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Original research

Serum 25-hydroxyvitamin D but not dietary vitamin D intake is associated with hemoglobin in women of reproductive age in rural northern Vietnam



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ABSTRACT

Background and objectives: Hypovitaminosis D and anemia are both prevalent in Vietnam, and low vitamin D status may be a risk factor for anemia. This study aimed to 1) describe vitamin D intake and its determinants, and 2) examine the associations of vitamin D intake and serum 25(OH)D concentrations with hemoglobin and anemia.

Methods and study design: We used data from the baseline survey of a pre-conceptual micronutrient supplementation trial in women of reproductive age (WRA) in Thai Nguyen, Vietnam (N = 4961). Vitamin D intake was estimated using a semi-quantitative food frequency questionnaire (FFQ). Multivariable regression models were used for the analyses.

Results: Median vitamin D intake was 0.2 µg/d (8.0 IU) [IQR: 0.4]. Age, being a farmer, food insecurity, and body mass index (BMI) were inversely associated with vitamin D intake, while socioeconomic status (SES), total energy intake, and education were positively associated with vitamin D intake. Vitamin D intake was not associated with hemoglobin concentration or anemia after adjusting for age, BMI, total energy intake, transferrin receptor, C-reactive protein, α₁-acid glycoprotein, SES, occupation, education, ethnicity, and food insecurity ($P = 0.56$ and $P = 0.65$ for hemoglobin and anemia, respectively). Controlling for the same covariates, 25(OH)D <50 nmol/L (vs. ≥50 nmol/L) was associated with decreased hemoglobin concentrations ($\beta = -0.91$ (SE:0.42), $P = 0.03$), but not with anemia ($P = 0.11$).

Conclusions: Low vitamin D status may be linked to reduced hemoglobin concentrations, but the role of diet in this association was not evident in this population of WRA in Vietnam where dietary vitamin D intake was very low.

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Introduction

Anemia is a substantial public health problem throughout the world, present in nearly a third of the global population [1]. The burden is higher in certain population groups and regions such as in Southeast Asia where the prevalence of anemia among non-pregnant women is estimated to be over 40% [2]. In women of

reproductive age (WRA) anemia is of particular concern as it has been associated with maternal and perinatal mortality [3] and adverse birth outcomes [4]. Furthermore, anemia is associated with decreased work capacity in adults, potentially resulting in economic consequences [5,6].

Iron deficiency is a major cause of anemia but other factors including infection, inflammation, and other nutrient deficiencies

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AGP, α₁-acid glycoprotein; AMDR, acceptable macronutrient distribution range; CRP, C-reactive protein; EAR, estimated average requirement; IOM, Institute of Medicine; IQR, interquartile range; RBP, retinol binding protein; SD, standard deviation; SE, standard error; SES, socioeconomic status; sTfR, soluble transferrin receptor; WHO, World Health Organization; WRA, women of reproductive age.

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have been recognized as important contributors to its etiology as well [5,7,8]. In a previous analysis from our group, we reported that nearly 20% of the non-pregnant WRA in this Vietnamese population were anemic, but iron deficiency anemia was relatively low, occurring in only 1.9% of women [9]. Similarly, Siridamrongvattana et al. [8], found that among pregnant women in the Thua Thien Hue province in the north central coast of Vietnam, the prevalence of anemia was nearly 20%, but only 6% had iron deficiency anemia. Given the burden of anemia but the low prevalence of iron deficiency in these studies of Vietnamese women, investigation of other factors contributing to anemia is warranted.

One such factor that has recently been described is vitamin D deficiency. Vitamin D deficiency among WRA has been linked to adverse maternal and child outcomes including risk factors for female infertility and neonatal respiratory complications [10,11]. Large population-based studies have also linked vitamin D deficiency to anemia [12–14], in particular anemia of inflammation [15,16]. The biochemical mechanism underlying this relationship may be explained by vitamin D's role in anti-inflammatory responses and gene expression [17]. Vitamin D has been reported to directly suppress transcription of hepcidin, the major iron-regulatory hormone [18], and lower pro-inflammatory cytokines known to stimulate hepcidin expression [19]. Hepcidin operates by preventing iron release from cells and limiting iron absorption during times of iron sufficiency. However, if hepcidin is pathologically elevated such as in response to a prolonged inflammatory stimulus, iron may become sequestered within cells, leaving insufficient iron available to support erythropoiesis, potentially leading to anemia [17]. Vitamin D, by lowering hepcidin may therefore prevent anemia under those conditions.

