REVIEW

Vitamin D Status in Anorexia Nervosa: A Meta-Analysis

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ABSTRACT

Objective: In anorexia nervosa (AN), osteoporosis and osteopenia are common, which have been associated with low circulating levels of vitamin D (VitD) in other settings. We aimed to meta-analyze cross-sectional studies reporting on VitD parameters in patients with AN and healthy controls (HCs).

Method: Electronic PubMed search from database inception until December 31, 2013 and meta-analysis of cross-sectional studies comparing serum levels of 25-hydroxyvitamin D (25OH-D), 1,25-dihydroxyvitamin D (1,25OH-D) and dietary VitD between patients with AN and HCs, before or after VitD supplementation. We calculated random effects standardized mean differences (SMDs) ±95% confidence intervals (CIs) as effect size measures.

Results: Out of 1,739 initial hits, 15 studies with a total of 927 participants (AN = 408 and HCs = 519) were meta-analyzed. In the unsupplemented state, both serum 25OH-D (studies = 4; $n = 168$; SMD = −0.43; 95%CI: −0.83 to −0.03; $p = .03$) and 1,25OH-D levels (studies = 4; $n = 113$; SMD = −1.06; 95%CI: −1.47 to −0.66; $p < .00001$) were significantly lower in AN than HCs. In AN patients treated with cholecalciferol supplementation, serum 25OH-D levels were significantly higher than in HCs (studies = 5; $n = 449$; SMD = 0.66; 95%CI: 0.01–1.31; $p = .05$). Paradoxically, despite lower 25OH-D and 1,25OH-D levels, AN patients reported similar intake of VitD compared to HCs (studies = 6; $n = 314$; SMD = 0.33; 95%CI: −0.16, 0.81; $p = .19$).

Discussion: Although AN patients reported similar dietary VitD intake compared to HCs, AN patients had significantly lower 25OH-D and 1,25OH-D levels without supplementation. Conversely, supplementation with cholecalciferol fully normalized VitD serum levels. Future studies are needed to clarify the role of VitD supplementation in AN for improving bone health. © 2014 Wiley Periodicals, Inc.

Keywords: anorexia nervosa; vitamin D; eating disorders; 25 hydroxyvitamin D

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Introduction

Anorexia Nervosa (AN) is a disease with many organic complications, such as bradycardia, myocardial fibrosis, cardiac arrhythmias, constipation, and infections.[1, 2] Among the most common complications, bone metabolism alterations play a central role.[3]

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Adolescence is the highest incidence age of AN, which matches the highest incidence age for the increase of bone mass.[4] Thus, the loss of bone mineral density may be particularly large and may occur early in AN, resulting in a higher prevalence of osteoporosis/osteopenia and bone fractures in this population compared to other adolescents.[3–5]

Among reversible risk factors for bone metabolism alterations, poor vitamin D circulating levels play a role.[6] The interest in vitamin D has been increasing exponentially because in addition to the negative impact on bone metabolism, low vitamin D status seems to have important extra-skeletal effects.[7–15] In addition, a protective role of vitamin D in mood disorders and suicide has been suggested.[16–18] Because AN is highly comorbid with depressive symptomatology[19] and suicide,[20] the role of vitamin D role in AN should be examined in more depth.

However in AN, there are conflicting reports about vitamin D levels. While many factors, such as low bone mineral density, increased rate of fractures and undernutrition suggest that in AN vitamin D status is likely poor, several studies reported normal vitamin D status in these patients.[21–27]

Because to the best of our knowledge, no quantitative meta-analysis has been conducted about vitamin D levels in AN, we aimed to meta-analyze cross-sectional studies reporting on vitamin D circulating parameters and dietary vitamin D intake in patients with AN and healthy controls (HCs). On the basis of the available literature and the link between undernutrition and poor vitamin D status, we hypothesized that AN patients would have significantly lower dietary vitamin D intake and lower vitamin D levels than HCs, and that supplementation with cholecalciferol would be able to compensate this difference.

Method

Search Strategy

We conducted an electronic literature search using PubMed without language restriction from database inception until 12/31/2013 for studies comparing levels of 25-hydroxyvitamin D (25OH-D), the active 1,25-dihydroxyvitamin D (1,25OH-D) and dietary vitamin D intake between patients with AN and HCs. Controlled vocabulary terms (MeSH) and the following keywords regarding were used in the search strategy: "(anorexia OR bulimia OR "eating disorder") AND (vitamin* OR retinol OR carotene* OR thiamin OR korsakoff OR riboflavin OR niacin* OR pellagra OR pantothen* OR pyridoxine OR biotin OR folate OR folic* OR folacin OR cobalamin OR hydroxycobalamin OR methylcobalamin OR megaloblastic anemia OR "ascorbic acid" OR ascorbate OR scurvy OR cholecalciferol OR ergocalciferol OR hypovitaminosis OR rickets OR osteoporosis OR osteomalacia OR osteopenia OR osteoporosis OR "bone density" OR gamma-tocopherol OR alpha-tocopherol OR tocotrienols OR phylloquinone OR phytomenadione OR phytonadione OR menaquinones)". Reference lists of included articles and those relevant to the topic were hand-searched for identification of additional, potentially relevant articles.

