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Strong relationship between vitamin D status and bone mineral density in anorexia nervosa

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ABSTRACT

Background: Anorexia nervosa (AN) is associated with impaired bone health and low bone mineral density (BMD) as a consequence of an inadequate peak bone mass in adolescence and bone loss in young adulthood. The vitamin D status with its implications for bone health in patients affected by AN has only been examined previously in small studies.
Objective: To evaluate the prevalence of vitamin D deficiency and test the hypothesis that patients with AN and vitamin D deficiency might have worse bone metabolism and lower bone density as compared with AN with adequate vitamin D repletion.
Design: We analysed the vitamin D status and bone metabolism in a large cohort ($n = 89$) of untreated patients affected by AN, with amenorrhoea.
Results: Vitamin D deficiency is widespread in untreated patients with AN: 16.9% had 25OH vitamin D levels below 12 ng/ml, 36% below 20 ng/ml and 58.4% below 30 ng/ml. PTH values were higher and BMD at both femoral sites were lower in patients with vitamin D < 20 ng/ml. Progressively higher values of BMD were observed by 4 ranks of 25 OH vitamin D values (severe deficiency: <12 ng/ml, deficiency: ≥12 ng/ml and <20 ng/ml, insufficiency: ≥20 and <30 ng/ml and normal: ≥30 ng/ml). In patients with severe vitamin D deficiency BMD at the hip were significantly lower than that measured in groups with values over 20 ng/ml ($p < 0.001$ for trend). The level of significance did not change for values adjusted for BMI or body weight.
Conclusion: We found a strong relationship between vitamin D status and hip BMD values with additional benefits for those with 25OHD levels above 20 ng/ml. Our results support the design of a randomized placebo-controlled clinical trial on the effect of vitamin D on BMD in patients with AN. The second point, whether 25OHD should be above 20 or 30 ng/ml remains a discussion point.

Introduction

Anorexia nervosa (AN) starts typically in mid-teenage years [1] and it is generally associated with amenorrhoea [2]. AN increases the risk for impaired bone health and for low bone mineral density (BMD) as a consequence of an inadequate peak bone mass in adolescence and bone loss in young adulthood [3].

The vitamin D status with its implications for bone health in patients with AN has only been examined previously in small studies [4–8] usually comparing the concentration of 25-hydroxyvitamin D (25 OHD) of AN group with that of a healthy controls. Kiriike et al. [9] found that most of 29 patients with AN had normal vitamin D concentrations but normality was defined as 25 OHD >16 ng/ml. Haagensen et al. [10] showed a very low prevalence of vitamin D deficiency (<20 ng/ml) in the AN group (2% vs. 24% among healthy controls), but the patients affected by AN had been encouraged to consume 400 IU vitamin D supplements daily by their primary eating disorder provider.

Recently, Veronese et al. [11] reported the results of a meta-analysis involving 15 studies with a total of 927 participants. They found that patients with AN have significantly lower serum levels of 25OHD and 1,25(OH)2D compared to healthy controls, while vitamin D introduced with diet was reported to be similar.

In this study we analysed the vitamin D status in a large cohort of untreated patients with AN to evaluate the prevalence of deficiency and tested the hypothesis that patients affected by AN with vitamin D deficiency might have worse bone metabolism and lower bone density as compared with AN with adequate vitamin D repletion. Moreover, we included in the assessment of bone metabolism both bone turnover markers, sclerostin (SOST) and Dickkopf-1 (DKK1) that we recently evaluated in a number of clinical conditions [12–16]. Wnt/ß-catenin

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Table 1
Main characteristics and distribution of 25 OHD levels in anorexia nervosa patients[1].

	Whole population	25 OHD <12 ng/ml	25 OHD ≥12 and <20 ng/ml	25 OHD ≥20 and <30 ng/ml	25 OHD ≥30 ng/ml
Number (%)	89/89 (100%)	15/89 (16.9%)	17/89 (19.1%)	20/89 (22.4%)	37/89 (41.6%)
Age ± SD (years)	23.6 ± 7.8	27.7 ± 8.0	23.8 ± 8.1	21.5 ± 8.6	23.0 ± 6.7
Weight ± SD (kg)	39.6 ± 5.4	37.4 ± 6.0	40.1 ± 5.9	39.6 ± 5.2	40.2 ± 4.9
BMI ± SD (kg/m^2)	15.2 ± 1.6	15.1 ± 1.5	15.0 ± 1.9	15.1 ± 1.7	15.3 ± 1.4
Current other Axis I Disorder[2]					
Major depressive episode, n (%) present	32/89 (35.9%)	5/89 (5.6%)	9/89 (10.1%)	8/89 (9.0%)	10/89 (11.2%)
Any anxiety disorder, n (%) present	11/89 (12.3%)	1/89 (1.1%)	2/89 (2.2%)	4/89 (4.5%)	4/89 (4.5%)

[1] The weight, BMI and age (±standard deviation: SD) were not statistically different in patients divided by 25 OHD levels (Anova and Bonferroni post hoc analysis).
[2] The structured clinical interview for DSM-IV was used to assess depression and anxiety disorders.

signalling pathway represents a major promoter of bone formation through effects on osteoblast number, maturation and progenitor differentiation and these actions are opposed by various intracellular and secreted factors [17]. The secreted Wnt antagonists SOST and DKK1 block Wnt signalling by binding to Wnt co-receptors, such as low-density lipoprotein receptor-related protein 5(LRP5) and LRP6, and by inhibiting the canonical Wnt/b-catenin signalling pathway [17].

