ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Nutritional Requirements and Status

Inflammation Adjustments to Serum Retinol and Retinol-Binding Protein Improve Specificity but Reduce Sensitivity when Estimating Vitamin A Deficiency Compared with the Modified Relative Dose-Response Test in Ghanaian Children

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ABSTRACT

Background: Serum retinol and retinol-binding protein (RBP) concentrations are commonly used biomarkers of vitamin A deficiency (VAD); however, evidence indicates that they are not always accurate, especially in populations with high exposure to inflammation.

Objective: The aim was to assess sensitivity and specificity of serum retinol and RBP concentrations to predict VAD, with and without adjustment for inflammation (using categorical and regression-adjusted approaches), using the modified relative dose-response (MRDR) as the reference standard for liver reserves.

Methods: This secondary analysis of diagnostic accuracy used inflammation and RBP data and analyzed serum retinol and MRDR from a subsample of women of reproductive age ($n = 178$) and preschool children ($n = 166$) in the cross-sectional 2017 Ghana Micronutrient Survey.

Results: Inflammation (elevated C-reactive protein and/or α_1-acid glycoprotein) was present in 41% of children and 16% of women. Among children, estimates of VAD prevalence were as follows: 7% (MRDR), 40% (serum retinol), 29% (categorical-adjusted serum retinol), 24% (RBP), 13% (categorical-adjusted RBP), and 7% (regression-adjusted RBP). Sensitivity (95% CI) ranged from 22.2% (2.81%, 60.0%; both adjusted RBPs) to 80.0% (44.4%, 97.5%; serum retinol), whereas specificity ranged from 63.3% (54.7%, 71.3%; serum retinol) to 93.5% (88.0%, 97.0%; regression-adjusted RBP). Among women, VAD prevalence ranged from 1% (RBP) to 4% (all others); sensitivity was 0% and specificity was >96% for all indicators.

Conclusions: Serum retinol and RBP had varying accuracy in estimating VAD, especially in children; adjustment for inflammation increased accuracy by increasing specificity at the expense of sensitivity. Effects of inflammation adjustment in the context of high inflammation and VAD prevalence need to be further explored. Especially in populations with high inflammation, the MRDR test should accompany serum retinol or RBP measurements in a subsample of subjects in population-based surveys. This trial was registered with the Open Science Framework registry (doi: 10.17605/OSF.IO/J7BP9). *Curr Dev Nutr* 2021;5:nzab098.

Keywords: biomarkers, modified relative dose-response, RBP4, sensitivity and specificity, serum retinol, vitamin A deficiency, children, women of reproductive age

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Abbreviations used: AGP, α_1-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia; CRP, C-reactive protein; GMS, Ghana Micronutrient Survey; MRDR, modified-relative dose response; RBP, retinol-binding protein; TLR, total liver vitamin A reserve; VAD, vitamin A deficiency.

Introduction

Vitamin A is an essential nutrient for growth, reproduction, vision, and immune function, with particular importance throughout pregnancy and early childhood. Assessing vitamin A status of vulnerable populations is important to guide public health interventions, such as high-dose vitamin A supplementation, food fortification, and biofortification. While there are several biomarkers of vitamin A status, each has advantages and drawbacks [reviewed in depth by Tanumihardjo et al. (1)]. The gold-standard biomarker is total liver vitamin A reserves (TLRs; reported in μmol vitamin A/g liver), where "total" refers to the sum of interconvertible retinol and retinyl esters; TLRs can be directly assayed by liver biopsy or estimated by retinol isotope dilution (2, 3). Neither of these methods are currently feasible in population-based health surveys and, thus, more practical biomarkers are applied. The most commonly used biological indicators of vitamin A deficiency (VAD) in large surveys are circulating concentrations of retinol and retinol-binding protein (RBP) in the blood serum/plasma of subjects.

However, in otherwise healthy individuals, these biomarkers are insensitive to mild-to-moderate deficiency because they are homeostatically maintained in the blood until TLRs are nearly exhausted (4). Furthermore, concentrations of both indicators are temporarily decreased by inflammation and infection (5, 6), which may overestimate VAD in some populations.

The modified-relative dose response (MRDR) test is not affected by the acute phase response to inflammation as assessed by C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) (7, 8), and the complexity of the laboratory analysis techniques is similar to measuring serum retinol concentrations by HPLC. The biological basis of the MRDR test is that hepatic *apo*-RBP is continually synthesized, but secretion is diminished during vitamin A depletion [defined as low TLR (1)], leading to accumulation in the absence of ligand (4). When a dose of retinol (i.e., vitamin A_2 in the MRDR test) is administered, accumulated RBP binds the ligand and the complex is secreted, causing a large amount of vitamin A_2 to appear in the serum within 5 h if the person has VAD (9). Conversely, a vitamin A–sufficient subject will not have accumulated *apo*-RBP and thus will have a low amount of vitamin A_2 secreted into serum by 5 h post-dose due to RBP turnover (10). The MRDR value is measured as the serum ratio of vitamin A_2 to endogenous vitamin A in the blood 5 h after dosing with vitamin A_2, determined by HPLC (1). The MRDR test is a reliable indicator of low TLRs and was validated with liver biopsy in animals (11–16) and in comparison to its predecessor, the relative dose-response test, which is based on the same biological principle and validated using human liver biopsy (17, 18). In addition, intervention studies in humans have demonstrated that the MRDR value changes in response to VA supplementation (19–23). In Zambian children, adjusting for inflammation improved the specificity of VAD estimates when compared with TLR determined by retinol isotope dilution (24).

