# Vitamin D Status Is Associated with Hepcidin and Hemoglobin Concentrations in Children with Inflammatory Bowel Disease

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**Background:** Anemia, iron deficiency, and hypovitaminosis D are well-known comorbidities in inflammatory bowel disease (IBD). Epidemiologic studies have linked vitamin D deficiency with increased risk of anemia, and in vitro studies suggest that vitamin D may improve iron recycling through downregulatory effects on hepcidin and proinflammatory cytokines.

**Methods:** We aimed to investigate the association of vitamin D status with inflammation, iron biomarkers, and anemia in pediatric IBD. Cross-sectional data were obtained from N = 69 patients with IBD aged 5 to <19 years. Iron biomarkers (ferritin, soluble transferrin receptor), and 25-hydroxyvitamin D (25(OH)D), inflammatory biomarkers (C-reactive protein and α-1-acid glycoprotein), hepcidin, and hemoglobin were collected. Iron biomarkers were regression corrected for inflammation. Multivariable logistic/linear models were used to examine the associations of 25(OH)D with inflammation, iron status, hepcidin, and anemia.

**Results:** Approximately 50% of subjects were inflamed (C-reactive protein >5 mg/L or α-1-acid glycoprotein >1 g/L). Iron deficiency prevalence (inflammation-corrected ferritin <15 μg/L or soluble transferrin receptor >8.3 mg/L) was 67%; anemia was 36%, and vitamin D insufficiency (25(OH)D <30 ng/mL) was 77%. In linear regression models, vitamin D insufficiency was associated with increased hepcidin levels (β [SE] = 0.6 [0.2], $P = 0.01$) and reduced hemoglobin (β [SE] = −0.9 [0.5], $P = 0.046$), controlling for age, sex, race, insurance status, body mass index for age, inflammation, disease diagnosis (ulcerative colitis versus Crohn's disease), and disease duration, compared with 25 (OH)D ≥30 ng/mL.

**Conclusions:** Our results suggest that concentrations of 25(OH)D ≥30 ng/mL are associated with lower hepcidin and higher hemoglobin levels. Further research is needed to clarify the association of vitamin D with inflammation, iron status, and anemia in pediatric IBD.

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**Key Words:** iron deficiency, anemia, inflammatory bowel disease, vitamin D, hepcidin

Inflammatory bowel disease (IBD) is a chronic intestinal condition consisting of Crohn's disease or ulcerative colitis and is currently thought to develop as a result of an inappropriate immune response to an environmental stimulus in genetically susceptible individuals.[1,2] Patients with IBD may experience several nutrition-related complications affecting the quality of life and overall health. These include growth failure, weight loss, and nutrient deficiencies resulting from inadequate dietary intake and malabsorption.[2,3] Another such complication of IBD is anemia. Recent studies have reported that the prevalence of

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anemia among children at IBD diagnosis is 55% to 72%.[4–6] Major contributors to anemia in IBD include inflammation and nutrient deficiencies, namely iron deficiency.[3,6] More recently, vitamin D deficiency, which is common in IBD,[7,8] has also been linked to anemia.[9–13]

Epidemiologic studies in other chronic disease populations including chronic kidney disease and heart disease have shown vitamin D status to be positively associated with hemoglobin and inversely associated with odds of anemia.[10,13,14] Studies in generally healthy adults and the elderly have further characterized this association, suggesting that the association between vitamin D and anemia may be specific to anemia of inflammation.[12,15] Indeed, the mechanism underlying this association is likely related to the influence of vitamin D on proinflammatory cytokines and hepcidin, the major iron regulatory hormone.[16] Proinflammatory cytokines that are often elevated in IBD[17,18] stimulate hepatic hepcidin expression, and subsequent elevations in hepcidin may have deleterious effects on iron recycling because of decreased iron absorption from the small intestine and iron sequestration within macrophages.[18,19] This may lead to reduced iron bioavailability to support hemoglobin synthesis and erythropoiesis; should the inflammatory stimulus persist, anemia may occur.[19,20]

In vitro studies have shown vitamin D to decrease hepcidin-stimulatory proinflammatory cytokines and act directly on the hepcidin antimicrobial peptide gene to lower hepcidin mRNA expression.[21,22] Moreover, recent studies from our group also found that treatment with high-dose vitamin D reduced circulating hepcidin concentrations in healthy adults[23] and increased hemoglobin concentrations in critically ill adults.[24] The anti-inflammatory and hepcidin-lowering effects of vitamin D may therefore increase iron bioavailability for erythropoiesis and hemoglobin synthesis, possibly improving anemia in individuals with vitamin D insufficiency.

