BMJ Nutrition, Prevention & Health

Maternal prenatal, with or without postpartum, vitamin D3 supplementation does not improve maternal iron status at delivery or infant iron status at 6 months of age: secondary analysis of a randomised controlled trial

Karen M O'Callaghan ,[1,2] Huma Qamar,[2] Alison D Gernand,[3] AK Onoyovwi,[2] Stanley Zlotkin,[2,4,5] Abdullah A Mahmud,[6] Tahmeed Ahmed,[6] Farhana K Keya,[6] Daniel E Roth[2,4,5]

To cite: O'Callaghan KM, Qamar H, Gernand AD, *et al*. Maternal prenatal, with or without postpartum, vitamin D3 supplementation does not improve maternal iron status at delivery or infant iron status at 6 months of age: secondary analysis of a randomised controlled trial. *BMJ Nutrition, Prevention & Health* 2023;**6**:e000758. doi:10.1136/bmjnph-2023-000758

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bmjnph-2023-000758).

For numbered affiliations see end of article.

Correspondence to
Dr Karen M O'Callaghan; karen.ocallaghan@kcl.ac.uk

Received 24 August 2023
Accepted 31 October 2023
Published Online First 14 November 2023

ABSTRACT

Background Vitamin D may modify iron status through regulation of hepcidin and inflammatory pathways. This study aimed to investigate effects of maternal vitamin D supplementation on iron status in pregnancy and early infancy.

Methods In a trial in Dhaka, Bangladesh, women (n=1300) were randomised to one of five vitamin D_3 regimens from 17 to 24 weeks' gestation until 26 weeks postpartum (prenatal; postpartum doses): 0;0, 4200;0, 16 800;0, 28 000;0 or 28 000;28 000 IU/week. All participants received standard iron-folic acid supplementation. In this secondary analysis (n=998), we examined effects of prenatal;postpartum vitamin D on serum ferritin and other biomarkers of maternal iron status (transferrin saturation, total iron binding capacity, soluble transferrin receptor and hepcidin) at delivery, and infant ferritin and haemoglobin at 6 months of age. Using linear regression, we estimated per cent mean differences between each intervention group and placebo with 95% CIs, with and without adjustment for baseline ferritin or inflammatory biomarkers (C reactive protein and α-1-acid glycoprotein (AGP)).

Results At delivery, ferritin concentrations were similar between each intervention group and placebo in unadjusted (n=998) and baseline ferritin-adjusted analyses (n=992; p>0.05). Compared with placebo, AGP was lower in each intervention group (per cent difference (95% CI) = −11% (−21 to −1.0), −14% (−23 to −3.5) and −11% (−19 to −2.0) in the 4200 IU/week, 16 800 IU/week and 28 000 IU/week groups, respectively; n=779). In the subgroup of women with baseline 25-hydroxyvitamin D < 30 nmol/L, ferritin was lower in each intervention group versus placebo (−23% (−37 to −5.0), −20% (−35 to −1.9) and −20% (−33 to −4.1) in the 4200 IU/week, 16 800 IU/week and 28 000 IU/week groups, respectively; n=645); effects were slightly attenuated after adjustment for inflammation (n=510). There were no effects of vitamin D on other iron biomarkers among women at delivery or infants aged 6 months.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The potential for vitamin D to aid in the prevention and treatment of iron deficiency has been attributed to the proposed role of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) in the regulation of erythropoiesis and suppression of hepcidin. Trial evidence for an effect of vitamin D on biomarkers of iron status is limited, particularly among perinatal populations with a high prevalence of both iron and vitamin D deficiency.

WHAT THIS STUDY ADDS

⇒ In a population in which iron deficiency was common (26% with ferritin < 15 µg/L), prenatal vitamin D supplementation did not affect biomarkers of iron transport (transferrin saturation), regulation (hepcidin) or iron-deficiency erythropoiesis (soluble transferrin receptor), nor was there an effect of prenatal, with or without postpartum, vitamin D supplementation on infant ferritin or haemoglobin at 6 months of age. Among women with vitamin D deficiency (25-hydroxyvitamin D < 30 nmol/L) prior to intervention, an observed negative effect of prenatal high-dose vitamin D supplementation on serum ferritin at delivery may reflect an anti-inflammatory effect of vitamin D.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings do not support the use of prenatal, with or without postpartum, vitamin D supplementation as an adjunctive measure to improve maternal iron status in late pregnancy or infant iron status in early life. Prenatal high-dose vitamin D supplementation may suppress inflammation among women at delivery, but the clinical implication of this effect remains to be established.

Conclusion These findings do not support improvement of iron status by vitamin D. The effect of prenatal vitamin D supplementation on ferritin may reflect an anti-inflammatory mechanism.

INTRODUCTION

Both low iron and vitamin D status have been recognised as public health concerns among pregnant women and young children, with disproportionately high rates of deficiencies reported in low-income and middle-income countries.[1–3] While the advantage of universal vitamin D supplementation during pregnancy is uncertain,[4] daily iron supplementation is recommended by the WHO as part of routine antenatal care to reduce the risks of iron-deficiency anaemia (IDA), puerperal sepsis, premature delivery and low birth weight.[5] Underlying biological mechanisms for the association of iron and vitamin D metabolism suggest vitamin D regulates iron homeostasis by indirectly promoting intestinal iron absorption and mobilisation of iron stores.[6 7] The metabolic interplay of iron and vitamin D may therefore be an important contributor for the promotion of iron availability.

Encoded by the HAMP gene, hepcidin is an iron-regulatory hormone and acute phase reactant that inhibits intracellular iron egress by binding to ferroportin at the basolateral membrane.[8] In vitro analysis of human cells supports direct suppression of hepcidin by 1,25-dihydroxyvitamin D (1,25(OH)$_2$D), the most biologically active metabolite of vitamin D, hence providing evidence that vitamin D facilitates iron transport and utilisation.[6] Although vitamin D is considered to exert anti-inflammatory properties,[9] whether vitamin D also plays an indirect role in hepcidin regulation through attenuation of the acute phase response has not been well examined[7] and pooled analysis suggests little benefit of vitamin D supplementation for reducing low-grade systemic inflammation.[10] Results of observational studies of the association between 25-hydroxyvitamin D (25(OH)D) and markers of iron status are inconclusive.[11–14] Though promising, trial evidence to support the use of vitamin D as an adjunct therapy for prevention of iron deficiency (ID) stem primarily from patients receiving treatment for chronic kidney disease,[15–17] with limited evidence from at risk but otherwise healthy populations,[18] including pregnant women.[19]

Given the evidence that vitamin D is involved in hepcidin-ferroportin regulation, and that low 25(OH)D and ID often coexist,[3] we analysed data from a previously reported randomised trial in Bangladesh[20 21] to examine the impact of prenatal, with or without postpartum, vitamin D supplementation on biomarkers of iron status in women at delivery and their infants at 6 months of age.