To our knowledge, no studies have previously compared estimates of VAD prevalence using serum retinol and RBP, with and without adjustment for inflammation, against the MRDR test. A random MRDR test subsample supports population surveys by better defining the underlying vitamin A status (1), and this study adds to the evidence concerning the accuracy of vitamin A biomarkers. This study used data on the micronutrient status of Ghanaian women and preschool children to compare the sensitivity and specificity of inflammation-adjusted serum retinol and RBP in estimating VAD in this population by using the MRDR test as the reference standard for low liver vitamin A reserves.

Methods

Participants and enrollment

This secondary analysis of diagnostic accuracy used demographic information and inflammation and vitamin A status biomarker data from a subsample of nonpregnant women of reproductive age and preschool children in the cross-sectional 2017 Ghana Micronutrient Survey (GMS; registered with Open Science Framework, doi: 10.17605/OSF.IO/J7BP9). Methodological details related to stratification, cluster selection, and recruitment of households and individuals have been described elsewhere (25, 26). Children were eligible if they were between 6 and 59 mo at the time of data collection, considered a household member, and had written consent from their mother or caretaker. Women were eligible if they were between 15 and 49 y at the time of data collections, nonpregnant by self-report, considered a household member, and gave written consent. The full survey successfully recruited 1165 children and 973 women. In each census enumeration area, the first 2 eligible children ≥18–59 mo of age and the first 2 nonpregnant women who were enrolled in the GMS were recruited for MRDR testing. In total, 166 children and 178 nonpregnant women were recruited for the MRDR analysis. Eligible children were older than the standard survey participants due to the increased blood volume required for MRDR analysis.

Dosing, blood sampling, and analysis

Participants undergoing the MRDR test received 5.3 (children) or 8.8 (women) μmol 3,4-didehydroretinyl acetate dissolved in soybean oil dispensed orally by positive displacement pipette ~5 h before blood sampling. Following administration of the dose, participants were asked to consume 15 g of a fatty snack (Nutella®; Ferrero) on a biscuit to facilitate the absorption of didehydroretinyl acetate. Blood was collected by venipuncture into 6-mL polyethylene terephthalate (PET) serum tubes containing clot activator (Becton Dickinson). No adverse events were reported from performing the blood draw. Immediately after collection, blood collection tubes were placed into a dark cold box at ~4°C. Whole-blood samples were centrifuged at 2800 x *g* for 7 min at room temperature on the same day; serum was separated, separated into aliquots, and immediately stored frozen at −20°C. Samples were later stored at the University of Ghana at −20°C until shipment on dry ice to international laboratories. Serum RBP, CRP, and AGP were analyzed in a single run at the VitMin-Lab (Wilstaett, Germany) using a sandwich ELISA method (27). Because blood samples were drawn after the dose of 3,4-didehydroretinyl acetate was given, the measured RBP concentrations captured both the RBP that was bound to retinol and the RBP that was bound to 3,4-didehydroretinyl.

Serum aliquots from these subgroups of children and women were further analyzed by JS, who was blinded from other study information at the time of analysis, for the MRDR test at the University of Wisconsin-Madison (Madison, WI, USA), with the intent of comparing the results with serum retinol and RBP data by DJS to support the findings of the survey. For the MRDR test, serum 3,4-didehydroretinol and retinol were measured simultaneously by HPLC as previously described (11). Briefly, 250 μL (or all available) serum was separated into aliquots into disposable glass tubes. Forty microliters C23-β-*apo*-carotenol was added as an internal standard to determine extraction efficiency, and 250 μL ethanol was added to denature proteins. Organic compounds were extracted 3 times by addition of 300 μL hexane, mixed with a vortex, and centrifuged. Pooled hexane layers were dried under N_2; the residue was resuspended into 40 μL 75:25 methanol:dichloroethane and 35 μL was manually injected onto an HPLC system with a reverse-phase Waters Resolve C18 column (5-μm, 3.9 × 150 mm) with 89:11 methanol:water (0.73 g/L trimethylamine) solvent run isocratically at 1 mL/min. Both retinol and 3,4-didehydroretinol were measured at 350 nm and quantified using calibration curves constructed with HPLC-purified standards.

