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Reduced Amylin Levels Are Associated with Low Bone Mineral Density in Women with Anorexia Nervosa

Monica H Wojcik, Erinne Meenaghan, Elizabeth A Lawson, Madhusmita Misra, Anne Klibanski, and Karen K Miller

Neuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Abstract

Context—Anorexia nervosa, characterized by extreme low body weight due to reduced nutrient intake, is associated with severe bone loss. Peptide hormones, including amylin, GIP, and GLP2, are released immediately after nutrient intake and may be involved in the regulation of bone turnover.

Objective—To investigate fasting levels of amylin, GIP, and GLP2 and their relationships with bone mineral density (BMD) in women with anorexia nervosa compared to healthy controls.

Design—Cross-sectional

Setting—Clinical Research Center

Study Participants—15 women with anorexia nervosa and 16 healthy controls

Intervention—None

Main Outcome Measures—Fasting serum amylin, GIP, and GLP2, and BMD

Results—Women with anorexia nervosa had significantly lower fasting serum amylin and GIP levels than healthy controls. Fasting serum GLP2 levels were not significantly different between groups. Fasting amylin levels were positively associated with BMD and Z-score at the PA spine, total hip, and femoral neck. Fasting amylin levels were also positively associated with weight and percent fat; after controlling for these variables, amylin was still a significant predictor of BMD and Z-score at the femoral neck and of Z-score at the total hip. In the anorexia nervosa group, there was a trend toward an inverse association between amylin and C-terminal telopeptide (CTX) levels ($R = -0.47$, $p = 0.08$). GIP and GLP2 levels did not predict BMD at any site.

Conclusion—Decreased secretion of amylin may be a mechanism through which reduced nutrient intake adversely affects BMD in anorexia nervosa.

Keywords

Amylin; GIP; GLP2; Anorexia nervosa; Bone mineral density

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Corresponding Author:, Karen K. Miller, Neuroendocrine Unit, Bulfinch 457B, Massachusetts General Hospital, Boston, MA 02114, KKMILLER@Partners.org, Tel: 617-726-3870, Fax: 617-726-5072.

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Introduction

Anorexia nervosa, characterized by extreme low body weight due to reduced nutrient intake, is associated with bone loss in the majority of such women, with osteopenia present in up to 90% [1,2]. Many factors are believed to contribute to this bone loss, including endocrine dysregulation, particularly IGF-1 and gonadal steroid deficiency, and hypercortisolemia. However, other factors contributing to the variability of bone mineral density (BMD) observed in these patients remain unknown. It has been suggested that pancreatic and enteric hormones may play a role in bone homeostasis, mediating the effect of acute nutritional status on bone turnover [3]. Peptide hormones, including amylin, glucose-dependent insulintropic polypeptide (GIP), and glucagon-like peptide-2 (GLP2), released immediately after nutrient intake, may be involved in the regulation of bone turnover by stimulating bone formation and decreasing bone resorption post-prandially [3,4]. Chronic nutrient deprivation, as seen in women with anorexia nervosa, may thus lead to lower bone mass in part via decreased levels of such hormones.

Amylin, a peptide hormone co-secreted with insulin from pancreatic beta cells [5,6], has been shown to stimulate bone formation and decrease bone resorption, via its action on osteoblasts and osteoclasts, respectively, in animal models and cell culture experiments [7,8]. Amylin has been demonstrated to cause osteoblast proliferation in both rodent [9–12] and human [13–15] osteoblast cell cultures. Amylin has also been shown to compromise osteoclast motility [16,17] and differentiation [18,19] in rodent cell cultures, and to decrease osteoclast resorptive activity [18,20]. The net effect of these complementary actions of amylin on bone may be to influence the balance between bone formation by osteoblasts and resorption by osteoclasts in favor of increasing bone mass. Indeed, amylin-deficient mice have a low bone mass phenotype [19] and decreased trabecular volume and number [21]. Furthermore, systemic administration of amylin has been shown to increase bone mass as well as increase histomorphometric indices of bone formation and reduce indices of bone resorption in mice [10,22].

In humans, amylin levels are severely reduced in patients with type I diabetes mellitus [23], another disease complicated by decreased BMD [24–26], and amylin has been posited to be a possible contributor to the bone loss observed in these patients [27]. Fasting amylin levels were lower in a study of adult men and post-menopausal women with osteoporosis than in controls, even after adjusting for weight [28]. Amylin levels have not been reported in women with AN, nor have their potential relationship with bone density been investigated.