Materials and methods

The AN sample was recruited from consecutive referrals to the eating disorder inpatient unit of Villa Garda Hospital (Northern Italy). Patients had to be Caucasian females aged between 13 and 45 years, to fulfil the DSM-5 diagnostic criteria for AN [18] plus secondary amenorrhoea for at least 6-months, as judged by an eating disorder specialist (R.D.G.), and to require inpatient treatment either as a result of failure of outpatient treatment or because the eating disorder could not be managed safely on an outpatient basis. Patients receiving hormonal or other medications known to affect bone metabolism or vitamin D supplements were excluded. None of the patients were on vitamin D supplements.

Body weight was measured to the nearest 0.1 kg using precision scales (Salus, Milan, Italy) with subjects wearing light clothing and no shoes; height was measured without shoes and recorded to the nearest millimetre using a stadiometer (Salus, Milan, Italy).

Blood samples were collected before starting the treatment.

Serum samples were collected when the patients were admitted in the inpatient unit before starting the treatment. They were aliquoted, and stored at −50 °C until they were assayed for intact N-propeptide of type I collagen (P1NP), C-terminal telopeptide of type I collagen (CTX), intact parathyroid hormone (PTH), 25 OHD, SOST and DKK1 in the laboratory of Rheumatology Unit of the University of Verona.

Bone turnover markers (P1NP and CTX), PTH and 25OHD, were measured by the IDS-ISYS Multi-Discipline automated analyser (Immunodiagnostic System, Boldon, UK) based on chemiluminescence technology. The coefficients of variation (CV) intra-assay measured in our laboratory were 3.0% for intact-P1NP (inter-assay CV 5%) and 3% for CTX (inter-assay CV 7%), 8.0% for 25OHD (inter-assay CV 12%) and 2.7% for PTH (1–84) (inter-assay CV 5.5%).

Serum DKK1 and SOST were measured by ELISA (Biomedica Medizinprodukte, Vienna, Austria) with a sensitivity of 0.38 and 2.6 pmol/L and intra-assay CV of 7 and 5% (inter-assay CV 8.2% and 6.9%) respectively.

BMD were measured by DXA scans (Prodigy Primo Lunar, A223040501 General Electric Company, Madison. WI 53707-7550, USA-EnCORE TM 2009 (v13.31) software) at the lumbar spine (LS) and hip (neck and total hip) in 82 of the patients.

Coexisting Axis I Psychiatric Disorders were assessed at baseline with the Structured Clinical Interview for DSM-IV (SCID) [19].

The study was approved by the institutional review board of the Medical School of Verona and informed consent was obtained from all subjects or their parents.

All statistical analyses were performed per protocol by SPSS Version 17 (SPSS, Inc., Chicago, IL, USA). Analysis of variance (ANOVA) with post hoc analysis (Bonferroni) and a two-sided Student's t test were used to estimate the absolute differences between groups.

Results

The study sample consisted of 89 women affected by AN, age 14–40 years (mean 23.3 ± 7.7). Table 1 shows the main characteristics and the prevalence of vitamin D deficiency on the basis of cut-off recently established by the Institute of Medicine (IOM) [20].

First, we divided the population into 2 groups on the basis of 25 OHD levels considered adequate (≥20 ng/ml) or insufficient (<20 ng/ml) [20], then for severe deficiency (<12 ng/ml), deficiency (≥12 ng/ml and <20 ng/ml), insufficiency (≥20 and <30 ng/ml) (threshold for adequacy for IOM) and normal (≥30 ng/ml) [21] to compare the consequences of vitamin D repletion on bone metabolism and BMD (Tables 1 and 2).

PTH values were higher in patients with vitamin D deficiency (Table 2) while no differences were found in bone turnover markers and in Wnt pathway inhibitors (Table 2).

SOST was negatively correlated with body weight ($p < 0.03$) and BMI ($p < 0.001$) and positively correlated with DKK1 ($p = 0.03$) only in patients with 25 OHD ≥20 ng/ml (data not shown). The positive correlation with DKK1 was confirmed after adjustment for body weight. All these correlations were not significant in the whole study population and in subjects with 25 OHD <20 ng/ml.

BMD at both femoral sites were lower in the vitamin D deficient patients (Table 2). Progressively higher values were observed by 4 ranks of 25 OHD values (Table 3). In patients with severe vitamin D deficiency both femoral neck and total hip BMD were statistically significantly lower (ANOVA and Bonferroni's post hoc analysis) than those measured in groups with values over 20 ng/ml ($p < 0.001$ for trend) (Fig. 1). The level of significance did not change for values adjusted for BMI or body weight.

Table 2
Bone turnover markers and bone mineral density (BMD) in anorexia nervosa patients with 25 OHD lower or higher than 20 ng/ml[1].

Bone parameters	25 OHD <20 ng/ml (n° 32)	25 OHD ≥20 ng/ml (n° 57)	p (Student t test)
25 OHD (ng/ml)	12.4 ± 5.1	34.7 ± 8.8	p < 0.001
PTH (pg/ml)	39.1 ± 21.1	27.5 ± 12.9	p < 0.01
CTX (μg/L)	0.78 ± 0.43	0.92 ± 0.48	n.s.
P1NP (ng/ml)	47.7 ± 33,5	49.3 ± 35.0	n.s.
DKK1 (pmol/L)	25.2 ± 11.6	23.6 ± 10.52	n.s.
SOST (pmol/L)	42.6 ± 25.4	36.5 ± 15.6	n.s.
BMD spine (mg/cm^2)	925 ± 157	973 ± 145	n.s
BMD femoral neck (mg/cm^2)	755 ± 111	879 ± 104	p < 0.001
BMD total hip (mg/cm^2)	749 ± 102	889 ± 99	p < 0.001

[1] BMD values were available for 30 and 48 patients with 25OHD values <20 and ≥20 ng/ml, respectively.