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Pilot Study of the Effect of Cholecalciferol Supplementation on Hepcidin in Children with Chronic Kidney Disease: Results of the D-fense Trial

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Abstract

Background—Hepcidin is a key mediator of the anemia of chronic kidney disease (CKD). There is emerging evidence that 25-hydroxyvitamin D (25D) regulates hepcidin production.

Methods—A randomized controlled trial of daily vitamin D supplementation for 12 weeks testing the effects of 4000 vs. 400 IU of cholecalciferol on serum hepcidin levels in children with non-dialysis CKD recruited at a tertiary care children's hospital. Hepcidin was quantified using a validated competitive enzyme-linked immunosorbent assay. 25D levels were measured using the chemiluminescence Liaison 25(OH)D assay system. Co-variables included hemoglobin, C-reactive protein, ferritin, and serum calcium and phosphorus for safety monitoring.

Results—Thirty-four subjects were randomized; 26.5% female, mean (SD) age 10.9 (5.8) years, 23.5% African American, mean (SD) baseline glomerular filtration rate was 60 (17.6) ml/min/1.73m², and mean (SD) baseline 25D level was 29.7 (11.5) ng/ml. Fifty percent of subjects were 25D deficient at baseline. There were no significant differences in baseline characteristics between the intervention and controls. Treatment with 4000 IU cholecalciferol was not associated with a significant change in hepcidin level at 4 or 12 weeks, and multivariable generalized estimating equation regression demonstrated no significant difference in change in hepcidin over the treatment period in either arm. Median CRP decreased significantly at 12 weeks in the intervention group.

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DISCLOSURES

None

Conclusions—These results do not suggest that daily nutritional vitamin D supplementation modifies serum hepcidin levels in children with CKD. Further study will be required to determine whether supplementation may be effective in children with more advanced CKD or on dialysis.

Keywords

Anemia; Hepcidin; Vitamin D; Cholecalciferol; Children; Chronic Kidney Disease

Introduction

The iron-regulatory protein hepcidin has emerged as a potentially modifiable mediator of the anemia of CKD, including erythropoiesis stimulating agent (ESA)-resistant anemia. Although ESAs are effective for raising hemoglobin, clinical trials in adults have demonstrated that escalating ESA dose is associated with an increased risk for adverse cardiovascular events, stroke, and death [1,2], and more than 20% of children with advanced CKD demonstrate hypo-responsiveness to ESA therapy [3]. There is a need for lower-toxicity, alternative anemia therapies to adjunctively treat the anemia of CKD. Modification of hepcidin is an attractive potential target for this therapy.

Hepcidin, encoded by the *HAMP* gene, is synthesized as an 84 amino acid prepropeptide in the liver and then cleaved by the prohormone convertase furin to create the active 25 amino acid form [4]. Hepcidin production in humans is down-regulated by hypoxia and anemia and up-regulated by iron-loading and inflammation [5–7]. Hepcidin regulates intestinal iron absorption and body iron distribution through its post-translational suppression of cell-membrane ferroportin expression [4]. When bound by hepcidin, ferroportin is internalized and degraded, leading to down-regulation of dietary iron absorption by intestinal enterocytes and inhibition of the release of intracellular (in hepatocytes, monocytes) iron stored in ferritin for utilization in erythropoiesis [8–10]. The primary mechanism for clearance of circulating hepcidin is glomerular filtration and proteolysis in the proximal tubule, which is decreased in the setting of reduced glomerular filtration rate (GFR) [11]. Hepcidin levels are elevated in both adults and children with CKD and on dialysis [10,12,13], and a recent analysis conducted within the Chronic Kidney Disease in Children (CKiD) longitudinal cohort study showed that in children with non-dialysis CKD, increased hepcidin levels are associated with decreasing hemoglobin and increased risk for incident anemia [14].

Vitamin D has emerged as a potential novel regulator of hepcidin production, with localized 25-hydroxyvitamin D (25D) metabolism modulating cytokine production [15,16]. Treatment of hepatocytes or monocytes with 25D has been shown to suppress *HAMP* expression *in vitro*, and high-dose vitamin D2/ergocalciferol supplementation has been shown to decrease circulating hepcidin levels in healthy subjects [17]. However, no study has tested the effect of daily nutritional vitamin D supplementation on hepcidin levels in children with CKD. We conducted a randomized, controlled trial to evaluate the efficacy of cholecalciferol supplementation to modify serum hepcidin levels in children with non-dialysis CKD.

Research Design and Methods

Study Design and Study Population

This study was approved by the Johns Hopkins Institutional Review Boards. All participants 18 years and older provided written informed consent in adherence with the Declaration of Helsinki. For participants < 18 years, written informed consent was provided by a parent/legal guardian. We performed a single-center, open-label, randomized, controlled trial of nutritional vitamin D3 supplementation as cholecalciferol at a dose of 4000 IU/day vs. the estimated average requirement (EAR) of 400 IU/day [18]. The recruitment period was June 2012 – December 2014 in the Johns Hopkins outpatient pediatric nephrology clinic via in-person approach of eligible subjects by a study coordinator during a routine clinic visit. The total treatment period was 12 weeks, with visits at baseline, 4, and 12 weeks. The clinicaltrials.gov registration number was NCT01532349.