Study Selection

We included studies comparing vitamin D levels in serum (measured as 25OH-D or 1,25OH-D) or in diet between patients with AN and HCs. Although 25OH-D is considered the most reliable marker of vitamin D status, we also included additional markers of vitamin D status, such as 1,25OH-D and dietary vitamin D intake. This was done in order to assess vitamin D status in patients with AN in a broad way due to an overall limited database. Studies were excluded if they: (1) did not use clear diagnostic criteria for AN, (2) measured only *in vitro* parameters, (3) did not measure parameters regarding vitamin D status in both patients and HCs.

We contacted authors asking for further information if: (1) data were not usable for a meta-analysis (i.e., no mean and SD or equivalent were provided for both AN and HCs), (2) no information about vitamin D supplementation was available (only in studies regarding 25OH-D). This systematic review was conducted following the strengthening the reporting of observational studies in epidemiology (STROBE) criteria for the quality assessment of included studies[28] and the indications of Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement.[29]

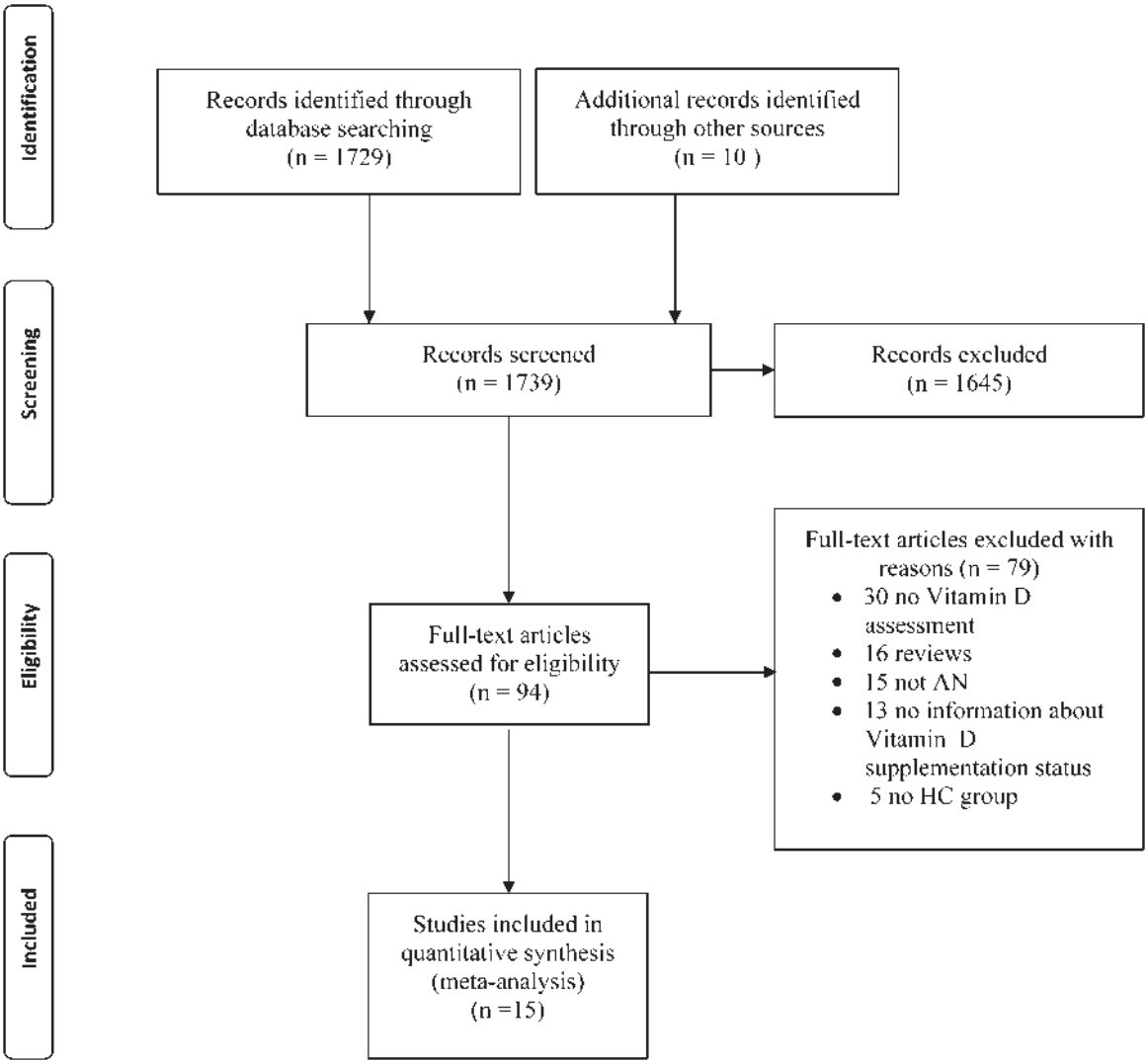
Data Extraction

Two authors (M.S. and N.V.) independently extracted data from the selected studies into a standardized Microsoft Excel spreadsheet. Any disagreement was resolved by consensus. The following information was extracted: (i) study population characteristics (e.g., sample size, demographics, comorbidities, anthropometric data), and if supplemented or not with cholecalciferol; (ii) serum 25-dihydroxyvitamin D (25OH-D) and 1,25-dihydroxyvitamin D (1,25OH-D); (iii) vitamin D introduced with diet (not considering vitamin D supplementation); and (iv) quality indicators used for the STROBE assessment.

Statistical Analysis

Because serum 25OH-D levels strongly depend on supplementation with cholecalciferol, we compared patients affected by AN with HCs according to supplementation status. Comparisons regarding 1,25OH-D were not divided