METHODS

Data source

Data for this secondary analysis was drawn from participants of the Maternal Vitamin D for Infant Growth (MDIG) trial, a randomised, placebo-controlled, dose-ranging trial of prenatal and postpartum vitamin D$_3$ supplementation in Dhaka, Bangladesh. A full description of the trial methods and primary outcomes is reported elsewhere.[20 21] Briefly, women having an uncomplicated singleton pregnancy were enrolled from the antenatal clinic at the Maternal and Child Health Training Institute in Dhaka and randomised to 1 of 5 similarly sized trial arms (n=1300) comprising a prenatal;postpartum regimen of 0;0 (placebo), 4200;0, 16 800;0 or 28 000;0 or 28 000;28 000 IU vitamin D$_3$/week from 17 to 24 weeks' gestation until 6 months postpartum. All participants were provided with standard iron-folic supplements (66 mg/d elemental iron and 350 µg/d folic acid) in line with usual care, in addition to daily calcium supplementation (500 mg/d as calcium carbonate). Vitamin D and placebo tablets were administered under direct supervision by study personnel during weekly home visits. Adherence was quantified as the proportion of scheduled doses received. Adherence to iron-folic acid and calcium cointerventions was not monitored but assumed to be similar across trial arms given the randomised design. Women with a haemoglobin (Hb) concentration < 70 g/L were considered at high risk for pregnancy complications and excluded prior to randomisation. The MDIG trial and secondary analysis were registered at clinicaltrials.gov (NCT01924013 and NCT04764955, respectively).

Health and sociodemographic data were collected at baseline (17–24 weeks' gestation) through interviewer-administered questionnaires. Maternal anthropometric measurements were obtained according to standardised protocols.[22] Body mass index was calculated at enrolment based on mid-pregnancy weight, as prepregnancy weight was unavailable. Non-fasting venous blood samples were processed to serum or plasma and stored at ≤ −70°C until analysis.

Participant inclusion in this secondary analysis depended on the availability of ≥ 1 biomarker of interest measured for women at delivery (ferritin, soluble transferrin receptor (sTfR), transferrin saturation, total iron binding capacity (TIBC) and/or plasma hepcidin) or infants at 6 months of age (ferritin and/or whole-blood Hb).

Laboratory analysis

Maternal serum ferritin was measured at enrolment using an Abbott Architect chemiluminescent microparticle immunoassay (CMIA) at the Clinical Biochemistry Laboratory (CBL) at International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). Ferritin at delivery was measured by electrochemiluminescence sandwich immunoassay on a Roche automated immunoassay analyzer (Cobas e601) using commercial kits at the Nutritional Biochemistry Laboratory at icddr,b. Cross-validation performed on 4% of analysed samples (n=84/2016) showed strong correlation between laboratories (*rho*=0.99; p<0.001) with acceptable inter-assay coefficient of variation (CV) (9.9%). Infant serum ferritin was analysed at 6 months of age by CMIA at CBL.

Maternal serum iron, transferrin and sTfR at enrolment were measured at CBL using commercial kits. TIBC was calculated as follows: TIBC (µmol/L) = 25.1 × transferrin (g/L). Transferrin saturation was calculated as the ratio of serum iron to TIBC and expressed as a percentage. Hb was measured in women at enrolment and infants at 6 months of age using a portable point-of-care haemoglobinometer (HemoCue Hb 201+) (online supplemental table 1).

Analysis of maternal and infant plasma C reactive protein (CRP) was first performed by sandwich ELISA at the Hospital for Sick Children, Toronto. To maximise sample availability for data analysis, additional plasma samples were quantified for CRP by automated spectrophotometry at CBL; correlation between laboratories was high (n=25; *rho*=0.97; p<0.001; CV=13%). High-sensitivity CRP was analysed in a subset of maternal samples with values < 1.6 mg/L, the lower limitation of quantification (LLoQ) for quantitative CRP at CBL. Plasma α–1-acid glycoprotein (AGP) and hepcidin at enrolment and delivery were analysed by ELISA. Serum 25(OH)D was analysed by LC-MS/MS, as described previously[21] (online supplemental table 1).

Outcomes

Serum ferritin reflects iron stores and is recommended by the WHO for assessment of iron status, with consideration of inflammation in high-risk settings.[23] Primary outcomes were therefore determined a priori as ferritin concentrations among (1) women at delivery and (2) infants at 6 months of age. ID was defined as ferritin < 15 µg/L in women and < 12 µg/L in infants. IDA was defined as ID plus Hb < 110 g/L. To delineate potential mechanisms of interaction between vitamin D and iron, secondary outcomes included a range of iron-related biomarkers at delivery including transferrin saturation, TIBC, sTfR and hepcidin, and infant Hb. Given the randomised design, we did not expect to find between-group differences in the distributions of inflammatory markers (ie, CRP and/or AGP) at enrolment; therefore, primary analysis was conducted without adjustment for inflammation to estimate the overall (ie, total) effect of vitamin D on iron status. In supplementary analysis, we included CRP and/or AGP (measured at the same time-point as the outcome) as covariates in the model to test whether concurrent inflammation explained the effect of vitamin D on ferritin.

Statistical analysis

Biomarker concentrations below the LLoQ were assigned objective values equal to or half the LLoQ (online supplemental table 1); we did not identify biologically implausible values. Biomarker variables with right-skewed distributions (ferritin, sTfR, hepcidin, CRP and AGP) were natural log (ln)-transformed to approximate normality. Bivariate relationships between biomarkers were examined using scatterplots and pairwise correlations were assessed using Spearman correlation coefficients. Geometric means with 95% CIs were presented where applicable to reflect central tendencies of skewed distributions. Participant characteristics, including biomarker concentrations at enrolment, were examined across intervention groups using analysis of variance with Tukey's post-hoc or χ^2 tests for continuous or categorical variables, respectively. Where data departed from normality, a Kruskal-Wallis was used. P<0.05 was considered statistically significant.

