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Serum FGF-21 levels are associated with worsened radial trabecular bone microarchitecture and decreased radial bone strength in women with anorexia nervosa

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Abstract

BACKGROUND—Anorexia nervosa (AN) is a psychiatric disorder characterized by self-induced starvation and low body weight. Women with AN have impaired bone formation, low bone mass and an increased risk of fracture. FGF-21 is a hormone secreted by the liver in starvation and FGF-21 transgenic mice have significant bone loss due to an uncoupling of bone resorption and bone formation. We hypothesized that FGF-21 may contribute to the low bone mass state of AN.

SUBJECTS AND METHODS—We studied 46 women: 20 with AN (median age [interquartile range]: 27.5 [25, 30.75] years) and 26 normal-weight controls (NWC) of similar age (25 [24, 28.5] years). We investigated associations between serum FGF-21 and 1) aBMD measured by dual energy X-ray absorptiometry, 2) parameters of bone microarchitecture in the distal radius and tibia measured by high-resolution peripheral quantitative CT and 3) bone strength, estimated by microfinite element analysis.

RESULTS—FGF-21 levels were similar in AN and NWC (AN: 33.1 [18.1, 117.0] pg/ml vs NWC: 57.4 [23.8, 107.1] pg/ml; $p=0.54$). There was a significant inverse association between log FGF-21 and trabecular number in the radius in both AN ($R=-0.57$, $p<0.01$) and NWC ($R=-0.53$, $p<0.01$) and a significant positive association between log FGF-21 and trabecular separation in the radius in AN ($R=0.50$, $p<0.03$) and NWC ($R=0.52$, $p<0.01$). Estimates of radial bone strength were inversely associated with log FGF-21 in AN ($R=-0.50$, $p<0.03$ for both stiffness and failure

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load). There were no associations between FGF-21 and aBMD, cortical parameters or tibial parameters in the AN or NWC groups.

CONCLUSIONS—FGF-21 may be an important determinant of trabecular skeletal homeostasis in AN.

Keywords

FGF-21; anorexia nervosa; bone microarchitecture; bone strength

Introduction

Anorexia nervosa (AN), a psychiatric disorder with a lifetime prevalence of 2.2% in women [1], is characterized by an inability to maintain a normal body weight. AN is associated with a number of medical co-morbidities including profound bone loss. Nearly 85% of women with AN have bone mineral density (BMD) values more than one-standard deviation below an age-comparable mean [2], and importantly this low bone mass is associated with a seven-fold increased risk of fracture [3].

A number of hormonal adaptations help minimize energy utilization in AN but also contribute to the loss of bone mass. For example, women with AN are growth hormone (GH) resistant [4] -- having normal or elevated GH levels coupled with low IGF-I levels, allowing for the maintenance of euglycemia [5] and mobilization of fat stores [6] while minimizing energy expenditure on growth-- and low IGF-I levels have been associated with low BMD in women with AN [7]. Hypogonadotropic hypogonadism, which prevents energy expenditure on reproduction, is also a characteristic finding in AN and states of estrogen deficiency result in increased bone resorption [8], thereby contributing to the loss of bone mass.

FGF-21 is a hormone produced in the liver [9] and in adipocytes [10] and starvation markedly upregulates FGF-21 production in the liver in animal models [11, 12]. Mice that over-express FGF-21 have a similar phenotype as women with AN -- compared to wild-type littermates, they weigh less, have a lower core body temperature and are GH resistant [13]. Importantly, these FGF-21 transgenic mice also have profound bone loss due to a marked increase in bone resorption and a decrease in bone formation [14]. We undertook this study to investigate the relationship between FGF-21 and bone in AN and hypothesized that FGF-21 would be associated with decreased bone mass and strength in AN.