FIGURE 1 PRISMA flow diagram of study selection process.



according to this criterion, since 1,25OH-D seems to be less affected by supplementation with cholecalciferol.[30] The meta-analysis was performed using Review Manager (RevMan) Version 5.1 for Windows (Cochrane Collaboration, http://ims.cochrane.org/revman).[31] When combining studies, the random effects model[32] was used to account for study heterogeneity. For continuous data, standardized mean difference (SMD) with its 95% confidence interval (CI) was used. Study heterogeneity was measured using the chi-squared and I-squared statistics, with chi-squared $p < .05$ and I-squared $>50\%$ indicating heterogeneity.[33] Moreover, for vitamin D markers reported in ≥ 5 studies and with significant heterogeneous results (i.e., I-squared $>50\%$), we conducted meta-regression analyses to explore if differences in age, BMI, country where the study was conducted, or duration of illness were significant moderators of group differences between AN participants and HCs. Meta-regression analyses were conducted using Comprehensive Meta-Analysis V3 (http://www.meta-analysis.com/index.php). Finally, funnel plots were inspected visually to assess the possibility of publication bias.

Results

The search identified 1,739 potentially eligible studies, of which 1,645 were excluded after title and abstract review. Full texts of 94 articles were read, and references of relevant papers were screened. Altogether, 15 studies were included in the meta-analysis (**Fig. 1**).

Study and Patient Characteristics

Studies and patients characteristics are summarized in **Tables 1** and **2**. The 15 analyzed studies[21,22,34–46] included a total of 927 participants (AN = 408

TABLE 1. Studies of serum 25-hydroxyvitamin D (25OH-D) and 1,25-dihydroxyvitamin D (1,25 OHD) with or without supplementation with cholecalciferol in patients with anorexia nervosa (AN) vs. healthy controls (HC)

