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Peptide YY in Adolescent Athletes with Amenorrhea, Eumenorrheic Athletes and Non-Athletic Controls

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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 ng/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.

Keywords

Athletes; Adolescents; Peptide YY; Bone density; Bone turnover

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The authors have no conflict of interest to declare

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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 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 [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 hours of aerobic weight-bearing training of the legs weekly, (ii) ≥ 30 miles of running weekly or (iii) ≥ 4 hours 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 21–35 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: $[\text{weight (in kg)} / [\text{height (in meters)}]^2]$. 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 WB 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; intraassay 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 mcg/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 $\pm$ 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.

RESULTS

Baseline Characteristics

Baseline body composition, bone density and some hormonal characteristics of these subjects have been previously reported [3], and are summarized in Table 1. However, we have not previously reported levels of PYY and adiponectin, or their association with testosterone, estradiol and bone density measures. Specifically, AA, EA, and healthy controls did not differ for age, bone age, height, and lean mass. Differences in weight, BMI, and fat mass were significant between the three groups, although all girls were within a normal weight range. BMI was lower in the AA compared with the EA, and a higher percentage of AA reported a history of disordered eating. Activity scores did not differ between AA and EA, and as expected, were greater than in controls. Leptin was lower and log ghrelin higher in AA than EA. Testosterone was lower in AA compared with EA and controls. Data were similar for the free androgen index (derived from total testosterone and sex hormone binding globulin levels) and are not reported. The groups did not differ for IGF-1 and adiponectin. Lumbar BMD, BMAD and whole body BMD were highest in EA, followed by AA and non-athletic controls (lumbar BMD: 1.01 ± 0.07 , 0.91 ± 0.09 and 0.96 ± 0.08 g/cm², $p=0.01$; lumbar BMAD: 0.27 ± 0.02 , 0.25 ± 0.02 and 0.26 ± 0.02 g/cm³, $p=0.004$; whole body BMD: 1.07 ± 0.06 , 0.98 ± 0.06 and 1.04 ± 0.07 g/cm², $p=0.002$). Similarly, EA had the highest BMD Z-scores of the three groups, whereas AA had the lowest (Table 1). Lumbar BMAD Z-scores were lower in AA than the other two groups, and lumbar and whole body BMD Z-scores were lower in AA than EA and trended lower than in controls. The bone formation marker PINP was significantly lower in the AA compared with controls.

Although all subjects with primary amenorrhea had been evaluated for other causes of amenorrhea prior to study referral, in order to be certain that athletes with primary amenorrhea did not differ from those with secondary amenorrhea, we also performed subset analysis comparing these two groups, and they did not differ for BMI-SDS, activity scores or bone density measures. In addition, we compared athletes with secondary amenorrhea with eumenorrheic athletes and non-athletic controls, and the differences reported in the previous paragraph in hormones and bone density persisted in this subset analysis (data not shown). We thus report girls with primary and secondary amenorrhea as a single group.

Peptide YY and adiponectin

The AA group had significantly higher PYY levels when compared with EA (111.2 ± 51.5 in AA versus 61.4 ± 21.2 in EA and 81.8 ± 54.8 pg/ml in controls, $p=0.02$) (Figure 1), and AA differed from EA for PYY even after adjusting for multiple comparisons using the Tukey-Kramer test. Adiponectin levels, in contrast, did not differ between the groups. Linear regression analyses did not reveal any significant associations of PYY or adiponectin with BMI-SDS, percent body fat, or exercise scores, all of which are surrogate measures of energy availability. However, on multivariate analysis that included these three potential predictors of PYY, significant predictors of PYY were BMI-SDS ($p=0.047$) and percent body fat ($p=0.046$), which together accounted for 10.3% of the variability in PYY. Interestingly, BMI-SDS was a negative predictor of PYY levels, whereas percent body fat was a positive predictor. Similar associations were observed within the AA group. Importantly, the differences in PYY levels between groups remained significant even after controlling for BMI-SDS ($p=0.007$), percent body fat ($p=0.001$) and activity scores ($p=0.01$) separately, and for these parameters taken together ($p=0.004$). Within girls with AA, there was an inverse association of PYY with log ghrelin ($r = -0.50$, $p=0.047$) and IGF-1 ($r = -0.50$, $p=0.046$).

