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RESEARCH ARTICLE

Predictors of vitamin D status in undernourished and well-nourished children 6–59 months old, in the JB Marks Municipality of South Africa

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Objective: to investigate the predictors of vitamin D (vitD) status of undernourished and well-nourished children aged under five years in the North West Province of South Africa.
Design: this cross-sectional study assessed sociodemographic data, nutritional supplement intake, vitD-rich food consumption, and sunlight exposure via a structured questionnaire. Venous blood samples were collected to evaluate vitD, iron, and inflammatory markers.
Setting: the maternal and child wellness departments of six community clinics in the JB Marks Municipality.
Participants: 121 undernourished and 51 well-nourished children, 6–59 months old.
Results: The prevalence of serum 25(OH)D < 30 ng/ml was 20.3%, 19.9%, and 21.6% in the total, undernourished, and well-nourished groups, respectively. The total mean 25(OH)D concentration was 38.41 ± 9.64 ng/ml. Age showed a negative association trend with 25(OH)D in the well-nourished group (β: −0.172, 95% CI −0.353, 0.010, $p = 0.063$), while household income was inversely associated with 25(OH)D ($\beta = -1.86$, 95%CI = −2.99, −0.733, $p = 0.001$) in the total group. Among the undernourished children, iron-deficiency anaemia (IDA) was associated with almost five times greater odds for 25(OH)D < 30 ng/ml (OR: 4.646, 95% CI 1.339, 16.123, $p = 0.016$). Intake of vitD supplements, therapeutic foods, multivitamins, and formula milk was associated with significantly higher 25(OH)D concentrations and was adjusted for in subsequent analyses. Additionally, consumption of eggs more than once a month was associated with higher 25(OH)D levels in the well-nourished children aged 24–59 months. Nutritional status, inflammation, and sunlight exposure were not associated with 25(OH)D concentrations.
Conclusion: vitD insufficiency exists in both the undernourished and the well-nourished. Age, egg consumption, and IDA should be considered in the correction of vitD insufficiency in children.
Trial registration: Pan African Clinical Trial Registry identifier: PACTR202110646172601..

Keywords: Vitamin D status, undernutrition, predictors, iron, anaemia, inflammation, diet, sunshine

Introduction

Vitamin D deficiency/insufficiency is emerging as a widespread health concern in both healthy and undernourished children in Africa. A 25% vitamin D deficiency (VDD) prevalence has been reported in children across Africa.[1] Similarly, in undernourished children, a 31% and 44% prevalence has been observed in Tanzania and Uganda, respectively.[2,3] There is increasing evidence of the role of vitamin D (vitD) in immune function and infection prevention.[4] Aside from impairment of skeletal growth, VDD in children may lead to increased susceptibility to infectious diseases, which is one of the leading causes of morbidity and mortality in children aged under five years.[5] The literature suggests that vitD status is influenced by a number of factors, including but not limited to the level of sunshine exposure, dietary intake, genetic variations, season, and skin pigmentation.[6–9] In South Africa (SA), Poopedi et al. observed the influence of season on vitD status in white but not black 10-year-old children, with 25-hydroxyvitamin D (25(OH)D) levels highest in summer and autumn.[10] Vitamin D levels have also been reported to increase with age in a study involving Malawian infants from birth to 24 months.[11] A number of studies in adults have associated VDD with elevated systemic and intestinal inflammation, which is common in undernourished children.[12,13] However, in a study by Mogire et al. inflammation was associated with higher 25 (OH)D concentration in African children aged between 0 and 8 years.[14] Vitamin D deficiency has been associated with iron deficiency (ID), as the latter is reported to inhibit the activity of the enzymes 25- and 1α-hydroxylase involved in the metabolism of vitD[15]. In South African children aged 6–59 months, little is known about the determining factors of vitD status and the factors contributing to VDD. Therefore, we aimed to describe the prevalence of VDD and insufficiency and investigate the predictors of vitD status among undernourished and well-nourished children aged under five years in a peri-urban area in SA.

Materials and methods

Study population and setting

This was a cross-sectional study including undernourished and well-nourished black African children, 6–59 months old. The study was conducted at the maternal and child wellness units in six community clinics in the JB Marks Municipality in the North West Province of SA (26.7145° S, 27.0970° E). The participants were children reporting to the clinics for routine growth monitoring or immunisation. Data were collected within one season (i.e. between May and August 2022) to prevent seasonal variations in the vitD levels. We employed consecutive sampling; therefore all children visiting the clinics for growth monitoring or immunisation were invited to participate and were recruited after informed consent was obtained until the estimated sample size was achieved.

Eligibility criteria

Inclusion criteria

Children aged 6–59 months whose parents provided consent were included. In the undernourished group, children who were underweight (weight-for-age z-score (WAZ) < −2 SD), or wasted (weight-for-height/length z-score (WHZ) < −2 SD, or had a mid-upper arm circumference (MUAC) < 12.5 cm), or were stunted (height/length-for-age z-score (HAZ) < −2 SD), or those with bilateral pitting oedema + or ++ were included. In the well-nourished group, children with WAZ and WHZ > −1 SD but < 2 SD and HAZ > −1 SD were included.

Exclusion criteria

In both groups, children who were on treatment for VDD, on medication for chronic illness, fever (axillary temperature > 38 °C), persistent cough in the past 2 weeks, persistent diarrhoea or vomiting in the past 3 days, medical record of a diagnosis of conditions associated with vitD hypersensitivity (i.e. sarcoidosis, primary hyperparathyroidism), blood transfusion in the last month, liver and kidney disease, or refusal of parent/caregiver to consent were excluded.

Ethics approval, consent, and registration

The study was approved by all the relevant authorising institutions. Parents of all prospective participants were verbally informed about the study and interested parents were provided with the information leaflet on the study in their preferred language. Written informed consent was obtained from interested parents before screening for eligibility into the study. The principles of the 2013 World Medical Association Declaration of Helsinki were meticulously followed throughout the study. To ensure confidentiality, all study procedures were carried out in privacy, and all data were anonymised.

Outcome measures

The primary outcome measures were serum 25(OH)D concentration, and prevalence of VDD and insufficiency.

Exposure measures

These included biomarkers of iron status, i.e. haemoglobin (Hb), soluble transferrin receptor (sTfR) and ferritin, and biomarkers of inflammation including C-reactive protein (CRP) and alpha–1 acid glycoprotein (AGP) concentrations. Data on anthropometry, sociodemographic information, dietary intake of vitD from supplements and food sources, medication use, and sunlight exposure were also assessed.