Effect of maternal vitamin D supplementation on iron status

Participants randomised to receive 28 000 IU vitamin D/week prenatally (with or without 28 000 IU/week postpartum) were aggregated into a single 'high-dose' prenatal vitamin D group to test the effect of the prenatal intervention on maternal biomarkers at delivery. Linear regression models were fitted using the assigned prenatal trial arm as the categorical exposure variable and ln-transformed ferritin at delivery as the (continuous) outcome variable. Effect estimates were back-transformed and reported as mean per cent differences and 95% CIs of each intervention group relative to placebo. In a multivariable model, we adjusted for baseline ferritin concentrations to control for between-group differences at enrolment. Effects of vitamin D on TIBC, transferrin saturation, sTfR and hepcidin at delivery were analysed by linear regression and reported as mean differences (for outcomes on their original scales) or per cent mean differences (for ln-transformed outcomes). In separate models, maternal CRP and AGP were regressed on vitamin D intervention group. For analyses of infant outcomes, the two high-dose prenatal vitamin D groups (28 000 IU/week) were considered separately for comparisons of each prenatal;postpartum intervention group to placebo using similar regression models as described above.

Maternal data were included in primary analyses if the delivery blood sample was: (1) prenatal, ≥ 37 weeks gestation; (2) prenatal, < 37 weeks gestation but within (≤) 14 days prior to delivery; or (3) postpartum, ≤ 14 days after delivery. Infant samples were included if obtained within (±) 4 weeks of the infant reaching 6 months of age, corresponding to the time of cessation of the intervention. Analysis was conducted as intention to treat.

Auxiliary analyses

Sensitivity analyses of maternal data included restriction to women who gave birth to a live-born infant. For infant outcomes, restricted analyses included term-born infants (≥ 37 weeks' gestation) due to the greater risk of ID among infants born premature.[24]

In supplementary analysis, we included concurrent CRP and/or AGP as covariates in the linear regression model; if inflammation mediated the association between vitamin D and ferritin, we expected the inclusion of CRP/AGP to attenuate the effect estimates for vitamin D. As an alternative approach to account for effects of inflammation, ferritin was replaced with a value 'corrected' for inflammation, using the approach recommended by the

Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) consortium.[25]

In prespecified subgroup analyses, effects of the intervention were examined by maternal vitamin D status (25(OH)D ≥ 30 nmol/L vs < 30 nmol/L) at enrolment. Subgroup analyses were conducted for infant outcomes stratified by sex.

Statistical analysis was performed using Stata V.17.1.

RESULTS

Participant characteristics

Of 1300 participants enrolled in the MDIG trial, 998 women (77%) and 743 infants (57%) contributed data to analyses at delivery and 6 months of age, respectively. Data were distributed equally among intervention groups, but analytical sample sizes differed by biomarker and timepoint (online supplemental figure 1). Sociodemographic characteristics were similar across trial arms (table 1 and online supplemental table 2). Mean maternal 25(OH)D concentrations were low prior to intervention, as expected based on previous findings.[21] ID, anaemia and IDA were observed in 26% (257/992), 61% (613/998) and 17% (172/992) of women at enrolment, respectively. Concurrent ID and vitamin D deficiency (25(OH)D < 30 nmol/L) was present in 17% (171/985) of women and combined IDA and vitamin D deficiency occurred in 11% (108/985) of women. Adherence to the intervention was high across all trial arms (table 1).

Maternal nutritional status and inflammation at delivery

Serum ferritin was weakly correlated with CRP (n=942; *rho*: 0.28; p<0.001) and AGP (n=779; *rho*: 0.26; p<0.001) at delivery, and there was a moderate correlation between CRP and AGP (n=779; *rho*: 0.53; p<0.001). Biomarker concentrations for women at delivery, by group, are shown in table 2. Prenatal vitamin D supplementation did not affect CRP but significantly reduced AGP at delivery (online supplemental table 3)

Effect of prenatal vitamin D supplementation on maternal iron status at delivery

Relative to placebo, geometric mean ferritin at delivery was lower in each vitamin D intervention group (table 2), but pairwise differences between each intervention arm and placebo were not statistically significant (table 3) and a dose-dependent relation was not observed (figure 1). Findings were similar on adjustment for baseline ferritin (table 3). Although 22% (n=219) of participants were missing CRP and/or AGP at delivery, the characteristics of the subset of participants who contributed to inflammation-adjusted analyses were similar to the complete group included in primary analysis (online supplemental table 4). In this subset, ferritin was significantly lower in each intervention group compared with placebo without adjustment for inflammation, and effects were attenuated towards the null on adjustment for CRP and AGP; while the magnitude of the attenuation was similar in analyses using the BRINDA approach and using CRP and AGP as covariates in the regression model, the difference in ferritin between placebo and each intervention group only remained statistically significant for the 28 000 IU/week group when using the BRINDA approach (online supplemental table 5). Inferences for primary analyses were unchanged when restricted to women who gave birth to a live-born infant (online supplemental table 6).

In subgroup analyses of women with 25(OH)D < 30 nmol/L at enrolment, ferritin was lower in each vitamin D intervention group compared with placebo (table 3; figure 1); with adjustment for baseline ferritin, the per cent mean differences were attenuated in the low-dose and mid-dose groups, but remained statistically significant and of similar magnitude in the high-dose group (28 000 IU/week prenatally) (table 3). Adjustment for CRP and AGP at delivery slightly attenuated the effect estimates but did not alter most inferences; however, sample sizes for these analyses were lower and the difference in ferritin between the placebo and 16 800 IU/week group was no longer significant (online supplemental table 7).

Transferrin saturation and sTfR were similar among trial arms at delivery (table 2), such that the per cent differences did not differ from placebo (table 4). A greater TIBC was observed for the low-dose group (4200 IU/week) only, but effect estimates were attenuated and no longer significant in analyses restricted to women with vitamin D deficiency at enrolment (data not shown). There were no effects of the vitamin D intervention on hepcidin (table 2 and table 4).

Effect of maternal vitamin D supplementation on infant iron status

Biomarker concentrations for infants at 6 months of age are shown in table 5. In primary unadjusted analyses, there was no difference in ferritin between each intervention group and placebo (table 6). Inferences were unchanged following adjustment for CRP (online supplemental table 8) and in analyses restricted to term-born infants (n=659; data not shown). Differences in Hb between each intervention group and placebo were not evident (table 4). Inferences were unchanged when stratified by sex (online supplemental table 9).