Although the association between vitamin D and anemia has been described in other disease populations,[10,13,25] the association between vitamin D, iron status, inflammation, and anemia has not been well characterized in patients with IBD, a condition characterized by high rates of anemia and inflammation. Therefore, the aims of this paper were to (1) explore the associations of vitamin D with markers of inflammation and hepcidin; and (2) determine whether vitamin D status was associated with hemoglobin and anemia in children with IBD. We hypothesized that vitamin D status assessed by plasma 25-hydroxyvitamin D (25 (OH)D) concentrations would be inversely associated with inflammation, hepcidin, odds of anemia and positively associated with hemoglobin concentrations.

## MATERIALS AND METHODS

### Study Population

Subjects included in this analysis were part of a cross-sectional study designed to investigate the association of inflammation with novel iron biomarkers in children with IBD.[26] Briefly, children presenting to the pediatric IBD clinics, outpatient infusion clinics, emergency department, and the inpatient gastroenterology service at Children's Healthcare of Atlanta between May and November 2014 were screened for eligibility. Our inclusion criteria were as follows[1]: 5 to 18 years of age[2] and confirmed diagnosis of IBD (Crohn's disease, ulcerative colitis, or IBD unclassified) by a pediatric gastroenterologist. Patients were excluded based on the following criteria[1]: any surgeries or infection requiring hospitalization within a 1-month period before entry,[2] inherited blood disorders (thalassemia, sickle cell anemia, or trait),[3] receipt of packed red blood cell infusion within 120 days of study enrollment,[4] pregnancy,[5] and mean corpuscular volume (MCV) >100 fL. Given that the parent study was designed to investigate the association of anemia and iron biomarkers with inflammation, we stratified our enrollment using clinical laboratory testing in the month before the study visit and enrolled subjects such that they would be categorized equally as follows: with/without inflammation (using C-reactive protein [CRP] >5 mg/L) and with/without iron deficiency (using MCV <75 fL/cell or elevated RDW >14.5% or presence of anemia). Of the children screened to be included in the study, n = 2 had recent blood transfusion and 1 had an MCV >100. The remaining exclusions were because we had already enrolled healthy (not inflamed and not iron deficient) subjects and were screening per our stratified enrollment for subjects who were inflamed and/or iron deficient (Fig. 1).

### Sample Collection and Laboratory Assays

Venous blood was collected from all subjects at the time of enrollment in a K2EDTA-coated tube (Beckman Dickinson). Whole blood samples of 3 to 5 mL each were immediately sent for the following laboratory tests per standard clinical protocol: complete blood count (Advia 2120 and 120; Siemens, Erlangen, Germany) and comprehensive metabolic panel (Vista 500; Siemens). Plasma was

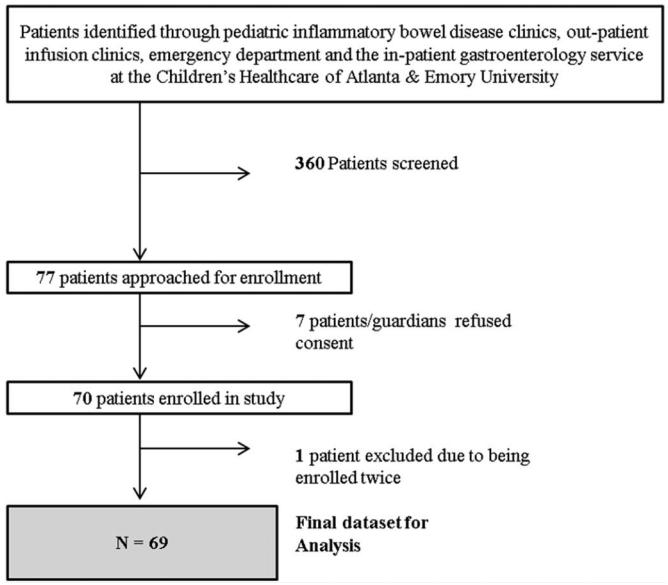


FIGURE 1. Flow of study participants during 6-month enrollment period from May 2014 to November 2014.

obtained by centrifuging the tube according to the manufacturer's specifications and then aliquoted and stored at −80°C. Frozen samples were shipped to the VitMin laboratory (Willstatt, Germany) for measurement of ferritin, CRP, α-1-acid glycoprotein (AGP), soluble transferrin receptor (sTfR), and retinol-binding protein using a novel sandwich enzyme-linked immunosorbent assay (ELISA) technique.[27] Plasma hepcidin concentrations were determined using the Human Hepcidin ELISA kit (TSZ Scientific) at Emory University according to the manufacturer's instructions. The average coefficient of variation of hepcidin among 11 subsamples was 18.8% (SD: 18.1, range: 1.1%–64.6%). An automated chemiluminescent technique (Automated IDS-iSYS System; Immunodiagnostic Systems, Fountain Hills, Arizona) was used to measure plasma 25(OH)D concentrations in a laboratory which participates in the vitamin D external quality assessment scheme (DEQAS, site 606) and the NIST/NIH Vitamin D Metabolites Quality Assurance Program (VitDQAP).