Data collection

Weight, height/length, and mid-upper arm circumference (MUAC) of each participant were measured by trained fieldworkers, and weight-for-age (WAZ), weight-for height (WHZ), and height-for-age (HAZ) z-scores were estimated using the WHO Anthro software (version 3.2, WHO, Geneva, Switzerland) to screen for undernutrition or good nourishment. The study nurse assessed participants for bilateral pitting oedema and signs of infection, including fever, diarrhoea, cough, and vomiting, by reviewing the participant's medical record booklet, physical assessment, and interviewing the parent. Children who were eligible for inclusion in the study were enrolled. At enrolment, a structured sociodemographic questionnaire and a non-quantitative vitD food frequency questionnaire were administered to assess the intake of vitD-containing foods over the past month. Additionally, information on participants' medication history, nutritional supplement, and therapeutic food intake was obtained from their medical record booklet and interviewing the parent. The level of sunlight exposure of participants was assessed using a sunlight exposure questionnaire. A detailed description of the study methods has been published elsewhere.[16]

Biomarker analysis of vitamin D status, inflammation, and iron status

A 2 ml venous blood sample was taken from each participant. Vitamin D status was measured as serum 25(OH)D concentration using chemiluminescence immunoassay, standardised with the National Institute of Standards and Technology (NIST) standard reference materials 972a on Beckman DxI 800 (https://www.beckmancoulter.com/products/immunoassay/dxi-800).[17,18] Markers of systemic inflammation and iron status including CRP, AGP, sTfR, and ferritin were measured using Q-Plex™ Human Environmental Enteric Dysfunction (11-Plex) assay from Quansys Biosciences (Logan, UT, USA)[19]. Haemoglobin concentration in whole blood was analysed with a portable Hb201 + HemoCue system (HemoCue, Angelholm, Sweden).[20] Details of biomarker analysis have been previously described.[16]

Definitions and cut-offs

Vitamin D deficiency, insufficiency, and sufficiency were defined as serum 25(OH)D concentrations of < 20 ng/ml, 20–29.9 ng/ml, and ≥3 0 ng/ml, respectively.[21] We further classified vitD status of 25(OH)D < 30 ng/ml as suboptimal, and 25 (OH)D ≥ 30 ng/ml as optimal. Inflammation was defined as CRP > 5 mg/l and AGP > 1 g/l.[22] Iron deficiency was defined as ferritin concentrations of < 12 µg/l after being corrected for inflammation using the BRINDA method,[23] and iron deficiency erythropoiesis (IDE) as sTfR ≥ 8.3 mg/l corrected for inflammation using the BRINDA method.[23–25] Anaemia was defined as Hb < 11.0 g/dl and ID anaemia (IDA) as the presence of anaemia and sTfR ≥ 8.3 mg/l and/or corrected ferritin concentration < 12 µg/l.[26,27] Hb concentrations were adjusted for altitude > 1 000 m above sea level by subtracting 0.2 g/dl (27). Stunting was defined as HAZ < −2 SD, wasting as WHZ < −2 SD or MUAC < 12.5 cm, and underweight as WAZ < −2 SD[28].

Statistical analysis

The sample size was calculated using the G*power 3.1.9.2 statistical program (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower) based on correlation (bivariate normal model), 5% probability of error, and a power of 80% and an effect size of 0.21; a sample size of 175 was required. Full details of sample size calculation are explained elsewhere.[16] Data analysis was performed using the Statistical Package for the Social Sciences software (version 27; IBM Corp, Armonk, NY, USA). The difference in continuous outcome measures between the two groups was analysed using independent *t*-tests when the data were normally distributed or Mann–Whitney U-tests for non-normally distributed. Chi-square and Fisher's exact tests were used to determine associations between categorical variables. Univariable and multivariable linear and logistic regression analyses were used to assess the association of sociodemographic, dietary, anthropometric, and environmental factors (age, sex, wasting, underweight, stunting, iron status and anaemia, inflammation, level of sunlight exposure, dietary vitD intake, and nutritional supplement intake) with serum 25(OH)D concentration and vitD status (deficiency, insufficiency and sufficiency, or optimal and suboptimal status).

Results

Characteristics of study participants

A total of 172 children, consisting of 121 undernourished and 51 well-nourished children with a median age of 18.2 (12.0, 30.1) months, and 53.5% girls, were included in the study (Table 1). In the undernourished group, the prevalence of stunting, underweight, and wasting was 90.1%, 43.8%, and 11.6%, respectively.

Vitamin D, inflammation, and iron status of the undernourished and well-nourished children

The total mean serum 25(OH)D concentration was 38.41 ± 9.64 ng/ml (Table 2). The undernourished group had a significantly higher 25(OH)D than the well-nourished children (p = 0.016). However, among children not taking any form of supplemental vitD, the serum 25(OH)D concentration was not significantly different between the undernourished and well-nourished groups (p = 0.185). The total prevalence of VDD (25(OH)D < 20 ng/ml) and insufficiency (25(OH)D = 20–29.9 ng/ml) was 1.7% and 18.6%, respectively (Table 2), with no difference between the undernourished and well-nourished children (p = 0.925). The mean Hb concentration of the total group was 10.61 ± 1.20 g/dl, with the undernourished group recording a concentration of 10.56 ± 1.24 g/dl, compared with 10.73 ± 1.11 g/dl in the well-nourished group (p = 0.409). The total prevalence of anaemia was 60.5%; 62.0% in the undernourished and 56.9% in the well-nourished children.

Potential factors contributing to vitamin D concentrations in undernourished and well-nourished children

Sociodemographic factors

Serum 25(OH)D differed among the household income groups in the total and undernourished groups (both p = 0.002) and was higher among children in the lowest household income category than those in the 3001–6000 ZAR category (p = 0.001 and p = 0.002, respectively) (Table 3). The concentration of 25(OH)D was borderline higher in undernourished boys than girls (p = 0.055).

Nutritional status

Severely stunted children had a higher 25(OH)D concentration than those were not stunted (p = 0.047). However, in a subgroup analysis of children who took no vitD supplements, therapeutic foods, multivitamins, and formula milk, there was no significant difference in the concentration of 25(OH)D between the stunted and non-stunted (38.05 ± 10.03 vs 36.02 ± 8.68 ng/ml , p = 0.275).

Iron status

Anaemic undernourished children had lower 25(OH)D concentrations than non-anaemic children (p = 0.028) (Table 3). The proportion of children with VDD and insufficiency was higher in those who had IDA compared with those who did not have IDA (p = 0.033) (Supplementary figure).

Table 1: Sociodemographic and anthropometric characteristics of undernourished and well-nourished children

Characteristics	Total (n = 172)	Undernourished (n = 121)	Well-nourished (n = 51)	p-value[1]
Sociodemographics				
Age (months)[a]	18.2 (12.0, 30.1)	17.3 (12.0, 28.4)	21.8 (12.0, 37.0)	0.176
Sex, *n* (%)				
Male	80 (46.5)	55 (45.5)	25 (49.0)	0.739
Female	92 (53.5)	66 (54.5)	26 (51.0)	
Monthly household income, ZAR *n* (%)				
< 500	30 (17.4)	22 (18.2)	8 (15.7)	0.424
500–1 000	32 (18.6)	26 (21.5)	6 (11.8)	
1 001–3 000	55 (32.0)	38 (31.4)	17 (33.3)	
3 001–6 000	34 (19.8)	23 (19.0)	11 (21.6)	
> 6 000	21 (12.2)	12 (9.9)	9 (17.6)	
Toilet facility *n* (%)				
Pit latrine	42 (24.6)	36 (30.0)	6 (11.8)	**0.026**
Public flush	35 (20.5)	25 (20.8)	10 (19.6)	
Private flush	94 (55.0)	59 (49.2)	35 (68.6)	
Anthropometry				
Weight (kg)[a]	9.09 (7.77, 11.26)	8.57 (7.37, 9.96)	11.69 (9.60, 14.15)	< **0.001**
MUAC (cm)[b]	14.17 ± 1.35	13.66 ± 1.12	15.39 ± 1.50	< **0.001**
WAZ[b]	−1.25 ± 1.24	−1.87 ± 0.82	0.23 ± 0.69	< **0.001**
WHZ[b]	−0.25 ± 1.13	−0.55 ± 1.12	0.47 ± 0.76	< **0.001**
HAZ[a]	−2.23 (−2.87, −0.76)	−2.59 (−3.22, −2.16)	−0.41 (−0.74, 0.22)	< **0.001**
Underweight, *n* (%)	53 (30.8)	53 (43.8)	-	
Stunted, *n* (%)	109 (63.4)	109 (90.1)	-	
Wasted, *n* (%)	14 (8.1)	14 (11.6)	-	