Associations of PYY and Adiponectin with Gonadal Steroids and Bone Metabolism Parameters

On linear regression analyses, only weak associations were observed between PYY or adiponectin and levels of testosterone, estradiol, and markers of bone metabolism (Table 2). Given the potential for confounding effects of other variables such as ghrelin, leptin, adiponectin and percent body fat masking an association of PYY with testosterone and estradiol, we also performed regression analysis using these variables and PYY in the model to determine independent predictors of testosterone and estradiol (Table 3). PYY was an independent predictor of testosterone in this regression model, and other independent predictors were percent body fat, adiponectin, and ghrelin accounting for 41% of the variability in testosterone levels. Log estradiol was predicted by percent body fat and IGF-1 with 24% of the variability explained.

We next performed regression modeling to determine independent predictors of bone turnover markers and bone density Z-scores and entered PYY, log ghrelin, leptin, IGF-1, testosterone, log estradiol and lean mass as the independent variables in this model (Table 3). PYY was an independent and negative predictor of PINP, as were log estradiol, log ghrelin, and testosterone. IGF-1 was a positive predictor of PINP. Log NTX was not predicted by PYY but was negatively predicted by log ghrelin. PYY was a negative predictor of lumbar bone mineral apparent density (BMAD) Z-scores, and with leptin and estradiol accounted for 42% of its variability. PYY did not predict lumbar and whole body BMD Z-scores. Lean mass and leptin positively predicted lumbar BMD Z-scores accounting for 16% of the variability, and lean mass and log estradiol positively predicted whole body BMD Z-scores accounting for 19% of its variability. Adiponectin did not predict BMD or BMAD Z-scores in this regression model.

DISCUSSION

We report significantly higher levels of PYY in adolescent AA when compared with EA, and that PYY is an independent negative predictor of testosterone levels after controlling for confounding effects of ghrelin, leptin, adiponectin, and percent body fat. This is consistent with animal studies which suggest that elevated PYY levels lead to a decrease in GnRH mediated gonadotropin secretion [8,18]. We also show that PYY is an independent negative predictor of PINP levels and lumbar BMAD Z-scores in this population (but not lumbar and whole body BMD Z-scores), suggesting that high PYY may be a contributor to low lumbar BMAD in athletes with decreased energy availability. This is consistent with our previous

study in which a significant negative correlation of PYY with bone turnover makers was found in adolescents with anorexia nervosa, an extreme state of low energy availability [6] and between PYY and bone mass in adults with anorexia nervosa [29]. Thus, endurance athletes with low energy availability and elevated PYY are more likely to be hypogonadal with low BMD. contrast, adiponectin levels do not differ between groups and do not predict BMD or BMAD.

Neuroendocrine factors that differentiate AA from EA are still not completely understood, although it has been previously postulated that hormones such as ghrelin and leptin may modulate reproductive function, and link low energy states to reduced GnRH pulsatility [4,5, 30–32]. Consistent with this postulate, we have previously reported increased ghrelin and decreased leptin in AA compared with EA, and have shown that these hormones predict levels of gonadal steroids [3]. Importantly, PYY, although an anorexigenic hormone, is elevated in anorexia nervosa [6] and reduces GnRH stimulated gonadotropin secretion in rodent models by acting through the Y2 receptors of NPY [8,18]. In addition, adiponectin inversely modulates gonadotropin secretion in vitro [9] and is inversely associated with energy availability [19, 20]. PYY and adiponectin may thus be other important neuroendocrine links between energy balance and reproductive function, which ultimately have an effect on bone metabolism. Until now, PYY and adiponectin levels had not been assessed in adolescent endurance athletes, and it was not known whether levels of these hormones differed in AA versus EA.