DISCUSSION

In this secondary analysis of a dose-ranging placebo-controlled trial, prenatal vitamin D supplementation did not improve iron status by delivery, nor was there evidence for an effect of prenatal, with or without postpartum, supplementation on ferritin or Hb concentrations in early infancy. Rather, the findings suggest a negative effect of high-dose vitamin D supplementation on serum ferritin among women with habitual vitamin D deficiency that may be explained by down-regulation of inflammation. These findings do not

Table 1 Characteristics of participants who contributed data to primary or secondary analysis of effects of prenatal vitamin D supplementation on maternal iron status at delivery by intervention group*

Prenatal vitamin D (IU/week)	0 n=203	4200 n=202	16 800 n=205	28 000 n=388	P value
Age at enrolment (years)†	23 (18, 38)	23 (18, 40)	22 (18, 35)	23 (18, 38)	0.60
Height at enrolment (cm)‡	151.2±5.4	151.2±5.2	150.6±5.5	151.0±5.6	0.67
BMI at enrolment (kg/m^2)‡§	24.1±4.2	23.5±4.3	23.8±4.0	23.9±3.9	0.51
Gestational age at enrolment (weeks)†	20.4 (17, 24)	20.1 (17, 24)	20.3 (17, 24)	20.4 (17, 24)	0.88
Asset index quintile (*n*, %)¶**					
Q1	43 (21)	41 (20)	28 (14)	83 (22)	0.22
Q2	34 (17)	43 (21)	50 (25)	64 (17)	
Q3	49 (24)	34 (17)	40 (20)	79 (21)	
Q4	37 (18)	35 (17)	44 (22)	81 (21)	
Q5	40 (20)	48 (24)	42 (21)	77 (20)	
Education level (*n*, %)					
Secondary school complete or higher††	44 (22)	53 (26)	43 (21)	89 (23)	0.60
Enrolment serum ferritin (µg/L)‡‡§§	27.4 (24.2-31.0)	24.6 (21.7-27.9)	25.1 (22.0-28.7)	27.9 (25.6-30.4)	0.29
Enrolment serum ferritin <15 µg/L (*n*, %)§§	45 (22)	60 (30)	61 (30)	91 (24)	0.12
Enrolment whole-blood Hb (g/L)‡	106±11	107±12	106±11	107±11	0.75
Enrolment whole-blood Hb <110 g/L (*n*, %)	125 (62)	118 (58)	130 (63)	240 (62)	0.77
Enrolment plasma CRP (mg/L)‡‡¶¶	4.9 (4.2-5.8)	4.1 (3.5-4.8)	4.5 (3.9-5.3)	4.5 (4.0-5.1)	0.43
Enrolment plasma CRP ≥5 mg/L (*n*, %)¶¶	109 (54)	84 (42)	87 (43)	186 (48)	0.054
Enrolment plasma hepcidin (ng/mL)‡‡***	4.25 (3.64-4.98)	3.59 (3.07-4.20)	3.34 (2.84-3.94)	3.78 (3.35-4.25)	0.20
Enrolment serum 25(OH)D (nmol/L)‡†††	27.4±14.0	26.6±13.2	28.2±13.9	26.8±14.1	0.61
Enrolment serum 25(OH)D <30 nmol/L (*n*, %)†††	125 (62)	133 (66)	126 (62)	261 (68)	0.38
Adherent to trial supplementation (*n*, %)‡‡‡	202 (99.5)	201 (99.5)	203 (99.0)	381 (98.2)	0.37
Live-born infant (*n,* %)	198 (98)	199 (99)	203 (99)	376 (97)	0.33
Gestational age at delivery (weeks)‡	38.9±1.7	39.1±1.4	38.9±1.6	38.9±1.8	0.44
Delivery method (*n*, %)					
Vaginal	93 (46)	76 (38)	97 (47)	178 (46)	0.17
C-section	110 (54)	126 (62)	108 (53)	210 (54)	

*Participants were considered eligible for inclusion in the present analysis provided a biomarker of interest (serum ferritin, transferrin saturation, soluble transferrin receptor or hepcidin) was available, and the delivery blood sample was collected as follows: (1) any prenatal sample ≥ 37 weeks gestation; (2) a prenatal sample at < 37 weeks gestation but within (≤) 14 days prior to delivery; or (3) any postpartum sample ≤ 14 days after delivery. P values for differences between groups by Kruskal-Wallis, analysis of variance (ANOVA) or $\chi 2$ test, where appropriate.
†Data are presented as median (min, max) (all such values).
‡Data are presented as mean±SD (all such values).
§N in each group: 0 IU/week, 203; 4200 IU/week, 202; 16 800 IU/week, 205; 28 000 IU/week, 388 due to missing data.
¶Determined by ownership of household items, using principal components analysis.
**Data are presented as number (%) (all such values).
††Defined as the achievement of a primary school certification, equivalent to at least 10 years of schooling, at the time of enrolment to the MDIG trial.
‡‡ Data presented as geometric means (95% CI) (all such values).
§§ N in each group: 0 IU/week, 202; 4200 IU/week, 200; 16800 IU/week, 204; 28000 IU/week, 386 due to missing data.
¶¶ N in each group: 0 IU/week, 203; 4200 IU/week, 201; 16800 IU/week, 204; 28000 IU/week, 385 due to missing data.
***N in each group: 0 IU/week, 176; 4200 IU/week, 177; 16800 IU/week, 175; 28000 IU/week, 324 due to missing data.
†††N in each group: 0 IU/week, 201; 4200 IU/week, 201; 16800 IU/week, 204; 28000 IU/week, 385 due to missing data.
‡‡‡Defined a priori as consumption of ≥ 80% of scheduled tablets.
BMI, body mass index; CRP, C-reactive protein; Hb, Haemoglobin; 25(OH)D, 25-hydroxyvitamin D.

Table 2 Biochemical measurements among women at delivery who contributed data to primary or secondary analysis of effects of maternal vitamin D supplementation on maternal iron status, by intervention group