Materials and Methods

Subjects

We studied 46 women: 20 with AN (median [interquartile range]: 27.5 [25, 30.75] years) and 26 normal-weight controls (NWC) of comparable age, (25.0 [24, 28.5] years). BMD data from a subset of the AN and NWC were previously published [15]. Women with AN were recruited through referrals from local eating disorder providers and on-line advertisements and NWC were recruited through on-line advertisements. Subjects with AN met DSM-IV weight and psychiatric criteria. None of the AN subjects were receiving

hyperalimentation therapy or tube feeds at the time of their participation. None of the subjects received estrogen within 3 months of the study. All NWC subjects had a normal BMI, a history of regular menstrual cycles and were receiving no medications known to affect bone mass. NWC did not have a past or present history of an eating disorder. Subjects with abnormal thyroid function tests, chronic diseases known to affect bone mineral density (other than AN) or diabetes mellitus were excluded from participation and all subjects had normal liver function.

All subjects were examined and blood was drawn for laboratory studies at a study visit at our Clinical Translational Science Center. Height was measured as the average of 3 readings on a single stadiometer, and subjects were weighed on an electronic scale while wearing a hospital gown.

The study was approved by the Partners Institutional Review Board and complied with the Health Insurance Portability and Accountability Act guidelines. Written informed consent was obtained from all subjects.

Biochemical Assessment

FGF-21 was measured by ELISA (R&D Systems, Minneapolis, MN) with a detection limit of 4.67 pg/mL. The inter-assay coefficient of variation (CV) was 7.5% and the intra-assay CV was 3.4%. A marker of bone formation, N-terminal propeptide of type 1 procollagen (PINP), and a marker of bone resorption, C-terminal collagen cross-links (CTX), were measured by a luminescent immunoassay analyzer (ISYS Analyzer; Immunodiagnostics Corporation, Woburn, MA). The detection limit for PINP was 1 ng/mL, with an intra-assay CV of 2.9% and an inter-assay CV of 4.6%. The detection limit for CTX was 0.023 ng/mL with an intra-assay CV of 3.2% and an inter-assay CV of 6.2%.

Radiologic Imaging

All subjects underwent dual energy X-ray absorptiometry (DXA) to measure aBMD of the posterior-anterior (PA) lumbar spine (L1-L4), total hip, total body and body composition including fat mass (kg) and % body fat using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA). Coefficients of variation of DXA have been reported as <1% for bone [16] and <2.7% for fat mass [17]. All participants underwent high-resolution peripheral quantitative CT (HR-pQCT) of the non-dominant distal radius and tibia (Xtreme CT; Scanco Medical AG, Bruttisellen, Switzerland) with an isotropic voxel size of 82 μm^3 as previously described [18]. An extended cortical analysis was performed, by methods previously described [19], to calculate cortical thickness.

Microfinite Element Analysis

We used linear microfinite element analysis (FEA) of HR-pQCT images in order to estimate the biomechanical properties of the distal radius and the distal tibia under uniaxial compression loading, as previously described [20-22]. The outcomes from the micro-FEA included both stiffness (kN/mm) and failure load (kN). A prior study reported strong associations between the biomechanical properties derived from microFEA and those measured directly via *ex vivo* testing of elderly human cadaveric radii [23].

Statistical Analysis

Statistical analysis was performed using JMP Pro 11.0 (SAS Institute, Cary, NC) software. Means and standard error of the mean (SEM) measurements are reported and compared using the Student's t-test unless the data were non-normally distributed, in which case medians and the interquartile range are reported and compared using the Wilcoxon test. As FGF-21 was the variable of interest and not normally distributed, we used log transformation to normalize its distribution. Pearson correlation coefficients, or Spearman's coefficients, if the data were not normally distributed, were calculated to assess univariate relationships. Multivariable analyses were performed using least-squares linear regression to control for confounders. A *p*-value of < 0.05 was used to indicate significance.

Results

Clinical characteristics

Clinical characteristics of the study subjects are listed in Table 1. aBMD was significantly lower in AN compared to NWC at all measured sites. PINP levels were significantly lower in AN compared to NWC, whereas CTX levels were similar in both groups. Median FGF-21 levels were lower in AN compared to NWC, although this difference was not significant (AN: 33.6 [18.1, 117] vs NWC: 57.4 [23.8, 107.1]; $p=0.54$) and this difference remained non-significant after controlling for total body fat mass ($p=0.62$).