GIP is secreted from K-cells in the duodenum [29], whereas GLP-2 is secreted by L-cells of the distal jejunum, ileum and colon shortly after nutrient intake [30], and these enteric hormones may be involved in mediating the postprandial reduction in bone resorption [31,32]. GIP receptors have been found on osteoblasts [33] and osteoclasts [34], and mice that overexpress GIP have increased BMD [35], whereas GIP receptor-knockout mice have reduced BMD [36]. GIP administration has also been shown to reduce bone loss in ovariectomized rats [37]. In humans, GLP2 administration has been shown to reduce markers of nocturnal bone resorption [38]. In addition, it inhibits bone resorption in ileostomy patients [39] and inhibits bone resorption and increases BMD in patients with short-bowel syndrome [40]. Thus, GIP and GLP2 may play a role in the preservation of bone mass following nutrient intake, when bone resorption is suppressed.

We hypothesized that women with anorexia nervosa, a disease characterized by low weight due to markedly depressed nutrient intake, would have lower fasting serum amylin levels as well as lower fasting serum levels of GIP and GLP2, and that this could contribute to the low bone mass seen in this population. We therefore investigated fasting levels of amylin, GIP, and

GLP2 in 15 women with anorexia nervosa and in 16 normal-weight healthy controls, and the association of these hormones with BMD.

Methods

Subjects

We studied 31 women: 1) women with anorexia nervosa (AN) (N=15), and 2) healthy women controls (HC) (N=16). All subjects were recruited from the community through advertisements and referrals from health care providers to participate in bone health studies.

Women with anorexia nervosa fulfilled Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [41] psychiatric and weight diagnostic criteria for anorexia nervosa, including intense fear of gaining weight, emphasis on body shape, and weight less than 85% of ideal body weight (IBW) as determined by the 1979 Department of Health, Education, and Welfare table [42]. Healthy controls had body mass index equal to or greater than 20 kg/m² and less than 30 kg/m², and had regular menses. Current or past use of medications known to affect bone density, including glucocorticoids, bisphosphonates and anticonvulsants, or current use of oral contraceptives, were exclusion criteria for all groups. Baseline characteristics on 3 of the controls have been previously published [43,44].

Methods

This study was approved by the Institutional Review Boards at Partners Health Care, Inc and the Massachusetts Institute of Technology, and written informed consent was obtained from all subjects. Each subject was admitted to the General Clinical Research Centers at Massachusetts General Hospital and Massachusetts Institute of Technology for an outpatient visit. Nutritional evaluation, including weight in a gown, height, frame size, and calculation of percent IBW (% IBW) and body mass index (BMI), was performed by research dietitians. A study investigator interviewed each study participant and obtained menstrual history, duration of anorexia nervosa, and medication use. BMD and body composition were measured using dual energy x-ray absorptiometry (DXA) with a Hologic 4500 densitometer (Hologic, Incorporated, Waltham, MA). This technique has a precision of 0.01g/cm² at the lumbar spine, 3% for fat mass and 1.4% lean body mass [45,46]. BMD data at sites other than total body were not available for two healthy controls, and at the lateral spine for three women with anorexia nervosa and one additional healthy control. Lateral spine Z-scores were available for ten women with anorexia nervosa and ten healthy controls. Serum was collected at 8 AM after an overnight fast and stored at -80°C for subsequent measurement of endocrine variables.

Assays

Serum amylin, GIP, and GLP2 were measured by radioimmunoassay (Phoenix Pharmaceuticals, Inc., Burlingame, CA) with an intra-assay percent coefficient of variation of 7.90–11.90% for amylin, 9.31%–10.90% for GIP, and 8.87%–11.2% for GLP2. Serum C-terminal telopeptide (CTX) was measured by enzyme-linked immunosorbent assay (Immunodiagnostic Systems Ltd., Boldon, UK), with a detection limit of 0.020 ng/ml and intraassay variation of 1.7–3.0%. Serum N-terminal propeptide (PINP) was measured by radioimmunoassay (Orion Diagnostica, Espoo, Finland), with a detection limit of 2 µl and an intraassay coefficient of variation of 6.5–10.2%.

