High-dose vitamin D-3 reduces circulating hepcidin concentrations: A pilot, randomized, double-blind, placebo-controlled trial in healthy adults

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High-dose vitamin D₃ reduces circulating hepcidin concentrations: A pilot, randomized, double-blind, placebo-controlled trial in healthy adults

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Abstract

Background & Aims—In vitro studies suggest that vitamin D may reduce hepcidin expression and pro-inflammatory cytokine release from monocytes. However, data assessing the vitamin D-mediated effects on iron recycling in healthy individuals are lacking. We aimed to examine the effect of high-dose vitamin D₃ on plasma hepcidin, inflammatory cytokine, and ferritin concentrations in healthy adults.

Methods—This was a pilot, double-blind, placebo-controlled trial in healthy adults (N=28) randomized to receive a one-time oral dose of 250,000 IU of vitamin D₃ or placebo. Between- and within-group differences in plasma hepcidin, pro-inflammatory cytokine [interleukin (IL)-1β, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1)], and ferritin concentrations at baseline and 1 week were determined using two-sample and paired t-tests, respectively.
Results—At baseline, plasma 25-hydroxyvitamin D [25(OH)D], hepcidin, pro-inflammatory cytokine, and ferritin concentrations did not differ between the two groups, and greater than 70% of subjects in both groups were vitamin D deficient (25(OH)D < 20 ng/mL). After 1 week, plasma hepcidin concentrations decreased by 73% from baseline in those who received vitamin D₃ (geometric mean ratio [GMR] = 0.27 (95% CI: 0.11-0.62); P = 0.005); there was no significant change in the placebo group (GMR = 0.73 (95% CI: 0.49-1.09); P = 0.11). Plasma cytokine and ferritin concentrations did not change significantly in either group.

Conclusions—High-dose vitamin D₃ significantly reduced plasma hepcidin concentrations in healthy adults 1 week post-dosing, without a change in plasma pro-inflammatory cytokine or ferritin concentrations. These data suggest that vitamin D may have a role in regulating iron recycling by acting independently of changes in pro-inflammatory markers.

Keywords
vitamin D; hepcidin; inflammation; anemia; iron

1. Introduction

Vitamin D deficiency and anemia are both prominent nutrition-related public health concerns. In the United States, it has been reported that 32% of adults have vitamin D deficiency as defined by 25-hydroxyvitamin D [25(OH)D] concentrations < 20 ng/mL [1]. In 2010, it was estimated that nearly one third of the global population had anemia [2]. Co-existence of vitamin D deficiency and anemia is not uncommon, as poor diets and illness are contributing factors to both conditions [3, 4], and chronic diseases, including chronic kidney disease (CKD) and cardiovascular disease, incur high rates of both [5-8]. Recently, vitamin D deficiency was identified as a potential risk factor for anemia, particularly anemia of inflammation, in the general population [9, 10].

Anemia of inflammation may develop due to disturbances in iron recycling secondary to pro-inflammatory cytokine-induced increases in the hepatic production of hepcidin, the major iron-regulatory hormone [11]. Elevations in hepcidin promote iron sequestration within cells of the reticuloendothelial system, thus limiting iron availability for erythropoiesis and hemoglobin synthesis [12]. Recent in vitro studies suggest a role for vitamin D in down-regulating both pro-inflammatory cytokines and hepcidin [13]. Treatment of cultured human monocytes with vitamin D has been shown to decrease the release of pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-1β (IL-1β) and down-regulate the expression of hepcidin mRNA [14, 15]. Furthermore, the hepcidin antimicrobial peptide (HAMP) gene has been found to contain a vitamin D response element, suggesting a mechanism for transcriptional regulation of hepcidin by vitamin D [15].

Despite the strong biological plausibility for the association between vitamin D status and anemia, rigorous randomized controlled trials examining the effect of high-dose vitamin D supplementation on specific biomarkers involved in the pathophysiology of anemia of inflammation, namely pro-inflammatory cytokines and hepcidin, are lacking. It remains unclear whether vitamin D-mediated effects on iron recycling occur due to reductions in
inflammation or through direct action on hepcidin expression. Moreover, the down-stream effects of vitamin D on makers of iron status have not yet been elucidated. Therefore, the purpose of this study was to examine the acute effect of high-dose vitamin D₃ supplementation on plasma hepcidin, inflammatory cytokine, and ferritin concentrations in healthy adults to better understand the mechanism by which vitamin D may influence iron recycling. We hypothesized that treatment with vitamin D₃ would reduce circulating hepcidin and plasma inflammatory cytokine concentrations, and increase plasma ferritin concentrations.