MUAC: mid-upper arm circumference; HAZ: height/length-for-age; WAZ: weight-for-age z-score; WHZ: weight-for-height/length z-score; ZAR: South African Rand; [a] median (25th, 75th percentile),[b] mean ± standard deviation [1] Undernourished and well-nourished groups were compared with independent *t*-test, Mann–Whitney U-test and chi-square test. P < 0.05 was considered significant and bolded in table.

Table 2: Comparison of vitamin D concentrations, inflammation, and iron status between undernourished and well-nourished children

Characteristics	Total ($n = 172$)	Undernourished ($n = 121$)	Well-nourished ($n = 51$)	p-value[1]
Vitamin D (ng/ml) (mean ± SD)				
Serum 25(OH)D	38.41 ± 9.64	39.55 ± 9.78	35.70 ± 8.81	0.016
Adjusted serum 25(OH)D[a]	37.3 ± 9.60[b]	38.1 ± 9.70[c]	35.5 ± 9.20[d]	0.185
Vitamin D status classification, *n* (%)				
Vitamin D sufficiency (≥ 30 ng/ml)	137 (79.7)	97 (80.2)	40 (78.4)	0.925
Vitamin D insufficiency (20–29.9 ng/ml)	32 (18.6)	22 (18.2)	10 (19.6)	
Vitamin D deficiency (< 20 ng/ml)	3 (1.7)	2 (1.7)	1 (2.0)	
Inflammation, *n* (%)				
CRP > 5 mg/l	37 (22.0)	24 (20.0)	13 (27.1)	0.317
AGP > 1 g/l	128 (76.2)	90 (75.0)	38 (79.2)	0.567
Iron and anaemia status, *n* (%)				
Anaemia	104 (60.5)	75 (62.0)	29 (56.9)	0.609
Severe anaemia	1 (0.6)	1 (0.8)	0 (0.0)	0.578
Moderate anaemia	42 (24.4)	33 (27.3)	9 (17.6)	
Mild anaemia	61 (35.5)	41 (33.9)	20 (39.2)	
Iron-deficiency anaemia	80 (47.3)	59 (49.2)	21 (42.9)	0.500
Iron deficiency	73 (43.5)	48 (40.0)	25 (52.1)	0.171

[a]Children consuming supplemental vitamin D (vitamin D supplements, formula milk, therapeutic food, or multivitamins) were excluded ([b] $n = 114$; [c] $n = 78$, [d] $n = 36$); [1] Undernourished and well-nourished groups were compared with independent *t*-test, Mann–Whitney U-test, and chi-square test. $P < 0.05$ was considered significant

Dietary intake

Children in the total and undernourished group who took vitD supplements ($p = 0.003$ and $p = 0.01$), therapeutic foods ($p < 0.001$ and $p < 0.001$), multivitamins (overall group $p = 0.053$), and formula milk ($p = 0.028$ and $p = 0.031$) had higher 25(OH)D than those who did not. Well-nourished children who consumed at least two dairy products daily had a lower 25(OH)D concentration compared with those who consumed a higher frequency of dairy products ($p = 0.016$) (Table 3). There was no difference in the vitD status of children who consumed fish or egg once a month compared with those who consumed these more frequently in the total group (Table 3). However, the well-nourished children aged 24–59 months who consumed eggs more than once a month had a significantly higher serum 25(OH)D concentration of 35.09 ± 6.16 IU compared with 28.33 ± 5.02 in those who had eggs once or less in a month ($p = 0.027$) (results not shown in a table).

Sociodemographic factors and nutritional status associated with vitamin D concentration

Among the well-nourished children, age showed a trend of negative association with 25(OH)D ($\beta = -0.17$, 95% CI = −0.353, 0.010, $p = 0.063$) (Table 4). Undernourished girls showed a trend of more than twice the odds of having suboptimal vitD levels compared with boys (OR = 2.42, 95% CI = 0.89, 6.60, $p = 0.084$) (Table 5). In the total group, 5.9% of the variability in 25(OH)D was explained by household monthly income, which was inversely associated with 25(OH)D ($\beta = -1.86$, 95% CI = −2.99, −0.733, $p = 0.001$). A unit decrease in HAZ was associated with 1.17 ng/ml ($p = 0.029$) increase in 25(OH)D concentration in the total group. This inverse relationship remained after excluding children who took supplemental sources of vitD ($\beta = -1.40$, 95% CI = −2.69, −0.11, $p = 0.034$) (Supplementary Table 1).

Association of iron status and inflammation with vitamin D

In the undernourished group, Hb concentrations showed a positive borderline association with 25(OH)D ($\beta = 1.40$, 95% CI = −0.01, 2.80, $p = 0.051$) (Table 4). Table 5 indicates that among the undernourished children, IDA was associated with almost five times increased odds of suboptimal vitD level (OR = 4.65, 95% CI = 1.34, 16.12, $p = 0.016$), while anaemia was associated with borderline increased odds of suboptimal vitD level (OR = 3.55, 95% CI = 0.90, 14.13, $p = 0.072$). Conversely, in the well-nourished group, anaemia was associated with reduced odds of suboptimal vitD level (OR = 0.135, 95% CI = 0.022, 0.820, $p = 0.030$) (Table 5). CRP and AGP were not associated with 25(OH)D. However, in the vitD deficient (25(OH)D < 20 ng/ml) children, there was a borderline inverse association between CRP > 5 mg/l and 25(OH)D concentration ($\beta = -2.750$, 95% CI = −6.051, 0.551, $p = 0.060$) (results not shown in table).