In a recent study, Laillou et al. [20] found that 90% of women in Vietnam had 25-hydroxyvitamin D [25(OH)D] concentrations <75 nmol/L and 40% had 25(OH)D concentrations <50 nmol/L, indicating a fairly high prevalence of vitamin D insufficiency among Vietnamese women. In that study, the mean vitamin D intake among women was 0.15 µg/d based on a household level food consumption survey. This estimated intake is much lower than the Recommended Dietary Allowances (RDA) for vitamin D intake for WRA of 5 µg and 15 µg in Vietnam and the United States, respectively [21,22]. Therefore, given the burden of anemia in WRA in Vietnam, the low rate of iron deficiency anemia, the very low estimated vitamin D intake, and the link between vitamin D status and anemia in other populations, we hypothesized that vitamin D intake and serum 25(OH)D concentrations would be positively associated with hemoglobin concentrations, and inversely associated with odds of anemia. The objectives of this study were to 1) describe vitamin D intake and its determinants, and 2) examine the associations between dietary vitamin D intake and hemoglobin concentration and anemia in WRA in the Thai Nguyen region of Vietnam. To overcome the concerns that dietary vitamin D intake may not be a good proxy for vitamin D status, we also examined the association of serum 25(OH)D concentrations with hemoglobin concentration and anemia in a subset of women (n = 88) in this population. Given that vitamin D deficiency and anemia are both highly prevalent nutrition-related public health concerns, this study contributes to the literature by evaluating the link between low vitamin D status and anemia in a population at risk for both conditions, and by exploring the role of diet in this association to potentially inform public health interventions.

Methods and materials

Study population

The data for this analysis came from the baseline survey of the PRECONCEPT study, a large double-blind randomized controlled

pre-conceptual micronutrient supplementation trial in the Thai Nguyen region of northern Vietnam (NCT01665378). This trial aimed to improve maternal and infant health and a detailed description of the methodology has been previously published [23]. Briefly, WRA (18–40 years) who were planning to become pregnant within a year of enrollment were recruited from 20 communes located in four of the nine districts of the Thai Nguyen province between November 2011 and April 2012 (winter to early spring in Vietnam). Women were excluded if they were pregnant, had recently used or were currently using iron and folic acid or multiple micronutrient supplements, were severely anemic (hemoglobin < 7 g/dL), had a history of a high risk pregnancy, or had a chronic hematologic disease. The study was approved by the Vietnam Institute of Social and Medical Studies and the Emory University Institutional Review Boards. Written informed consent was obtained from all participants at enrollment.

Data collection and processing

Dietary intake

Dietary vitamin D intake was estimated using a semi-quantitative food frequency questionnaire (FFQ) which included a list of 107 common foods and beverages consumed in Vietnam. This FFQ was previously validated by Vietnam's National Institute of Nutrition [24]. Trained interviewers asked participants to recall the frequency and portion size of listed foods and beverages consumed over the three months prior to the interview. Nutrient intake was estimated using the FFQ data and Vietnamese food composition tables [25]. Complex dishes not included in the food table were broken down into ingredients based on a Vietnamese recipe book and nutrient contents were calculated [26].

Biochemical and anthropometric measurements

Hemoglobin concentration was measured in capillary blood using a portable hemoglobin analyzer, HemoCue® Hb 301. Venous blood samples (5 mL) were collected by trained nurses, stored in an icebox, and transported within 4 h to Thai Nguyen University of Pharmacy and Medicine (TUMP) Hematology Department where they were centrifuged at 1500×g. Plasma ferritin, soluble transferrin receptor (sTfR), retinol binding protein (RBP), C-reactive protein (CRP) and α₁-acid glycoprotein (AGP) were measured via sandwich ELISA [27]. The intra-assay coefficient of variation (CV) was <3.0%. Due to budget constraints, 25(OH)D concentrations were measured in only a subset (n = 88) of participants. The subset of women for 25(OH)D assessment was selected based on reported dietary vitamin D intake. Women were categorized into deciles based on their intake and 8–10 women from each decile were randomly selected for the analysis. Serum 25(OH)D concentrations were measured using an automated chemiluminescent technique (IDS-iSYS automated machine, Immunodiagnostic Systems, Inc., Fountain Hills, AZ) in a laboratory which participates in the Vitamin D External Quality Assessment Scheme (DEQAS, site #606) and the National Institute of Standards and Technology/NIH Vitamin D Metabolites Quality Assurance Program to ensure the accuracy of 25(OH)D measurements. Stool samples were analyzed using the Kato-Katz method for evaluation of intestinal helminth infection [28]. Height and weight were measured twice via standard methods [29].