2. Materials and Methods

2.1. Subjects and Protocol

Subjects were participants in a double-blind, randomized, placebo-controlled trial designed to evaluate the impact of a large bolus dose of vitamin D₃ given prior to winter, on 25(OH)D concentrations year round in healthy adults [16]. Briefly, adults between the ages of 18 and 65 who were healthy by self-report were recruited from the Emory University campus in Atlanta, GA between August and December 2012. Participants were excluded if they were currently pregnant or breastfeeding, had granulomatous conditions, a history of kidney or liver disease, diabetes, a history of malignancy, thyrotoxicosis, a history of calcium or bone abnormalities including hyperparathyroidism, osteoporosis, and Paget's disease, an inability to ambulate, an intake of greater than 1000 mg/day of calcium, and/or used medications including antihypertensives, barbituates, anticonvulsants, or steroids. Sex, race, height, weight, time spent outdoors, and vitamin D supplement use were collected via participant self-report. Participants were asked to refrain from taking any additional vitamin D supplementation during the course of the study. A total of 28 participants were randomized to receive a one-time oral bolus dose of 250,000 IU of vitamin D₃ (Biotech Pharmacal, Fayetteville, AR) or matching placebo (Biotech Pharmacal, Fayetteville, AR). This study was approved by the Emory University Institutional Review Board, and is registered at clinicaltrials.gov (NCT01924910). All participants provided written informed consent upon enrollment. The current study uses samples drawn from participants at baseline (n=14 in the vitamin D group; n=14 in the placebo group) and approximately 1 week (5-10 days) later (n=13 in the vitamin D group; n=11 in the placebo group).

2.2. Analytical procedures

Plasma 25(OH)D concentrations were determined using an automated chemiluminescent technique (IDS-iSYS automated machine, Immunodiagnostic Systems, Inc., Fountain Hills, AZ), as previously described [16]. Plasma pro-inflammatory cytokines, interleukin (IL)-1β, IL-6, and IL-8, were measured using a high-sensitivity magnetic bead-based Luminex Performance Assay multiplex kit (R&D Systems, Minneapolis, MN) with a Bioplex analyzer (Bio-Rad, Hercules, CA). Plasma monocyte chemoattractant protein-1 (MCP-1) concentrations were assayed using a bead-based Luminex Performance assay kit (R&D Systems, Minneapolis, MN) on a Bioplex analyzer (Bio-Rad, Hercules, CA). Plasma ferritin concentrations were determined via ELISA (ab108698 – Ferritin Human ELISA kit, Abcam Inc., Cambridge, MA) following manufacturer instructions; the intra-assay CV was 2.48%. A ferritin cut-off value of < 12 ng/mL was used to define low iron stores [17].
Plasma hepcidin concentrations were determined using an electrochemiluminescence immunoassay as previously described [14, 18, 19]. Briefly, streptavidin-coated and blocked 96-well plates were incubated with 25 μL of biotin-labeled capture antibody (4 μg/mL) for 1 hour. Plasma samples were diluted 1:50 in assay buffer, added to their respective washed wells, and incubated at room temperature for 1 hour. Captured plasma hepcidin was detected with 25 μL of 0.1 μg/mL ruthenium-labeled conjugate hepcidin-specific detection antibody, and hepcidin concentrations were interpolated against a standard curve of reference standard hepcidin (Eli Lilly and Company, Indianapolis, IN, USA) [18, 19].