Weight, BMI, fat mass, and percent body fat were significantly lower in the AA when compared with EA, although activity scores did not differ between these two groups. In addition, the AA group had a higher percentage of reported disordered eating behavior. These data suggest lower energy availability in AA compared with the other two groups. We observed that PYY levels were higher in AA compared with EA, and were inversely related to BMI-SDS after controlling for fat mass, consistent with the concept that PYY levels reflect energy status and may be related to menstrual status of athletes. Higher PYY levels have also been reported in adult exercising women who are amenorrheic [33]. In contrast, adiponectin levels did not differ between the groups.

We also assessed testosterone and estradiol levels in AA, EA and non-athletic controls as indicators of the functionality of the H-P-G axis, and found significantly lower testosterone levels in AA compared with EA and controls. Estradiol levels did not differ between groups, likely because menstruating subjects were evaluated during the early follicular phase of their cycles, when estradiol levels are the lowest. Percent body fat was the most important predictor of levels of testosterone and estradiol, and the association of body fat with the H-P-G axis is well-known [34]. Importantly, PYY emerged as an independent negative predictor of testosterone levels after controlling for the confounding effects of percent body fat, log ghrelin, leptin, IGF-1, and adiponectin. Interestingly, female rodent models indicate stimulatory GABA mediated effects of testosterone on the firing of GnRH neurons and pulsatile GnRH release, and low testosterone levels in AA may further contribute to reduced GnRH pulsatility and hypogonadotropic hypogonadism [35,36]. It is also possible that other mechanisms account for the association between PYY and testosterone levels. For example, PYY is known to stimulate ACTH and cortisol secretion, and cortisol can affect the H-P-G axis by reducing GnRH pulsatility and the responsiveness of gonadotropes to GnRH secretion [37–39]. One limitation of this study is that we evaluated testosterone and estradiol levels as markers of GnRH and gonadotropin secretion, and did not assess gonadotropin pulsatility or cortisol levels.

The AA group had lower bone density measures including BMAD than EA. We assessed BMAD in addition to traditional measures of bone density in order to adjust for body size because our subjects were children and some had not completed growth. Areal bone density

assessed by DXA (unlike volumetric bone density) can underestimate BMD in short children and overestimate BMD in tall children. BMAD, which is computed using measures of bone mineral content and bone area reported by DXA, is a surrogate measure of volumetric bone density, and not affected by body size [28]. Lumbar BMAD and BMAD Z-scores were lowest in AA followed by non-athletic controls and EA. Of importance, lumbar BMD and BMAD measures have been reported to consistently predict upper limb fracture risk in children 9–17 years old [40].

PYY acts through the Y2 receptor to reduce NPY secretion, and may have effects on bone metabolism mediated through the Y2 receptor. of the Y2 receptor (also found on osteoblasts) leads to increased bone mass in rodents [10], which suggests that elevated PYY could contribute to decreased bone formation and low BMD. Consistent with this hypothesis, PYY has been reported to be a negative independent predictor of bone formation markers [6] and bone density [29] in extreme low energy states. Two groups of investigators have reported conflicting data regarding the PYY knock-out mouse. Whereas one group indicated that this mouse model of PYY deficiency is osteopenic [41], another group has recently reported higher bone density in the PYY knock-out mouse model [42]. In addition to PYY, other hormones associated with a state of energy deficit, such as leptin, ghrelin, adiponectin and IGF-1 have also been related to bone metabolism. Correction of leptin deficiency causes increases in bone formation markers in adults with hypothalamic amernorrhea [5], and ghrelin administration increases osteoblast proliferation in tissue cultures [43]. Clinical studies report that adiponectin is an inverse predictor of bone density in humans [19,21], although *in vitro* studies indicate that adiponectin activates both osteoclasts and osteoblasts [11,12]. Finally, IGF-1, a bone trophic hormone, decreases with weight loss [44].