## Assessment of Nutrition and Health Status

Measures of anthropometrics included measurements of weight and height using standardized techniques by trained clinical nurses. World Health Organization Child Growth Standards (World Health Organization Anthro, Geneva, Switzerland) were used to calculate age- and sex-adjusted z-scores for anthropometric measurements. Further categorization of z-scores was as follows: stunting as a height-for-age z-score $<-2$, wasting as body mass index for age z-score (BAZ) $<-2$, overweight as a BAZ $>2$, and obesity as a BAZ $>3$. The following thresholds were used to define abnormal values for these biochemical indicators: (1) iron deficiency: ferritin $<15$ mg/L,[28] sTFR $>8.3$ mg/L[29] and (2) vitamin D insufficiency: 25(OH)D $<30$ ng/mL[30]; anemia was defined using the following thresholds as per World Health Organization guidelines,[31] Hb $<11.5$ g/mL for children aged 5 to 11.99 years, Hb $<12.0$ g/mL for children aged 12 to 14.99 years, Hb $<12.0$ g/mL for females aged $\geq 15.0$ years, and Hb $<13.0$ g/mL for males aged $\geq 15.0$ years. CRP and AGP are commonly measured acute phase proteins that assess the presence of inflammation. CRP concentrations increase quickly in response to an acute insult, peaking at approximately 48 hours and decreasing within a week with a half-life of 19 hours. In contrast, AGP concentrations increase more slowly and remain elevated for a longer period of time.[32] Taken together, CRP and AGP measurements can be used to classify individuals who have inflammation spanning from incubation, early convalescence to late convalescence. Therefore, we explored the association of inflammation with growth by defining systemic inflammation as a composite variable of either CRP $>5$ mg/L[33] or AGP $>1.0$ g/L.[34] The following data on each participant were collected: demographics (age, sex, and self-reported race), socioeconomic status as measured by insurance status (state-provided Medicaid or private insurance), and clinical disease information (disease type, duration of disease, disease location, history of previous surgeries, disease activity, and previous and current medical therapy).

## Statistical Methods

Descriptive statistics were evaluated for all variables and presented as means ± SD or median (interquartile range) for continuous variables and as "n" (percent) for categorical variables. Differences in study variables by vitamin D status (dichotomized as plasma 25(OH)D $\geq 30$ ng/mL compared with plasma 25(OH)D $<30$ ng/mL) were examined using 2-sample independent $t$ tests for normally distributed continuous variables, Wilcoxon–Mann–Whitney tests for nonnormally distributed continuous variables, and $\chi^2$ or Fisher's exact tests for categorical variables. In the absence of the gold standard for iron deficiency as defined by Prussian blue staining of bone marrow iron stores, we used regression modeling to adjust ferritin and sTfR for inflammation, the methods of which have been previously published.[26,35] Briefly, adjustment was performed using the following equation: adjusted ferritin/sTfR = unadjusted ferritin/sTfR $- \beta_1 (CRP_{obs} - CRP_{ref}) - \beta_2 (AGP_{obs} - AGP_{ref})$, where a CRP and AGP reference value (e.g., maximum of lowest decile) was used so that ferritin/sTfR was not overadjusted at the lower values for CRP and AGP.

Simple linear regression was used to examine the unadjusted association of vitamin D status with continuous outcomes, hepcidin, and hemoglobin. Multivariable linear regression was used to further evaluate the association between vitamin D status (independent variable) with hepcidin and hemoglobin (dependent variables), with age, sex, race, Medicare status, BAZ, inflammation, disease diagnosis (Crohn's disease and ulcerative colitis), and disease duration included as covariates. Hepcidin was the only nonnormally distributed continuous variable used in our regression analysis and was log transformed. Logistic regression was used to evaluate the association between vitamin D status (independent variable) and binary outcomes, inflammation, and anemia (dependent variables), with similar adjustment for a priori covariates listed above. For the anemia models, we evaluated the interaction between vitamin D status and race, given literature suggesting differences in vitamin D status and anemia prevalence by race.[12,36–38] Statistical analyses were performed using SAS v. 9.4 (SAS Institute Inc., Cary, NC), with a 2-sided alpha of 0.05 used to define statistical significance.