Statistical Analysis

Statistical analysis was performed using JMP Statistical Discoveries, version 4.0.2 (SAS Institute, Inc, Cary, NC). All variables were tested for normality by the Shapiro-Wilk test. Clinical characteristics and bone densities were compared using ANOVA for normally

distributed variables. All variables that were not normally distributed in all groups (weight, number of drinks/week, BMD at posteroanterior spine, total fat mass, total fat-free mass, GLP2) were compared using the Wilcoxon rank sum test. Univariate regression analyses were performed to investigate pancreatic and enteric hormone levels as potential predictors of BMD and body composition parameters; Spearman coefficients are reported. Multivariate least-squares regression models were constructed to control for weight and percent body fat. Data are reported as mean $\pm$ standard error of the mean (SEM). Significance is defined as $p < 0.05$, and “trend” is defined as $0.05 < p < 0.10$.

Results

Clinical characteristics of study subjects

Clinical characteristics of the two groups were compared in Table 1. The mean age did not differ between the two groups (anorexia nervosa range: 19–41 years; healthy controls range: 17–41 years). As expected, mean weight, BMI, total fat mass, % fat and fat free mass were all lower in women with anorexia nervosa compared with healthy controls. No subjects in either group reported cigarette smoking, and the frequency of alcohol consumption was similar between groups (anorexia nervosa: 1.2 ± 0.6 drinks/week, healthy controls: 1.0 ± 0.7 drinks/week, $p = 0.84$).

Absolute BMD and Z-scores are compared in Table 2. Mean BMDs at all skeletal sites were lower in women with anorexia nervosa compared to healthy controls, and mean Z-score was lower at all sites except the lateral spine, at which there was a trend.

Hormone Levels

Mean fasting serum levels of the pancreatic and enteric hormones are shown in Table 3. Mean fasting amylin and GIP levels were lower in women with anorexia nervosa compared to healthy controls. Fasting GLP2 levels were similar in the two groups.

Pancreatic and enteric hormones as predictors of BMD and body composition

Non-parametric univariate regression models were constructed for fasting amylin, GIP, and GLP2 levels on BMD, body weight, and body composition variables; the results are shown in Table 4. The analyses revealed significant positive associations between fasting amylin and BMD at the PA spine, total hip, and femoral neck (Figure) as well as with Z-scores at these skeletal sites. Fasting amylin levels were also significant positive predictors of weight, fat mass and percent body fat (% fat). There was a trend toward a positive association between GLP2 and total hip Z-score. There were no other significant associations between amylin, GIP, GLP2 and BMD and body weight and composition variables. Fasting amylin remained a significant predictor of BMD at the femoral neck ($p = 0.02$) and total hip ($p = 0.04$) and of Z-score at both the femoral neck ($p = 0.01$) and total hip ($p = 0.02$), but not of PA spine BMD ($p = 0.15$) or PA spine Z-score ($p = 0.10$), after controlling for percent fat (Table 5). Comparable results were obtained after controlling for weight (Table 5).

There was no association between age and fasting amylin, GLP2, or BMD at any site. There was a trend ($R = -0.34$, $p = 0.06$) toward an inverse association between age and GIP.

Markers of bone metabolism in the anorexia nervosa group demonstrated a trend toward an inverse association between fasting amylin levels and C-terminal telopeptide (CTX) ($R = -0.47$, $p = 0.08$), but not between amylin levels and N-terminal propeptide (PINP) ($R = -0.37$, $p = 0.17$).

Discussion

To our knowledge, we are the first to report that fasting serum amylin levels are lower in women with anorexia nervosa than in normal-weight healthy controls and to report a significant positive association between amylin levels and BMD in this population. Importantly, this relationship remained significant at the femoral neck and total hip after controlling weight and after controlling for percent body fat. This suggests that amylin may be an independent predictor of low bone density and may be involved in the pathogenesis of bone loss in this population. In addition, we report a trend toward an inverse association between fasting serum amylin levels and C-terminal telopeptide (CTX), a marker of bone resorption among women with anorexia nervosa. Our data do not support a role for GIP or GLP2 in bone loss in this population. In sum, our data suggest that lower levels of amylin, but not GIP or GLP2, may contribute to the osteopenia and osteoporosis observed in women with anorexia nervosa through effects on bone resorption. Our study is preliminary as it was limited by its small size and cross-sectional design. Because anorexia nervosa primarily affects young women and is complicated by an extremely high prevalence of osteopenia and osteoporosis, further studies are warranted to investigate the potential relationship between amylin and bone loss in this population.