2.3. Statistical Analyses

Descriptive statistics were performed for all variables and reported as mean ± SD or geometric mean (95% confidence interval (CI)) for continuous variables, and number (%) for categorical variables. Variables which were not normally distributed were transformed to the natural logarithmic scale; in the case of variables with values of zero (IL-1β and IL-6), a constant of 0.01 was added to all non-missing values prior to log-transformation. Baseline comparisons between the vitamin D and placebo groups were examined using two sample t-tests for continuous variables, and χ² or Fisher's exact test for categorical variables. Between- and within-group differences in plasma 25(OH)D, cytokine, ferritin, and hepcidin concentrations from baseline to 1 week were evaluated using two sample independent t-tests, and paired t-tests, respectively. For variables requiring log-transformation, the results were back-transformed so as to be expressed in the original unit of measurement, as geometric means and their corresponding 95% CI. The mean differences between groups and between time points of the log-transformed data were exponentiated (back-transformed) to generate geometric mean ratios (GMR). A GMR of 1 indicates no treatment effect. All analyses were performed in SAS version 9.4 (SAS Institute Inc, Cary, NC) using a two-sided P-value of 0.05 to define statistical significance.

3. Results

3.1. Participant characteristics

Baseline demographic and biochemical characteristics for this study population are shown in Table 1. This was a young and predominantly female cohort. The mean body mass index (BMI) was within the normal range for both groups. Most participants were Caucasian and reported spending less than 10 hours outdoors per week. Very few participants (n=5 total) reported regular intake of vitamin D supplements prior to the start of the study. Baseline demographic and health status characteristics did not differ significantly between vitamin D and placebo groups. As previously reported [16], geometric mean baseline plasma 25(OH)D concentrations for the vitamin D and placebo groups were in the vitamin D deficient range with greater than 70% of participants in either group having a baseline 25(OH)D concentration < 20 ng/mL. Plasma 25(OH)D concentrations increased 150% relative to baseline after 1 week in the group that received high-dose vitamin D₃, compared to no significant change in the placebo group [GMR = 2.5 (95% CI: 2.0-3.0), P < 0.001 for the vitamin D group vs. GMR = 1.1 (95% CI: 0.9-1.1), P = 0.38 for the placebo group]. Baseline geometric mean ferritin concentrations in both groups were greater than the cut-off value of 12 ng/mL used to define low iron stores. Plasma pro-inflammatory cytokine, hepcidin, and
ferritin concentrations did not differ significantly between vitamin D and placebo groups at baseline.

3.2. Effect of high-dose vitamin D on pro-inflammatory cytokine concentrations

Geometric means of plasma IL-6, IL-1β, IL-8, and MCP-1 concentrations with their corresponding 95% confidence intervals at baseline and approximately 1 week later are shown in Fig. 1. For any of the plasma cytokines, geometric means did not differ significantly between vitamin D and placebo groups 1 week after dosing ($P = 0.23$-$0.87$). Likewise, there were no significant differences in plasma cytokines within groups from baseline to 1 week ($P = 0.14$-$0.99$).

3.3. Effect of high-dose vitamin D on plasma hepcidin concentrations

Plasma hepcidin concentrations are shown in Fig. 2. There were no significant differences between groups at baseline [GMR = 0.72 (95% CI: 0.29, 1.80), $P = 0.47$]. By the 1 week time point plasma hepcidin concentrations were 73% lower in the vitamin D group relative to the placebo group [GMR = 0.27 (95% CI: 0.08-0.96), $P = 0.04$]. The within-group difference was also statistically significant in the vitamin D group such that hepcidin decreased by 73% from baseline to 1 week [GMR = 0.27 (95% CI: 0.11-0.62); $P = 0.005$]. There was no significant change in hepcidin from baseline to 1 week in the placebo group [GMR = 0.73 (95% CI: 0.49-1.09), $P = 0.11$].

3.4. Effect of high-dose vitamin D on plasma ferritin concentrations

Plasma ferritin concentrations are shown in Fig. 3. Baseline and 1 week ferritin concentrations did not differ significantly between the vitamin D and placebo groups ($P = 0.35$ and $P = 0.44$, respectively). Neither group had significant changes in plasma ferritin concentrations from baseline to 1 week ($P = 0.95$; $P = 0.55$, for vitamin D and placebo groups, respectively).

4. Discussion

In this cohort of healthy adults with a high prevalence of vitamin D deficiency, treatment with a single large dose of vitamin D$_3$ significantly reduced plasma hepcidin concentrations after 1 week, but did not have a statistically significant effect on plasma concentrations of pro-inflammatory cytokines or ferritin. To our knowledge, this study is the first to evaluate the effect of high-dose vitamin D supplementation on markers involved in the pathophysiology of anemia of inflammation in healthy adults in the context of a randomized controlled trial. Mechanisms by which vitamin D has been proposed to influence iron recycling and anemia are summarized in Fig. 4. Our findings suggest that even in the absence of inflammatory conditions, vitamin D may act directly on hepcidin, independent of inflammatory cytokines.