In this study, consistent with studies from Baldock *et al.* and Wong *et al.* [42,45], PYY was an independent inverse predictor of PINP, a surrogate bone formation marker, and also inversely predicted BMAD Z-scores in a regression model that included potential confounders of the association between PYY and bone, such as ghrelin, leptin, IGF-1, testosterone, estradiol and lean mass. Other important predictors of low BMD and BMAD Z-scores were low estradiol and leptin, and low lean mass, consistent with known effects of estradiol, leptin and lean mass on bone [46–48]. IGF-1 was a positive predictor of PINP, consistent with its bone anabolic effects [49], and testosterone and estradiol inversely predicted PINP, consistent with their known inhibitory effects on bone turnover [47]. In contrast to our expectations [43], ghrelin was an inverse predictor of PINP, and adiponectin did not predict bone turnover markers or bone density. It is possible that significant alterations in adiponectin levels are required before effects on bone density or bone turnover markers are observed. A limitation of our findings is the cross-sectional design of the study, and the associations we report cannot determine causation. Prospective studies are necessary to establish a causal relationship between high PYY levels and hypogonadism and low BMD in the adolescent athlete.

To conclude, we have shown that PYY, but not adiponectin, is elevated in adolescent AA when compared with EA and predict lower levels of testosterone and bone formation markers and lower lumbar BMAD Z-scores. These findings suggest that increased PYY is likely one of many anabolic and catabolic signals that impact bone mineralization and the complex neural signaling pathways affecting GnRH output in athletes with decreased energy availability.

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References

1. De Souza MJ, West SL, Jamal SA, Hawker GA, Gundberg CM, Williams NI. The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. *Bone* 2008;43:140–8. [PubMed: 18486582]
2. Christo K, Prabhakaran R, Lamparello B, Cord J, Miller K, Goldstein M, Gupta N, Herzog D, Klibanski A, Misra M. Bone Metabolism in Adolescent Amenorrheic and Eumenorrheic Athletes and Controls. *Pediatrics* 2008;121:1127–36. [PubMed: 18519482]
3. Christo K, Cord J, Mendes N, Miller K, Goldstein M, Klibanski A, Misra M. Acylated Ghrelin and Leptin in Adolescent Athletes with Amenorrhea, Eumenorrheic Athletes and Controls: A Cross-sectional Study. *Clin Endocrinol* 2008;69:628–33.
4. Kluge M, Schussler P, Uhr M, Yassouridis A, Steiger A. Ghrelin suppresses secretion of luteinizing hormone in humans. *J Clin Endocrinol Metab* 2007;92:3202–5. [PubMed: 17550958]
5. Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A, Mantzoros CS. Recombinant Human Leptin in Women with Hypothalamic Amenorrhea. *N Engl J Med* 2004;351:987–997. [PubMed: 15342807]
6. Misra M, Miller KK, Tsai P, Gallagher K, Lin A, Lee N, Herzog DB, Klibanski A. Elevated Peptide YY Levels in Adolescent Girls with Anorexia Nervosa. *J Clin Endocrinol Metab* 2005;91:1027–33. [PubMed: 16278259]
7. Cambuli VM, Musiu MC, Incani M, Paderi M, Serpe R, Marras V, Cossu E, Cavallo MG, Mariotti S, Loche S, Baroni MG. Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children. *J Clin Endocrinol Metab* 2008;93:3051–7. [PubMed: 18492759]
8. Pinilla L, Fernandez-Fernandez R, Roa J, Castellano JM, Tena-Sempere M, Aguilar E. Selective role of neuropeptide Y receptor subtype Y2 in the control of gonadotropin secretion in the rat. *Am J Physiol Endocrinol Metab* 2007;293:E1385–92. [PubMed: 17785504]
9. Rodriguez-Pacheco F, Martinez-Fuentes AJ, Tovar S, Pinilla L, Tena-Sempere M, Dieguez C, Castano JP, Malagon MM. Regulation of pituitary cell function by adiponectin. *Endocrinology* 2007;148:401–10. [PubMed: 17038552]
10. Baldock PA, Sainsbury A, Couzens M, Enriquez RF, Thomas GP, Gardiner EM, Herzog H. Hypothalamic Y2 receptors regulate bone formation. *J Clin Invest* 2002;109:915–21. [PubMed: 11927618]
11. Luo XH, Guo LJ, Xie H, Yuan LQ, Wu XP, Zhou HD, Liao EY. Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway. *J Bone Miner Res* 2006;21:1648–56. [PubMed: 16995820]
12. Luo XH, Guo LJ, Yuan LQ, Xie H, Zhou HD, Wu XP, Liao EY. Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway. *Exp Cell Res* 2005;309:99–109. [PubMed: 15963981]
13. Huda MS, Wilding JP, Pinkney JH. Gut peptides and the regulation of appetite. *Obes Rev* 2006;7:163–82. [PubMed: 16629873]
14. Ballantyne GH. Peptide YY(1–36) and peptide YY(3–36): Part I. Distribution, release and actions. *Obes Surg* 2006;16:651–8. [PubMed: 16687037]
15. Grandt D, Teyssen S, Schimiczek M, Reeve JR Jr, Feth F, Rascher W, Hirche H, Singer MV, Layer P, Goebell H, et al. Novel generation of hormone receptor specificity by amino terminal processing of peptide YY. *Biochem Biophys Res Commun* 1992;186:1299–306. [PubMed: 1324662]
16. Keire DA, Mannon P, Kobayashi M, Walsh JH, Solomon TE, Reeve JR Jr. Primary structures of PYY, [Pro(34)]PYY, and PYY-(3–36) confer different conformations and receptor selectivity. *Am J Physiol Gastrointest Liver Physiol* 2000;279:G126–31. [PubMed: 10898754]
17. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR. Inhibition of food intake in obese subjects by peptide YY3–36. *N Engl J Med* 2003;349:941–8. [PubMed: 12954742]