Inclusion criteria were age ≥ 1 and < 21 years, and a clinical diagnosis of stage 2–5 non-dialysis CKD with estimated glomerular filtration rate (eGFR) between 15 and 90 ml/min/1.73m² using the bedside Schwartz equation [19]. Nutritional vitamin D supplements were discontinued at randomization when applicable and replaced with the study drug. Medication data including prescription of ESAs and iron supplements was collected for all subjects, and doses remained stable throughout the study period. Exclusion criteria were history of hypercalcemia or nephrolithiasis; gastrointestinal tract discontinuity; current pregnancy or pregnancy within the last 12 months; kidney, liver, or heart transplant within the last 12 months; history of sickle cell anemia or thalassemia; or children with 25D levels < 5 ng/ml or > 60 ng/ml at baseline.

Randomization and Interventions

The intervention model was parallel assignment. Randomized assignments were generated using block stratification and a pseudorandom number generator. Intervention subjects were provided with a 4-week supply of 4000 IU/day cholecalciferol tablets at the baseline visit and an additional 8-week supply of tablets at the 4 week visit. Controls received 400 IU cholecalciferol tablets and received appropriate quantities at the 4 and 8 week visits. If unable to tolerate tablets, participants in both arms were supplied with Enfamil™ D-Vi-Sol™ 400 IU/ml liquid in the appropriate dose and volume. Randomization and drug dispensation were handled by the Pharmacy and Investigational Drug service at The Johns Hopkins Hospital. All participants, study staff, and analytic team members were masked to the randomization sequence.

Data Collection and Study Outcomes

Participants provided blood samples at each visit, and urine samples at the baseline and 4 week visits. Serum aliquots were stored at –80°C. Serum hepcidin was the primary outcome variable and was quantified at all visits. Previously frozen serum samples were batch shipped overnight to Intrinsic Lifesciences (La Jolla, California) and hepcidin-25 was quantified using a validated competitive enzyme-linked immunosorbent assay (ELISA), with sensitivity of 5 ng/mL and interassay variability of 12%, in a single laboratory [20]. Hemoglobin was a secondary outcome and was measured locally at Johns Hopkins. Serum

vitamin D concentrations were measured in the Immunology Laboratory at the Johns Hopkins Hospital using the fully automated chemiluminescence immunoassay (DiaSorin, Stillwater, MN; www.diasorin.com), which measures both D2 and D3 and is reported as a total serum 25D concentration. The intraassay coefficient of variation was less than 10% [21]. C-reactive protein (CRP) as a marker of inflammation (baseline, 4 and 12 weeks), and ferritin (baseline and 12 weeks), which has been shown to be correlated with hepcidin in previous studies, were measured locally in the Johns Hopkins Clinical Laboratory. In a sensitivity analysis, human interleukin 6 (IL-6) was measured in a subgroup of previously frozen serum samples using a commercially available enzyme-linked immunosorbent assay kit, the Quantikine® Human IL-6 Immunoassay (R&D Systems, Inc., Minneapolis, MN) with minimum detectable dose of <0.07 pg/ml, interassay coefficient of variation 3.8–6.4% and intraassay coefficient of variation 2–4.2% [22]. Serum samples were tested in duplicate according to the manufacturer's instructions, and the plates were read immediately after application of the stop solution. The optical density of each sample was determined using VERSAmax tunable microplate reader (Molecular Devices, Sunnyvale, CA).

Safety Monitoring

Serum calcium, phosphorus, and albumin (to calculate corrected total calcium level if albumin < 3.5 g/dl), and urine calcium:creatinine ratios were measured at the baseline and 4-week visits to monitor for vitamin D toxicity. If subjects randomized to the intervention demonstrated hypercalcemia (serum total calcium > 10.8 mg/dl in 1–5 year-olds, > 10.3 mg/dl in 6–12 year-olds, or > 10.2 mg/dl in 13–20 year-olds), hyperphosphatemia (serum phosphorus > 6.5 mg/dl in 1–5 year-olds, > 5.8 in 6–12 year-olds, or > 4.5 in 13–20 year-olds), or hypercalciuria (urine calcium:creatinine ratio > 0.2) at the baseline visit, they were unenrolled from the study [22]. In addition, if intervention subjects demonstrated hypercalcemia, hyperphosphatemia, or hypercalciuria at the 4-week visit, the study drug was discontinued; these subjects remained in the study and 12-week data was collected. Subjects with 25D levels < 5 ng/ml at the baseline visit were unenrolled, as the clinically indicated vitamin D would be a higher dose than available in the trial [23]. Finally, if 25D levels > 60 ng/ml were noted at the 4-week visit in intervention subjects, supplements were stopped due to increased risk for vitamin D toxicity, but 12-week data was still collected.

Statistical Considerations

All analyses were based on intention-to-treat. Sample size for this study was based on estimates of the difference in change in serum hepcidin. We anticipated that a sample size of 34 participants would result in 90% power to detect a difference between the intervention and control groups in serum hepcidin of 50% at an α of 0.05, and would also provide 95% power to detect a 1 g/dL increase in hemoglobin with supplementation. Statistical significance was $p < 0.05$. All analyses were performed using Stata version 14.0 (Stata Corporation, College Station, TX). Distributions of continuous variables were assessed for normality and were log transformed if indicated to assess correlations between variables. For normally distributed data, mean $\pm$ SD was reported and changes between baseline and 4- and 12-week data assessed using the paired t test. For skewed data, median and IQR were reported and differences assessed using the Wilcoxon signed-rank test. Multivariable generalized estimating equation (GEE) regression analysis was used to evaluate percent

change from baseline in hepcidin, 25D, hemoglobin, and CRP in control vs. intervention groups. Variables which were not normally distributed were log-transformed for GEE analysis. GEE models utilized a Huber and White robust variance estimator [24], which assumed an exchangeable working correlation matrix.