While few studies have explored the association of vitamin D with hepcidin, our findings are consistent with the data of Bacchetta et al. [15] in which seven healthy volunteers given 100,000 IU vitamin D$_3$ had a 33% reduction in hepcidin concentrations 72 hours post-dosing. Similarly, in patients with CKD, treatment with vitamin D has been associated with...
reductions in hepcidin mRNA expression in peritoneal macrophages [20] and circulating hepcidin concentrations [14]. Results from observational studies have been mixed. In a study of pregnant adolescents, circulating 25(OH)D concentrations were not associated with hepcidin concentrations [21], while a study in CKD patients showed that lower levels of 1,25(OH)2D were associated with increased hepcidin concentrations [22]. Taken together, our data and the previous reports provide evidence for hepcidin-lowering by vitamin D as a potential mechanism by which vitamin D may influence iron recycling. The recent discovery of a vitamin D response element on the HAMP gene also lends strong biological plausibility to our results [15]. Further long-term studies are needed to establish the clinical implications of inflammation-independent changes in circulating hepcidin concentrations.

Concentrations of pro-inflammatory cytokines did not change significantly in response to high-dose vitamin D supplementation in this healthy cohort. This is in contrast to epidemiologic studies, which have reported increased odds of anemia of inflammation with lower vitamin D status [9, 10]. Vitamin D has also been shown to have anti-inflammatory effects in various in vitro studies, observational studies, and clinical trials [14, 23]. However, these studies have largely been conducted in populations with chronic conditions or infections, where elevations in pro-inflammatory cytokines are more likely to be observed. In utilizing healthy volunteers for this study, we were able to demonstrate that vitamin D may affect hepcidin concentrations even without altering inflammatory cytokine concentrations.

Despite the significant reduction in hepcidin, circulating ferritin concentrations were unchanged in response to high-dose vitamin D3 supplementation. However, given the physiological response to iron recycling in the presence of elevated hepcidin concentrations, this is not entirely unexpected. Any effects of hepcidin reduction on iron egress from cells and iron bioavailability may have been better captured as transport iron, using markers such as serum iron, total iron binding capacity, and transferrin saturation as opposed to ferritin, the storage form of iron [12, 24]. In a previous observational study, we found that 25(OH)D status was significantly positively associated with hemoglobin and serum iron concentrations in a cohort of generally healthy adults, while serum ferritin concentrations did not differ between participants who were vitamin D deficient and those who were not [10]. Similarly, Thomas et al. [21] found that 25(OH)D status was significantly positively associated with hemoglobin concentrations and serum iron among pregnant adolescents, but did not observe a significant association between vitamin D status and ferritin concentrations.

Our findings may have potential implications for vitamin D as a therapy in combatting anemia. As hepcidin concentrations have been inversely associated with hemoglobin concentrations and positively associated with risk for anemia [25], reducing hepcidin levels in the blood may be a target for anemia therapies. However, very few trials have directly evaluated the effect of vitamin D treatment on hemoglobin concentrations or anemia. Among those that have, the results have been mixed, likely due to the differences in dosage and form of vitamin D administered, and the population studied [26-28]. In studies of patients with CKD, vitamin D or its analogues have been shown to increase hemoglobin concentrations [27, 29-31]. Larger studies examining the effect of vitamin D administration
on circulating hepcidin concentrations, and the subsequent impact on hemoglobin concentrations or anemia in both healthy and diseased populations at risk for anemia are necessary.