Prenatal vitamin D (IU/week)				
	0	**4200**	**16800**	**28000**
Serum ferritin (µg/L)*, geometric mean (95% CI)	50.4 (45.0 to 56.4)	43.4 (38.4 to 49.1)	45.0 (40.5 to 49.9)	45.2 (41.5 to 49.1)
Serum ferritin <15 µg/L*, *n* (%)	20 (9.9)	24 (12)	22 (11)	50 (13)
Inflammation-adjusted serum ferritin (µg/L)†, geometric mean (95% CI)	34.9 (31.0 to 39.2)	29.3 (25.6 to 33.6)	30.5 (27.1 to 34.2)	29.6 (26.9 to 32.4)
Inflammation-adjusted serum ferritin <15 µg/L†, *n* (%)	24 (15)	34 (22)	28 (17)	65 (22)
Serum transferrin‡ (mg/dL), mean (95% CI)	377.2 (363.7 to 390.8)	398.7 (383.9 to 413.5)	385.9 (373.2 to 398.6)	376.5 (367.1 to 385.9)
TIBC (µmol/L)§‡, mean (95% CI)	94.7 (91.3 to 98.1)	100.1 (96.4 to 103.8)	96.9 (93.7 to 100.0)	94.5 (92.1 to 96.9)
sTfR (nmol/L)‡, geometric mean (95% CI)	14.4 (13.2 to 15.7)	15.1 (13.9 to 16.5)	14.1 (13.1 to 15.2)	14.7 (13.8 to 15.5)
Serum transferrin saturation (%)¶, geometric mean (95% CI)	13.3 (11.7 to 15.3)	12.6 (11.4 to 13.8)	12.9 (11.7 to 14.3)	13.4 (12.2 to 14.7)
Plasma CRP (mg/L)**, geometric mean (95% CI)	9.8 (8.2 to 11.9)	9.9 (8.3 to 11.8)	9.0 (7.5 to 10.7)	9.8 (8.5 to 11.2)
Plasma CRP ≥5 mg/L**, *n* (%)	144 (72)	134 (70)	135 (70)	244 (68)
Plasma AGP (g/L)††, geometric mean (95% CI)	1.05 (0.98 to 1.14)	0.93 (0.85 to 1.02)	0.91 (0.84 to 0.98)	0.94 (0.89 to 0.99)
Plasma AGP ≥1 g/L††, *n* (%)	85 (52)	67 (42)	66 (40)	131 (44)
Plasma hepcidin (ng/mL)‡‡, geometric mean (95% CI)	10.7 (8.8 to 13.0)	9.8 (8.0 to 12.0)	10.1 (8.3 to 12.1)	11.0 (9.5 to 12.6)
Serum 25(OH)D (nmol/L)§§, mean (95% CI)	24.2 (21.4 to 27.0)	69.7 (66.3 to 73.2)	100.1 (96.1 to 104.1)	112.1 (108.8 to 115.4)
Serum 25(OH)D <30 nmol/L§§, *n* (%)	98 (76)	2 (1.6)	0 (0)	1 (0.4)

*N in each group: 0 IU/week, 203; 4200 IU/week, 202; 16 800 IU/week, 205; 28 000 IU/week, 388.
†Serum ferritin adjusted for CRP and AGP using a regression-correction approach adapted from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia consortium. N in each group: 0 IU/week, 162; 4200 IU/week, 158; 16 800 IU/week, 163; 28 000 IU/week, 296.
‡N in each group: 0 IU/week, 95; 4200 IU/week, 103; 16 800 IU/week, 118; 28 000 IU/week, 199.
§Calculated as: 25.1× transferrin (g/L).
¶Calculated as: (serum iron/TIBC) x 100. N in each group: 0 IU/week, 89; 4200 IU/week, 95; 16 800 IU/week, 109; 28 000 IU/week, 185.
**N in each group: 0 IU/week, 202; 4200 IU/week, 191; 16 800 IU/week, 192; 28 000 IU/week, 357.
††N in each group: 0 IU/week, 162; 4200 IU/week, 158; 16 800 IU/week, 163; 28 000 IU/week, 296.
‡‡N in each group: 0 IU/week, 176; 4200 IU/week, 173; 16 800 IU/week, 173; 28 000 IU/week, 324.
§§N in each group: 0 IU/week, 129; 4200 IU/week, 123; 16 800 IU/week, 137; 28 000 IU/week, 252.
AGP, α-1-acid glycoprotein; CRP, C reactive protein; 25(OH)D, 25-hydroxyvitamin D; sTfR, soluble transferrin receptor; TIBC, total iron binding capacity.

support the use of vitamin D supplementation as an adjunct therapy for improvement of iron status among pregnant women receiving standard iron-folic acid supplementation.

In vitro studies have provided evidence for a role of $1,25(OH)_2D$ in the regulation of iron homeostasis by indirectly promoting the export of intracellular iron.[6] However, few randomised trials have examined changes in ferritin or hepcidin across a range of vitamin D doses and limited results from trials in generally healthy populations were mixed.[6 7 18] Our findings align with those of the small trial (n=7) by Bacchetta, who reported a minor decrease in serum ferritin following a bolus dose of vitamin D_2 (100 000 IU) that was accompanied by a fall in hepcidin.[6] In contrast, a larger trial by Madar *et al*[18] showed no effect of daily vitamin D supplementation (400 or 1000 IU) on various iron-related biomarkers (ferritin, Hb, serum iron and transferrin) in a population with habitually low iron and vitamin D status. Unlike the earlier

Table 3 Per cent mean difference in maternal serum ferritin concentrations at delivery by intervention group relative to placebo*

	All participants						Participants with 25(OH)D <30 nmol/L at enrolment					
	Unadjusted			Adjusted†			Unadjusted			Adjusted†		
Intervention group	n	% difference (95% CI)	P value	n	% difference (95% CI)	P value	n	% difference (95% CI)	P value	n	% difference (95% CI)	P value
0 IU/week	203	Ref	Ref	202	Ref	Ref	125	Ref	Ref	124	Ref	Ref
4200 IU/week	202	−14 (−27, 1.3)	0.07	200	−10 (−23, 4.4)	0.16	133	−23 (−37 to −5.0)	0.01	132	−17 (−31, 1.2)	0.07
16 800 IU/week	205	−11 (−24, 4.9)	0.17	204	−7.8 (−21, 7.3)	0.30	126	−20 (−35 to −1.9)	0.03	126	−17 (−31, 1.5)	0.07
28 000 IU/week	388	−10 (−22, 3.2)	0.13	386	−11 (−22, 1.7)	0.90	261	−20 (−33 to −4.1)	0.02	260	−19 (−32 to −4.4)	0.01

*Effect estimates for between-group differences were calculated using linear regression models with placebo (0 IU/week) as the reference group, whereby intervention group reflects the prenatal vitamin D dose provided in IU/week from randomisation (17–24 weeks of gestation) to delivery.
†Adjusted models include adjustment for ln-transformed serum ferritin concentrations at enrolment (17–24 weeks of gestation).
25(OH)D, 25-hydroxyvitamin D.