## Ethical Considerations and Institutional Oversight

This interventional clinical study was conducted in accordance with the principles of the Declaration of Helsinki and with appropriate approval and oversight by the Institutional Review Boards of Emory University and Children's Healthcare of Atlanta.

## Access to Study Data

All authors had access to the study data and have reviewed and approved this final manuscript.

# RESULTS

## Participant Characteristics

Demographic and clinical characteristics of the study population as a whole and stratified by 25(OH)D status (25(OH)D $\geq 30$ ng/mL versus $<30$ ng/mL) are summarized in Table 1. With the exception of stunting, the demographic,

**TABLE 1.** Demographic, Anthropometric, and Clinical Characteristics of the Study Population, n = 69

| Characteristics | Total Population, Mean ± SD, or n (%) | 25(OH)D ≥30 ng/mL (n = 16), Mean ± SD, or n (%) | 25(OH)D <30 ng/mL (n = 53), Mean ± SD, or n (%) | $P^a$ |
|---|---|---|---|---|
| Demographics | | | | |
| Age, yrs | 15 ± 3 | 14 ± 4 | 15 ± 3 | 0.34 |
| Age, yrs | | | | |
| 5 to <10 | 7 (10) | 4 (6) | 3 (4) | 0.05 |
| 10–18 | 62 (90) | 12 (17) | 50 (72) | |
| Sex | | | | |
| Females | 31 (45) | 7 (44) | 24 (45) | 0.91 |
| Race | | | | |
| African American | 38 (55) | 7 (10) | 31 (45) | 0.23 |
| Asian | 3 (4) | 1 (1) | 2 (3) | |
| Caucasian | 27 (39) | 7 (10) | 20 (29) | |
| Hispanic | 1 (1) | 1 (1) | 0 (0) | |
| Insurance | | | | |
| Medicaid | 17 (25) | 4 (6) | 13 (19) | 1.00 |
| Private insurance | 52 (75) | 12 (17) | 40 (58) | |
| Anthropometrics | | | | |
| Stunting (HAZ < −2) | 4 (6) | 3 (19) | 1 (2) | 0.04 |
| Wasting (BAZ < −2) | 3 (4) | 1 (6) | 2 (4) | 0.55 |
| Overweight (BAZ >2) | 7 (10) | 0 (0) | 7 (13) | 0.19 |
| Clinical features | | | | |
| Disease type | | | | |
| Crohn's disease | 49 (71) | 9 (13) | 40 (58) | 0.21 |
| Ulcerative colitis | 20 (29) | 7 (10) | 13 (19) | |
| Disease location | | | | |
| Ileocolon | 32 (46) | 6 (9) | 26 (38) | 0.67 |
| Colon | 4 (6) | 1 (1) | 3 (4) | |
| Colon and upper GI | 7 (10) | 2 (3) | 5 (7) | |
| Pancolitis (ulcerative colitis) | 15 (22) | 5 (7) | 10 (14) | |
| TI | 5 (7) | 0 (0) | 5 (7) | |
| TI and upper GI | 1 (1) | 0 (0) | 1 (1) | |
| Left sided (ulcerative colitis) | 5 (7) | 2 (3) | 3 (4) | |
| Current medication(s) | | | | |
| Anti-TNF | 42 (61) | 9 (13) | 33 (48) | 0.50 |
| Anti-TNF + 5-ASA | 1(1) | 1 (1) | 0 (0) | |
| Anti-TNF + MTX | 3 (4) | 1 (1) | 2 (3) | |
| Anti-TNF + thiopurine | 1 (1) | 1 (1) | 0 (0) | |
| CST | 3 (4) | 0 (0) | 3 (4) | |
| CST + 5-ASA | 2 (3) | 1 (1) | 1 (1) | |
| CST + Anti-TNF | 1 (1) | 0 (0) | 1 (1) | |
| CST + thiopurine + 5-ASA | 1 (1) | 0 (0) | 1 (1) | |
| Thiopurine | 6 (9) | 1 (1) | 5 (7) | |
| Thiopurine + 5-ASA | 2 (3) | 0 (0) | 2 (3) | |
| 5-ASA | 3 (4) | 1 (1) | 2 (3) | |
| None | 4 (6) | 1 (1) | 3 (4) | |