A major strength of this study was the double-blind, randomized, placebo-controlled clinical trial design. Also, the healthy, young study population allowed us to ascertain the effects of vitamin D on iron recycling in the absence of potentially confounding conditions. However, we were limited in that we were unable to measure hemoglobin concentrations in our study participants to determine the influence of vitamin D treatment on hemoglobin and anemia status. Given the high prevalence of vitamin D deficiency in this population, the generalizability of our results to other populations with a lower prevalence of vitamin D deficiency may be limited. However, as a pilot efficacy study, the high prevalence of vitamin D deficiency in this population may have been advantageous in allowing us to ascertain the impact of vitamin D therapy on our outcomes. Other limitations of this study include the lack of measures of iron status other than ferritin. With our relatively short duration of observation and the small sample size we were possibly underpowered to detect changes in inflammatory markers and ferritin, and the generalizability of our findings may be limited. However, the fact that we observed a statistically significant reduction in hepcidin concentrations in response to vitamin D supplementation in spite of the short duration and small sample size suggests a robust effect of vitamin D on hepcidin concentrations, and lends merit to our findings.

In summary, this pilot study addresses a gap in the literature related to the mechanism underlying the association between vitamin D status and anemia observed in several epidemiologic studies. Supplementation with high-dose vitamin D\textsubscript{3} significantly reduced circulating hepcidin concentrations after 1 week among healthy adults without chronic or inflammatory disease and independent of circulating cytokine markers of inflammation. The down-stream effects of vitamin D on markers of iron status and anemia require further examination in larger studies of longer duration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References