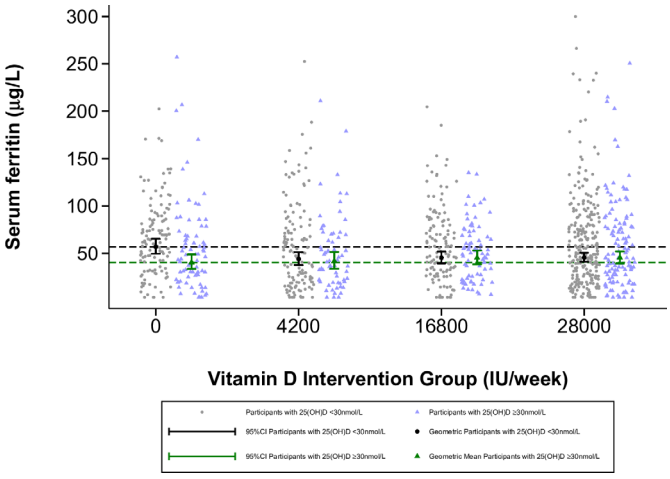

Figure 1 Maternal serum ferritin concentrations at delivery in participants with 25(OH)D concentrations ≥ 30 nmol/L (blue) and < 30 nmol/L (grey) at enrolment. For ease of visualisation, ferritin concentrations > 300 µg/L are excluded from the distribution but included in calculated geometric mean and 95% CI (n=2 in 0 IU/week, n=3 in 4200 IU/week and n=1 in 16 800 IU/week group). *N* for all participants: 0, n=203; 4200, n=202; 16800, n=205; 28000, n=388. *N* for participants with 25(OH)D < 30 nmol/L: 0, n=125; 4200, n=133; 16800, n=126; 28 000, n=261. *N* for participants with 25(OH)D ≥ 30 nmol/L: 0, n=76; 4200, n=68; 16800, n=78; 28000, n=124. Intervention group reflects the vitamin D dose provided in IU/week, represented as a prenatal supplementation regimen assigned at randomisation (17–24 weeks of gestation). 25(OH)D, 25-hydroxyvitamin D.

results by Bacchetta *et al*,[6] we did not find evidence that the change in ferritin was due to downregulation of hepcidin by vitamin D. Rather, the decrease in AGP, coupled with the slight attenuation of effects in inflammation-adjusted analyses, suggests vitamin D may be acting through anti-inflammatory mechanisms. The challenge of interpretation of ferritin in the presence of inflammation is well acknowledged[26]; given the lack of intervention effects on surrogate biomarkers of iron status, the reduction in ferritin observed in the present study therefore likely reflects suppression of the acute phase response by vitamin D rather than a reduction in stored iron.

We are aware of only one randomised controlled trial that has explored the effect of vitamin D supplementation on iron status during pregnancy[19]; in a relatively vitamin D replete population (mean 25(OH)D at enrolment of 46 nmol/L) in the UK-based Maternal Vitamin D Osteoporosis Study (MAVIDOS), there was no effect of prenatal vitamin D_3 supplementation (1000 IU/d) on ferritin, hepcidin or inflammatory markers.[19] Variations in the intervention dose as well as ethnic and sociodemographic differences between the present cohort and that of the MAVIDOS trial may limit between-study comparisons. While our findings are consistent with the hypothesis that high-dose vitamin D supplementation indirectly reduces circulating ferritin through anti-inflammatory

Table 4 Differences in maternal iron related biomarkers at delivery by intervention group relative to placebo*

Intervention group	TIBC (µmol/L)			Transferrin saturation (%)			sTfR (nmol/L)			Hepcidin (ng/mL)		
	n	Mean difference (95% CI)	P value	n	% difference (95% CI)	P value	n	% difference (95% CI)	P value	n	% difference (95% CI)	P value
0 IU/week	95	Ref	Ref	89	Ref	Ref	95	Ref	Ref	176	Ref	Ref
4200 IU/week	103	5.38 (0.51 to 10.24)	0.03	95	−5.8 (−21 to 12)	0.49	103	5.1 (−6.5 to 18.1)	0.40	173	−8.3 (−30 to 20)	0.53
16 800 IU/week	118	2.17 (−2.54 to 6.89)	0.37	109	−3.3 (−18 to 14)	0.69	118	−2.1 (−12.6 to 9.5)	0.71	173	−6.1 (−28 to 23)	0.65
28 000 IU/week	199	−0.19 (−4.46 to 4.08)	0.93	185	0.26 (−14 to 17)	0.97	199	1.80 (−8.08 to 12.74)	0.73	324	2.4 (−19 to 30)	0.84

*Effect estimates for between-group differences were calculated using linear regression models with placebo (0 IU/week) as the reference group, whereby intervention group reflects the prenatal vitamin D dose provided in IU/week from randomisation (17–24 weeks of gestation) to delivery.
sTfR, soluble transferrin receptor; TIBC, total iron binding capacity.

pathways, the intervention effect was relatively more pronounced among participants with vitamin D deficiency at enrolment. Cautious interpretation of this finding is warranted given the reduction in sample size, but if confirmed in other populations, this effect may have important implications for populations or individuals in which chronic inflammation, low iron and low vitamin D status are common and concurrent. In agreement with the MAVIDOS trial, we did not observe meaningful differences in ferritin or inflammation between the placebo and lower dose supplementation arms. While optimal dosing strategies for prevention of multiple micronutrient deficiencies require consideration of competing or reciprocal nutrient–nutrient interactions,[27] particularly for supplement doses that exceed the DRV, we interpret our findings with reservation given the lack of a dose–response relationship.

Serum (extracellular) ferritin reflects intracellular iron storage such that a low ferritin concentration represents only the first stage of ID and does not reflect iron demand or functional iron capacity.[26] We therefore explored a range of biomarkers to examine potential pathways that may explain the metabolic interplay of vitamin D and iron. In contrast to the negative effect of vitamin D on maternal ferritin, we did not observe meaningful differences in markers of iron transport (transferrin saturation), regulation (hepcidin) or ID erythropoiesis (sTfR). The greater TIBC among women receiving low-dose vitamin D supplementation in our primary analytical approach corroborates the trend for a reduction in ferritin, yet this difference is interpreted cautiously given that it was no longer present in analyses restricted to women with vitamin D deficiency at enrolment, and given the multiple testing in this study. We did not expect low dietary iron to be a limiting factor in our analyses as all women were provided with standard iron-folic acid supplementation throughout the study period. However, given the high prevalence of ID in the placebo group at delivery, it is possible that overall iron uptake (ie, absorbed iron) was insufficient to meet iron demands. The lack of a decrease in sTfR does not support the hypothesis that the reduction in serum ferritin reflects diversion of stored iron to the bone marrow for erythropoiesis, despite possible effects of vitamin D on the expression of the erythropoietin receptor.[28]

A limitation of this study is that it was a secondary analysis of a randomised trial which was not designed in tandem with the original trial and there was a reduction in sample size in the present analysis. However, given the similarity of participant characteristics across intervention arms and relative consistency in the number of participants per arm who contributed biomarker data, we do not believe selection bias to have influenced our results. Due to the eligibility criteria of the MDIG trial, findings may not

