peptide YY, ghrelin, leptin, estradiol and testosterone. EA and non-athletic controls were evaluated during the early follicular phase of the menstrual cycle. Screening history included an evaluation of the use of performance enhancing drugs (by self-report) and was verified by an interview with the subjects' primary care physician when considered necessary. None of the enrolled subjects used performance enhancing drugs as determined by our screening methods. Bone density and body composition were assessed using dual energy X-ray absorptiometry (DXA) using a Hologic 4500 scanner (Waltham, MA). The coefficients of variation (CV) for spine and whole body BMD using this instrument at our institution are 1.1% and 0.8%, and for fat and lean mass 2.1% and 1.0% respectively. *Z*-scores for the spine (L1–L4) were obtained using the Hologic reference data base [27]. We calculated bone mineral apparent density (BMAD) from lumbar bone mineral content and area to correct for body size [28].

Biochemical analysis

An enzyme immunoassay was used to measure PYY (Linco Research; St Charles, MO; intra-assay CV 1.0–5.8%, sensitivity 1.4 pg/ml) and NTX by (Osteomark-Wampole Laboratories, Inverness Medical Professional Diagnostics, Princeton, NJ; detection limit 2.5 nM bone collagen equivalent (BCE), CV 4.6%). High-molecular-weight adiponectin was measured using an enzyme-linked immunosorbent assay (Millipore; Billerica, MA; intra-assay CV of 3.0–8.8%, sensitivity of 0.4 ng/ml), and PINP using a radioimmunoassay (Orion Diagnostica Oy, Espoo, Finland; detection limit 2 μg/l; CV of 6.5–10.2%). Specific details for the analysis of ghrelin, leptin, testosterone and estradiol were previously reported by our group [3]. Samples were stored at −80 °C until analysis, and were run in triplicate.

Statistical methods

Data were analyzed using the JMP program (version 4, SAS Institute Inc., Cary, NC) and are presented as mean ± SD. When data were not normally distributed logarithmic conversions were performed to approximate a normal distribution. This was necessary for ghrelin, estradiol and NTX. ANOVA followed by the Tukey–Kramer test was performed to determine differences between groups and to correct for multiple comparisons. A p value of <0.05 was considered significant. Univariate and mixed model stepwise regression analyses were used to determine predictors of gonadal steroids, bone turnover markers, and bone mineral density. Variables included in the regression models were hormones and body composition parameters expected to predict the dependent variable based on *in vitro* and animal models, regardless of whether or not significant associations were observed of these variables with the dependent variable on univariate analysis. This approach was chosen in order to account for confounding effects of various variables and to rule out the masking of associations of various independent variables with the dependent variable because of confounders.