Demographic data

Demographic data were obtained using an interviewer-administered structured questionnaire. Socioeconomic status (SES) was assessed using the World Bank asset questionnaire for developing countries (and adapted for the local context in Vietnam), which includes questions about home and land ownership, house construction materials, access to services such as water and electricity, and household assets such as livestock [30]. Socioe-

conomic status was categorized into quintiles for this analysis. Household food insecurity was measured using the FANTA/USAID Household Food Insecurity Access Scale (HFIAS), and categorized by level of food security: food secure, mildly food insecure, moderately food insecure, and severely food insecure [31]. Other demographic variables used in this analysis included education, ethnicity, and occupation. Education was categorized based on highest grade completed (0–5th grade, 6–9th grade, 10–12th grade, or greater than 12th grade). Ethnicity was dichotomized into majority and minority groups, with majority defined as those of Kinh ethnicity. Finally, occupation was dichotomized as farmer or other occupation.

Definitions

Anemia was defined based on the World Health Organization (WHO) criteria for non-pregnant WRA, as hemoglobin concentration <12 g/dL [2]. Vitamin D status was categorized as 25(OH)D <50 nmol/L and 25(OH)D >75 nmol/L for descriptive and modelling purposes based on commonly accepted cut-offs for vitamin D insufficiency [21,32]. To further characterize the population by potential contributors to anemia etiology, namely inflammation and nutrient deficiency, we categorized inflammation as CRP concentrations >5 mg/L or AGP concentrations >1 g/L [33], and nutrient deficiency was defined as iron deficiency (plasma ferritin <12 µg/L) or vitamin A deficiency (RBP <1.05 mmol/L) [9].

Statistical analyses

Descriptive statistics were examined for all variables. Continuous variables were reported as means ± standard deviation (SD) for normally distributed variables or medians and interquartile range (IQR) for non-normally distributed variables; categorical variables were presented as percentages. Non-normally distributed variables were transformed to the natural logarithmic scale or categorized into tertiles for regression analyses. Because some values were zero, a constant of 0.01 was added to all non-missing values prior to log transformation. Multivariable linear regression with stepwise elimination was used to evaluate significant determinants of vitamin D intake and 25(OH)D concentration.

Spearman correlation was used to assess the simple correlation between vitamin D intake and hemoglobin concentrations. Multivariable linear regression was then used to examine the association between vitamin D intake (log-transformed) and hemoglobin concentration. Multivariable logistic regression was used to assess the association between vitamin D intake, categorized into tertiles (independent variable), and anemia (dependent variable). All models were controlled for potential confounders, age, BMI, total energy intake, iron status (sTfR) and inflammatory variables (CRP, AGP) in model 1, with socioeconomic variables (food insecurity, education, ethnicity, occupation, and SES) added in model 2. A two-stage least squares analysis was applied to assess indirect relationships among independent variables with hemoglobin concentration and anemia.

The relationship between dietary vitamin D intake and serum 25(OH)D concentrations was assessed via linear regression. Multivariable regression was used to assess the association between 25(OH)D (defined both as a continuous variable and categorized as 25(OH)D <50 nmol/mL vs. 25(OH)D ≥50 nmol/L) and hemoglobin concentration as well as anemia, controlling for the potential confounders noted above.

Results were presented as β coefficients and standard errors (SE) for linear regression models or odds ratio (OR) with 95% confidence intervals (CI) for logistic regression models. All analyses were performed using SAS v 9.4 (SAS Institute, Inc., Cary, NC), with a two-sided significance level of 0.05. This secondary analysis used

all available data from the parent study for examining the associations between vitamin D intakes and hemoglobin/anemia. The subsample for 25(OH)D assays was determined by available resources.

Results

Population characteristics

Of the 5011 women recruited for the PRECONCEPT Study, 4961 who had hemoglobin and dietary intake data were included in the present analysis. The sociodemographic, biochemical, and dietary intake characteristics of this sample of Vietnamese WRA are presented in Table 1. The mean age was approximately 26 years, and mean BMI was in the normal range at 19.6 ± 2.0 kg/m². The mean hemoglobin concentration was 13.0 ± 1.4 g/dL, and 19.6% of the women were anemic. Approximately 7% of women had nutrient deficiency as evidenced by low ferritin and/or low RBP, and a similar proportion had evidence of inflammation (elevated CRP and/or elevated AGP). The majority of women were farmers and approximately half reported being of an ethnic minority. Twelve percent of the women reported completing greater than a 12th grade education and about 20% were moderately or severely food insecure.