Dietary factors associated with vitamin D status

Intake of multivitamins accounted for approximately 2.2% variability in 25(OH)D concentration ($R^2 = 0.022$) and was associated with 77% reduced odds for suboptimal vitD status in the total study group ($p = 0.049$) in model 1. However, after adjusting for therapeutic food, formula milk, and vitD supplement intake, the significance of the association was lost ($p = 0.134$) (Table 5). In the total group, intake of vitD supplement and formula milk were positively associated with 25(OH)D ($\beta = 7.83$, 95% CI = 2.64, 13.02, $p = 0.003$ and $\beta = 3.84$, 95% CI = 0.414, 7.270, $p = 0.028$, respectively) and accounted for 5% and 2.8% of the variability in 25(OH)D concentration. After adjusting for other sources of supplemental vitD (i.e. multivitamin, therapeutic food), vitD supplement intake but not formula milk intake remained positively associated with 25(OH)D concentration ($\beta = 6.8$, 95% CI = 0.021, 13.64, $p = 0.049$ and $\beta = 2.45$, 95% CI = −1.30, 6.21, $p = 0.199$) (data not shown in table). The frequency of intake of dairy products and margarine were negatively associated with 25(OH)D in the well-nourished group ($\beta = -0.42$, 95% CI = −0.78, −0.05, $p = 0.025$ and $\beta = -0.97$, 95% CI = −1.85, −0.09, $p = 0.031$) (Table 4).

Generally, the frequency of intake of vitD-containing foods such as fish and egg was low (Figure 1). The frequency of fish intake was inversely associated with 25(OH)D in the total group ($\beta = -0.45$, 95% CI = −0.89, −0.01, $p = 0.044$) and well-nourished

Table 3: Vitamin D concentration by sociodemographic factors, nutritional, iron and inflammation status, and dietary intake among undernourished and well-nourished children

Characteristics	Total (*n* = 172) Mean ± SD 25(OH)D ng/ml	*p*-value	Undernourished group (*n* = 121) Mean ± SD 25(OH)D ng/ml	*p*-value	Well-nourished group (*n* = 51) Mean ± SD 25(OH)D ng/ml	*p*-value
Sociodemographics						
Age categories (months) [a]						
< 12	40.42 ± 10.11	0.546	40.03 ± 10.90	0.860	41.27 ± 8.50	0.112
≥ 12– < 24	37.71 ± 10.05		38.65 ± 9.91		34.54 ± 10.20	
≥ 24– < 36	38.86 ± 10.59		41.29 ± 10.61		32.18 ± 7.57	
≥ 36– < 48	37.64 ± 6.56		40.36 ± 7.02		33.84 ± 3.68	
≥ 48	36.43 ± 6.78		38.73 ± 5.67		33.87 ± 7.29	
Sex[b]						
Boys	39.81 ± 9.35	0.077	41.42 ± 9.30	0.055	36.26 ± 8.60	0.661
Girls	37.20 ± 9.78		38.00 ± 9.97		35.16 ± 9.14	
Household monthly income [a]						
< 500	42.86 ± 9.86*	**0.002**	43.44 ± 8.96*	**0.002**	41.25 ± 12.56	0.250
500–1 000	40.44 ± 9.39		41.01 ± 9.36		38.00 ± 10.00	
1 001–3 000	37.77 ± 9.59		39.71 ± 9.43		33.44 ± 8.72	
3 001–6 000	33.74 ± 7.73*		32.70 ± 7.82*		35.91 ± 7.42	
> 6 000	38.18 ± 9.79		41.89 ± 11.45		33.23 ± 3.36	
Toilet facilities [a]						
Pit latrine	39.95 ± 8.47	0.344	41.03 ± 8.17	0.570	33.43 ± 7.85	0.239
Public flush	39.13 ± 8.61		38.85 ± 8.10		39.84 ± 10.23	
Private flush	37.47 ± 10.49		38.99 ± 11.34		34.90 ± 8.41	
Nutritional status [a]						
No stunting	36.23 ± 8.40*	**0.047**	38.50 ± 6.15	0.571	-	-
Moderate stunting	39.02 ± 10.17		39.02 ± 10.17		-	-
Severe stunting	40.99 ± 10.02*		40.99 ± 10.02		-	-
No underweight	37.90 ± 10.01	0.172	39.55 ± 10.59	0.306	-	-
Moderate underweight	38.55 ± 8.90		38.55 ± 8.90		-	-
Severe underweight	43.85 ± 6.86		43.85 ± 6.86		-	-
No wasting	38.33 ± 9.91	0.719	39.59 ± 10.20	0.919	-	-
Wasting	39.30 ± 6.01		39.300 ± 6.01		-	-
Iron status [b]						
No anaemia	39.53 ± 8.53	0.219	41.86 ± 7.58	**0.028**	34.66 ± 8.51	0.472
Anaemia	37.68 ± 10.28		38.14 ± 10.73		36.48 ± 9.10	
No ID	37.81 ± 9.35	0.264	38.87 ± 9.36	0.282	34.50 ± 8.71	0.348
ID	39.49 ± 9.97		40.83 ± 10.31		36.92 ± 8.92	
No IDA	38.97 ± 8.99	0.476	41.02 ± 8.58	0.122	34.50 ± 8.33	0.350
IDA	37.90 ± 10.39		38.25 ± 10.73		36.92 ± 9.55	
Inflammation [b]						
CRP > 5 mg/l	37.79 ± 8.74	0.590	39.18 ± 8.25	0.790	35.22 ± 9.37	0.796
CRP ≤ 5 mg/l	38.76 ± 9.89		39.78 ± 10.14		35.97 ± 8.73	
AGP > 1 g/l	38.73 ± 9.90	0.661	40.24 ± 9.95	0.254	35.13 ± 8.91	0.339
AGP ≤ 1 g/l	37.96 ± 8.81		37.89 ± 9.07		38.16 ± 8.42	
Dietary intake [b]						
No therapeutic food intake	38.31 ± 9.66	**< 0.001**	39.43 ± 9.82	**< 0.001**	-	-
Therapeutic food intake	46.60 ± 0.42		46.60 ± 0.42		-	-
No multivitamin intake	37.74 ± 9.53	0.053	38.78 ± 9.56	0.074	35.38 ± 9.12	0.554
Multivitamin intake	41.44 ± 9.75		42.84 ± 10.26		37.41 ± 7.18	
No vit D supplement intake	37.77 ± 9.39	**0.003**	38.84 ± 9.44	**0.010**	35.33 ± 8.90	0.243
Vit D supplement intake	45.60 ± 9.84		46.72 ± 10.71		41.50 ± 4.92	

(*Continued*)

Table 3: Continued.