Parameters of Microarchitecture and Estimated Bone Strength (Table 1)

In the radius, trabecular bone volume fraction (BV/TV), trabecular number (TbN), trabecular thickness (TbTh) and cortical thickness (CtTh) were significantly lower and trabecular separation (TbSp) was significantly higher in AN compared to NWC. Estimates of bone strength -- stiffness and failure load -- were also significantly lower in AN compared to NWC.

Similarly, in the tibia, BV/TV, TbN, CtTh and estimated stiffness and failure load were all significantly lower in AN compared to NWC. Tibial TbSp was also significantly higher in AN compared to NWC.

Association between FGF-21 and BMD, Bone Microarchitecture, Estimated Bone Strength and Bone Turnover Markers

Bone Mineral Density—There were no significant associations between aBMD and log FGF-21 in the group as a whole or in the AN or NWC groups separately.

Bone Microarchitecture—In the group as a whole, there was a significant inverse association between log FGF-21 and radial TbN ($R = -0.39$, $p < 0.01$) (Figure 1) and a positive association between log FGF-21 and radial TbSp ($R = 0.42$, $p < 0.01$). These relationships remained significant after controlling for total body fat mass and total body lean mass ($p = 0.01$ for TbN and $p < 0.01$ for TbSp). In the group as a whole, there was also a significant inverse association between log FGF-21 and TbTh in the tibia ($R = -0.29$, $p < 0.05$) which did not remain significant after controlling for total body fat mass and total body lean mass ($p = 0.53$).

In AN, there were significant inverse associations between log FGF-21 and radial BV/TV ($R = -0.50$, $p = 0.02$) (Figure 2A) and TbN ($R = -0.57$, $p < 0.01$) (Figure 2B). There was also a significant positive association between log FGF-21 and radial TbSp ($R = 0.50$, $p < 0.03$) (Figure 2C). These associations all remained significant after controlling for duration of amenorrhea (BV/TV: $p < 0.04$; TbN: $p = 0.01$ and TbSp: $p < 0.02$) as well as total body fat mass and total body lean mass (BV/TV: $p < 0.04$; TbN: $p < 0.03$, TbSp: $p = 0.02$). There were no associations between log FGF-21 and tibial parameters in AN.

In NWC, in the radius, there was a significant inverse association between log FGF-21 and TbN ($R = -0.53$, $p < 0.01$) (Figure 3A) and a significant positive association between log FGF-21 and TbSp ($R = 0.52$, $p < 0.01$) (Figure 3B). The relationship between log FGF-21 and TbN did not remain significant after controlling for total body fat mass and total body lean mass ($p = 0.18$) or after controlling for age and BMI ($p = 0.16$). There was a trend towards significance in the relationship between log FGF-21 and TbSp after controlling for total body fat mass and total body lean mass ($p = 0.10$) but the relationship did not remain significant after controlling for age and BMI ($p = 0.12$). There were no associations between log FGF-21 and tibial parameters.

Estimated Bone Strength—In AN, there were significant inverse associations between log FGF-21 and radial stiffness ($R = -0.50$, $p < 0.03$) (Figure 2D) and failure load ($R = -0.50$, $p = 0.02$) (Figure 2E). There were no associations between log FGF-21 and parameters of estimated bone strength in the tibia. There were no associations between log FGF-21 and parameters of estimated bone strength in NWC.

Bone Turnover Markers—In NWC, the marker of bone resorption, CTX was positively associated with log FGF-21 ($R = 0.45$, $p = 0.02$) (Figure 3C). There were no associations between log FGF-21 and P1NP in NWC. There were no significant associations between CTX or P1NP and log FGF-21 in AN.

Discussion

We have shown that FGF-21, a hormone which is associated with bone loss due to an uncoupling of bone resorption and bone formation in animal models, is inversely associated with trabecular microarchitectural parameters in women with AN and these associations remain significant after controlling for possible confounders including duration of amenorrhea and total body fat and lean mass. We have also shown that FGF-21 is inversely associated with estimates of bone strength in women with AN. Therefore, FGF-21 may be an important determinant of trabecular skeletal homeostasis in women with AN.