Table 5 Biochemical measurements among infants at 6 months of age who contributed data to primary or secondary analysis of effects of maternal vitamin D supplementation on infant iron status, by intervention group

Vitamin D (prenatal; postpartum IU/week)					
	0;0	4200;0	16 800;0	28 000;0	28 000;28 000
Serum ferritin (µg/L)*, geometric mean (95% CI)	24.9 (21.7 to 28.7)	24.3 (20.8 to 28.4)	21.8 (19.0 to 25.0)	23.4 (20.2 to 27.0)	24.0 (20.6 to 27.9)
Serum ferritin <12 µg/L*, *n* (%)	29 (20)	30 (20)	35 (25)	29 (20)	28 (20)
Whole blood Hb (g/L)†, mean (95% CI)	106.9 (105.1 to 108.8)	105.1 (103.3 to 107.0)	106.4 (104.7 to 108.2)	105.3 (103.4 to 107.2)	106.2 (104.5 to 108.0)
Whole blood Hb <110 g/L†, *n* (%)	84 (56)	92 (63)	92 (64)	84 (58)	83 (59)
Plasma CRP (mg/L)‡, geometric mean (95% CI)	0.94 (0.68 to 1.30)	0.93 (0.67 to 1.29)	0.96 (0.69 to 1.35)	0.80 (0.59 to 1.06)	1.04 (0.73 to 1.49)
Plasma CRP ≥5 mg/L‡, *n* (%)	7 (12)	4 (10)	9 (17)	4 (8)	3 (7)
Serum 25(OH)D (nmol/L)§, mean (95% CI)	52.4 (46.1 to 58.6)	53.5 (47.4 to 59.6)	54.8 (48.2 to 61.3)	49.5 (43.6 to 55.3)	81.8 (75.5 to 88.2)
Serum 25(OH)D <30 nmol/L§, *n* (%)	15 (21)	14 (19)	12 (17)	16 (23)	1 (1.6)

*N in each group: 0;0 IU/week, 146; 4200;0 IU/week, 148; 16 800;0 IU/week, 141; 28 000;0 IU/week, 143; 28 000; 28 000 IU/week, 141.
†N in each group: 0;0 IU/week, 150; 4200;0 IU/week, 145; 16 800;0 IU/week, 143; 28 000;0 IU/week, 145; 28 000; 28 000 IU/week, 140.
‡N in each group: 0;0 IU/week, 58; 4200;0 IU/week, 42; 16 800;0 IU/week, 53; 28 000;0 IU/week, 53; 28 000; 28 000 IU/week, 41.
§N in each group: 0;0 IU/week, 71; 4200;0 IU/week, 72; 16 800;0 IU/week, 69; 28 000;0 IU/week, 71; 28 000; 28 000 IU/week, 61.
CRP, C-reactive protein; Hb, Haemoglobin; 25(OH)D, 25-hydroxyvitamin D.

be generalisable to pregnant women presenting with severe anaemia (Hb < 70 g/L). The panel of iron-related biomarkers facilitated examination of the relationship between vitamin D and several aspects of iron homeostasis in pregnant women, but data were comparatively limited for infants (only ferritin and Hb). Measurements of erythropoietin and Hb at delivery may have enabled further clarification of the relationship between vitamin D and anaemia.[14] Lastly, while point-of-care testing of capillary Hb can bias estimates relative to venous blood and laboratory methods,[29] we expected such bias to be similar across trial arms.

CONCLUSION

In a population with a high prevalence of vitamin D deficiency, ID and anaemia, prenatal vitamin D supplementation did not improve maternal iron status at delivery. Evidence for an effect of continued postpartum supplementation on infant iron status at 6 months of age was also not observed. However, prenatal vitamin D supplementation reduced maternal AGP at delivery. The extent to which high-dose vitamin D supplementation may indirectly modify micronutrient status through attenuation of the acute phase response should be further explored in view of establishing effective intervention

Table 6 Difference in infant serum ferritin and whole-blood haemoglobin concentrations at 6 months of age by intervention group relative to placebo*

	Serum ferritin (µg/L)			Haemoglobin (g/L)		
Intervention group	n	% difference (95% CI)	P value	n	Mean difference (95% CI)	P value
0;0 IU/week	146	Ref	Ref	150	Ref	Ref
4200;0 IU/week	148	−2.5 (−20, 19)	0.81	145	−1.8 (−4.3, 0.73)	0.16
16 800;0 IU/week	141	−12.7 (−29, 7.2)	0.19	143	−0.50 (−3.0, 2.0)	0.70
28 000;0 IU/week	143	−6.3 (−24, 15)	0.53	145	−1.6 (−4.2, 0.89)	0.20
28 000;28 000 IU/week	141	−4.0 (−22, 18)	0.70	140	−0.70 (−3.2, 1.9)	0.59

*Effect estimates for between-group differences were calculated using linear regression models with placebo (0;0 IU/week) as the reference group, whereby intervention group reflects the maternal prenatal;postpartum vitamin D dose provided in IU/week from randomisation (17–24 weeks of gestation) to 6 months postpartum.

programmes for prevention of both iron and vitamin D deficiency.

Author affiliations
[1]Department of Nutritional Sciences, King's College London, London, UK
[2]Centre for Global Child Health and SickKids Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada
[3]Department of Nutritional Sciences, The Pennsylvania State University, University Park, Pennsylvania, USA
[4]Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
[5]Department of Paediatrics, The Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada
[6]Nutrition Research Division, International Centre for Diarrhoeal Disease Research Bangladesh, Dhaka, Bangladesh

Acknowledgements The authors would like to thank Ulaina Tariq for her contributions to data analysis at the early stages of this work.

Contributors DER designed the study, is the principal investigator and guarantor. AAM supervised field study activities and data collection. FKK conducted clinical follow-up. DER, ADG, SZ and TA provided methodological oversight. AKO performed laboratory analyses. HQ and KMOC conducted statistical analysis. KMOC and DER wrote the manuscript, and KMOC is responsible for the final content. All authors reviewed and contributed to the final manuscript and agree to be accountable for all aspects of the work.

Funding Funding for this research was provided to DER by the Bill and Melinda Gates Foundation under grant agreement OPP1176987. Under the grant conditions of the Bill and Melinda Gates Foundation, a Creative Commons Attribution 4.0 Generic License has been assigned to the Author Accepted Manuscript version that might arise from this submission. The funding agency was not involved in the design, implementation, analysis or interpretation of the data.

Competing interests None declared

Patient consent for publication Not applicable.