Characteristics	Total ($n = 172$) Mean ± SD 25(OH)D ng/ml	*p*-value	Undernourished group ($n = 121$) Mean ± SD 25(OH)D ng/ml	*p*-value	Well-nourished group ($n = 51$) Mean ± SD 25(OH)D ng/ml	*p*-value
No formula milk	37.54 ± 9.62	**0.028**	38.45 ± 9.86	**0.031**	35.56 ± 8.86	0.811
Formula milk	41.38 ± 9.23		42.89 ± 8.88		36.34 ± 9.06	
Dairy intake ≤ 14 [b,c]	39.74 ± 9.78	0.064	39.80 ± 10.09	0.748	39.51 ± 8.80	**0.016**
Dairy intake > 14	37.02 ± 9.36		39.20 ± 9.45		33.43 ± 8.12	
Low fish freq ≤ 5 [b,d]	38.96 ± 9.67	0.080	39.74 ± 9.75	0.520	36.61 ± 9.17	0.257
High fish freq > 5	35.43 ± 9.08		37.82 ± 10.37		33.51 ± 7.73	
Egg intake freq ≤ 1[e]	39.05 ± 9.65	0.544	39.23 ± 9.50	0.796	38.57 ± 10.36	0.134
Egg intake freq > 1[f]	38.10 ± 9.67		39.72 ± 9.98		34.50 ± 7.93	

[a]ANOVA, [b] independent *t*-test, * ANOVA (Tukey Post-hoc test) [c] Dairy intake ≤ 14 refers to consuming no more than two different dairy products every day of the week. Dairy intake > 14 refers to intake of more than two different dairy products daily (the different milk products listed in the FFQ included milk, cheese, mageu, amasi, or yogurt), [d] 1 fish product once a month, [e] intake of egg once a month; [f] intake of egg twice or more per month. Freq: frequency, CRP: C-reactive protein, AGP: alpha-1 acid glycoprotein, ID: iron deficiency, IDA: iron deficiency anaemia. $P < 0.05$ was considered significant and bolded in table.

group ($\beta = -0.81$, 95% CI = −1.54, −0.09, $p = 0.029$) in the crude model but not in the adjusted model ($p = 0.127$ and $p = 0.172$, respectively).

The reported frequency of egg consumption was not associated with 25(OH)D in the total study group ($p = 0.899$). However, in the well-nourished children between 24 and 59 months old, the frequency of egg consumption showed a positive trend of association with 25(OH)D ($\beta = 1.25$ 95% CI = −0.05, 2.56, $p = 0.059$) (results not shown in table).

Sunlight exposure and vitamin D status

In our study, all participants (100%) were reported to have some level of sunlight exposure. The weekly sun exposure score and weekly time in sun score in the total group were 20 (IQR 145, 25) and 20 (IQR 12, 25), respectively. The median weekly sun exposure score, i.e. 20 (IQR 15, 25) in the undernourished group vs 20 (IQR 10, 25) in the well-nourished group did not differ significantly between the groups ($p = 0.313$). Similarly, there was no difference in the weekly median time in sun score between the undernourished and well-nourished groups, i.e. 20 (IQR 15, 25) vs 20 (IQR 10, 25) ($p = 0.885$), respectively. However, children in the lowest household income category (< 500 ZAR), had a higher amount of sun exposure score compared with the 500–1000 ZAR category (19.0 ± 1.11 vs 13.3 ± 1.11, $p = 0.018$), and borderline higher than the 3001–6000 ZAR category (14.6 ± 1.11, $p = 0.078$). The daily sunlight exposure, which is a product of the level of the body exposed and the amount of time spent outdoors per day, was not significantly associated with 25(OH)D concentration and predicted only 0.8% variation in 25(OH)D concentration ($\beta = -0.282$, 95% CI = −0.774, 0.209, $p = 0.258$, $R^2 = 0.008$). Similarly, the time of day spent outdoors accounted for < 0.1% of the variation in vitD levels and was not significantly associated with 25 (OH)D concentration ($\beta = 0.10$, 95% CI = −1.848, 2.05, $p = 0.918$, $R^2 < 0.001$). Additionally, we observed that the weekly sun exposure score and weekly time in sun score was not associated with the 25(OH)D concentration in the total as well as the undernourished and well-nourished groups (Table 4).

Discussion

In this study, we investigated the influence of sociodemographic, nutritional, and environmental factors, iron status, and inflammation on vitD status among undernourished and well-nourished children. Iron deficiency anaemia was associated with almost five times increased odds of suboptimal vitD among the undernourished children. The prevalence of suboptimal vitD status (25(OH)D < 30 ng/ml) in our study was 19.9% among the undernourished, and 21.6% among the well-nourished children. Contrary to our findings, a higher suboptimal vitD level prevalence of 43.7% and 36.5% was reported in undernourished and well-nourished children, aged 6–24 months, respectively, in Uganda[3]. The mean 25(OH)D concentration of 35.7 ng/ml estimated in the well-nourished group in the present study was similar to the 32.2 ng/ml reported in well-nourished children in the Ugandan study; nonetheless, for the undernourished children, we observed a higher concentration of 39.6 ng/ml compared with 32.5 ng/ml in the Ugandan study.[3] This could be due to the higher intake of multivitamins, therapeutic food, and formula milk among the undernourished compared with the well-nourished children in our study. Furthermore, children from lower socioeconomic households had higher 25(OH)D concentrations compared with those from the middle- and high-income households. This may be attributed to our finding of higher amount of sun exposure of the children from the lowest income households compared with the middle- and higher-income groups. Children from higher socioeconomic households are more likely to spend time engaging in indoor activities such as watching television, compared with those from lower socioeconomic households.[29]

We observed that 25(OH)D concentrations showed a declining trend with age in the well-nourished group of our study. A previous study, which included children aged six days to eight years from five African countries, reported that 25(OH)D concentrations reduced with increasing age, and each added year increased the odds of VDD by 69% and insufficiency by 43%.[14] Additionally, girls tended to have lower 25(OHD) concentrations than boys, especially among the undernourished. An account of the influence of sex on vitD status has been given in previous studies. Girls were observed to have 32% increased odds of VDD compared with boys in a multinational study involving 4 509 children from five African countries[14]. Conversely, Poopedi et al. observed no influence of sex on 25 (OH)D concentrations in SA.[10] It has been hypothesised that gender differences in vitD status may be linked to androgen-related variances in vitD-binding protein levels or disparity in the production of 7-dehydrocholesterol in the skin or its 25-hydroxylation in the liver, or gender-related differences in body fat.[30]

In the present study, stunted children had a higher 25(OH)D concentration, which was primarily driven by their intake of

Table 4: Associations of age, plus nutritional and environmental factors with 25(OH)D concentrations (multivariable linear regression, β-values, and 95% confidence intervals)