Results

Study Participants

We screened 1472 potential participants during the recruitment period. Of 145 eligible subjects, 34 were randomized. Figure 1 summarizes the flow of subjects. Baseline characteristics are presented in Table 2. Mean (SD) baseline 25D level was 29.7 (11.5), and 50% of subjects were noted to be vitamin D insufficient/deficient at baseline with 25D level < 30 ng/ml. A small number of subjects, 3 in each arm, had baseline 25D levels < 20 ng/ml. There were no statistically significant baseline differences in demographic or clinical characteristics between the control and intervention groups, although the difference in proportion of subjects prescribed iron supplements at baseline by treatment arm approached significance (17.7% in the control arm vs. 41.2% in the intervention arm, $p=0.13$) (Table 1).

Laboratory Parameters

Figure 2 displays 25D levels at baseline and 12 weeks by treatment arm. At baseline the only significantly correlated variables were log hepcidin and log ferritin ($r=0.58$, $p<0.001$). Log hepcidin was also correlated with ferritin at the 12-week visit ($r=.71$, $p<0.001$; $r=0.45$, $p=0.02$). Baseline and 4-week values by treatment arm are presented in Table 2a. In the control arm, no significant differences were noted in any clinical parameters at the 4-week visit. In the intervention arm, median (IQR) 25D level increased from 32 (23, 30) to 38.5 (31, 50) ng/ml ($p=0.001$) and there was a borderline-significant decrease in the prevalence of vitamin D insufficiency/deficiency from 47.1% to 14.3% ($p=0.05$). Baseline and 12-week values by treatment arm are presented in Table 2b; here were no significant differences noted in any clinical parameters of interest in the control arm. A significant increase in median (IQR) 25D level was again seen in the intervention arm – 32 (23, 39) to 43 (31, 51) ng/ml, $p=0.01$, and a statistically significant decrease in median (IQR) CRP from 0.5 (0.3, 2.2) to 0.3 (0.1, 0.5) mg/l ($p=0.03$) was also noted. In light of the observed significant change in CRP level at 12 weeks in the intervention arm, a post-hoc sensitivity analysis was performed in a randomly-selected subgroup of 12 subjects (6 intervention and 6 control). No significant change in IL-6 level from baseline to 12 weeks was noted in either arm; intervention 2.66 (1.56, 4.38) to 3.16 (1.21, 9.79) pg/ml, $p=0.99$; control 1.04 (0.84, 1.86) to 1.62 (1.02, 1.84) pg/ml, $p=0.52$. In a subgroup analysis conducted only among intervention subjects with 25D levels < 30 ng/mL at baseline ($n=8$), the difference in median hepcidin from the baseline to the 12-week visit still did not reach significance (84.9 [38.3, 130.4] vs. 63[31.8, 146.3], $p=0.87$).

There were no significant differences in the observed changes in hepcidin, 25D, hemoglobin, or CRP noted between baseline and the 4- or 12-week follow up visits in the control group (Table 3). In the intervention group, 25D increased significantly 48.4% and 34.5% at both the 4- and 12-week visits compared to baseline, and the difference in the change in 25D

levels was significantly different in the intervention vs. control group ($p = 0.003$). Compared to the control group, the supplementation intervention significantly increased 25D levels (+41.8%; 95% CI 12.9 to 78.2%). A significant decrease greater than 60% in CRP between the baseline and 12-week visits was noted in the intervention group (−60.6%; 95% CI −83.5 to −6.4%); however, the difference in the change in CRP between the intervention and control groups did not reach statistical significance (−31.5%; 95% CI −76.3 to 97.5%).

Compliance and Safety

25D levels were significantly increased at 4 and 12 weeks in the intervention group and unchanged in the control group, suggesting compliance with the intervention. No subjects randomized to the control group demonstrated cholecalciferol-associated adverse events (AE) at the 4 week study visit. Three intervention subjects were unenrolled after the baseline visit due to safety parameters. Five subjects in the intervention arm had the study drug discontinued at the 4 week visit due to AE (Table 4). No significant difference in baseline 25D levels in those with and without AE was noted, although the trend was for a higher level among those who sustained an AE.

Discussion

In this randomized, controlled trial, daily supplementation with 4000 IU cholecalciferol compared with the EAR of 400 IU/day, with a resulting significant increase in 25D levels in the intervention group, did not result in a significant change in serum hepcidin level after 4 or 12 weeks of treatment in children with non-dialysis CKD. Hemoglobin did not change significantly in either arm. A significant decrease in CRP was noted between baseline and 12-week visits in the intervention group, but the overall difference in change in CRP by study arm did not reach statistical significance.

Previous *in vitro* studies have demonstrated that treatment of cultured cells with 25D or 1,25-dihydroxyvitamin D results in decreased hepcidin mRNA expression via direct suppression of *HAMP* gene expression mediated by locally activated or administered 1,25-dihydroxyvitamin binding to the vitamin D receptor [25,17]. *In vivo*, short-term suppression of hepcidin production by vitamin D has been previously reported by Bacchetta et al. who demonstrated that treatment of 7 healthy adults with a single oral dose of 100,000 IU of vitamin D₂/ergocalciferol resulted a 34% decrease in circulating hepcidin levels at 24 hours and a 33% decrease at 72 hours, notably without any significant change in circulating “activated” 1,25-dihydroxyvitamin D levels [17]. Our study utilized cholecalciferol rather than ergocalciferol supplementation, but given that cholecalciferol effectiveness in raising 25D levels is high, and in some studies has been demonstrated to be higher, compared to ergocalciferol (vitamin D₂), the difference in formulation likely does not account for our observed lack of change in hepcidin concentration.[26,27] However, the supplemental dose utilized by Bacchetta et al. far exceeds dietary reference values. Although vitamin D treatment is generally safe, it has been associated with increase urinary and serum calcium [28], and in light of the present study in which 5 subjects treated with 4000 IU/day cholecalciferol demonstrated either hyperphosphatemia, hypervitaminosis D, or nephrolithiasis, it is not without risk. Notably, the magnitude of the change in circulating