**TABLE 1.** (*Continued*)

| Characteristics | Total Population, Mean ± SD, or n (%) | 25(OH)D ≥30 ng/mL (n = 16), Mean ± SD, or n (%) | 25(OH)D <30 ng/mL (n = 53), Mean ± SD, or n (%) | $P^a$ |
|---|---|---|---|---|
| Before surgery | | | | |
| No surgery | 49 (71) | 13 (19) | 36 (52) | 0.24 |
| Anal surgery | 4 (6) | 1 (1) | 3 (4) | |
| Colonic resection | 3 (4) | 0 (0) | 3 (4) | |
| Ileocolonic resection | 12 (17) | 1 (1) | 11 (16) | |
| Ileocolonic resection + anal surgery | 1 (1) | 1 (1) | 0 (0) | |
| Duration of disease, yrs | 3 ± 3 | 3 ± 2 | 3 ± 2.6 | 0.71 |

[a]Two-sample independent *t* tests for normally distributed continuous variables, Wilcoxon–Mann–Whitney tests for nonnormally distributed continuous variables, and $\chi^2$ or Fisher's exact tests for categorical variables, comparing 25(OH)D ≥30 ng/mL with 25(OH)D <30 ng/mL.
ASA, amino salicylic acid; CST, corticosteroids; GI, gastrointestinal; HAZ, height for age z-score; MTX, methotrexate; TI, terminal ileum; TNF, tumor necrosis factor.

anthropometric, and clinical characteristics of our study population did not differ significantly by vitamin D status.

## Inflammation, Iron Deficiency, Anemia, and Vitamin D Status

Nutritional and inflammation status of the study population is summarized in Table 2 (see Table 1, Supplemental Digital Content 1, http://links.lww.com/IBD/B543). The prevalence of vitamin D deficiency (25(OH)D <20 ng/mL) was approximately 38% (n = 26) and vitamin D insufficiency (25(OH)D <30 ng/mL) was approximately 77% (n = 53) (see Table 1, Supplemental Digital Content 1, http://links.lww.com/IBD/B543). As would be expected with our stratified enrollment, nearly 50% of our subjects were inflamed (elevated CRP or AGP), and the prevalence of anemia was 36% (n = 25). Iron deficiency (using inflammation-adjusted biomarkers) was common whether measured by low ferritin or elevated TfR or both, affecting approximately 67% (n = 46) subjects; 19 patients (28%) had iron deficiency anemia.

**TABLE 2.** Nutrient Status in the Study Population, n = 69

| Biomarkers as Categorical Variables | Total Population, n (%) | 25(OH)D ≥30 ng/mL (n = 16), n (%) | 25(OH)D <30 ng/mL (n = 53), n (%) | $P^a$ |
|---|---|---|---|---|
| Iron deficiency (biomarkers not corrected for inflammation) | | | | |
| Low ferritin <15 μg/L | 22 (32%) | 7 (44%) | 15 (28%) | 0.25 |
| Elevated TfR >8.3 mg/L | 28 (41%) | 8 (50%) | 20 (38%) | 0.38 |
| Low ferritin or elevated TfR | 36 (52%) | 10 (63%) | 26 (49%) | 0.35 |
| Iron deficiency (biomarkers regression correction for inflammation) | | | | |
| Low ferritin <15 μg/L | 31 (50%) | 11 (16%) | 22 (32%) | 0.06 |
| Elevated TfR >8.3 mg/L | 28 (45%) | 9 (13%) | 22 (32%) | 0.29 |
| Low ferritin or elevated TfR | 46 (67%) | 13 (19%) | 33 (48%) | 0.16 |
| Inflammation | | | | |
| Elevated CRP >5 mg/L | 28 (41%) | 9 (56%) | 19 (36%) | 0.15 |
| Elevated AGP >1.0 g/L | 30 (44%) | 10 (63%) | 20 (38%) | 0.08 |
| Elevated CRP or AGP | 34 (49%) | 11 (69%) | 23 (43%) | 0.08 |
| Hepcidin, ng/mL, median (interquartile range) | 608 (491) | 414 (412) | 661.6 (423) | 0.09 |
| Anemia | | | | |
| All | 25 (36%) | 5 (31%) | 20 (38%) | 0.64 |
| Iron deficiency anemia | 19 (28%) | 4 (25%) | 15 (28%) | 0.25 |

[a]Wilcoxon–Mann–Whitney tests for nonnormally distributed continuous variables, and $\chi^2$ or Fisher's exact tests for categorical variables, comparing 25(OH)D ≥30 ng/mL with 25(OH)D <30 ng/mL.

