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Rapid Communication

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 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.

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 comorbidities 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

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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 (P1NP), 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 P1NP 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. P1NP 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 microarchitecure 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 (Table 2)

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 (Table 2).

Bone microarchitecture

In the group as a whole, there was a significant inverse association between log FGF-21 and radial TbN ($R = -0.39$, $p < 0.01$) (Fig. 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 ($R = -0.50$, $p = 0.02$) (Fig. 2A) and TbN ($R = -0.57$,