Author	*N;* Patients with AN	*N*; Healthy Controls (Variables Matched on)	Exclusion Criteria	Mean Age ± SD (Range); AN (Range) vs. HC (Range)	Mean; Duration of Illness (Months) (Range)	Mean BMI ± SD (Range); AN (Range) vs. HC (Range)	Supplementation with Vitamin D %)	Measurement Method
25OH-D not Supplemented with Cholecalciferol								
DiVasta, 2011; (USA)[21]	12	12	Diseases or drugs affecting bone health	AN: 19.6 ± 2.0; HC: 20.0 ± 2.4	NA	AN:16.5 ± 1.4; HC: 22.7 ± 1.0	—	Liquid chromatography tandem mass spectrometry
Fonseca, 1988; (UK)[34]	17	17	NA	AN: 23 ± 5.1; (13–47); HC: NA	51 ± 23 (6–96)	AN: NA; HC: NA	—	Competitive protein binding assay
Van Binsbergen, 1988; (The Netherlands)[35]	20	20	Age lower than 18 or over 35; amenorrhea at least 6 mo; oral contraception; other psychiatric diseases	AN:24.7 ± 3.9; HC: 25.8 ± 3.9	75 ± 23 6–144)	AN:14.4 ± 1.6; HC: 19.6 ± 2.0	—	Competitive protein binding assay
Viapiana, 2007; (Italy)[36]	55	15	Drugs affecting bone metabolism	AN:25.2 ± 6.5; HC: 24.6 ± 7.9	96.7 ± 68.0	AN:14.3 ± 1.7; HC: 22.6 ± 2.7	—	ELISA
Total; (means, SDs and percentages are weighted with n values)	104	64		AN: 24.1 ± 5.7; HC: 23.9 ± 5.7	73 ± 44	AN: 14.6 ± 1.8; HC: 21.3 ± 2.5	—	Competitive protein binding assay: 2 studies; ELISA; 1 study; liquid chromatography tandem mass spectrometry: 1 study
25OH-D supplemented with cholecalciferol								
Faje, 2012 (USA)[37]	44	25	Psychosis, suicidality, history of substance abuse, drugs affecting bone metabolism.	AN:16.7 ± 0.2; HC: 15.7 ± 0.2	NA	AN: 17.2 ± −0.2; HC: 21.1±0.6	AN=100%HC=100%	Chemiluminescent assay
Faje, 2013; (USA)[38]	21	23	Pregnancy, previous bone fractures, history of substance abuse, medications affecting bone metabolism.	AN: 19.3 ± 0.5; HC: 19.1 ± 0.2	33.1 ± 5.9	AN: 17.8 ± 0.2; HC: 22.4 ± 0.5	AN = 100%; HC = 100%	Chemiluminescent assay
Grinspoon, 1999; (USA)[39]	30	30	Abnormal TSH levels; elevated FSH, PRL or testosterone levels, a ratio LH/FSH above 2.5 were excluded.	AN: 24 ± 1; HC: 24 ± 1	NA	AN: 16.7 ± 0.3; HC: 21.1 ± 0.3	AN = 62%; HC = 13%	RIA
Haagensen, 2008; (USA)[22]	50	200	NA	AN 18.4 ± 3.9; (15–26); HC: 14.9 ± 1.6 (11–18)	NA	AN: 18.0 ± 1.6; HC: 24.2 ± 6.0	AN = 86%; HC = 14%	Competitive binding assay
Soyka, 1999; (USA)[40]	11	15	Drugs affecting bone metabolism	AN:16.0 ± 0.4; HC: 15.1 ± 0.4	37 ± 18 (2–72)	AN: 16.5 ± 0.4; HC: 21.8 ± 0.4	AN = 35%; HC = 28%.	RIA
Total (means, SDs and percentages are weighted with *n* values)	156	293		AN:18.9 ± 3.5; HC: 16.2 ± 3.2	34 ± 11	AN:17.4 ± 1.1; HC: 23.4 ± 5.1	AN:84%; (35–100%); HC: 29% (13–100%)	Chemiluminescent assay: 2 studies; RIA: 2 studies; competitive binding assay: 1 study
1,25-dihydroxyvitamin D Not Supplemented								
DiVasta, 2011; (USA)[21]	12	12			NA		—	Radioassay

TABLE 1. *Continued*

Author	*N;* Patients with AN	*N*; Healthy Controls (Variables Matched on)	Exclusion Criteria	Mean Age ± SD (Range); AN (Range) vs. HC (Range)	Mean; Duration of Illness (Months) (Range)	Mean BMI ± SD (Range); AN (Range) vs. HC (Range)	Supplementation with Vitamin D %)	Measurement Method
			Diseases or drugs affecting bone health	AN:19.6 ± 2.0; HC: 20.0 ± 2.4		AN:16.5 ± 1.4; HC: 22.7 ± 1.0		
Fonseca, 1988; (UK)[34]	17	17	NA	AN: 23 ± 5.1; (13–47); HC: NA	51 ± 23 (6–96)	AN: NA; HC: NA	—	Radioassay
Kiriike, 1992; (Japan)[41]	11	10	NA	AN: 20.1 ± 5.8; HC: 22.4 ± 2.1	32 ± 58	AN: 13.7 ± 1.5; HC: 19.5 ± 0.8	NA	RIA
Olmos, 1991; (Spain)[42]	12	22	NA	AN: 17 ± 3; (14–24); HC: NA	18 ± 14 (6–46)	AN: NA; HC: NA	NA	Radioassay
Total (means, SDs and percentages are weighted with n values)	52	61		AN:20.4 ± 5.3; HC: 22.4 ± 2.1	43 ± 31	AN: 13.7 ± 1.5; HC: 19.5 ± 0.8	two studies without Vitamin D supplementation	Radioassay: 3 studies; RIA: 1 study

Footnotes: NA = not available; AN = anorexia nervosa; HC = healthy controls; 25OH-D = 25-hydroxyvitamin D; 1,25OH-D: 1,25 dihydroxyvitamin D; RIA = radioimmunoassay.

and HCs = 519). Six studies[34–36, 42, 44, 45] (40.0%) were conducted in Europe, 8 in the US[21, 22, 37–40, 43, 46] (53.3%), and one (6.7%) was conducted in Japan. The mean age of patients with AN and of HCs was 19.47 ± 5.00 years and 17.90 ± 4.42 years, respectively. The mean body mass index (BMI) in patients and HCs was 16.16 ± 2.02 and 22.29 ± 3.98, respectively. In patients with AN, the mean duration of illness was 40.40 ± 33.49 months.