Among the 25 anemic patients, 76% had iron deficiency anemia, 80% were African American, 72% were inflamed (see Table 2, Supplemental Digital Content 2, http://links.lww.com/IBD/B543), and 60% had duration of disease ≥2 years. There was no significant difference in the prevalence of anemia by vitamin D insufficiency status ($P = 0.64$).

## Associations of Vitamin D Status with Inflammation and Hepcidin

In a multivariable logistic model, 25(OH)D concentrations <30 ng/mL were not significantly associated with inflammation (defined as CRP >5 mg/L or AGP >1.0 g/L) ($P = 0.14$). Those with 25(OH)D concentrations <30 ng/mL had significantly higher plasma hepcidin concentrations (Table 3) controlling for age, sex, race, inflammation, insurance, BAZ score, and disease duration (β [SE] = 0.6 [0.2], $P = 0.01$), compared with those with plasma 25(OH)D concentrations ≥30 ng/mL (Fig. 2). Both models (hepcidin and inflammation as outcomes) were also dichotomized by plasma 25(OH)D cutoff points for vitamin D deficiency (25(OH)D <20 ng/mL versus ≥20 ng/mL), but no significant associations were observed ($P = 0.13$ and $P = 0.39$, respectively).

## Associations of Vitamin D Status with Hemoglobin and Anemia

Those with plasma 25(OH)D concentrations <30 ng/mL had significantly lower hemoglobin concentrations, compared with those with 25(OH)D ≥30 ng/mL, (Table 3) controlling for age, sex, race, insurance, BAZ score, and disease duration (β [SE] = −0.9 [0.5]), $P = 0.046$. In multivariable logistic models, 25(OH)D concentrations <30 ng/mL were associated with increased odds of anemia compared with 25(OH)D concentrations ≥30 ng/mL (OR 3.2; 95% confidence interval, 0.5–22.7), but this was not statistically significant ($P = 0.24$). The interaction between race and 25(OH)D status in the fully adjusted model with anemia as the outcome was not statistically significant ($P = 0.63$). Both models (hemoglobin and anemia as outcomes) were dichotomized by plasma 25(OH)D cutoff points for vitamin D deficiency (25(OH)D <20 ng/mL versus ≥20 ng/mL), but no significant associations were observed ($P = 0.17$ and $P = 0.95$, respectively).

# DISCUSSION

This study investigated the potential associations of vitamin D status with inflammation, hepcidin, hemoglobin, and anemia in a chronically inflamed population of children with IBD. Notable findings included[1]: plasma 25(OH)D concentrations <30 ng/mL were associated with elevations in hepcidin, compared with 25(OH)D concentrations ≥30 ng/mL[2]; plasma 25(OH)D concentrations <30 ng/mL were associated with decreased hemoglobin, compared with those with 25(OH)D concentrations ≥30 ng/mL[3]; No significant associations were observed between 25(OH)D concentrations and either inflammation or anemia.

The hepcidin results presented are consistent with in vitro and clinical studies in humans. In a series of in vitro studies, our group demonstrated that treatment with $1,25(OH)_2D$, the active form of vitamin D can downregulate hepcidin mRNA expression and upregulate ferroportin, the cellular iron exporter, in cultured human monocytes.[22] Bacchetta et al[21] have also shown that treatment of hepatocytes and monocytes with both 25(OH)D and $1,25(OH)_2D$ resulted in decreased expression of hepcidin mRNA. This group subsequently identified a vitamin D response element on the hepcidin antimicrobial peptide gene, lending strong biological plausibility of the association between vitamin D and hepcidin. Furthermore, treatment with high-dose vitamin D has been found

**TABLE 3.** Association of 25(OH)D <30 ng/mL with Inflammation, Anemia, Hepcidin, and Hemoglobin

| Serum 25(OH)D <30 ng/mL (Y Versus N) | | | | |
|---|---|---|---|---|
| Logistic Regression, Outcome Variable | Odds Ratio (95% Confidence Interval) | *P* | Adj Odds Ratio (95% Confidence Interval)[a] | *P* |
| Inflammation[b] | 0.3 (0.1–1.1) | 0.08 | 0.4 (0.1–1.4) | 0.14 |
| Anemia | 1.3 (0.4–4.4) | 0.64 | 3.2 (0.5–22.7) | 0.24 |
| **Serum 25(OH)D <30 ng/mL (Y Versus N)** | | | | |
| Linear Regression, Outcome Variable | Beta (SE) | *P* | Adj Beta (SE)[a] | *P* |
| Hepcidin[c] | 0.3 (0.2) | 0.14 | 0.6 (0.2) | 0.01 |
| Hemoglobin | −0.2 (0.6) | 0.63 | −0.9 (0.5) | 0.046 |

n = 69.