AN is a psychiatric disease associated with significant bone loss [2] and an increased risk of fracture [3]. The body's response to a state of negative energy balance is to preserve energy through a number of hormonal alterations, including hypogonadotropic hypogonadism and GH resistance, and these alterations result in a loss of bone mass. Women with AN have a sustained increased risk of fracture even many years after weight restoration [24], and therefore understanding the pathophysiology of bone loss in AN is critical to reducing the long-term risk of fracture and its associated morbidity.

FGF-21 is a hormone whose production in the liver is markedly upregulated in response to starvation [11, 12]. FGF-21 is induced by peroxisome proliferator-activated receptor (PPAR)- α – a nuclear receptor activated by fatty acids during starvation – and may be necessary for the production of ketones and gluconeogenesis during periods of prolonged fasting [11, 12, 25]. FGF-21 is also a mediator of insulin-independent glucose uptake [26] and its therapeutic potential is currently an active area of investigation [27].

Although one might expect levels of FGF-21 to be elevated in a state of chronic starvation such as AN, prior studies have demonstrated that women with AN have lower levels of FGF-21 as compared to NWC [28], likely due to the fact that fat stores are low in women with AN and therefore free-fatty acids, which serve as the ligand for PPAR- α and are therefore potentially necessary for FGF-21 production, are low in AN. In adolescent girls, we have previously shown that FGF-21 levels are higher than NWC only after controlling for both percent body fat and insulin resistance, but unadjusted FGF-21 levels were similar in both groups [29]. Importantly, in women with AN, levels of FGF-21 *decrease* after two-months of realimentation therapy [28], suggesting that the levels of FGF-21 are maximally induced in this state of chronic starvation. In this study, we found levels of FGF-21 to be lower in AN than NWC, although not significantly so, which is likely due to the fact that our AN subjects had a higher mean BMI, and therefore likely more fat mass, as compared to the women in the prior study (BMI: 17.1 vs 15.9 kg/m²) [28].

Although *in vitro* models suggest that FGF-21 may stimulate bone formation [30], in animal models, FGF-21 is an important negative regulator of bone mass. Transgenic mice that over-express FGF-21 have a low bone mass phenotype and mice treated with pharmacologic doses of FGF-21 lose bone mass due to a decrease in bone formation and a significant increase in bone resorption [14]. FGF-21 may also mediate the loss of bone mass during lactation [31]. A prior study investigating the effects of FGF-21 on BMD in humans found a positive relationship between FGF-21 and total body and spine BMD in a group of 16 women [32]. This group was older and heavier than our group (mean age: 34 years and mean BMI: 26.8 kg/m²) and included a postmenopausal subject; parameters of bone microarchitecture were also not measured [32]. FGF-21 is positively associated with BMI [10] -- thought to be due to FGF-21 resistance -- and therefore perhaps a positive relationship between FGF-21 and bone was found in the prior study due to resistance to the effects of FGF-21 at the level of the bone.

In our group of low-weight women with AN and our leaner, normal-weight group, we found an inverse association between FGF-21 and trabecular bone parameters, consistent with the animal models demonstrating negative effects of FGF-21 on bone; our data suggest that FGF-21 may be a determinant of trabecular skeletal homeostasis. Importantly, after controlling for possible confounders, the relationship between FGF-21 and trabecular microarchitectural parameters in NWC did not remain significant whereas they remained significant in the AN group. It is therefore possible that FGF-21 may be a more important determinant of bone mass in states of under-nutrition as compared to normal-weight states. Interestingly, we did not find a relationship between FGF-21 and cortical bone parameters, and our associations were observed predominantly at the radius, not the tibia. This suggests that FGF-21 may have a more pronounced effect on trabecular bone in humans and that

perhaps the negative effects of FGF-21 on bone can be overcome at weight-bearing sites and therefore FGF-21 has a more significant effect on non-weight-bearing sites.