Table 1
Baseline descriptive characteristics for women in PRECONCEPT Study (N = 4961).^a

	Mean ± SD or n (%)
Socio-demographic and health status characteristics	
Age (years)	26.2 ± 4.6
Education (completed 12th grade) [†]	592 (12.0)
Occupation (farmer) [†]	3991 (80.6)
Ethnicity (minority) [†]	2448 (49.5)
Food insecurity (moderately or severely food insecure) [†]	936 (18.9)
BMI (kg/m ²) [†]	19.6 ± 2.0
Gravidity [†]	1.3 ± 0.8
Dietary intake	
Vitamin D intake (µg/d)	0.2 (0.4) ^b
Iron intake (mg/d)	15.7 (8.3) ^b
Total energy intake (kcal/d)	2,104.1 (842.9) ^b
Intake of foods containing vitamin D in last 3 months	
Any milk [†]	1676 (33.8)
A glass of milk at least once per week	1035 (20.9)
Any eggs [†]	4464 (90.0)
An egg at least once per week	3639 (73.4)
Any pork [†]	2285 (46.1)
A small piece at least once per week	1466 (29.6)
Biochemical markers	
Serum 25(OH)D (nmol/L) [†]	57.4 ± 10.7
25(OH)D < 50 nmol/L [†]	18 (20.5)
25(OH)D > 75 nmol/L [†]	82 (93.2)
Hemoglobin (g/dL)	13.0 ± 1.4
Anemia ^c	974 (19.6)
Ferritin (µg/L) [†]	68.2 (66.3) ^b
sTfR (mg/L) [†]	4.5 (1.5) ^b
RBP (µmol/L) [†]	1.6 (0.5) ^b
Nutrient deficiency ^d	337 (6.8)
AGP (g/L) [†]	0.7 ± 0.2
CRP (mg/L) [†]	0.3 (0.7) ^b
Inflammation ^e	337 (6.8)
Hookworm (proportion of women with any eggs) [†]	953 (21.5)

Values are Means ± SD or n (%) unless otherwise noted.

25(OH)D: 25-hydroxyvitamin D; sTfR, soluble transferrin receptor; RBP, retinol binding protein; AGP, α₁-acid glycoprotein; CRP, C-reactive protein.

^a N = 4961 women with hemoglobin measurements and dietary vitamin D intake data.

^b median (IQR) for non-normally distributed variables.

^c anemia defined as hemoglobin <12 g/dL.

^d ferritin <12 µg/L or RBP <1.05 mmol/L.

^e AGP >1 g/L or CRP >5 mg/L.

[†] n = 88 for 25(OH)D; n = 4960 for AGP, CRP, ferritin, RBP, sTfR; n = 4959 for intake of milk, eggs, pork; n = 4958 for BMI; n = 4948 for gravidity; n = 4951 for occupation, education, food insecurity; n = 4949 for ethnicity; n = 4432 for hookworm.

Distribution and determinants of dietary vitamin D intake

The median estimated dietary vitamin D intake was 0.2 µg/d [8.0 IU] (IQR: 0.4), Table 1. The distribution of dietary vitamin D intake is shown in Fig. 1. The majority of women consumed less than 1 µg/d on average of dietary vitamin D, less than 1% of women met the Vietnamese RDA of 5 µg/d, and none met the United States' RDA of 15 µg/d [21,22]. Dietary sources of vitamin D included milk, eggs, and pork ribs, which contained only 1.0, 0.88, and 0.69 µg (40, 35.2, and 27.6 IU) of vitamin D per serving, respectively as determined by the Vietnamese food composition table [25]. A third of the women reported consuming any milk in the three months prior to interview, and about 20% reported consuming a glass of milk at least once per week. Approximately 90% of women reported consuming eggs over the three months prior to interview, and nearly 75% reported consuming them at a frequency of at least once per week. Approximately 46% of women reported eating pork at all, and about 30% consumed it at least once per week. Other potential dietary sources of vitamin D including mushrooms and fish did not contribute to dietary vitamin D intake in this population. Mushrooms were consumed in negligible amounts, and the fish in this region is not the fatty fish known to be a source of dietary vitamin D.

Significant determinants of vitamin D intake are presented in Table 2. In multivariable linear regression analysis, older age, farming as an occupation, increased food insecurity, and higher BMI were statistically significantly associated with lower vitamin D intake ($P < 0.001$ for all), while higher socioeconomic status, higher energy intake, and higher educational attainment were associated with higher vitamin D intake ($P < 0.001$ for all). Ethnic minority, hookworm infection, and gravidity were not significantly associated with dietary vitamin D intake.

Associations of vitamin D intake with hemoglobin and anemia

Vitamin D intakes were significantly correlated with hemoglobin concentration in bivariate analysis (Spearman $r = 0.03$, $P = 0.02$). However, dietary vitamin D intake was no longer significantly associated with hemoglobin concentration ($\beta = -0.01$ (SE: 0.02), $P = 0.56$) in the multivariable linear regression model that adjusted for age, BMI, energy intake, sTfR, CRP, AGP, ethnicity, occupation, food insecurity, education level and socioeconomic status (Table 3).