[a]Multivariable regression models for inflammation, anemia, hepcidin, and hemoglobin outcomes with parameter estimates comparing 25(OH)D concentrations <30 ng/mL versus ≥30 ng/mL, adjusted for the following covariates—age, sex, race (AA versus all others), inflammation, insurance (Medicaid versus all others), BAZ, disease duration, and diagnosis (ulcerative colitis versus Crohn's disease).

[b]CRP >5 mg/L or AGP >1.0 g/L, inflammation not included as covariate in model.

[c]Variable log transformed.

AA, African American.

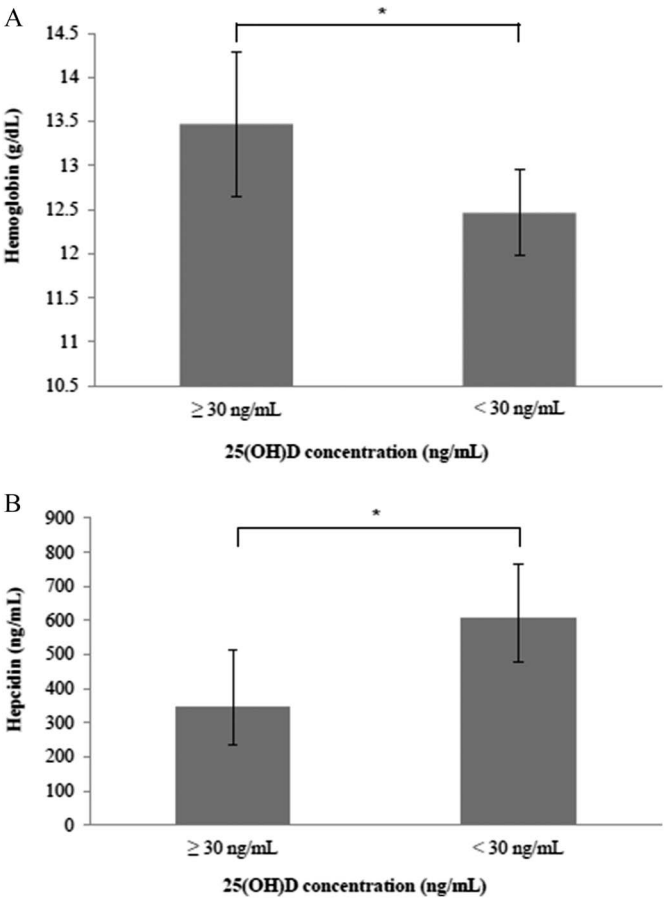


FIGURE 2. Hemoglobin (A) and hepcidin (B) concentrations with corresponding 95% confidence intervals, by 25(OH)D concentration. Panel A shows least-squares mean hemoglobin concentration controlling for age, sex, race, Medicaid status, BAZ, inflammation, and disease duration, by 25(OH)D concentration. Those with 25(OH)D concentrations <30 ng/mL had significantly lower hemoglobin concentrations compared with those with 25(OH)D concentrations ≥30 ng/mL ($P = 0.03$). Panel B shows the geometric mean hepcidin concentrations controlling for age, sex, race, Medicaid status, BAZ, inflammation, and disease duration, by 25(OH)D concentration. Those with 25(OH)D concentrations <30 ng/mL had significantly higher serum hepcidin concentrations compared with those with 25(OH)D concentrations ≥30 ng/mL ($P = 0.01$). *$P < 0.05$.

to significantly reduce circulating hepcidin concentrations among healthy adults.[23] The results from the current study suggest that an inverse association between vitamin D status and hepcidin may exist in the pediatric IBD population as well.

Plasma 25(OH)D concentrations <30 ng/mL were also associated with reduced hemoglobin concentrations, compared with 25(OH)D concentrations ≥30 ng/mL, in the current study. This is consistent with several studies in chronic kidney disease, cardiovascular disease, and the general population, which have described a positive association between vitamin D status and hemoglobin and/or an inverse association between vitamin D status and anemia.[9–13,15,39,40] Data from clinical trials have been mixed,[39–42] but recent studies in chronic kidney disease and critically ill adult patient populations found that treatment with vitamin D or its analogues resulted in significant increases in hemoglobin concentrations.[24,43] This association has not been previously studied in IBD animal models or clinical studies. Our finding that vitamin D insufficiency was associated with increased hepcidin concentrations and reduced hemoglobin concentrations supports the hepcidin-lowering role of vitamin D in improving iron recycling in a pediatric IBD population.