25D levels observed by Bacchetta et al (27 ± 2 to 43.5 ± 3 ng/ml) was similar to that seen with our intervention [17]. However, the more gradual increase in 25D observed in our trial was not associated with a decrease in hepcidin. Fibroblast growth factor 23 (FGF23) levels increased as well in Bacchetta et al.'s study, a potentially undesirable effect in CKD patients [17]. Our findings did not demonstrate a significant decrease in circulating hepcidin level with supplementation using a dosing scheme that is commonly applied in clinical practice. With regard to our hypothesis that vitamin D3 supplementation could decrease hepcidin-mediated iron-restricted erythropoiesis, a trend for decreased median ferritin was noted between the baseline and 12 week visits in the intervention subjects which did not reach statistical significance (Table 2b); this could reflect an increase in accessible, utilized iron in these subjects and is consistent with the direction of the non-significant trend for decreased hepcidin observed in this group. However, the limited sample size for this study may have limited the significance of these observations.

Given the significant cost and potential adverse effects associated with escalating ESA therapy in CKD patients, vitamin D3 supplementation would be an attractive potential adjuvant therapy to modify hepcidin-mediated iron restriction. Vitamin D is now recognized for its multiple extra-skeletal effects including innate immune system regulation. Local (non-renal) 25D hydroxylation is known to regulate innate immune function via local metabolism in immune cells [15,16,29]. It has been reported that locally-activated 25D inhibits the production of inflammatory cytokines including IL-6 and TNF- α , and that inflammatory cytokines may serve to directly induce hepcidin production [12,15,16,30,31]. Alternatively, a recent study published by Smith et al. reported that high dose vitamin D3 supplementation reduced plasma hepcidin concentrations in healthy adults without change in plasma cytokine concentration, suggesting that vitamin D may play a role in the regulation of the hepcidin-iron axis independently of changes in pro-inflammatory cytokines [32]. There is accumulating evidence that nutritional 25D deficiency is a risk factor for anemia in both healthy people and in those with kidney disease, and hepcidin upregulation is likely a key mediator of anemia [33–35]. Vitamin D supplementation and correction of deficiency has been associated with increased hemoglobin, decreased ESA dose requirement, and improvement in ESA-resistant anemia in dialysis patients [36–39], although a more recent study did not confirm these associations [40].

We did observe a small but statistically significant decrease in CRP between baseline and 12 weeks in intervention subjects. We did not measure inflammatory cytokine levels concurrently with hepcidin and 25D levels in the course of the study and are unable to globally describe changes in markers of inflammation other than CRP in response to the intervention. Given the observed decrease in CRP level at 12 weeks in the intervention arm however, we did perform a post-hoc sensitivity analysis in a subgroup of intervention and control subjects, but found no significant change in serum IL-6 level from baseline to 12 weeks in either treatment arm; however, given the very small sample size and the relatively low IL-6 levels observed, this result should not be taken to suggest that vitamin D may not modify IL-6 levels under other conditions and would require further investigation. The absence of additional measures of inflammation remains a limitation of the current study.

Reference values for serum hepcidin in children have been difficult to define, in part due to variation in available hepcidin assays (including ELISA and mass spectrometry), limiting comparisons across studies [41,42]. A prior study using the same ELISA assay performed in the same laboratory as our study reported the median hepcidin level in healthy pediatric controls as 25.3 ng/mL, and in 48 children with stage 2–4 CKD as 127.3 ng/mL [12]. We previously described hepcidin levels in a cohort of 133 children with stage 2–4 CKD in the CKiD cohort study: median (IQR) baseline hepcidin level in D-fense subjects was higher at 63.2 (46.7, 130.6) ng/mL compared to 41.7 (21.6, 66.4) ng/mL in CKiD, despite lower median (IQR) GFR in the CKiD subjects (41.7 (21.6, 66.4) vs. 60.1 (48.7, 72.1) ml/min/1.73m² in D-fense) [14]. However, despite a distribution of hepcidin levels shifted to the left compared to the present study, increased risk for hemoglobin decline and incident anemia was noted in CKiD subjects with higher baseline hepcidin levels [14].

Fifty percent of subjects in this study were vitamin D deficient at baseline. The recognition of vitamin D deficiency and its associated risks (including extra-skeletal risks) by nephrology providers has increased substantially over the last 10 years, with clinical practice guidelines for the diagnosis and management published by the National Kidney Foundation Kidney Disease Outcomes and Quality Initiative in 2008 [23]. Using the currently established threshold for vitamin D insufficiency and deficiency, our data suggest this is an ongoing problem requiring vigilant monitoring and treatment. The separate and important question of whether current vitamin D thresholds are associated with patient outcomes is beyond the scope of this analysis.