Vitamin D intakes were also significantly associated with anemia in bivariate analysis. Women in the highest tertile of vitamin D intake were 20% less likely to be anemic compared to those in the lowest tertile (OR: 0.80, 95% CI: 0.67, 0.95; $P = 0.01$), and these

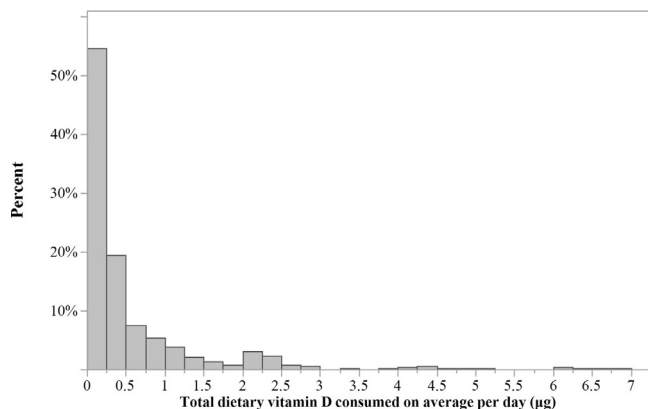


Fig. 1. Distribution of dietary vitamin D intake among women of reproductive age in northern Vietnam ($n = 4961$).

Table 2

Determinants of dietary vitamin D intake^a in women of reproductive age in northern Vietnam.^b

	β (SE)	P
Age (years)	-0.01 (0.004)	<0.001
BMI (kg/m ²)	-0.05 (0.01)	<0.001
Total energy intake (kcal/d) ^a	1.50 (0.05)	<0.001
Occupation (farmer)	-0.39 (0.05)	<0.001
SES quintile	0.15 (0.01)	<0.001
Lowest	Ref	
Low	0.13 (0.05)	0.01
Middle	0.24 (0.05)	<0.001
High	0.40 (0.06)	<0.001
Highest	0.60 (0.06)	<0.001
Food insecurity category ^c	-0.15 (0.02)	<0.001
Food secure	Ref	
Mildly food insecure	-0.22 (0.05)	<0.001
Moderately food insecure	-0.31 (0.05)	<0.001
Severely food insecure	-0.43 (0.10)	<0.001
Level of education ^d	0.15 (0.03)	<0.001
0–5th grade	Ref	
6–9th grade	0.19 (0.06)	0.004
10–12th grade	0.40 (0.07)	<0.001
Greater than 12th grade	0.37 (0.09)	<0.001

Values are β coefficients and SE, $n = 4961$.

SES: socioeconomic status.

^a Transformed to natural logarithmic scale.

^b Results from multivariable linear regression analysis with step-wise elimination.

^c Categories of food insecurity: food secure, mildly food insecure, moderately food insecure, severely food insecure.

^d Levels of education: 0–5th grade, 6–9th grade, 10–12th grade, or greater than 12th grade.

associations remained significant after controlling for age, total energy intake, sTfR (or ferritin), CRP, and AGP (Model 1: OR: 0.78, 95% CI: 0.64, 0.94; $P = 0.01$) (Table 3). However, the association between vitamin D intake and anemia was attenuated and no longer statistically significant when ethnic minority, occupation, education, food insecurity, and SES quintile, were added to the model (Model 2: OR: 0.95, 95% CI: 0.78, 1.17; $P = 0.65$). The results of the two stage least square analysis also showed that there was no residual association between vitamin D intake and hemoglobin concentration ($P = 0.97$) or anemia ($P = 0.19$), after accounting for the association of vitamin D intake with SES (results not shown).

Association of dietary vitamin D intake with 25(OH)D status

The mean serum 25(OH)D concentrations in the subset of women ($n = 88$) was 57.4 ± 10.7 nmol/L (Table 4). Approximately 20% of this sample had 25(OH)D concentrations < 50 nmol/L and 93% had 25(OH)D concentrations < 75 nmol/L. This subset of women was similar to the larger study population in terms of sociodemographic, health status, and biochemical characteristics (Table 4). Dietary vitamin D intake was not significantly associated with serum 25(OH)D concentrations in linear regression analysis controlling for total energy intake ($\beta = 0.48$ (SE: 0.35), $P = 0.17$).

Determinants of 25(OH)D concentration and association of 25(OH)D with hemoglobin and anemia

None of the predictors of dietary vitamin D intake, including age, BMI, total energy intake, occupation, food insecurity, education and socioeconomic status, were significantly associated with serum 25(OH)D concentrations ($P > 0.05$ for all).

After full adjustment for all covariates mentioned above, women with serum 25(OH)D < 50 nmol/L had significantly lower hemoglobin concentration compared to women with serum 25

Table 3
Multivariable regression analysis of vitamin D intake with hemoglobin and anemia.