The lower levels of fasting amylin that we have observed in women with anorexia nervosa compared to healthy controls may contribute to the lower BMD seen in the anorexia nervosa group. Our group has previously found an acute decrease in markers of bone formation after a 4-day fast, independent of acid-base status [47]. Bone resorption has been shown to be acutely suppressed upon nutrient intake [4,31,48] and in a rodent model, administering food in multiple small portions was observed to decrease bone resorption and increase BMD relative to a comparable nutrient load given once-a-day [49]. In women with anorexia nervosa, which is characterized by chronic starvation, bone resorption is increased and bone formation low [50]. Because anorexia nervosa is characterized by chronic undernutrition and decreased insulin secretion [51,52] we hypothesized that amylin levels would be lower in women with anorexia nervosa compared to healthy controls.

We also hypothesized that GIP and GLP2 levels would also be decreased in women with anorexia nervosa. We report a trend toward lower mean GIP but could not confirm low GLP2 levels in this group. Two previous studies are contradictory with regard to fasting GIP levels in adolescent girls with AN, and to our knowledge there are no published data of GIP levels in adults with AN. Tomasik *et al.* demonstrated higher [53], and Stock *et al.* demonstrated lower [54], mean fasting GIP levels in adolescents with anorexia nervosa compared to controls. The cause of the contrasting results is not clear, as the subjects in the two studies were of generally comparable ages, of significantly lower weight than the controls, and in a fasting state. In addition, an increased GIP response to an oral glucose tolerance test [53,55] or a mixed meal [53–55] has been demonstrated in adolescent girls with anorexia nervosa compared to controls. Additional studies are needed to determine whether mean levels of these peptides are reduced in anorexia nervosa. Although studies have suggested that GIP and GLP2 may be involved in regulating bone homeostasis in response to nutrient intake, we found no relationship between GIP or GLP2 levels and BMD in women with anorexia nervosa.

Limitations of this study include its small size and cross-sectional design. In addition, we did not measure insulin, which is secreted with amylin. Moreover, we did not measure GIP or GLP2 levels in response to food intake, which may be more important than basal fasting levels given the effects of feeding and food fractionation on enteric hormones and bone metabolism [3,31,49]. Therefore, it is possible that a relationship exists between BMD and GIP and/or GLP2 in women with anorexia nervosa, but that we were unable to detect it. Furthermore, we recognize that many hormonal and other factors influence bone density, including IGF-1,

cortisol, gonadal steroids, PTH and calcium, and although we were unable to include these variables in our model, further larger studies that incorporate these variables in a more complex model are warranted.

In conclusion, we have shown that mean fasting levels of amylin are significantly lower in women with anorexia nervosa than controls, and that amylin is a significant predictor of BMD and Z-scores at the femoral neck and total hip even after controlling for weight or fat mass. Decreased levels of amylin are likely a result of low nutritional intake of these women, and may represent one mechanism underlying bone loss in anorexia nervosa. Although weight may be a confounding factor, there is evidence to suggest that amylin may exert a weight-independent effect on bone mass, providing a link between nutrient intake and bone turnover. Studies are warranted to further examine the effect of amylin, GIP, and GLP2 on bone mass in anorexia nervosa.

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Figure.
Amylin levels are positively associated with BMD at the PA spine, total hip, and femoral neck.

Table 1

Clinical characteristics

	Anorexia Nervosa	Healthy Controls	<i>p</i>
Age (years)	28.0 ± 2.0	25.0 ± 1.7	0.26
Height (cm)	163.2 ± 1.4	163.8 ± 2.1	0.82
Weight (kg)	47.0 ± 1.0	63.0 ± 2.3	<0.001
BMI (kg/m ²)	17.6 ± 0.2	23.5 ± 0.6	<0.001
Total fat-free mass (kg)	36.6 ± 1.0	44.2 ± 1.3	<0.001
Total fat mass (kg)	9.2 ± 0.6	18.9 ± 1.5	<0.001
% fat	19.3 ± 1.3	28.6 ± 1.4	<0.001
Age at menarche (years)	13.4 ± 0.4	12.4 ± 0.4	0.08

Data are reported as mean ± SEM. BMI, body mass index.