Strengths of this pilot study include its prospective, randomized, controlled design. The relatively short intervention period allowed for assessment of the acute effects on serum hepcidin without significant changes in GFR or therapeutic regimen in children with stable non-dialysis CKD. The major limitations are small sample size which was determined in part with an estimated 50% change in hepcidin in the intervention group; although serum hepcidin levels have been shown to decrease promptly and significantly in patients undergoing hemodialysis [13], the potential reduction may be significantly smaller in response to vitamin D supplementation. The analysis was also limited by increased rates of post-randomization ineligibility and adverse events in the intervention group leading to discontinuation of the intervention after 4 weeks of treatment in 5 subjects. Additionally, intra-individual and diurnal variability in hepcidin levels has been reported [43]. Healthy subjects demonstrate iron-independent diurnal variation in hepcidin, with levels being lowest in the morning and increasing steadily throughout the day [44,45]. Given the challenges of scheduling patients for study visits in the context of school and parents' work schedules, we were unable to collect blood samples for hepcidin at standardized times across the study; this may have increased the variability of the measurements resulting in underestimation of the effect size of any change in hepcidin observed in the intervention group. In addition we observed a non-significant trend for more intervention patients prescribed iron supplements compared to controls; since iron treatment can increase hepcidin levels [42], this could have contributed to underestimation of change in hepcidin in the intervention group.

In conclusion, in this randomized controlled clinical trial, the first such trial conducted in children with CKD, effective vitamin D₃ supplementation failed to modify the primary

outcome of hepcidin level; these results do not support a short-term benefit of supplementation as an adjunctive treatment for anemia. Nevertheless, the measurement of hepcidin in patients with CKD, and investigation of interventions that could serve to modify its production, remains an attractive strategy in the development novel targets for anemia therapy. Further study will be required to determine whether this intervention may be more effective in children with advanced CKD or on dialysis, or in patients supplemented for longer periods of time. Going forward, multicenter trials will be critical to amass sample sizes required to detect even small differences in hepcidin concentration.

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Study data were collected and managed using REDCap electronic data capture tools hosted at Johns Hopkins

(Paul A. Harris, Robert Taylor, Robert Thielke, Johnathan Payne, Nathaniel Gonzalez, Jose G. Conde, Research electronic data capture (REDCap) (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources.

References

1. Pfeffer MA, Burdmann EA, Chen CY, Cooper ME, de Zeeuw D, Eckardt KU, Feyzi JM, Ivanovich P, Kewalramani R, Levey AS, Lewis EF, McGill JB, McMurray JJ, Parfrey P, Parving HH, Remuzzi G, Singh AK, Solomon SD, Toto R. TREAT Investigators. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med*. 2009; 361:2019–2032. [PubMed: 19880844]
2. Unger EF, Thompson AM, Blank MJ, Temple R. Erythropoiesis-stimulating agents--time for a reevaluation. *N Engl J Med*. 2010; 362:189–192. [PubMed: 20054037]
3. Atkinson MA, Martz K, Warady BA, Neu AM. Risk for anemia in pediatric chronic kidney disease patients: a report of NAPRTCS. *Pediatr Nephrol*. 2010; 25:1699–1706. [PubMed: 20464428]
4. Poli M, Asperti M, Ruzzenenti P, Regoni M, Arosio P. Hepcidin antagonists for potential treatments of disorders with hepcidin excess. *Front Pharmacol*. 2014; 586:1–13.
5. Mercadel L, Metzger M, Haymann JP, Thervet E, Boffa JJ, Flamant M, Vrtovsni F, Houillier P, Froissart M, Stengel B. the NephroTest Study Group. The relation of hepcidin to iron disorders, inflammation and hemoglobin in chronic kidney disease. *PLoS One*. 2014; 9:e99781. [PubMed: 24978810]
6. Babitt JL, Lin HY. Molecular mechanisms of hepcidin regulation: Implications for the anemia of CKD. *Am J Kidney Dis*. 2010; 55:726–744. [PubMed: 20189278]
7. Malyszko J, Mysliwiec M. Hepcidin in anemia and inflammation in chronic kidney disease. *Kidney Blood Press Res*. 2007; 30:15–30. [PubMed: 17215586]
8. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *J Clin Invest*. 2004; 306:2090–2093.
9. Roy CN, Andrews NC. Anemia of inflammation: The hepcidin link. *Curr Opin Hematol*. 2005; 12:107–111. [PubMed: 15725899]
10. Atkinson MA, White CT. Hepcidin in anemia of chronic kidney disease: Review for the pediatric nephrologist. *Pediatr Nephrol*. 2012; 27:33–40. [PubMed: 21400189]