18. Fernandez-Fernandez R, Aguilar E, Tena-Sempere M, Pinilla L. Effects of polypeptide YY(3–36) upon luteinizing hormone-releasing hormone and gonadotropin secretion in prepubertal rats: in vivo and in vitro studies. *Endocrinology* 2005;146:1403–10. [PubMed: 15564330]
19. Misra M, Miller KK, Cord J, Prabhakaran R, Herzog DB, Goldstein M, Katzman DK, Klibanski A. Relationships between serum adipokines, insulin levels, and bone density in girls with anorexia nervosa. *J Clin Endocrinol Metab* 2007;92:2046–52. [PubMed: 17356044]
20. Pannacciulli N, Bunt JC, Ortega E, Funahashi T, Salbe AD, Bogardus C, Krakoff J. Lower total fasting plasma adiponectin concentrations are associated with higher metabolic rates. *J Clin Endocrinol Metab* 2006;91:1600–3. [PubMed: 16449334]
21. Lenchik L, Register TC, Hsu FC, Lohman K, Nicklas BJ, Freedman BI, Langefeld CD, Carr JJ, Bowden DW. Adiponectin as a novel determinant of bone mineral density and visceral fat. *Bone* 2003;33:646–51. [PubMed: 14555270]
22. Rickenlund A, Carlstrom K, Ekblom B, Brismar TB, Von Schoultz B, Hirschberg AL. Effects of oral contraceptives on body composition and physical performance in female athletes. *J Clin Endocrinol Metab* 2004;89:4364–70. [PubMed: 15328063]
23. Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics* 1997;99:505–12. [PubMed: 9093289]
24. Ogden CL, Kuczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R, Grummer-Strawn LM, Curtin LR, Roche AF, Johnson CL. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. *Pediatrics* 2002;109:45–60. [PubMed: 11773541]
25. Aaron DJ, Kriska AM, Dearwater SR, Cauley JA, Metz KF, LaPorte RE. Reproducibility and validity of an epidemiologic questionnaire to assess past year physical activity in adolescents. *Am J Epidemiol* 1995;142:191–201. [PubMed: 7598119]
26. Greulich, WW.; Pyle, SI. Radiographic atlas of skeletal development of the hand and wrist. Vol. 2d. Stanford, Calif: Stanford Univ. Press; 1959.
27. Kelly, T.; Specker, B.; Binkley, T.; Zemel, B.; Leonard, M.; Kalkwarf, H.; Moyer-Mileur, L.; Pan, H.; Cole, T.; Shepherd, J. Pediatric BMD Reference Database for U.S. White Children. Children's Bone Health Abstract; Sorrento, Italy. 2005.
28. Ward KA, Ashby RL, Roberts SA, Adams JE, Zulf Mughal M. UK reference data for the Hologic QDR Discovery dual-energy x ray absorptiometry scanner in healthy children and young adults aged 6–17 years. *Arch Dis Child* 2007;92:53–9. [PubMed: 16943261]
29. Utz AL, Lawson EA, Misra M, Mickley D, Gleysteen S, Herzog DB, Klibanski A, Miller KK. Peptide YY (PYY) levels and bone mineral density (BMD) in women with anorexia nervosa. *Bone* 2008;43:135–9. [PubMed: 18486583]
30. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gormelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lehouc Y, Froguel P, Guy-Grand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998;392:398–401. [PubMed: 9537324]
31. Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 2002;110:1093–103. [PubMed: 12393845]
32. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Genet* 1998;18:213–5. [PubMed: 9500540]
33. Scheid JL, Williams NI, West SL, VanHeest JL, De Souza MJ. Elevated PYY is associated with energy deficiency and indices of subclinical disordered eating in exercising women with hypothalamic amenorrhea. *Appetite* 2009;52:184–92. [PubMed: 18929607]
34. Misra M, Prabhakaran R, Miller KK, Tsai P, Lin A, Lee N, Herzog DB, Klibanski A. Role of cortisol in menstrual recovery in adolescent girls with anorexia nervosa. *Pediatr Res* 2006;59:598–603. [PubMed: 16549537]