In this study, we examined the relationship between FGF-21 and nineteen related bone parameters. As these are all related measures, we did not control for multiple testing and in fact, given our results of eleven statistically significant findings in the hypothesized direction, the likelihood of this happening by chance would be infinitesimally small. The major limitation of this study is its cross-sectional nature and therefore we cannot imply causation from the association between FGF-21 and bone but importantly, we have shown that FGF-21 is associated with worsened parameters of trabecular microarchitecture in women with AN. The relationship between FGF-21 and bone in humans will need to be further investigated as FGF-21's therapeutic potential continues to be explored.

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Highlights

- In animal models, FGF-21 is associated with significant bone loss due to an uncoupling of bone resorption and bone formation
- Serum FGF-21 was inversely associated with radial trabecular number in women with anorexia nervosa
- FGF-21 levels were inversely associated with estimates of radial bone strength in women with anorexia nervosa
- FGF-21 may be an important determinant of trabecular skeletal homeostasis in anorexia nervosa

Figure 1.

There was significant overlap in log FGF-21 levels in women with anorexia nervosa and the normal-weight controls. In the group as a whole, there was an inverse association between radial trabecular number (TbN) and log FGF-21.

Figure 2.

In women with anorexia nervosa, there was a significant inverse association between log FGF-21 and radial bone volume fraction (BV/TV) (panel A) and trabecular number (TbN) (panel B) and a significant positive association between log FGF-21 and radial trabecular separation (TbSp) (panel C). There was also a significant inverse association between radial stiffness (panel D) and failure load (panel E) -- two estimated measures of bone strength -- in women with anorexia nervosa.

Figure 3.

In normal-weight controls, there was a significant inverse association between log FGF-21 and radial trabecular number (TbN) (panel A) and significant positive associations between log FGF-21 and radial trabecular separation (TbSp) (panel B) and the marker of bone resorption, CTX (panel C).

Table 1

Clinical Characteristics of the Study Subjects

	Anorexia Nervosa (n=20)	Normal Weight Controls (n=26)	<i>p</i> -value
Age (years) *	27.5 [25, 30.8]	25 [24, 28.5]	0.1
BMI (kg/m ²) *	17.8 [15.9, 18.3]	22.4 [21.4, 23.2]	<0.0001
Weight (kg)	47.6 ± 1.4	61.9 ± 1.2	<0.0001
Height (cm)	166.7 ± 1.4	165.8 ± 1.4	0.64
Duration of anorexia nervosa (years)	10.3 ± 0.9	-----	
Duration of amenorrhea (months)	36 [15.75, 90.75]	-----	
Posterior-anterior spine BMD (g/cm ²)	0.83 ± 0.03	1.01 ± 0.02	<0.0001
Total Hip BMD (g/cm ²)	0.76 ± 0.02	0.98 ± 0.02	<0.0001
Total Body BMD (g/cm ²)	0.97 ± 0.02	1.07 ± 0.01	<0.0001
Fat Mass by DXA (kg)	9.3 ± 0.7	18.5 ± 0.8	<0.0001
% Body Fat by DXA	18.8 ± 1.1	29 ± 0.9	<0.0001
PINP (ng/ml) *	61.7 [47.2, 67.6]	73.9 [63, 95.7]	<0.03
CTX (ng/ml)	0.80 ± 0.07	0.81 ± 0.06	0.87
FGF-21 (pg/ml) *	33.6 [18.1, 117]	57.4 [23.8, 107.1]	0.54
<i>Microarchitecture parameters by HR-pQCT</i>			
Radius			
Total area (mm ²)	272 ± 10.1	270 ± 9.4	0.93
BV/TV (%)	0.11 ± 0.01	0.14 ± 0.01	0.001
Tb N (1/mm) *	1.74 [1.63, 1.95]	1.92 [1.82, 2]	0.03
Tb Th (mm)	0.06 ± 0.002	0.07 ± 0.003	<0.01
Tb Sp (mm) *	0.52 [0.45, 0.55]	0.45 [0.43, 0.48]	<0.01
Ct Th (mm)	0.79 ± 0.03	0.90 ± 0.03	<0.02
<i>Finite element analysis estimates of strength:</i>			
Stiffness (kN/mm)	62.4 ± 3.2	76.7 ± 2.7	<0.01
Failure load (kN)	3.1 ± 0.2	3.9 ± 0.1	<0.01
Tibia			
Total area (mm ²) *	613.5 [579, 747]	682.8 [603, 724]	0.50
BV/TV (%) *	0.13 [0.10, 0.16]	0.15 [0.14, 0.17]	<0.01
Tb N (1/mm)	1.69 ± 0.05	1.89 ± 0.06	<0.02