11. Preza GC, Pinon R, Ganz T, Nemeth E. Cellular catabolism of the iron-regulatory protein hepcidin. *PLoS One*. 2013; 8:e58934. [PubMed: 23536837]
12. Zaritsky J, Young B, Wang HJ, Westerman M, Olbina G, Nemeth E, Ganz T, Rivera S, Nissenson AR, Salusky IB. Hepcidin--a potential novel biomarker for iron status in chronic kidney disease. *Clin J Am Soc Nephrol*. 2009; 4:1051–1056. [PubMed: 19406957]
13. Zaritsky J, Young B, Gales B, Wang HJ, Rastogi A, Westerman M, Nemeth E, Ganz T, Salusky IB. Reduction of serum hepcidin by hemodialysis in pediatric and adult patients. *Clin J Am Soc Nephrol*. 2010; 5:1010–1014. [PubMed: 20299375]
14. Atkinson MA, Kim JY, Roy CN, Warady BA, White CT, Furth SL. Hepcidin and risk of anemia in CKD: a cross-sectional and longitudinal analysis in the CKiD cohort. *Pediatr Nephrol*. 2015; 30:635–643. [PubMed: 25380788]
15. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab*. 2008; 4:80–90. [PubMed: 18212810]
16. Baeke F, Gysemans C, Korf H, Mathieu C. Vitamin D insufficiency: implications for the immune system. *Pediatr Nephrol*. 2010; 25:1597–1606. [PubMed: 20180136]
17. Bacchetta J, Zaritsky JJ, Sea JL, Chun RF, Lisse TS, Zavala K, Nayak A, Wesseling-Perry K, Westerman M, Hollis BW, Salusky IB, Hewison M. Suppression of Iron-Regulatory Hepcidin by Vitamin D. *J Am Soc Nephrol*. 2014; 25:564–572. [PubMed: 24204002]
18. Institute of Medicine EAR. Dietary Reference Intakes for Calcium and Vitamin D. Institute of Medicine; http://iom.nationalacademies.org/~media/Files/Report%20Files/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D/calciumvitd_lg.jpg
19. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009; 20:629–637. [PubMed: 19158356]
20. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. *Blood*. 2008; 112:4292–4297. [PubMed: 18689548]
21. Havers FP, Detrick B, Cardoso SW, Berendes S, Lama JR, Sugandhavesa P, Mwelase NH, Campbell TB, Gupta A. ACTG A5175 PEARLS and NWCS319 Study Teams. Change in vitamin D levels occurs early after antiretroviral therapy initiation and depends on treatment regimen in resource-limited settings. *PLoS One*. 2014; 9(4):e95164. [PubMed: 24752177]
22. Lai S, Fishman EK, Lai H, Pannu H, Detrick B. Serum IL-6 levels are associated with significant coronary stenosis in cardiovascularly asymptomatic inner-city black adults in the US. *Inflamm Res*. 2009; 58(1):15–21.
23. KDOQI Work Group. KDOQI clinical practice guideline for nutrition in children with CKD: 2008 update. *Am J Kidney Dis*. 2009; 53:S11–104.
24. White H. A Heteroskedasticity-Consistent Covariance Matrix Estimator and a Direct Test for Heteroskedasticity. *Econometrica*. 1980; 48:817–838.
25. Zughaier SM, Alvarez JA, Sloan JH, Konrad RJ, Tangpricha V. The role of vitamin D in regulating the iron-hepcidin-ferroportin axis in monocytes. *J Clin Transl Endocrinol*. 2014; 1:19–25. [PubMed: 25097830]
26. Alvarez J, Wasse H, Tangpricha V. Vitamin D supplementation in pre-dialysis chronic kidney disease: A systematic review. *Dermatoendocrinol*. 2012; 4(2):118–127. [PubMed: 22928067]
27. Lehmann U, Hirche F, Stangl GI, Westphal S, Dierkas J. Bioavailability of vitamin D(2) and D(3) in healthy volunteers, a randomized placebo-controlled trial. *J Clin Endocrinol Metab*. 2015; 98(11):4339–4345.
28. Marckmann P, Agerskov H, Thineshkumar S, Bladbjerg EM, Sidelmann JJ, Jespersen J, Nybo M, Rasmussen LM, Hansen D, Scholze A. Randomized controlled trial of cholecalciferol supplementation in chronic kidney disease patients with hypovitaminosis D. *Nephrol Dial Transplant*. 2012; 27:3523–3531. [PubMed: 22822092]
29. Bikle D. Nonclassic actions of vitamin D. *J Clin Endocrinol Metab*. 2009; 94:26–34. [PubMed: 18854395]
30. Means RT. Hepcidin and cytokines in anaemia. *Hematology*. 2009; 9:357–362.

31. Nemeth E. Targeting the hepcidin-ferroportin axis in the diagnosis and treatment of anemias. *Adv Hematol.* 2010; 2010:750643. [PubMed: 20066043]
32. Smith EM, Alvarez JA, Kearns MD, Hao L, Sloan JH, Konrad RJ, Ziegler TR, Zughaier, Tangpricha V. High-dose vitamin D3 reduces circulating hepcidin concentrations; A pilot, randomized, double-blind, placebo-controlled trial in healthy adults. *Clin Nutr.* 2016 S0261–5614(16)30148-0.
33. Atkinson MA, Melamed ML, Kumar J, Roy CN, Miller ER 3rd, Furth SL, Fadrowski JJ. Vitamin D, race, and risk for anemia in children. *J Pediatr.* 2014; 164:153–158. [PubMed: 24112861]
34. Kendrick J, Targher G, Smits G, Chonchol M. 25-Hydroxyvitamin D deficiency and inflammation and their association with hemoglobin levels in chronic kidney disease. *Am J Nephrol.* 2009; 30:64–72. [PubMed: 19218791]
35. Smith EM, Tangpricha V. Vitamin D and anemia; insights into an emerging association. *Curr Opin Endocrinol Diabetes Obes.* 2015; 22:1–7. [PubMed: 25485518]
36. Saab G, Young DO, Gincherman Y, Giles K, Norwood K, Coyne DW. Prevalence of vitamin D deficiency and the safety and effectiveness of monthly ergocalciferol in hemodialysis patients. *Nephron Clin Pract.* 2007; 105:c132–138. [PubMed: 17228173]
37. Kumar VA, Kujubu DA, Sim JJ, Rasgon SA, Yang PS. Vitamin D supplementation and recombinant human erythropoietin utilization in vitamin D-deficient hemodialysis patients. *J Nephrol.* 2011; 24:98–105. [PubMed: 20563998]
38. Kiss Z, Ambrus C, Almasi C, Berta K, Deak G, Horonyi P, Kiss I, Lakatos P, Marton S, Molner MZ, Nemeth Z, Szabo A, Mucsi I. Serum 25(OH)-cholecalciferol concentration is associated with hemoglobin level and erythropoietin resistance in patients on maintenance hemodialysis. *Nephron Clin Pract.* 2011; 117:c373–378. [PubMed: 21071961]
39. Rianthavorn P, Boonyapapong P. Ergocalciferol decreases erythropoietin resistance in children with chronic kidney disease stage 5. *Pediatr Nephrol.* 2013; 28:1261–1266. [PubMed: 23420502]
40. Miskulin D, Majchrzak K, Tighiouart H, Muther RS, Kapoian T, Johnson DS, Weiner DE. Ergocalciferol Supplementation in Hemodialysis Patients with Vitamin D Deficiency: A Randomized Clinical Trial. *J Am Soc Nephrol.* 2016; 27:1801–1810. [PubMed: 26677862]
41. Macdougall IC, Malyszko J, Hider RC, Bansal SS. Current status of the measurement of blood hepcidin levels in chronic kidney disease. *Clin J Am Soc Nephrol.* 2010; 5:1681–1689. [PubMed: 20724518]
42. Cangemi G, Pistorio A, Miano M, Gattorno M, Acquila M, Bicocchi MP, Gastaldi R, Riccardi F, Gatti C, Fioredda F, Calvillo M, Melioli G, Martini A, Dufour C. Diagnostic potential of hepcidin testing in pediatrics. *Eur J Haematol.* 2013; 90:323–330. [PubMed: 23438060]
43. Ford BA, Eby CS, Scott MG, Coyne DW. Intra-individual variability in serum hepcidin precludes its use as a marker of iron status in hemodialysis patients. *Kidney Int.* 2010; 78:769–773. [PubMed: 20668427]
44. Troutt JS, Rudling M, Persson L, Stahle L, Angelin B, Butterfield AM, Schade AE, Cao G, Konrad RJ. Circulating Human Hepcidin-25 Concentrations Display a Diurnal Rhythm, Increase with Prolonged Fasting, and Are Reduced by Growth Hormone Administration. *Clin Chem.* 2012; 58:1225–1232. [PubMed: 22679180]
45. Schaap CCM, Hendriks JCM, Kortman GAM, Klaver SM, Kroot JJC, Laarakkers CMM, Wiegerinck ET, Tjalsma H, Janssen MCH, Swinkels DW. Diurnal Rhythm rather than Dietary Iron Mediates Daily Hepcidin Variations. *Clin Chem.* 2013; 59:527–535. [PubMed: 23232066]