Hemoglobin as outcome ^c	Model 1 ^a		Model 2 ^b	
	β (SE)	<i>P</i>	β (SE)	<i>P</i>
Vitamin D intake ^d	0.03 (0.02)	0.04	−0.01 (0.02)	0.56
Tertile of dietary vitamin D intake				
Low	Ref		Ref	
Middle	0.001 (0.05)	0.99	−0.03 (0.05)	0.55
Higher	0.11 (0.05)	0.04	−0.01 (0.05)	0.87
Anemia as outcome ^e	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Tertile of dietary vitamin D intake				
Low	Ref		Ref	
Middle	1.00 (0.84, 1.19)	1.00	1.07 (0.90, 1.27)	0.45
Higher	0.78 (0.64, 0.94)	0.01	0.95 (0.78, 1.17)	0.65

Values are β coefficients and SE for linear regression analyses and OR and 95% CI for logistic regression analyses, n = 4961.

^a Model 1: Association of vitamin D intake with hemoglobin or anemia, adjusted for age, BMI, total energy intake, and transferrin receptor, C-reactive protein, and α_1 -acid glycoprotein.

^b Model 2: Model 1 + adjustment for ethnicity, occupation, education level, food insecurity, and socioeconomic quintile.

^c Results from multivariable linear regression analysis.

^d Transformed to natural logarithmic scale.

^e Results from multivariable logistic regression analysis, BMI dropped from these models due to collinearity.

Table 4
Biochemical and sociodemographic characteristics of subset with available 25(OH)D.^a

	Mean \pm SD or n (%)
Sociodemographic and health status characteristics	
Age (y)	26.6 \pm 5.1
Education (completed 12th grade)	13 (14.8)
Occupation (farmer)	69 (78.4)
Ethnicity (minority)	44 (50.0)
Food insecurity (moderately or severely food insecure)	13 (14.8)
BMI (kg/m ²)	19.1 \pm 2.0
Gravidity	1.2 \pm 0.8
Dietary intake	
Vitamin D intake (μ g/d)	0.2 (0.4) ^b
Iron intake (mg/d)	15.9 (9.8) ^b
Total energy intake (kcal/d)	2149.9 (895.6) ^b
Biochemical markers	
Serum 25(OH)D (nmol/L)	57.4 \pm 10.7
25(OH)D < 50 nmol/L	18 (20.5)
25(OH)D \geq 50 nmol/L	82 (93.2)
Hemoglobin (g/dL)	12.9 \pm 1.5
Anemia ^c	21 (23.9)
Plasma Ferritin (μ g/L)	76.5 (58.5) ^b
Plasma sTfR (mg/L)	4.7 \pm 1.0
Plasma RBP (mmol/L)	1.7 (0.4) ^b
Nutrient deficiency ^d	5 (5.7)
Plasma AGP (g/L)	0.7 \pm 0.2
Plasma CRP (mg/L)	0.4 (0.6) ^b
Inflammation ^e	3 (3.4)
Hookworm (proportion of women with any eggs)	18 (22.5)

Values are Means \pm SD or n (%) unless otherwise noted.

25(OH)D, 25-hydroxyvitamin D; AGP, α_1 -acid glycoprotein; BMI, body mass index; CRP, C-reactive protein; RBP, retinol binding protein; sTfR, soluble transferrin receptor.

^a n = 88.

^b Median (IQR) for non-normally distributed variables.

^c Anemia defined as hemoglobin <12 g/dL.

^d Ferritin <12 μ g/L or RBP < 1.05 mmol/L.

^e AGP >1 g/L or CRP >5 mg/L.

(OH)D \geq 50 nmol/L (β = −0.91 (SE: 0.42), *P* = 0.03) (Table 5). In the models with anemia as the outcome, neither serum 25(OH)D as a continuous variable nor 25(OH)D <50 nmol/L were significantly associated with anemia in unadjusted or adjusted models (Table 5). Similar non-significant associations were observed with hemoglobin or anemia when 25(OH)D was categorized as <75 nmol/L vs. \geq 75 nmol/L (*P* = 0.62 and *P* = 0.72 for hemoglobin and anemia, respectively). Serum 25(OH)D modeled either continuously or dichotomized as 25(OH)D <50 nmol/L vs. 25(OH)D \geq 50 nmol/L

was not statistically significantly associated with other markers of iron status, plasma sTfR (*P* = 0.23 and *P* = 0.18, for serum 25(OH)D continuously and dichotomized, respectively) or plasma ferritin (*P* = 0.053 and *P* = 0.12, for serum 25(OH)D continuously and dichotomized, respectively), controlling for age, total energy intake, BMI, AGP, CRP, ethnicity, food insecurity, occupation, education, and SES.