**Abbreviations**

1. 25(OH)2D, 1,25-dihydroxyvitamin D

2. 25(OH)D, 25-hydroxyvitamin D

3. BMI, body mass index

4. CKD, chronic kidney disease

5. GMR, geometric mean ratio

6. IL-1β, interleukin-1β

7. IL-6, interleukin-6

8. IL-8, interleukin-8

9. MCP-1, monocyte chemoattractant protein-1
Fig. 1.
Geometric means and 95% confidence intervals for plasma IL-1β, IL-6, IL-8, and MCP-1 concentrations at baseline and 1 week in the placebo- and vitamin D-treated subjects.
Concentrations of IL-1β (A), IL-6 (B), IL-8 (C), and MCP-1 (D) did not differ between vitamin D and placebo groups at baseline or 1 week, and there were no significant changes in concentrations of any of the cytokines from baseline to 1 week in either treatment group. Specific results for each cytokine are list below. IL-1β (A), difference between groups at 1 week: GMR = 0.56 (95% CI: 0.21-1.50), P = 0.23; within group difference from baseline to 1 week: GMR = 0.86 (95% CI: 0.55-1.33), P = 0.46; GMR = 0.92 (95% CI: 0.69-1.23), P = 0.53, for the vitamin D and placebo groups, respectively. IL-6 (B), difference between groups at 1 week: GMR = 0.88 (95% CI: 0.46-1.68), P = 0.69; within group difference from baseline to 1 week: GMR = 0.93 (95% CI: 0.77-1.12), P = 0.40; GMR = 0.80 (95% CI: 0.58-1.09), P = 0.14, for vitamin D and placebo groups, respectively. IL-8 (C), difference between groups at 1 week: GMR = 1.04 (95% CI: 0.74-1.48), P = 0.80; within group difference from baseline to 1 week: GMR = 1.05 (95% CI: 0.79-1.39), P = 0.73; GMR = 0.94 (95% CI: 0.75-1.17), P = 0.52, for vitamin D and placebo, respectively. MCP-1 (D), difference between groups at 1 week: GMR = 1.02 (95% CI: 0.81-1.29), P = 0.87; within group difference from baseline to 1 week: GMR = 1.00 (95% CI: 0.89-1.12), P = 0.99; GMR = 0.96 (95% CI: 0.89-1.05), P = 0.34, for vitamin D and placebo groups, respectively.
Fig. 2.  
Geometric means and 95% confidence intervals of plasma hepcidin concentrations at baseline and 1 week in the placebo- and vitamin D-treated subjects. Plasma hepcidin concentrations did not differ between groups at baseline (GMR = 0.72 (95% CI: 0.29, 1.80), P = 0.47). Plasma concentrations of hepcidin in the vitamin D and placebo groups differed significantly at 1 week (2.4 ng/mL (95% CI: 0.8-7.4) for the vitamin D group and 9.0 ng/mL (95% CI: 4.8-16.7) for placebo; GMR = 0.27 (95% CI: 0.08-0.96), P = 0.04). Plasma hepcidin concentrations in the vitamin D group decreased significantly from baseline values after 1 week (GMR = 0.27 (95% CI: 0.11-0.62), P = 0.005); there was no significant change from baseline in the placebo group (GMR = 0.73 (95% CI: 0.49-1.09), P = 0.11). **P < 0.01, *P < 0.05.
Fig. 3.
Geometric mean plasma ferritin concentrations with their 95% confidence intervals at baseline and 1 week in the placebo- and vitamin D-treated subjects. Vitamin D and placebo groups did not differ in plasma ferritin concentrations at baseline [GMR = 0.68 (95% CI: 0.29-1.57), \( P = 0.35 \)] or 1 week post-dosing [GMR = 0.71 (95% CI: 0.29-1.75), \( P = 0.44 \)]. Neither group had significant changes in plasma ferritin concentrations from baseline to 1 week [GMR = 0.99 (95% CI: 0.83-1.19), \( P = 0.95 \); GMR = 0.95 (95% CI: 0.80-1.14), \( P = 0.55 \), for vitamin D and placebo groups, respectively].
Fig. 4.
Proposed Role of Vitamin D in Enhancing Iron Recycling. Iron recycling, under non-pathologic conditions, involves transferrin-bound iron in circulation traveling to the bone marrow to support erythropoiesis. Upon senescence, red blood cells (RBCs) are engulfed by macrophages and iron is recycled back into circulation to support further erythropoiesis. Dietary iron may also enter the circulating pool from absorption in the duodenum based on the body’s needs. Elevations in pro-inflammatory cytokines suppress erythropoiesis in the bone marrow and shorten RBC lifespan due to increased macrophage activation and erythrophagocytosis. Pro-inflammatory cytokines IL-6 and IL-1β also stimulate the liver to up-regulate the expression of hepcidin antimicrobial peptide (HAMP). Hepcidin inhibits iron egress from cells of the reticuloendothelial system, including enterocytes and macrophages, by binding to and inducing the degradation of the cellular iron exporter, ferroportin, resulting in decreased iron absorption from the small intestine, and increased iron sequestration within the macrophage. Vitamin D has been shown to promote erythropoiesis and iron recycling by increasing erythroid progenitor proliferation, decreasing pro-inflammatory cytokines, and suppressing hepcidin expression. The results from the current study demonstrate that treatment with vitamin D may directly reduce circulating hepcidin concentrations independent of changes in inflammatory cytokines. Decreases in pro-inflammatory cytokines and hepcidin may increase iron bioavailability for erythropoiesis and hemoglobin synthesis by preventing iron sequestration in macrophages, and removing impairments on iron absorption, thus restoring iron recycling.
Figure adapted with permission from [13] Smith EM and Tangpricha V. Vitamin D and anemia: insights into an emerging association. Curr Opin Endocrinol Diabetes Obes 2015, 22:432–38.
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<th></th>
<th>Vitamin D (n = 14)</th>
<th>Placebo (n = 14)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Age (years), mean ± SD</td>
<td>28.2 ± 6.7</td>
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<td>Female, n (%)</td>
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<td>Hours spent outdoors per week&lt;sup&gt;b&lt;/sup&gt;, mean ± SD</td>
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<td>7.0 ± 5.4</td>
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<td>4 (28.6)</td>
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<td>Plasma 25-hydroxvitamin D [25(OH)D] (ng/mL)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.6 (13.6-20.2)</td>
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<td>Plasma 25(OH)D &lt; 20 ng/mL, n (%)</td>
<td>10 (71.4)</td>
<td>11 (78.6)</td>
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<td>Plasma ferritin (ng/mL)&lt;sup&gt;de&lt;/sup&gt;</td>
<td>24.5 (12.9-46.4)</td>
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<td>Plasma IL-1&lt;beta&gt; (pg/mL)&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Plasma MCP-1 (pg/mL)&lt;sup&gt;d&lt;/sup&gt;</td>
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Abbreviations: IL-1β: interleukin-1β; IL-6: interleukin-6; IL-8: interleukin-8; MCP-1: monocyte chemoattractant protein-1

<sup>a</sup> Two-sample t-test for continuous variables, χ<sup>2</sup> or Fisher's exact test for categorical variables

<sup>b</sup> n=13 for vitamin D group

<sup>c</sup> reported dosages ranged from 400-1000 IU/day

<sup>d</sup> geometric mean (95% confidence interval)

<sup>e</sup> n=13 for vitamin D group, n=11 for placebo group

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