Table 2

Bone mineral density

	Anorexia Nervosa	Healthy Controls	<i>p</i>
PA spine BMD (g/cm ²)	0.860 ± 0.022	1.027 ± 0.033	<0.001
PA spine Z-score	−1.6 ± 0.2	−0.1 ± 0.3	<0.001
PA spine mean vertebral area (cm ²)	54.08 ± 1.25	57.64 ± 1.50	0.08
Lateral spine BMD (g/cm ²)	0.700 ± 0.018	0.789 ± 0.035	0.03
Lateral spine Z-score	−1.2 ± 0.2	−0.1 ± 0.5	0.06
Total hip BMD (g/cm ²)	0.767 ± 0.027	0.984 ± 0.032	<0.001
Total hip Z-score	−1.5 ± 0.2	0.3 ± 0.2	<0.001
Femoral neck BMD (g/cm ²)	0.684 ± 0.022	0.865 ± 0.030	<0.001
Femoral neck Z-score	−1.5 ± 0.2	0.02 ± 0.3	<0.001
Total body BMD (g/cm ²)	1.043 ± 0.027	1.097 ± 0.018	0.10
Total body Z-score	−0.5 ± 0.3	0.1 ± 0.2	0.11

Data are reported as mean ± SEM. PA spine, posteroanterior spine; BMD, bone mineral density.

Table 3

Pancreatic and enteric peptides

	Anorexia Nervosa	Healthy Controls	<i>p</i>
Amylin (pg/mL)	3.3 ± 0.3 (2.0–5.6)	4.7 ± 0.3 (2.49–6.71)	0.001
GIP (pg/mL)	50.7 ± 3.0 (31.4–71.8)	60.5 ± 3.5 (36.6–85.2)	0.04
GLP2 (pg/mL)	92.2 ± 10.5 (43.6–152.0)	106.3 ± 11.6 (26.1–193.0)	0.39

Data are reported as mean ± SEM. (Range)

Table 4

Univariate regression analysis

	Amylin		GIP		GLP2	
	R	p	R	p	R	p
Weight (kg)	0.36	0.05	0.21	0.26	0.19	0.31
BMI (kg/m ²)	0.33	0.07	0.17	0.37	0.08	0.68
Fat mass (kg)	0.42	0.02	0.19	0.31	0.13	0.50
% fat	0.45	0.01	0.13	0.50	0.08	0.65
Fat-free mass (kg)	0.32	0.08	0.25	0.18	0.22	0.24
PA spine BMD (g/cm ²)	0.45	0.02	0.30	0.11	0.20	0.30
PA spine Z-score	0.46	0.01	0.32	0.10	0.18	0.36
Total hip BMD (g/cm ²)	0.53	<0.01	0.25	0.19	0.27	0.15
Total hip Z-score	0.53	<0.01	0.25	0.20	0.32	0.09
Femoral neck BMD (g/cm ²)	0.51	0.01	0.24	0.21	0.23	0.24
Femoral neck Z-score	0.54	<0.01	0.24	0.21	0.29	0.13

Significant associations in bold type.

Table 5

Multivariate regression analysis

Variables in model	β coefficient	F ratio	p	Cumulative R ²
PA spine BMD				
% fat	0.0003	0.62	0.44	0.14
amylin	0.0290	2.22	0.15	
weight	0.0078	10.89	<0.01	0.38
amylin	0.0186	1.33	0.26	
PA spine Z-score				
% fat	0.0280	0.75	0.39	0.18
amylin	0.2744	2.96	0.10	
weight	0.0656	11.66	<0.01	0.42
amylin	0.1902	2.11	0.16	
Total hip BMD				
% fat	0.0048	1.27	0.27	0.26
amylin	0.0454	4.63	0.04	
weight	0.0096	15.34	<0.01	0.51
amylin	0.0344	4.25	0.05	
Total hip Z-score				
% fat	0.0330	0.95	0.34	0.29
amylin	0.4202	6.31	0.02	

Variables in model	β coefficient	F ratio	p	Cumulative R ²
weight	0.0727	13.55	<0.01	0.51
amylin	0.3306	6.03	0.02	
Femoral neck BMD				
% fat	0.0036	0.98	0.33	0.28
amylin	0.0439	6.01	0.02	
weight	0.0084	17.43	<0.01	0.55
amylin	0.0331	5.78	0.02	
Femoral neck Z-score				
% fat	0.0200	0.43	0.52	0.29
amylin	0.4173	7.56	0.01	
weight	0.0638	12.69	<0.01	0.52
amylin	0.3220	6.95	0.01	

Significant associations in bold type.