Discussion

This paper reports the dietary vitamin D intake among WRA in the largely rural and mountainous Thai Nguyen province of northern Vietnam and its association with hemoglobin concentration and anemia. We found that reported dietary vitamin D intake was profoundly low in this population, with less than 1% of women reporting intakes of vitamin D meeting the Vietnamese RDA of 5 μ g/d, and none of the women reporting intakes of vitamin D meeting even the estimated average requirement (EAR) of 10 μ g/d (400 IU/day), as recommended by the Institute of Medicine (IOM) in the United States [21,22]. Dietary vitamin D intake was not significantly associated with hemoglobin concentrations or anemia after adjustment for sociodemographic variables; therefore, our hypothesis regarding the link between vitamin D intake and hemoglobin and anemia is rejected. However, in the subset of women with available 25(OH)D concentrations, serum 25(OH)D concentrations <50 nmol/L were significantly associated with a 0.91 g/dL reduction in hemoglobin concentration compared to serum 25(OH)D concentrations \geq 50 nmol/L, consistent with our hypothesis.

Low dietary vitamin D intake in this population is likely due to the dearth of food sources of vitamin D consumed by women in this region; the only food sources of vitamin D consumed were eggs, milk, and pork ribs. As the food supply in Vietnam is not fortified with vitamin D, the content of vitamin D in these foods is quite low. Though the overall intake of vitamin D was extremely low, we found that higher SES (higher quintile of SES, higher educational attainment, better food security, and non-farming occupation) was associated with increased dietary vitamin D intake. These findings are consistent with an earlier nationwide study that assessed vitamin D intake among WRA in Vietnam using a national household food intake survey [20]. Studies of food consumption patterns in Vietnam indicate that rural households were more likely to consume diets higher in carbohydrates, and lower in animal proteins and fats compared to those living in urban areas

Table 5
Multivariable regression analysis of 25(OH)D with hemoglobin and anemia.

Hemoglobin as outcome ^c	Model 1 ^a β (SE)	P	Model 2 ^b β (SE)	P
25(OH)D (continuous)	0.05 (0.04)	0.25	0.05 (0.04)	0.24
25(OH)D (categorical)				
25(OH)D ≥ 50 nmol/L	Ref		Ref	
25(OH)D < 50 nmol/L	−0.68 (0.42)	0.11	−0.91 (0.42)	0.03
Anemia as outcome ^d	OR (95% CI)	P	OR (95% CI)	P
25(OH)D (continuous)	0.94 (0.82, 1.07)	0.34	0.82 (0.66, 1.03)	0.08
25(OH)D (categorical)				
25(OH)D ≥ 50 nmol/L	Ref		Ref	
25(OH)D < 50 nmol/L	1.28 (0.36, 4.54)	0.70	5.44 (0.67, 43.98)	0.11

Values are β coefficients and SE for linear regression analyses and OR and 95% CI for logistic regression analyses, n = 88.

25(OH)D: 25-hydroxyvitamin D.

^a Model 1: Association of 25(OH)D with hemoglobin or anemia, adjusted for age, BMI, total energy intake, and transferrin receptor, C-reactive protein, and α₁-acid glycoprotein.

^b Model 2: Model 1 + adjustment for ethnicity, occupation, education level, food insecurity, and socioeconomic quintile.

^c Results from multivariable linear regression analysis.

^d Results from multivariable logistic regression analysis, BMI dropped from these models due to collinearity.

[34,35]. Previous findings from our study population showed that nearly 55% of women consumed carbohydrates in excess of the Acceptable Macronutrient Distribution Range (AMDR) as recommended by the IOM [36], and a similar proportion did not meet the recommended intakes from fats [37]. The high carbohydrate-low fat composition of the diet may explain why vitamin D intakes are low, since the primary natural dietary sources of vitamin D are foods rich in fats and proteins such as fatty fish and cheese.

Dietary vitamin D intake was not associated with hemoglobin or anemia after controlling for sociodemographic variables. One potential explanation is that vitamin D intake and anemia appeared to be largely determined by SES. This is supported by our two-stage least squares analysis in which we found that after controlling for the association between quintile of SES and vitamin D intake, residual vitamin D intake was not significantly associated with hemoglobin concentration or anemia. The association of vitamin D intake with SES is consistent with findings of other micronutrient intakes in this population [38], suggesting that those with higher SES may have more diverse diets, potentially translating to improved health outcomes, such as in the case of anemia.

Another explanation for why vitamin D intake was not associated with hemoglobin or anemia is that dietary vitamin D intake may not be reflective of an individual's vitamin D status, as measured by 25(OH)D concentrations. Approximately 90% of an individual's vitamin D requirement comes from sun exposure [39], and given the extremely low dietary vitamin D intake in our population, it is likely that dietary vitamin D intake was not a primary contributor to 25(OH)D status. Indeed, dietary vitamin D intake was not significantly associated with serum 25(OH)D concentrations in our study population among the group in which 25(OH)D was available.