Exposures	25(OH)D concentrations											
	Total, $n = 172$					Undernourished group, $n = 121$				Well-nourished group, $n = 51$			
	Model 1			Model 2		Model 1		Model 2		Model 1		Model 2	
	β (95% CI)	p-value	R^2	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Age [a]	−0.08 (−0.19, 0.02)	0.126	0.014	−0.03 (−0.14, 0.09)	0.651	−0.01 (−0.15, 0.12)	0.855	0.07 (−0.08, 0.2)	0.357	−0.15 (−0.30, 0.01)	0.062	−0.17 (−0.35, 0.01)	0.063
Nutritional status [b]													
HAZ	−1.56 (−2.62,−0.50)	**0.004**	0.047	−1.17 (−2.21, −0.12)	**0.029**	−1.38 (−3.48, 0.72)	0.197	−1.09 (−3.26, 1.07)	0.319	−1.88 (−6.04, 2.28)	0.369	−1.63 (−5.89, 2.63)	0.445
WAZ	−1.21(−2.37,−0.05)	**0.041**	0.024	−0.82 (−1.95, 0.31)	0.152	−0.76 (−2.92, 1.39)	0.485	−0.18 (−2.39, 2.03)	0.872	1.41 (−2.23, 5.04)	0.441	0.96 (−2.96, 4.88)	0.624
WHZ	−0.34 (−1.63, 0.96)	0.608	0.002	−0.33 (−1.60, 0.95)	0.615	−0.06 (−1.65, 1.53)	0.940	0.06 (−1.58, 1.69)	0.944	2.69 (−0.54, 5.91)	0.100	0.94 (−1.59, 5.84)	0.255
MUAC (cm)	−0.51 (−1.59, 0.56)	0.349	0.005	0.01 (−1.10, 1.11)	0.990	0.70 (−0.88, 2.27)	0.383	0.94 (−0.60, 2.48)	0.228	−0.50 (−2.89, 1.90)	0.678	0.70 (−2.22, 3.61)	0.631
Iron and anaemia status biomarkers [b]													
			$n = 168$				$n = 120$				$n = 48$		
Hb (g/dl)	0.62 (−0.60, 1.83)	0.317	0.006	1.12 (−0.09, 2.34)	0.070	1.30 (−0.12, 2.71)	0.073	1.40 (−0.01, 2.80)	0.051	−1.02 (−3.27, 1.24)	0.369	0.24 (−2.34, 2.82)	0.852
Ferritin (µg/l)	−0.04(−0.07, −0.00)	**0.038**	0.020	−0.03 (−0.07, −0.00)	0.075	−0.05 (−0.09, −0.00)	**0.042**	−0.04 (−0.08, −0.00)	0.075	−0.03 (−0.09, 0.04)	0.361	−0.01 (−0.09, 0.06)	0.736
sTfR (mg/l)	−0.02 (−0.08, 0.05)	0.598	0.002	−0.02 (−0.07, 0.05)	0.647	−0.03 (−0.11, 0.05)	0.456	−0.01 (−0.10, 0.07)	0.740	−0.02 (−0.13, 0.10)	0.788	−0.02 (−0.15, 0.11)	0.744
Inflammation [e]													
CRP (mg/l)	−0.003 (−0.03, 0.02)	0.779	0.000	−0.01 (−0.03, 0.02)	0.657	−0.001 (−0.03, 0.03)	0.949	−0.01 (−0.03, 0.02)	0.680	−0.01 (−0.05, 0.04)	0.790	−0.01 (−0.06, 0.04)	0.677
AGP (g/l)	−0.01 (−0.06, 0.04)	0.680	0.001	−0.01 (−0.06, 0.04)	0.711	0.01 (−0.05, 0.06)	0.802	0.01 (−0.05, 0.06)	0.774	−0.07 (−0.18, 0.04)	0.215	−0.06 (−0.17, 0.05)	0.288
Dietary factors[a]													
			n = 172				**n = 121**				**n = 51**		
Dairy intake	−0.15 (−0.34, 0.04)	0.126	0.014	−0.15 (−0.35, 0.04)	0.126	0.05 (−0.19, 0.28)	0.709	0.03 (−0.21, 0.27)	0.817	−0.46 (−0.76, −0.15)	**0.004**	−0.42 (−0.78, −0.05)	**0.025**
Margarine	−0.17 (−0.67, 0.32)	0.489	0.003	−0.29 (−0.79, 0.20)	0.244	0.25 (−0.34, 0.85)	0.400	0.15 (−0.46, 0.76)	0.633	−1.00 (−1.83, −0.17)	**0.019**	−0.97 (−1.85, −0.09)	**0.031**
Fish intake	−0.45 (−0.89, −0.01)	**0.044**	0.024	−0.35 (−0.79, 0.10)	0.127	−0.19 (−0.74, 0.36)	0.498	−0.05 (−0.60, 0.49)	0.851	−0.81 (−1.54, −0.09)	**0.029**	−0.58 (−1.43, 0.26)	0.172
Egg intake	−0.20 (−0.81, 0.42)	0.530	0.002	0.04 (−0.58, 0.66)	0.899	0.12 (−0.62, 0.87)	0.744	0.45 (−0.30, 1.21)	0.235	−0.78 (−1.86, 0.29)	0.149	−0.62 (−1.72, 0.48)	0.264
Sunshine exposure [a]													
Weekly sunshine exposure [c]	−0.04 (−0.13, 0.05)	0.383	0.004	−0.01 (−0.11, 0.08)	0.814	0.03 (−0.04, 0.10)	0.431	0.06 (−0.02, 0.13)	0.136	−0.01 (−0.11, 0.10)	0.884	−0.00 (−0.12, 0.11)	0.949
Weekly time in sun score [d]	0.03 (−0.16, 0.23)	0.739	0.001	0.11 (−0.10, 0.304)	0.303	0.04 (−0.04, 0.11)	0.341	0.06 (−0.02, 0.14)	0.125	0.02 (−0.12, 0.15)	0.826	0.02 (−0.13, 0.17)	0.810

Association between linear exposures and 25(OH)D concentrations was assessed with univariable and multivariable linear regression. $P < 0.05$ considered significant. Model 1: unadjusted, Model 2: adjusted. [a] Age, sex, intake of therapeutic foods, vitamin D supplement, and multivitamin intake and formula milk were used as covariates in the adjusted model. [b] Age, sex, intake of therapeutic foods, vitamin D supplement and multivitamin intake, formula milk, and monthly household income were used as covariates in the adjusted model. [c] Weekly sunshine exposure score = time spent outdoors x level of skin exposure x number of days spent outdoors in a week (maximum score = 100, minimum = 1). [d] Weekly time in sun score = time spent outdoors x number of days in a week spent outdoors (maximum = 25; minimum = 1). [e] Adjusted for age, sex, and medication intake. CRP: C-reactive protein, AGP: alpha-1 acid glycoprotein, Hb: haemoglobin, sTfR: soluble transferrin receptor, MUAC: mid-upper arm circumference, WAZ: weight-for-age-z-score, WHZ: weight-for-height/length z-score, HAZ: height-for-age z-score. $P < 0.05$ was considered significant and bolded in table.

Table 5: Association of sociodemographic, nutritional, and environmental factors with suboptimal vitamin D status (multivariable logistic regression, odds ratios, and 95% confidence intervals)