	Anorexia Nervosa (n=20)	Normal Weight Controls (n=26)	<i>p-value</i>
Tb Th (mm)	0.08 ± 0.003	0.08 ± 0.002	0.16
Tb Sp (mm)	0.53 ± 0.02	0.46 ± 0.01	<0.02
Ct Th (mm) *	0.98 [0.92, 1.12]	1.23 [1.18, 1.34]	<0.001
<i>Finite element analysis estimates of strength:</i>			
Stiffness (kN/mm)	171.4 ± 11	218.7 ± 5.7	<0.001
Failure load (kN)	8.7 ± 0.5	11 ± 0.3	<0.001

Mean ± SEM

* median [interquartile range]

Table 2

Correlation coefficients for univariate associations between log FGF-21 and bone parameters

	R-value for group as a whole	R-Value for women with anorexia nervosa	R-value for normal-weight controls
PA spine BMD (g/cm ²)	0.09 (p=0.57)	0.12 (p=0.61)	0.003 (p=0.99)
Total hip BMD (g/cm ²)	-0.04 (p=0.80)	-0.17 (p=0.47)	-0.09 (p=0.66)
Total body BMD (g/cm ²)	-0.03 (p=0.85)	0.08 (p=0.74)	-0.23 (p=0.26)
CTX (ng/ml)	0.15 (p=0.33)	-0.18 (p=0.46)	0.45 (p=0.02)
P1NP (ng/ml)	-0.07 (p=0.66)	-0.24 (p=0.33)	0.04 (p=0.86)
Radius:			
BV/TV (%)	-0.20 (p=0.19)	-0.50 (p=0.02)	-0.04 (p=0.83)
TbN (1/mm)	-0.39 (p<0.01)	-0.57 (p<0.01)	-0.53 (p<0.01)
TbTh (mm)	0.04 (p=0.77)	-0.18 (p=0.44)	0.11 (p=0.58)
TbSp (mm)	0.42 (p<0.01)	0.50 (p<0.03)	0.52 (p<0.01)
CtTh (mm)	0.08 (p=0.61)	-0.16 (p=0.51)	0.24 (p=0.25)
Stiffness (kN/mm)	-0.15 (p=0.34)	-0.50 (p<0.03)	0.10 (p=0.65)
Failure load (kN)	-0.17 (p=0.27)	-0.50 (p=0.02)	0.06 (p=0.78)
Tibia:			
BV/TV (%)	-0.17 (p=0.25)	-0.39 (p<0.10)	-0.12 (p=0.58)
TbN (1/mm)	0.07 (p=0.66)	-0.24 (p=0.31)	0.27 (p=0.18)
TbTh (mm)	-0.29 (p<0.05)	-0.34 (p=0.14)	-0.28 (p=0.17)
TbSp (mm)	0.02 (p=0.89)	0.25 (p=0.29)	-0.20 (p=0.34)
CtTh (mm)	0.04 (p=0.77)	0.17 (p=0.47)	-0.13 (p=0.54)
Stiffness (kN/mm)	-0.11 (p=0.49)	-0.26 (p=0.28)	-0.06 (p=0.78)
Failure load (kN)	-0.11 (p=0.45)	-0.18 (p=0.46)	-0.04 (p=0.84)