35. Pielecka J, Quaynor SD, Moenter SM. Androgens increase gonadotropin-releasing hormone neuron firing activity in females and interfere with progesterone negative feedback. *Endocrinology* 2006;147:1474–9. [PubMed: 16339200]
36. Sullivan SD, Moenter SM. Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. *Proc Natl Acad Sci U S A* 2004;101:7129–34. [PubMed: 15096602]
37. Inui A, Inoue T, Sakatani N, Oya M, Morioka H, Mizuno N, Baba S. Biological actions of peptide YY: effects on endocrine pancreas, pituitary-adrenal axis, and plasma catecholamine concentrations in the dog. *Horm Metab Res* 1987;19:353–7. [PubMed: 2822559]
38. Breen KM, Davis TL, Doro LC, Nett TM, Oakley AE, Padmanabhan V, Rispoli LA, Wagenmaker ER, Karsch FJ. Insight into the neuroendocrine site and cellular mechanism by which cortisol suppresses pituitary responsiveness to gonadotropin-releasing hormone. *Endocrinology* 2008;149:767–73. [PubMed: 17962347]
39. Oakley AE, Breen KM, Clarke IJ, Karsch FJ, Wagenmaker ER, Tilbrook AJ. Cortisol reduces gonadotropin-releasing hormone pulse frequency in follicular phase ewes: influence of ovarian steroids. *Endocrinology* 2009;150:341–9. [PubMed: 18801903]
40. Jones G, Ma D, Cameron F. Bone density interpretation and relevance in Caucasian children aged 9–17 years of age: insights from a population-based fracture study. *J Clin Densitom* 2006;9:202–9. [PubMed: 16785082]
41. Wortley KE, Garcia K, Okamoto H, Thabet K, Anderson KD, Shen V, Herman JP, Valenzuela D, Yancopoulos GD, Tschop MH, Murphy A, Sleeman MW. Peptide YY regulates bone turnover in rodents. *Gastroenterology* 2007;133:1534–43. [PubMed: 17920065]
42. Wong I, Enriquez R, Boey D, Sainsbury A, Herzog H, Eisman J, Baldock P. PYY deletion increases bone formation in mice. 18th Annual Scientific Meeting of the Australian & New Zealand Bone & Mineral Society; 2008. www.anzbums.org.au/asm2008
43. Fukushima N, Hanada R, Teranishi H, Fukue Y, Tachibana T, Ishikawa H, Takeda S, Takeuchi Y, Fukumoto S, Kangawa K, Nagata K, Kojima M. Ghrelin directly regulates bone formation. *J Bone Miner Res* 2005;20:790–8. [PubMed: 15824852]
44. Misra M, Miller KK, Bjornson J, Hackman A, Aggarwal A, Chung J, Ott M, Herzog DB, Johnson ML, Klibanski A. Alterations in growth hormone secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. *J Clin Endocrinol Metab* 2003;88:5615–23. [PubMed: 14671143]
45. Baldock PA, Sainsbury A, Couzens M, Enriquez RF, Thomas GP, Gardiner EM, Herzog H. Hypothalamic Y2 receptors regulate bone formation. *J Clin Invest* 2002;109:915–921. [PubMed: 11927618]
46. Misra M, Aggarwal A, Miller KK, Almazan C, Worley M, Soyka LA, Herzog DB, Klibanski A. Effects of anorexia nervosa on clinical, hematologic, biochemical, and bone density parameters in community-dwelling adolescent girls. *Pediatrics* 2004;114:1574–83. [PubMed: 15574617]
47. Riggs B. The mechanisms of estrogen regulation of bone resorption. *J Clin Invest* 2000;106:1203–4. [PubMed: 11086020]
48. Baek K, Bloomfield SA. Beta-Adrenergic Blockade and Leptin Replacement Effectively Mitigate Disuse Bone Loss. *J Bone Miner Res*. 2008
49. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev* 2008;29:535–59. [PubMed: 18436706]