Although we did not observe significant associations of dietary vitamin D intake with hemoglobin or anemia, we did find a significant inverse association between serum 25(OH)D concentrations <50 nmol/L and hemoglobin concentrations in a smaller subset of women. This finding is consistent with studies in those with chronic kidney disease which have found that treatment with vitamin D or its analogue resulted in significant increases in hemoglobin concentrations [40,41]. In our fully adjusted model, serum 25(OH)D concentrations <50 nmol/L were associated with a 0.91 g/dL reduction in hemoglobin concentrations, compared to serum 25(OH)D concentrations ≥50 nmol/L. This association is not inconsequential, as a meta-analysis by Stoltzfus et al. [3] found that a 1 g/dL increase in hemoglobin was associated with a 25% reduction in maternal mortality. Therefore, improvements in 25(OH)D con-

centrations to maintain a level of 50 nmol/L or greater (the level designated by the IOM as sufficient to maintain bone and overall health) [21], may have beneficial implications in terms of hemoglobin concentrations.

The potential mechanism underlying the association between vitamin D and hemoglobin likely involves hepcidin, the major iron-regulatory hormone [17]. When hepcidin concentrations are elevated, such as in response to an inflammatory stimulus, this prevents iron egress from cells of the reticuloendothelial system, limiting iron absorption and sequestering iron within macrophages [42]. Vitamin D has been shown to lower hepcidin-stimulatory pro-inflammatory cytokines and act directly on the hepcidin antimicrobial peptide gene to suppress hepcidin expression in hepatocytes and macrophages [18,19]. Thus, vitamin D, in lowering pro-inflammatory cytokines and hepcidin, may increase iron bioavailability for hemoglobin synthesis and erythropoiesis.

We did not observe a significant association between serum 25(OH)D concentrations with anemia in this population, despite reports of significant associations in other populations [14–16,43,44]. It is possible that while serum 25(OH)D concentrations <50 nmol/L were associated with reductions in hemoglobin, this was not severe enough to affect anemia status. In our small subset, we were unable to determine whether the relationship between 25(OH)D and anemia differed by inflammation or nutrient status. Previous studies have reported vitamin D status to be associated particularly with anemia of inflammation, and this is in line with the potential mechanism described above [15,16]. Furthermore, the lack of an association between either dietary vitamin D intake or serum 25(OH)D concentrations with anemia may suggest that anemia is largely determined by SES in this population. Indeed, in a previous analysis by our group exploring the multi-causal etiology of anemia in this study population, it was found that minority ethnicity, lower education, and lower SES quintile were all significant predictors of anemia [9].

Strengths of this analysis included a large sample size and use of a validated semi-quantitative FFQ to estimate nutrient intake at an individual level. However, there are important limitations of our study. First, the cross-sectional nature of our analysis precludes us from drawing causal inferences from the associations we observed. Second, FFQs rely on recall, which may be inaccurate and especially difficult in foods eaten infrequently (which may be the case for food sources of vitamin D). However, given that relatively few foods are good natural sources of vitamin D, and that the majority of women in our population eat a diet high in carbohydrates and low in fats, errors in recall are unlikely to affect our

vitamin D intake estimates. Another limitation is that we were unable to measure serum 25(OH)D concentrations in the entire study population due to limited resources. Although this sample had sufficient power to detect the observed association between vitamin D status and hemoglobin concentrations, we may have been under powered to detect an association between vitamin D status and anemia. Moreover, we cannot conclude that the associations observed with hemoglobin in the smaller subset, holds for the entire study population. However, the sociodemographic, health status, and biochemical characteristics of the subset were similar to those of the entire study population, and the serum 25(OH)D levels observed in our subset were similar to those reported in other studies of vitamin D status among Vietnamese women [20,45,46]. It is also possible that there may be residual confounding in our associations due to variables that we were unable to control for such as outdoor physical activity, so we cannot exclude the possibility that our results may be biased. Additionally, we were unable to examine the associations of other vitamin D metabolites including the active form of vitamin D (1,25-dihydroxyvitamin D) and free 25(OH)D concentrations with our outcomes which may have allowed us to more comprehensively evaluate the link between vitamin D and iron metabolism. Finally, this was a study in a primarily rural and mountainous region of northern Vietnam, where the majority of women worked as farmers; our findings may not be generalizable to men or the entire country of Vietnam, especially urban or southern areas.

In conclusion, we found that dietary vitamin D intake was very low in this population, and was inversely associated with SES, but not with hemoglobin concentrations or anemia. Serum 25(OH)D concentrations <50 nmol/L were significantly inversely associated with hemoglobin concentration, suggesting that achieving a vitamin D status ≥ 50 nmol/L may result in improvements in hemoglobin concentrations. Further research, including experimental studies, is warranted to fully evaluate the implications of this association, and to understand the role of vitamin D in iron metabolism.

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