Investigated serum vitamin D parameters included 25OH-D and 1,25OH-D. For 25-OH-D, measurement methods included competitive protein binding assay (three studies[22, 34, 35]), chemiluminescent assay (two studies[37, 38]), radio-immuno assay (RIA) (two studies[39, 40]), ELISA and liquid chromatography tandem mass spectrometry (1 study each[21, 36]). The 1,25OH-D was assessed with radioassay in three studies[21, 34, 42] and with RIA in one.[41] Regarding dietary vitamin D intake, two studies used a 4-day record,[40, 43] while the remaining studies used a variety of other dietary questionnaires (food-frequency, Youth/adolescent questionnaire, and 24-h or 48-h recall).[21, 44–46]

STROBE quality indicators of cross-sectional studies (**Table 3**) indicated that three studies (20.0%)[21, 36, 45] did not provide a clear definition of AN or a clear ascertainment of vitamin D, respectively, whereas only 1 study[35] (6.7%) did not provide a clear description of handling of AN and Vitamin D in the analysis.

Serum 25 Hydroxyvitamin D

Pooling data from four cross-sectional studies regarding serum 25OH-D without supplementation[21, 34–36] (AN = 104, HC = 64), AN patients had significantly lower 25OH-D levels than HCs (SMD = −0.43; 95%CI = −0.83 to −0.03; $p = .03$, $I^2 = 30\%$) (**Fig. 2**a; **Table 1**).

Conversely, in studies with participants supplemented with cholecalciferol (AN = 84% vs. HCs = 29%), 25OH-D was significantly higher in patients with AN than in HCs (studies = 5; $n = 449$; SMD = 0.66; 95%CI: 0.01–1.31; $p = .05$; $I^2 = 87\%$)[22, 37–40] (**Fig. 2**b; **Table 1**) leading to a significant lower proportion of people with hypovitaminosis D in AN (11/115 = 10%) vs. HCs (109/248 = 44%) ($p = .04$) in the three studies with available data.[22, 37, 38]

Meta-regression Analysis for Serum 25 Hydroxyvitamin D

Because a high heterogeneity was found in the studies in which AN patients were supplemented with cholecalciferol ($I^2 = 87\%$),[22, 37–40] we conducted a meta-regression analysis finding that neither age ($p = .08$) nor BMI ($p = .21$) were significant moderators of the difference in serum 25OH-D levels between AN patients and HCs. Because all studies supplemented with cholecalciferol were conducted in the USA and only two studies reported data on illness duration,[38, 40] these moderators were not examined.

Serum 1,25-dihydroxyvitamin D

Pooling data from four cross-sectional studies[21, 34, 41, 42] (AN = 52, HCs = 61), AN patients had significantly lower serum 1,25OH-D levels than HCs (SMD = −1.06; 95%CI: −1.47 to −0.66; $p < .00001$; $I^2 = 0\%$) (**Table 1**; Fig. **3**).

Dietary Vitamin D

Pooling data from six cross-sectional studies about usual diet without cholecalciferol supplementation,[21, 43–46] (AN = 154, HCs = 160), dietary vitamin D did not differ between patients with AN and HCs (SMD = 0.33; 95%CI: −0.16 to 0.81; $p = .19$; $I^2 = 75\%$) (**Table 2**; **Fig. 4**).

Meta-regression Analysis for Dietary Vitamin D

Because a high heterogeneity was found in studies reporting usual diet ($I^2 = 75\%$),[21, 40, 43, 44] we conducted a meta-regression analysis, finding that differences in country ($p = .89$), age ($p = .54$) and BMI ($p = .45$) were not significant moderators of the of the difference in serum dietary vitamin D intake between AN patients and HCs.

Publication Bias

Inspecting the funnel plots, there did not appear to be a publication bias for any of the studied outcomes.