Figure 1.

PYY levels in non-athletic controls, amenorrheic athletes and eumenorrheic athletes. PYY levels were higher in adolescent amenorrheic athletes compared to eumenorrheic athletes and non-athletic controls ($p=0.02$) and remained lower in amenorrheic compared to eumenorrheic athletes after adjusting for multiple comparisons. * $p<0.05$

Table 1

Baseline characteristics of non-athletic controls, amenorrheic athletes and eumenorrheic athletes

	Non-athletic controls N=16	Amenorrheic athletes N=16	Eumenorrheic athletes N=15	p
Age (years)	15.6±1.4	15.9±1.2	15.6±1.3	Ns
Bone age (years)	15.6±1.4	16.0±0.7	15.9±1.3	Ns
Height (cm)	164.3±5.7	164.9±5.2	163.8±5.6	Ns
Weight (kg)	55.2±6.5	51.7±4.0 [*]	58.5±7.6	0.01
BMI (kg/m ²)	20.5±2.3	19.0±1.3 [*]	21.8±2.7	0.004
BMI-SDS	-0.20±0.51	-0.62±0.32 ^{* °}	0.14±0.68	0.0008
Activity (hours/week)	9.6±4.7	24.3±7.2 ^{**}	28.6±9.8 ^{**}	<0.0001
Disordered eating (%)	0%	62.5%	6.7%	<0.0001
Fat mass (kg)	14.6±4.3	11.4±3.0 ^{* °}	16.3±4.4	0.004
Lean mass (kg)	40.1±3.6	39.9±3.9	42.3±4.8	Ns
% body fat	25.4±5.6	21.4±5.0 ^{* °}	26.6±4.7	0.02
Lumbar BMD Z-score	-0.11±0.65	-0.63±0.34 [*]	0.16±0.77	0.02
Lumbar BMAD Z-score	0.10±0.17	-0.02±0.20 ^{* °}	0.23±0.13	0.001
Whole body BMD Z-score	0.06±0.78	-0.55±0.72 ^{* °}	0.32±0.94	0.01
PINP (ng/ml)	191±149	99±53 ^{**}	136±63	0.047
NTX (nmol BCE)	2.26±0.37	2.03±0.23 [°]	2.22±0.25	0.08
IGF-1 (ng/ml)	674±215	545±204	583±146	Ns
Log ghrelin (pg/ml)	1.79±0.16	2.05±0.18 ^{* **}	1.69±0.25	<0.0001
Leptin (ng/ml)	12.4±6.3	7.0±3.4 ^{* °}	14.7±8.6	0.005
Adiponectin (ng/ml)	105±53	113±40	99±79	Ns
Testosterone (ng/dl)	25.0±8.4	17.9±7.6 ^{* **}	29.0±8.6	0.002
Log estradiol (pg/ml)	1.30±0.14	1.33±0.21	1.44±0.13	0.07

* p<0.05 compared with EA

** p<0.05 compared with controls

° p<0.10 compared with controls

Table 2

Associations between hormones, body composition, bone density measures and markers of bone turnover (all subjects)