Ethics approval The MDIG trial protocol was approved by the Research Ethics Committees of the Hospital for Sick Children in Toronto (REB #1000039072) and the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b; PR-13055). Approval for this secondary analysis was obtained at both sites (Hospital for Sick Children REB #1000061108; icddr,b PR-20098). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Data described in the manuscript, code book and analytic code will be made available upon reasonable request to the authors. Deidentified individual participant data will be provided for use in secondary data analyses approved by an independent research ethics board, and data requestors will be required to sign a data access agreement.

ORCID iD
Karen M O'Callaghan http://orcid.org/0000-0002-5853-3796

REFERENCES

1 Saraf R, Morton SMB, Camargo CA, *et al*. Global summary of maternal and newborn vitamin D status - a systematic review. *Matern Child Nutr* 2016;12:647–68.
2 Stevens GA, Finucane MM, De-Regil LM, *et al*. Global, regional, and national trends in Haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. *Lancet Glob Health* 2013;1:e16–25.
3 Stevens GA, Beal T, Mbuya MNN, *et al*. Micronutrient deficiencies among preschool-aged children and women of reproductive age worldwide: a pooled analysis of individual-level data from population-representative surveys. *Lancet Glob Health* 2022;10:e1590–9.
4 Bialy L, Fenton T, Shulhan-Kilroy J, *et al*. Vitamin D supplementation to improve pregnancy and perinatal outcomes: an overview of 42 systematic reviews. *BMJ Open* 2020;10:e032626.
5 World Health Organization. *WHO Recommendations on Antenatal Care for a Positive Pregnancy Experience*. World Health Organization, 2016. Available: https://www.who.int/publications/i/item/9789241549912 [accessed 4 Aug 2023].
6 Bacchetta J, Zaritsky JJ, Sea JL, *et al*. Suppression of iron-regulatory Hepcidin by vitamin D. *J Am Soc Nephrol* 2014;25:564–72.
7 Smith EM, Alvarez JA, Kearns MD, *et al*. High-dose vitamin D_3 reduces circulating Hepcidin concentrations: a pilot, randomized, double-blind, placebo-controlled trial in healthy adults. *Clin Nutr* 2017;36:980–5.
8 Ganz T, Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G199–203.
9 Christakos S, Dhawan P, Verstuyf A, *et al*. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev* 2016;96:365–408.
10 Calton EK, Keane KN, Newsholme P, *et al*. The impact of Cholecalciferol supplementation on the systemic inflammatory profile: a systematic review and meta-analysis of high-quality randomized controlled trials. *Eur J Clin Nutr* 2017;71:931–43.
11 Mogire RM, Muriuki JM, Morovat A, *et al*. Vitamin D deficiency and its association with iron deficiency in African children. *Nutrients* 2022;14:1372.
12 Masoud MS, Yakout SM, Al-Attas OS, *et al*. The association between iron and vitamin D status in Arab adolescents. *Public Health Nutr* 2020;23:1208–13.
13 Malczewska-Lenczowska J, Sitkowski D, Surała O, *et al*. The association between iron and vitamin D status in female elite athletes. *Nutrients* 2018;10:167.
14 Thomas CE, Guillet R, Queenan RA, *et al*. Vitamin D status is inversely associated with anemia and serum erythropoietin during pregnancy. *Am J Clin Nutr* 2015;102:1088–95.
15 Kumar VA, Kujubu DA, Sim JJ, *et al*. Vitamin D supplementation and recombinant human erythropoietin utilization in vitamin D-deficient Hemodialysis patients. *J Nephrol* 2011;24:98–105.
16 Rianthavorn P, Boonyapapong P. Ergocalciferol decreases erythropoietin resistance in children with chronic kidney disease stage 5. *Pediatr Nephrol* 2013;28:1261–6.
17 Zughaier SM, Alvarez JA, Sloan JH, *et al*. The role of vitamin D in regulating the iron-Hepcidin-Ferroportin axis in monocytes. *J Clin Transl Endocrinol* 2014;1:19–25.
18 Madar AA, Stene LC, Meyer HE, *et al*. Effect of vitamin D3 supplementation on iron status: a randomized, double-blind, placebo-controlled trial among ethnic minorities living in Norway. *Nutr J* 2016;15:74.
19 Braithwaite VS, Crozier SR, D'Angelo S, *et al*. The effect of vitamin D supplementation on Hepcidin, iron status, and inflammation in pregnant women in the United Kingdom. *Nutrients* 2019;11:190.
20 Roth DE, Gernand AD, Morris SK, *et al*. Maternal vitamin D supplementation during pregnancy and Lactation to promote infant growth in Dhaka, Bangladesh (MDIG trial): study protocol for a randomized controlled trial. *Trials* 2015;16:300.
21 Roth DE, Gernand AD, Al Mahmud A. Vitamin D supplementation in pregnancy and Lactation and infant growth. *N Engl J Med* 2018;379:1881.
22 Cheikh Ismail L, Knight HE, Bhutta Z, *et al*. Anthropometric protocols for the construction of new International fetal and newborn growth standards: the INTERGROWTH-21St project. *BJOG* 2013;120 Suppl 2:42–7.
23 World Health Organization. *WHO Guideline on Use of Ferritin Concentrations to Assess Iiron Status in Individuals and Populations*. 2020. Available: https://www.who.int/publications/i/item/9789240000124 [accessed 4 Aug 2023].

24 Siddappa AM, Rao R, Long JD, *et al*. The assessment of newborn iron stores at birth: a review of the literature and standards for Ferritin concentrations. *Neonatology* 2007;92:73–82.
25 Namaste SM, Aaron GJ, Varadhan R, *et al*. Methodologic approach for the biomarkers reflecting inflammation and nutritional determinants of anemia (BRINDA) project. *Am J Clin Nutr* 2017;106(Suppl 1):333S–347S.
26 Pfeiffer CM, Looker AC. Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. *Am J Clin Nutr* 2017;106(Suppl 6):1606S–1614S.
27 Christian P, Jiang T, Khatry SK, *et al*. Antenatal supplementation with micronutrients and biochemical indicators of status and Subclinical infection in rural Nepal. *Am J Clin Nutr* 2006;83:788–94.
28 Alon DB, Chaimovitz C, Dvilansky A, *et al*. Novel role of 1,25(OH)2D3 in induction of erythroid progenitor cell proliferation. *Exp Hematol* 2002;30:403–9.
29 Killilea DW, Kuypers FA, Larkin SK, *et al*. Blood draw site and analytic device influence hemoglobin measurements. *PLoS One* 2022;17:e0278350.