Discussion

In this meta-analysis, we have shown that patients with AN have significantly lower serum levels of 25OH-D and 1,25OH-D compared to HCs, while Vitamin D introduced with diet was reported to be similar between these two groups. In exploratory meta-regression analyses, neither country of study origin, differences in age or BMI between patients with AN and HCs nor study duration significantly moderated these findings. Among parameters investigating vitamin D status, serum 25OH-D is the best measure of vitamin D levels because it accounts for the main sources of vitamin D, i.e. diet and sunlight.[47] The finding that when not

TABLE 2. Studies of dietary vitamin D in patients with anorexia nervosa (AN) vs. healthy controls (HC)

Author	*N;* Patients with AN	*N*; Healthy Controls Variables (Matched on)	Exclusion Criteria	Mean Age ± SD (Range); AN (Range) vs. HC (Range)	Mean Duration of Illness (Months) (Range)	Mean BMI ± SD Range); AN (Range) vs. HC (Range)	Supplementation with Vitamin D (%)	Dietary Questionnaire
DiVasta, 2011 (USA)[21]	12	12	Diseases or drugs affecting bone health	19.6 ± 2.0 20.0 ± 2.4	NA	16.5 ± 1.4; 22.7 ± 1.0	—	Youth/adolescent Questionnaire
Misra, 2006 (USA)[43]	39	39	—	15.9 ± 0.3; 15.0 ± 0.3	11 ± 2	16.5 ± 0.2; 21.8 ± 0.5	44%; 23%	4-day record
Moreira-Varela, 1990 (Spain)[44]	43	22	—	16.5 ± 2.9; 17.3 ± 2.4	36 ± 37 (9–108)	17.4 ± 2.6; 21.3 ± 2.2	—	48-hr dietary recall
Soyka, 1999 (USA)[40]	17	18	Drugs affecting bone metabolism	16.0 ± 0.4; 15.1 ± 0.4	37 ± 18 (2–72)	16.5 ± 0.4; 21.8 ± 0.4	35%; 28%.	4-day food record + history of dietary intake over the month before
Sundgot-Borgen, 1993 (Norway)[45]	7	30	—	17 ± 1; (14–18); 21 ± 3 (13–25)	—	47.0 ± 4.6; 60.0 ± 5.6	—	24-hr dietary recall
Taylor, 2009 (USA)[46]	36	39	—	16.6 ± 0.3; 15.4 ± 0.2	—	17.4 ± 0.2; 21.4 ± 0.5	47%;15%	FFQ
Total (means, SDs and percentages are weighted with *n* values)	154	160		AN: 16.6 ± 1.9; HC: 16.9 ± 3.0	24 ± 25	AN: 16.8 ± 1.6; HC: 21.7 ± 1.0	AN: 42%; HC: 22%	4-day record: 2 studies; FFQ: 1 study; Youth/adolescent Questionnaire: 1 study; 24-h dietary recall: 1 study; 48-h dietary recall: 1 study

Footnotes: AN = anorexia nervosas; HC = healthy controls; IU = International Units; FFQ = food frequency questionnaire.

TABLE 3. Quality assessment based on guidelines from the STROBE statement

Study	Clear Description of Subject Eligibility and Sources/Methods of Subject Selection	Clearly Defined Exposure Ascertainment: Anorexia Nervosa	Clearly Defined Outcome Ascertainment: Vitamin D	Clear Description of Handling of Anorexia Nervosa and Vitamin D in the Analyses	Control for Potential Confounders by Exclusion or Statistical Adjustment
DiVasta, 2011[21]	X	X	X	X	X
Faje, 2012[37]	X	X	X	X	X
Faje, 2013[38]	X	X	X	X	X
Fonseca, 1988[34]	X			X	X
Grinspoon, 1999[39]	X	X		X	X
Haagensen, 2008[22]	X	X	X	X	X
Kiriike, 1992[41]	X	X		X	X
Misra, 2006[43]	X	X	X	X	X
Moreiras-Varela, 1990[44]	X	X	X	X	X
Olmos, 1991[42]	X	X	X	X	X
Soyka, 1999[40]	X	X	X	X	X
Sundgot-Borgen, 1993[45]	X		X	X	X
Taylor, 2009[46]	X	X	X	X	X
Van Birsbesgen, 1988[35]	X	X	X		X
Viapiana, 2007[36]	X		X	X	X
Total	15/15 (100%)	12/15 (80.0%)	12/15 (80.0%)	14/15 (93.3%)	15/15 (100%)

STROBE: strengthening the reporting of observational studies in epidemiology.

supplemented, patients with AN have significantly lower serum 25OH-D concentrations than HCs is seemingly paradoxical, since patients with AN reported similar dietary Vitamin D intake in our meta-analysis, since these patients have usually higher physical activity levels compared to age-matched HCs,[48] and since vitamin D is a fat soluble hormone. Because people with AN have decreased stores of fat mass, they should have higher circulating 25OH-D concentrations.[22, 49]