Figure 1. Flow diagram. More subjects in the intervention arm were ineligible post-randomization, or required discontinuation of the study drug due to labs outside safety parameters at the 4 week visit.

Figure 2.
25-hydroxyvitamin D levels at baseline and 12 week visits by study arm.

Table 1

Baseline patient characteristics overall and by treatment arm

	Overall	Control (n=17)	Intervention (n=17)	p-value [‡]
Age (years), mean (SD)	10.9 (5.8)	10.9 (6.3)	10.8 (5.5)	0.96
Female, n (%)	9 (26.5%)	6 (35.3%)	3 (17.7%)	0.24
Race, n (%)				0.59
African-American	8 (23.5%)	5 (29.4%)	3 (17.7%)	
Caucasian	20 (58.8%)	9 (52.9%)	11 (64.7%)	
Other	6 (17.7%)	3 (17.7%)	3 (17.6%)	
Hispanic, n (%)	1 (3%)	0 (0%)	1 (5.9%)	0.31
Glomerular Disease, n (%)	9 (26.5%)	6 (35.3%)	3 (17.7%)	0.24
eGFR (ml/min/1.73m ²), mean (SD)	60 (17.6)	57.6 (16.9)	62.5 (18.4)	0.42
eGFR < 30 ml/min/1.73m ² , n (%)	3 (8.8%)	1 (5.9%)	2 (11.7%)	0.55
25 hydroxyvitamin D (ng/ml), median (IQR)	29.7 (21, 36)	29 (20, 34)	32 (23, 39)	0.32
25 hydroxyvitamin D deficient, n (%)	17 (50%)	9 (52.9%)	8 (47.1%)	0.73
Hepcidin (ng/ml), median (IQR)	63.2 (46.7, 130.6)	54.9 (37.8, 105.4)	76.9 (47.2, 130.6)	0.39
Hemoglobin (g/dl), mean (SD)	12.7 (11.7, 13.6)	13.1 (2.4)	12.4 (1.4)	0.31
CRP (mg/L), median (IQR)	12.8 (2.0)	0.3 (0.1, 0.9)	0.5 (0.3, 2.2)	0.18
Ferritin (ng/ml), median (IQR)	53 (27, 114)	52 (27, 107)	53.5 (29.5, 125.5)	0.94
Summer and autumn enrollment, n (%)	19 (55.9%)	10 (58.8%)	9 (52.9%)	0.73
Prescribed iron supplements, n (%)	10 (29.4%)	3 (17.7%)	7 (41.2%)	0.13
Prescribed ESA, n (%)	2 (5.9%)	1 (5.9%)	1 (5.9%)	1.0

SD standard deviation, eGFR estimated glomerular filtration rate, IQR interquartile range, CRP C-reactive protein, ESA erythropoiesis stimulating agent

[‡]t-test, chi², or Wilcoxon rank-sum test

Table 2a

Baseline and 4-week laboratory parameters by treatment arm

	Control		p-value [‡]	Intervention		p-value [‡]
	Baseline n = 17	4-weeks		Baseline	4-weeks	
Hepcidin (ng/ml), mean (SD)	81.0 (65.5)	71.1 (80.6) (n=16)	0.70	108.9 (97.2)	101.3 (79.2) (n=14)	0.82
Hepcidin (ng/ml), median (IQR)	54.9 (37.8, 105.4)	47.3 (30, 76.8) (n=16)	0.53	76.9 (47.2, 130.6)	74.8 (58.2, 107.6) (n=14)	0.82
25D (ng/ml), median (IQR)	29 (20, 34)	29 (26.5, 33.5)	0.79	32 (23, 39)	38.5 (31, 50)	0.001
25D deficient, n (%)	9 (52.9%)	9 (56.2%)	0.85	8 (47.1%)	2 (14.3%)	0.05
Hemoglobin (g/dl), mean (SD)	13.1 (2.4)	13.0 (2.1) (n=11)	0.91	12.4 (1.4)	12.6 (1.5) (n=6)	0.77
CRP (mg/L), median (IQR)	0.3 (0.1, 0.9)	0.3 (0.1, 0.5) (n=15)	0.12	0.5 (0.3, 2.2)	0.9 (0.2, 3) (n=14)	0.61