Exposures	Suboptimal vitamin D status 25(OH)D < 30 ng/ml											
	Total				Undernourished group				Well-nourished group			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	n = 172				*n* = 121				*n* = 51			
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Female [e]	1.62 (0.76, 3.47)	0.215	1.60 (0.73, 3.51)	0.237	2.38 (0.91, 6.25)	0.079	2.42 (0.89, 6.60)	0.084	0.75 (0.20, 2.88)	0.679	0.60 (0.14, 2.50)	0.479
Nutritional status [f]												
Stunting	1.14 (0.52, 2.48)	0.747	1.05 (0.44, 2.48)	0.916	2.94 (0.36, 23.98)	0.313	0.32 (0.03, 3.07)	0.321	-		-	
Underweight	0.73 (0.32, 1.69)	0.465	0.88 (0.35, 2.21)	0.791	0.723 (0.29, 1.81)	0.488	1.04 (0.34, 3.24)	0.941	-		-	
Wasting	0.28 (0.04, 2.22)	0.229	0.34 (0.04, 2.86)	0.322	0.28 (0.04, 2.26)	0.233	0.35 (0.04, 3.10)	0.344	-		-	
Iron and anaemia status [f]												
	n = 168				*n* = 120				*n* = 48			
Anaemia	1.84 (0.82, 4.12)	0.141	1.24 (0.51, 2.98)	0.640	5.57 (1.56, 19.93)	**0.008**	3.55 (0.90, 14.13)	0.072	0.34 (0.09, 1.37)	0.130	0.14 (0.02, 0.82)	0.030
ID	1.29 (0.60, 2.76)	0.516	1.24 (0.54, 2.88)	0.615	1.19 (0.48, 3.00)	0.705	1.30 (0.44, 3.84)	0.641	1.50 (0.36, 6.18)	0.575	1.54 (0.28, 8.35)	0.618
IDA	2.43 (1.11, 5.32)	**0.026**	1.99 (0.83, 4.79)	0.126	4.92 (1.69, 14.33)	**0.004**	4.65 (1.34, 16.12)	**0.016**	0.71 (0.18, 2.82)	0.622	0.32 (0.05, 1.93)	0.215
Inflammation [g]												
CRP ≥ 5 mg/l	0.75 (0.28, 1.97)	0.553	0.81 (0.30, 2.19)	0.683	0.54 (0.15, 2.00)	0.359	0.67 (0.17, 2.55)	0.553	1.20 (0.26, 5.56)	0.816	1.09 (0.23, 5.17)	0.910
AGP >1 g/l	1.20 (0.48, 3.02)	0.696	1.36 (0.53, 3.51)	0.528	0.93 (0.33, 2.63)	0.893	1.07 (0.36, 3.15)	0.901	2.79 (0.31, 25.14)	0.360	2.52 (0.26, 24.26)	0.423
Dietary factors [e]												
	n = 172				*n* = 121				*n* = 48			
Therapeutic food intake	< 0.001	0.999	< 0.001	0.999	< 0.001	0.999	< 0.001	0.999	-		-	
Multivitamin intake	0.23 (0.05, 1.00)	**0.049**	0.21 (0.03, 1.63)	0.134	0.33 (0.07, 1.51)	0.153	0.36 (0.04, 2.99)	0.343	< 0.001	0.999	< 0.001	0.999
Vit D supplement intake	0.28 (0.04, 2.22)	0.229	0.33 (0.04, 2.70)	0.288	0.38 (0.05, 3.11)	0.366	0.51 (0.06, 4.41)	0.544	< 0.001	0.999	< 0.001	0.999
Formula milk	0.65 (0.25, 1.71)	0.384	0.79 (0.29, 2.13)	0.641	0.37 (0.10, 1.34)	0.131	0.44 (0.12, 1.63)	0.218	2.13 (0.44, 10.37)	0.351	2.63 (0.48, 14.24)	0.263
Dairy intake > 14 [a]	1.32 (0.63, 2.77)	0.471	1.48 (0.69, 3.18)	0.309	0.94 (0.38, 2.31)	0.885	1.20 (0.46, 3.10)	0.713	3.33 (0.64, 17.41)	0.155	4.05 (0.60, 27.19)	0.150
High fish frequency > 5 [b]	2.29 (0.92, 5.66)	0.073	2.21 (0.88, 5.55)	0.092	2.23 (0.61, 8.12)	0.226	2.05 (0.55, 7.70)	0.287	2.50 (0.63, 10.00)	0.195	2.33 (0.54, 9.99)	0.254
Egg intake frequency > 1 [h]	1.07 (0.48, 2.37)	0.873	1.01 (0.44, 2.31)	0.977	1.03 (0.40, 2.66)	0.949	0.82 (0.30, 2.24)	0.698	1.14 (0.26, 5.07)	0.861	1.20 (0.26, 5.64)	0.816
Sunshine exposure [e]												
Weekly sunshine exposure [c]	1.00 (0.98, 1.03)	0.948	1.00 (0.97, 1.02)	0.719	1.01 (0.98, 1.04)	0.618	1.00 (0.97, 1.04)	0.967	0.99 (0.96, 1.03)	0.689	0.99 (0.96, 1.03)	0.653
Weekly time in sun score [d]	0.98 (0.94, 1.03)	0.465	0.97 (0.92, 1.02)	0.274	0.98 (0.92, 1.03)	0.391	0.96 (0.90, 1.02)	0.177	1.00 (0.91, 1.09)	0.987	1.01 (0.91, 1.12)	0.882

Association between categorical exposures and vitamin D insufficiency with binary and multivariable logistic regression. [a] Daily intake of at least two dairy products, [b] one fish product once a month. [c] Weekly sunshine exposure score = time spent outdoors x level of skin exposure x number of days spent outdoors in a week (maximum score = 100, minimum = 1). [d] Weekly time in sun score = time spent outdoors x number of days in a week spent outdoors (maximum = 25; minimum = 1). [e] Age, sex, intake of therapeutic foods, vitamin D supplement and multivitamin intake, and formula milk were used as covariates in the adjusted model. [f] Age, sex, intake of therapeutic foods, vitamin D supplement and multivitamin intake, formula milk, and monthly household income were used as covariates in the adjusted model. [g] Adjusted for age, sex, and medication intake. [h] Intake of egg more than once a month. CRP: C-reactive protein, AGP: alpha-1 acid glycoprotein, ID: iron deficiency, IDA: iron deficiency anaemia. $P < 0.05$ was considered significant and bolded in table.

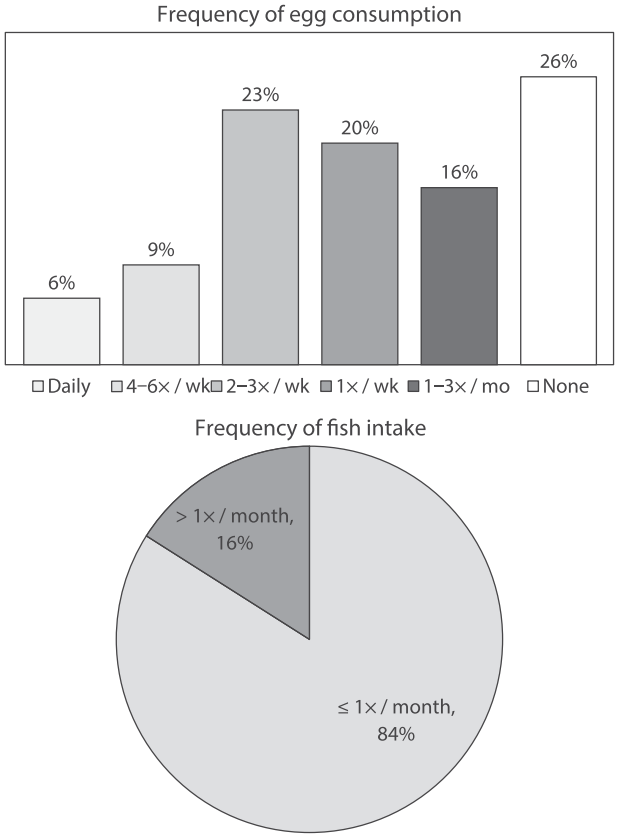

Figure 1: Frequency of intake of eggs and fish in the total study group. The bar and pie chart illustrates the frequencies of intake of eggs and fish in the overall study group.