	Testosterone			Estradiol*			Lumbar BMD Z			Lumbar BMAD Z			Whole body BMD Z			PINP			NTX*		
	r	p		r	p		r	p		r	p		r	p		r	p		r	p	
PYY	-0.29	0.04		-0.13	0.40		-0.13	0.38		-0.26	0.08		-0.20	0.18		-0.30	0.046		-0.08	0.60	
Ghrelin*	-0.33	0.03		-0.17	0.26		-0.16	0.30		-0.38	0.01		-0.23	0.13		-0.24	0.12		-0.31	0.04	
Leptin	0.40	0.005		0.31	0.04		0.24	0.11		0.53	0.0001		0.03	0.84		0.07	0.65		0.20	0.20	
Adiponectin	0.29	0.049		0.27	0.07		0.00	0.98		0.07	0.64		0.05	0.76		-0.09	0.56		-0.01	0.93	
IGF-1	0.01	0.94		0.35	0.02		0.18	0.24		0.28	0.07		0.03	0.87		0.33	0.03		0.06	0.69	
Testosterone				0.31	0.03		-0.02	0.87		0.14	0.37		0.16	0.28		-0.16	0.30		-0.08	0.60	
Estradiol*							0.15	0.32		0.39	0.008		0.17	0.26		-0.12	0.43		-0.04	0.82	
Lean mass	0.16	0.30		0.04	0.80		0.27	0.07		0.04	0.79		0.33	0.03		-0.00	0.95		0.29	0.06	
Fat mass (%)	0.46	0.001		0.43	0.003		0.18	0.24		0.41	0.005		-0.07	0.64		-0.06	0.58		0.09	0.58	

PINP: N-terminal propeptide of type 1 procollagen; CTX: C-telopeptide; BMAD: bone mineral apparent density; PYY: peptide YY

* Indicates log converted data

Table 3

Regression modeling to determine independent predictors of gonadal steroids, markers of bone turnover and measures of bone density (all subjects)

	Parameter estimate	F ratio	P value	r ²	Cumulative r ²
Parameters entered into model: % body fat, PYY, log ghrelin, leptin, IGF-1, adiponectin					
Testosterone					
% body fat	0.61	7.1	0.01	0.18	0.18
PYY	-0.059	7.1	0.01	0.13	0.31
Adiponectin	0.042	4.4	0.04	0.05	0.36
Log ghrelin	-9.06	3.5	0.07	0.05	0.41
Log estradiol					
% body fat	0.00026	4.9	0.03	0.14	0.14
IGF-1	0.0039	4.4	0.04	0.10	0.24
Parameters entered into model: PYY, log ghrelin, leptin, IGF-1, testosterone, log estradiol, lean mass					
PINP					
IGF-1	0.20	8.1	0.007	0.11	0.11
PYY	-0.84	10.5	0.003	0.08	0.19
Log estradiol	-211.6	4.9	0.03	0.09	0.28
Log ghrelin	-154.4	7.6	0.009	0.08	0.36
Testosterone	-3.41	4.5	0.04	0.07	0.43
Log NTX					
Log ghrelin	-0.38	4.3	0.04	0.10	0.10
Parameters entered into model: PYY, log ghrelin, leptin, IGF-1, testosterone, log estradiol, lean mass					
Lumbar BMD					
Z-score					
Lean mass	0.056	3.9	0.05	0.09	0.09
Leptin	0.030	3.1	0.09	0.07	0.16
Lumbar BMAD					
Z-score					
Leptin	0.013	10.2	0.003	0.31	0.31

	Parameter estimate	F ratio	P value	r ²	Cumulative r ²
Log estradiol	0.31	4.4	0.04	0.07	0.38
	PYY	4.3	0.045	0.04	0.42
Whole body					
BMD Z-score					
Lean mass	0.07	6.6	0.01	0.13	0.13
Log estradiol	1.30	3.2	0.08	0.06	0.19

PINP: N-terminal propeptide of type I procollagen; CTX: C-telopeptide; BMAD: bone mineral apparent density; PYY: peptide YY