SD standard deviation, IQR interquartile range, CRP C-reactive protein

[‡]t-test, chi², or Wilcoxon rank-sum test

Table 2b

Baseline and 12-week laboratory parameters by treatment arm

	Control			Intervention		
	Baseline n=17	12-weeks	p-value [‡]	Baseline	12-weeks	p-value [‡]
Hepcidin (ng/ml), mean (SD)	81.0 (65.5)	73.7 (75.4) (n=15)	0.77	108.9 (97.2)	85.9 (52.8) (n=12)	0.46
Hepcidin (ng/ml), median (IQR)	54.9 (37.8, 105.4)	48.2 (38, 63) (n=15)	0.28	76.9 (47.2, 130.6)	75.4 (38.6, 123.12) (n=12)	0.48
25D (ng/ml), median (IQR)	29 (20, 34)	29.7 (23, 38)	0.26	32 (23, 39)	43 (31, 51) (n=11)	0.01
25D deficient, n (%)	9 (52.9%)	9 (60%)	0.54	8 (47.1%)	2 (18.1%)	0.09
Hemoglobin (g/dl), mean (SD)	13.1 (2.4)	14.7 (8.0) (n=15)	0.44	12.4 (1.4)	12.2 (1.5) (n=12)	0.71
CRP (mg/L), median (IQR)	0.3 (0.1, 0.9)	0.3 (0.1, 1.6)	0.91	0.5 (0.3, 2.2)	0.3 (0.1, 0.5)	0.03
Ferritin (ng/ml), median (IQR)	52 (27, 107)	70 (25, 115) (n=15)	0.53	53.5 (29.5, 125.5)	45 (32.5, 77) (n=12)	0.08

SD standard deviation, IQR interquartile range, CRPC-reactive protein

[‡]t-test, chi², or Wilcoxon rank-sum test

Multivariable Generalized Estimating Equation (GEE) Regression Analyses of Changes in Laboratory Parameters from Baseline

Table 3

	Median (IQR) Control	% Change from baseline (95% CI) at 4 weeks Control	% Change from baseline (95% CI) at 12 weeks Control	Median (IQR) Intervention	% Change from baseline (95% CI) at 4 weeks Intervention	% Change from baseline (95% CI) at 12 weeks Intervention	Intervention vs. Control, Difference in Change (%) from Baseline (95%CI)	p value
Hepcidin	54.9 (37.8 to 105.4)	-15.9 (-39.7, 17.5)	-13.8 (-37.4, 18.7)	76.9 (47.2, 130.6)	-1.1 (-27.8, 35.5)	-14.0 (-41.6, 26.5)	9.7 (-29.2, 69.9)	0.68
Vitamin D 25	29.0 (20.0 to 34.0)	13.2 (-11.7, 45.1)	18.0 (-8.6, 52.2)	32.0 (23.0, 39.0)	48.4 (20.8, 82.3)	34.5 (2.6, 76.3)	41.8 (12.9, 78.2)	0.003
Hemoglobin	12.8 (12.1 to 14.0)	-1.7 (-8.3, 5.3)	6.0 (-11.2, 26.5)	12.5 (11.6, 13.0)	-1.3 (-6.3, 3.9)	-0.8 (-4.4, 2.9)	-4.4 (-15.1, 7.7)	0.46
CRP	0.3 (0.1 to 0.9)	-27.4 (-60.3, 32.8)	-0.2 (-41.9, 71.2)	0.5 (0.3, 2.2)	-4.6 (-70.3, 206.4)	-60.6 (-83.5, -6.4)	-31.5 (-76.3, 97.5)	0.48

Each variable is expressed as a 10% difference for variables that are natural log-transformed, or a one unit difference for variables that are untransformed in the GEE model.

IQR interquartile range, *CI* confidence interval, *Vitamin D 25* 25-hydroxyvitamin D, *CRP* C-reactive protein

Table 4

Comparison of baseline characteristics in subjects randomized to intervention arm (4000 IU/day cholecalciferol) with and without observed adverse events (hyperphosphatemia^{*}, hypervitaminosis D^{**}, or nephrolithiasis) during treatment period

Baseline Characteristic	Adverse Event (n=5)	No Adverse Event (n=12)	p-value [‡]
Age (years), mean (SD)	8.8 (6.3)	11.7 (5.2)	0.4
eGFR ^{***} , mean (SD)	73.7 (10.1)	57.8 (19.4)	0.04
25 hydroxyvitamin D (ng/ml), median (IQR)	36 (24, 41)	30.7 (22, 38.5)	0.53
Treated with activated vitamin D, n (%)	0 (0%)	1 (8.3%)	0.51

^{*} Serum phosphorus > 6.5 mg/dl in 1–5 year olds, > 5.8 in 6–12 year olds, or > 4.5 in 13–20 year olds

^{**} 25-hydroxyvitamin D levels > 60 ng/ml

IU international units, *SD* standard deviation, *eGFR* estimated glomerular filtration rate, *IQR* interquartile range

[‡] t-test, chi², or Wilcoxon rank-sum test