therapeutic food, formula milk, and multivitamin and vitD supplements, likely as part of undernutrition management. Wasting and underweight did not influence 25(OH)D concentration. In agreement with our findings, Mogire et al. in five African countries and Sudfeld et al. in Tanzania observed no relationship between stunting, wasting, and underweight and 25 (OH)D concentration among children.[14,31] Contrarily, VDD was associated with severe wasting among Kenyan children with rickets, but not with stunting.[32]

Some studies suggest that persistent inflammation and chronic infections may alter vitD metabolism, affecting 25(OH)D concentrations.[33,34] According to Edfeldt et al., inflammatory mediators increase the activation of CYP27B1 and vitD-binding receptors in macrophages, leading to rapid calcitriol synthesis and depletion of serum 25(OH)D.[34] In the present study, we observed no association between CRP or AGP and 25(OH)D concentrations in the total group, but an inverse trend of association between CRP > 5 mg/l and 25(OH)D among the VDD children. The lack of statistical significance in the association could be attributed to the limited inflammation range of our apparently healthy study sample. Previous studies on this association in children have yielded inconclusive findings. Our findings align with a cross-sectional survey from the BRINDA study, involving 9 880 children aged 6–59 months, which found no significant correlation between CRP or AGP and 25(OH)D concentration.[35] However, an inverse relationship between inflammation and 25(OH)D was observed in children with inflammatory bowel disease after vitD supplementation.[36] A US National Survey investigating the association between vitD and CRP in adults found an inverse relationship between 25 (OH)D and CRP when serum 25(OH)D was < 21 ng/ml, suggesting that the inverse correlation between CRP and 25 (OH)D might be limited to lower 25(OH)D concentrations.[37] In agreement with this finding, we observed a negative association between CRP and 25(OH)D among participants with 25 (OH)D < 20 ng/ml.

Iron status may influence 25(OH)D concentrations, as iron is a component of the cytochrome P450 enzymes, responsible for vitD metabolism. Hence, ID alters the activity of 25- and 1α-hydroxylases, and may lead to VDD.[15] However, this relationship has not been researched extensively in undernourished children. In our study, although there was an inverse trend between ferritin and 25(OH)D, we observed a positive association between IDA and anaemia with suboptimal vitD status. This suggests that ferritin levels may reflect increased inflammation rather than iron status in our study.[23] In support of the influence of iron on 25 (OH)D, a study by Heldenberg et al. revealed an increase in 25 (OH)D concentration after intramuscular injection of iron in infants with IDA.[38] A retrospective study of 120 IDA and 125 healthy children aged 1–15 years found significantly lower 25 (OH)D levels in children with IDA and a strong positive association between IDA and VDD.[39] The role of iron in vitD metabolism may explain the almost five and four times higher odds for suboptimal vitD observed in the undernourished children with IDA and anaemia in our study.

With regard to the impact of dietary vitD intake on serum 25 (OH)D concentrations, we found that children who consumed therapeutic food, primarily the lipid-based ready-to-use-therapeutic food, "plumpy nut", multivitamins, vitD supplements, and formula milk had a higher serum 25(OH)D concentration. In the multivariable model, however, intake of therapeutic foods did not influence 25(OH)D concentrations significantly. This observation could be due to the small number of participants (i.e. two) who took therapeutic foods in our study. Even after adjusting for multivitamin, formula milk, and therapeutic food intake, the consumption of vitD supplements remained associated with a 6.8-unit increase in 25(OH)D concentration. Among the undernourished children, formula milk intake accounted for a 4.2 ng/ml increase in 25(OH)D concentration.

Dairy product consumption showed no significant impact on 25 (OH)D levels in our overall study group. It is worth mentioning that the dairy products consumed by participants were primarily not fortified with vitD. This aligns with findings from a similar study among school children in Ireland, indicating that intake of unfortified milk does not contribute to serum 25(OH)D levels.[40] Additionally, it is known that fatty fish is an excellent source of vitD, thus intake of 2–3 servings per week (130 g/week) increased serum 25(OH)D concentrations.[8,41] In our study however, we observed no association between the frequency of fish consumption and 25(OH)D in the adjusted model. The reported frequency of fish (i.e. sardines, pilchard, mackerel, salmon, and hake) intake among our participants was generally very low. Given the age of our participants, it is likely that fish is not a significant part of their diet. Among the 16% who reported fish intake at least once a month, we did not collect detailed information on the quantity. In a trial with children aged 6–23 months, conducted in the same setting as our study, only 8.4% of the children consumed fish at least once a week.[42] Egg yolk, recognised as a vitD source, showed an association with higher 25(OH)D concentrations among the 24–59-month-old well-nourished children, as evidenced in this study.

We found no association of sunlight exposure with 25(OH)D in our study. Like our findings, a study examining predictors of

25(OH)D in healthy young adults in the Western Cape province of SA found no association between sun exposure, as assessed through a questionnaire, and 25(OH)D.[43] The absence of an association between sun exposure and 25(OH)D, alongside the prevalence of vitD insufficiency amidst ample sunshine in the present and other studies, warrants further research. Aside from sunlight exposure and dietary vitD intake, polymorphisms in vitD binding proteins have been reported as partly responsible for the variability of serum 25(OH)D concentrations, hence could have an influence on the 25(OH)D concentration of our study participants,[14,44] but this was unfortunately not investigated here. The fact that the data were collected during winter may have limited any findings related to sunshine exposure.

This study has some other limitations and, therefore, the results should be interpreted with caution, bearing in mind that the dietary and sunlight exposure were self-reported, and dependent on respondents' memory, hence subject to both under- and over-reporting. Although we aimed at assessing the frequency of intake of vitD-rich foods, a quantified food frequency questionnaire would have been preferable. Additionally, this was a cross-sectional study with a relatively small sample size, thus a causal relationship cannot be established, and the generalisability of the results may be limited. Additionally, we did not consider low birthweight or prematurity at birth, which could affect children's vitD status. These factors should be included in future studies. Nevertheless, this study provides useful insights into the predictors of vitD status in undernourished and well-nourished children in SA.

Conclusion

In conclusion, the prevalence of VDD and insufficiency, IDA, and anaemia among both undernourished and well-nourished children in this study highlights the widespread issue of hidden hunger, even among children who appear healthy based on their anthropometric measurements. This provides evidence that primary healthcare should promote and provide nutritional education at every contact session with children, irrespective of anthropometric status, to improve their micronutrient status. Given the high risk of suboptimal vitD levels among undernourished children with IDA and anaemia, vitD status should be considered in the management of anaemia, particularly in undernourished children. Additionally, intake of vitD supplement and egg contribute to vitD status, and should be encouraged in the management VDD in 6–59-month-old African children from this peri-urban community in SA.

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Ethical standards disclosure – This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Health Research Ethics Committee of the North-West University, South Africa (NWU-00253-21-A1) and the South African National and Provincial Department of Health. Written informed consent was obtained from all parents. The study is registered at the Pan African Clinical Trial Registry (PACTR202110646172601).

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