Several reasons could explain these findings. First, it is largely known that patients with AN tend to overestimate their food intake which could result in similar dietary vitamin D intake between ANs and HCs.[21] Although it has been proposed that patients with AN provide accurate self-reports of food,[50] studies comparing self-reported dietary intake with energy intake measured by a trained dietician suggest that patients with AN are prone to over-estimate their dietary intake.[51, 52] This bias can lead to an inconsistent evaluation of micronutrient intake. Moreover, not all physical activities have similar effects on maintaining optimal levels of 25OH-D, with outdoor leisure activities seeming to be particularly effective in this regard.[53] It is possible that patients with AN spend more time

FIGURE 2 Serum 25-hydroxyvitamin D (25OH-D) in patients with AN vs. healthy controls. (a) Without supplementation with cholecalciferol. (b) With supplementation with cholecalciferol. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

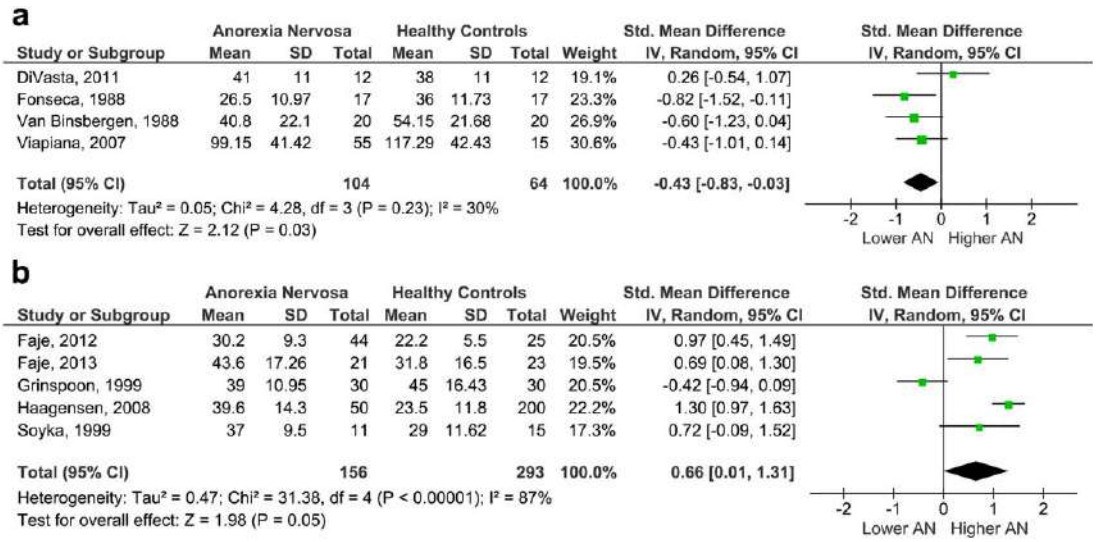
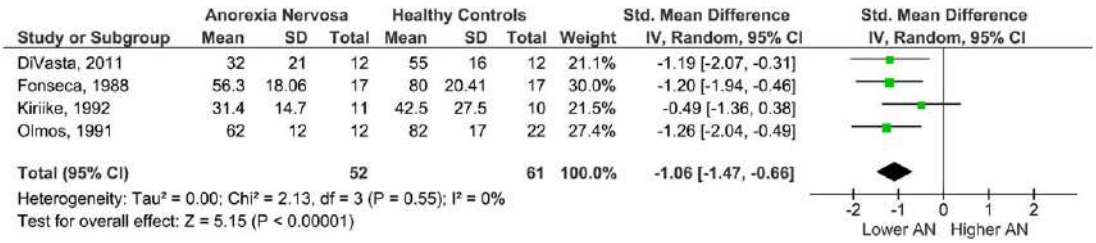


FIGURE 3 1,25-dihydroxyvitamin D (1,25OH-D) in patients with AN vs. healthy controls. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