We report a difference of approximately 1 unit in hemoglobin (1 g/dL) between those with plasma 25(OH)D concentrations <30 ng/mL versus those with ≥30 ng/mL. Although this magnitude is small, literature would suggest that it is clinically meaningful, especially in a population where improvements in hemoglobin are hard to achieve. In patients with IBD, Ananthakrishnan et al[44] have reported hemoglobin below 9 g/dL (HR 2.51; 95% confidence interval, 1.23–5.15) as a predictor of severe Clostridium difficile infection. In pregnant women, combining risk estimates from individual studies, Stoltzfus et al[45] reported a 1 g/dL increase in pregnancy hemoglobin being associated with a 25% reduction in maternal mortality (OR = 0.75; 95% confidence interval, 0.62–0.89). Similarly, Scott et al[46] estimate 1.8 million deaths in children aged 28 days to 5 years could be avoided each year by increasing Hb in these children by 1 g/dL.

Although we found an association between vitamin D insufficiency and hemoglobin levels, there was no association between vitamin D insufficiency and anemia. Epidemiologic studies in chronic kidney disease patients receiving hemodialysis have had varying results. In a cross-sectional study analyzing the relationship between 25(OH)D and inflammatory markers in hemodialysis patients, Mohiuddin et al[47] reported no significant association between 25(OH)D levels and hemoglobin levels. However, Bednarek-Skublewska et al[48] reported a significant positive correlation between 25(OH)D and hemoglobin in a similar cohort of hemodialysis patients. Several additional studies in chronic kidney disease, cardiovascular disease, and the general population have also described a positive association between vitamin D status and hemoglobin and/or an inverse association between vitamin D status and anemia.[9–13,15,49,50] We hypothesize our findings of a significant association of vitamin D status with hemoglobin but not with anemia may be reflective of a threshold effect in the relationship between 25(OH)D status and hemoglobin, such that the likelihood of having anemia plateaus once hemoglobin levels reach a particular value.

In addition, our small sample size precluded assessment of associations of vitamin D status with different subtypes of anemia including iron deficiency anemia and anemia of inflammation. Previous studies have suggested that the association between vitamin D status and anemia may be specific to anemia of inflammation,[12,15] which is consistent with the mechanism of action of vitamin D in iron recycling described above. However, iron deficiency anemia has been described as more prevalent in pediatric IBD populations than anemia of

inflammation.[6] Thus, a low prevalence of anemia of inflammation relative to iron deficiency anemia may explain the lack of association between vitamin D status and anemia in this study. Indeed, despite the significant association between vitamin D insufficiency and hepcidin concentrations, we did not observe a significant association between vitamin D status and markers of inflammation measured in this study (CRP and AGP). Further research in the pediatric IBD population is needed to clarify the relationship between vitamin D, inflammation, hepcidin, and anemia.

Strengths of our study were inclusion of study participants from across the IBD severity spectrum, measurement of hepcidin as a key intermediary of the effect of inflammation on iron stores and subsequent anemia, use of inflammation-adjusted estimates of iron deficiency, and our novel research question to investigate the association of vitamin D levels and iron status in the pediatric IBD population. However, there were several important limitations. First, the cross-sectional study design precludes us from establishing temporality between our biomarkers and making causal conclusions regarding our observed associations. One potential limitation of this analysis is that data on vitamin D supplementation among study participants were unavailable, preventing us from assessing whether our outcomes differed by supplementation status or dose. Further limitations include our lack of measurements of "gold standard" iron status by bone marrow biopsy and measures of enteric inflammation such as fecal calprotectin. In addition, of the 360 patients screened, even though our eligibility criteria were broad with many more subjects eligible for enrollment, because of stratified enrollment, only 77 patients were included in our study. Our results may therefore lack generalizability to pediatric patients with IBD with characteristics different than those included in our study.

In conclusion, we found that plasma 25(OH)D concentrations $<30$ ng/mL were associated with increased hepcidin concentrations and reduced hemoglobin concentrations, compared with plasma 25(OH)D concentrations $\geq 30$ ng/mL, in this population of pediatric patients with IBD. Our findings suggest that achieving vitamin D sufficiency may result in improved hemoglobin concentrations in this pediatric IBD population, possibly through vitamin D-mediated reductions in hepcidin. Further research is warranted to assess the therapeutic effect of vitamin D in increasing hemoglobin concentrations and to clarify the association of vitamin D with inflammation, iron status, and anemia in the pediatric IBD population.

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