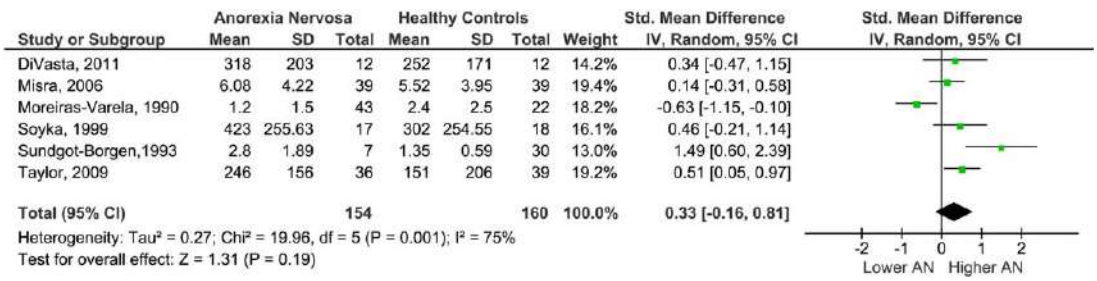


performing indoor than outdoor activities, or that they wear covering clothes minimizing light exposure having greater shame sensations and reduced thermogenesis than age-matched adolescents, resulting in lower serum 25OH-D.[54] Finally, even if low serum 25OH-D is a condition typical of obese people related to higher fat mass, increasing research has shown that low serum 25OH-D is also associated with emaciated states, like undernutrition, neoplastic cachexia, and AN.[22, 55, 56] Notably, low serum 25OH-D levels have been associated with both high, but also with low BMI values, suggesting that in particular conditions the relationship between vitamin D and fat mass may have an inverted U-shape.[22, 57] Therefore, our work supports the idea that in patients with AN supplementation with cholecalciferol can be useful to reverse the vitamin D deficit in serum, which is essential in order to avoid/counter bone loss. When supplemented with cholecalciferol, in fact, young women with AN had significantly higher serum 25OH-D concentrations than HCs, likely due to the anthropometric characteristics cited before and maybe due to higher use of non-caloric supplementations than in other adolescents.[22]

Low serum 25OH-D levels may lead to the bone loss that is typical of AN resulting in lower bone mineral density and the higher frequency of clinical and nonclinical fractures compared to healthy adolescents.[3, 58, 59] Therefore, given the crucial role of vitamin D in bone metabolism, vitamin D deficiencies should be corrected if patients do not abide by recommended intake values.[58] However, since cholecalciferol supplementation alone is not able to significantly increase bone mineral density as shown by a recent meta-analysis of general population subjects,[60] this intervention alone should not be considered sufficient by clinicians treating individuals with AN. Rather, other features, such as deficit in insulin growth factor-1, leptin and estrogens, might also strongly contribute to the imbalance in bone formation and resorption, finally leading to a significant impairment in both trabecular and cortical microarchitecture.[4] In addition, low serum 25OH-D concentrations are associated with mood disorders and suicide, co-morbidities strongly related to AN.[16–20] Unfortunately, even if there is increasing research about extra-skeletal effects of low vitamin D, these studies were mainly performed in post-menopausal women, and no specific studies are available in AN.

Finally, patients with AN also had lower serum levels of the active form of Vitamin D, i.e., 1,25-dihydroxyvitamin D. Serum levels of 1,25-dihyroxyvitamin D have little relationship to 25OH-D stores are being regulated primarily by parathyroid

FIGURE 4 Dietary vitamin D in patients with AN vs. healthy controls. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



hormone (PTH) levels: as part of a low serum 25OH-D status, the active form of vitamin D usually increases, instead of decreasing, as we observed in patients with AN.[61] Both 25 and 1,25-dihyroxyvitamin D decrease in the presence of some medical conditions, like hypoparathyroidism or end-stage kidney disease, mediated by an impairment in the renal 1-alpha hydroxylase. A possible explanation of this 25 and 1,25-dihyroxyvitamin D mismatch in AN is that AN patients usually have low serum estrogens levels and these hormones seem to be important agonists of the 1-alpha hydroxylase.[62]

The findings of our work should be considered in the context of its limitations. The main limitation of the study is the cross-sectional design of the meta-analyzed studies, as well as the often small sample size. Moreover, we had to exclude 5 potentially eligible studies with a HC group and 12 studies without a HC group because of lacking data regarding Vitamin D supplementation status. Furthermore, we were unable to conduct a meta-analysis of the prevalence of hypovitaminosis D in AN patients without supplementation because only one study[21] reported this categorical result, and other authors did not reply when we attempted to obtain this information. Another important limitation is that due to the still relatively low number of available studies, we pooled serum 25OH-D data without considering the different methods of measurement, although the methods of the measurement can influence 25OH-D levels.[63] Furthermore, in the evaluation of serum 25OH-D levels in supplemented patients, no distinction was made between vitamin D obtained with diet or from supplementation. Finally, data were lacking about daily dietary intake of Vitamin D in relationship to whether AN was newly diagnosed, in treatment or recovered, which may impact on dietary habits. Nevertheless, despite these limitations, this is the first meta-analysis of the important area of vitamin D dysfunction in AN compared to HCs that has overcome prior limitations of results from small and often inconclusive individual studies.

In conclusion, although AN patients subjectively reported comparable dietary vitamin D intake compared to HCs, patients with AN had significantly lower serum 25OH-D and 1,25OH-D levels without supplementation. Supplementation with cholecalciferol seems to normalize serum 25OH-D to values to levels similar to HCs. Future longitudinal and interventional studies tailored for patients with AN are needed in order to identify the role of vitamin D dysfunction and the potential utility of correction in the long-term outcomes of specific subgroups of AN patients.

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