Vitamin A and inflammation indicators

Vitamin A indicators in this analysis included serum retinol, RBP, and the MRDR test as a proxy for low TLRs. To determine VAD, the following cutoffs were used: serum retinol, <0.7 μmol/L (28); RBP, <0.7 μmol/L (29); MRDR value, serum molar ratio of

TABLE 1 Descriptive characteristics of Ghanaian children ($n = 166$) and women ($n = 178$) recruited for MRDR testing as a subsample of a national micronutrient survey[1]

	Children		Women	
	n	Values	*n*	Values
Age[2]	162	40.0 ± 12.2[3]	175	29.7 ± 8.8
Female	165	94 (57)[4]	—	—
Height-for-age *z*-score	154	−0.87 ± 1.56	—	—
Weight-for-height *z*-score	152	−0.51 ± 0.91	—	—
Weight-for-age *z*-score	155	−0.83 ± 1.11	—	—
BMI, kg/m^2	—	—	169	23.9 ± 4.7
Given a vitamin A capsule in past 6 mo[5]	166	20 (12)	—	—
Household has adequately fortified oil (≥10 ppm RE)	112	57 (51)	119	60 (50)
Inflammation category[6]	157		167	
None		93 (59)		140 (84)
Incubation		4 (3)		7 (4)
Early convalescence		19 (12)		8 (5)
Late convalescence		41 (26)		12 (7)

[1]AGP, α_1-acid-glycoprotein; CRP, C-reactive protein; MRDR, modified-relative dose response; RE, retinol equivalents.
[2]Age is presented in months for children and years for women.
[3]Mean ± SD (all such values).
[4]Frequency (%) (all such values).
[5]Other responses: No, 128 (77%); Don't know, 18 (11%).
[6]Inflammation categories based on cutoffs of CRP >5 mg/L and AGP > 1 g/L: none, neither CRP nor AGP elevated; incubation, elevated CRP only; early convalescence, elevated CRP and AGP; late convalescence, elevated AGP only.

3,4-didehydroretinol to retinol ≥0.060 (2). The WHO criteria (28) for severe, moderate, and mild public health problems using the serum retinol cutoff are ≥20%, 10–19%, and 2–9%, respectively.

The presence of inflammation was also taken into account for serum retinol and RBP concentrations, using a categorical adjustment method (both indicators) and a regression adjustment method (RBP only). For the categorical adjustment, elevated values of CRP (>5 mg/L) and AGP (>1 g/L) were used to determine 4 inflammation categories among children and women included in the MRDR subsample: no inflammation, incubation (elevated CRP only), early convalescence (elevated CRP and AGP), and late convalescence (elevated AGP only). Using these inflammation categories, serum retinol and RBP concentrations were adjusted using correction factors calculated according to the method of Thurnham and colleagues (5) for both the children's and women's values.

RBP concentrations were also adjusted using the regression approach developed by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project (30). Because RBP is an acute phase protein that temporarily decreases during an inflammatory response, the BRINDA approach adjusts (i.e., increases) individuals' RBP concentrations based on the severity of their inflammatory response. Specifically, the BRINDA approach yields a continuous adjustment that is based on an individual's CRP and AGP concentrations and the association, estimated using linear regression, between CRP and RBP and AGP and RBP. The BRINDA adjustment for RBP was made only on the children with RBP, CRP, and AGP values ($n = 147$). The BRINDA method only recommends that RBP be adjusted in preschool children (31), and as such, no inflammation adjustment was used for women in our analysis.

Ethics

Ethical approval was obtained from the Ghana Health Service Ethics Review Committee (GHS-ERC number: GHS-ERC 15/01/2017).

Statistical analysis

Descriptive statistics for the children and women included in this analysis were tabulated, including age, sex, nutritional status, selected vitamin A exposure variables, inflammation status, and vitamin A biomarkers. The unweighted prevalence of VAD was determined for each biomarker by calculating the percentage of children or women above or below the cutoff for deficiency; the VAD prevalence for serum retinol and RBP was calculated with and without adjustment for inflammation. Because our objective was to compare the results of multiple biomarkers of vitamin A status, no statistical weights were used during data analysis.

Sensitivity and specificity were calculated for deficiency cutoffs of unadjusted and inflammation-adjusted serum retinol and RBP using MRDR as the reference standard. Sensitivity refers to the probability of a test correctly identifying those who have the condition (i.e., VAD) among all who truly have the condition (as determined by a reference or gold standard), whereas specificity is the probability of a test correctly identifying people who do not have the condition among those who truly do not have it (32). Thus, a highly sensitive test will identify true positives while minimizing false negatives and a highly specific test will identify true negatives while minimizing false positives. Confidence intervals for sensitivity and sensitivity were calculated using exact confidence intervals for proportions. Data analysis was performed in Stata (version 15.1; StataCorp LLC).

Results

Subjects' descriptive characteristics are shown in **Table 1**. Nutritional status was in the normal range for children and women. Only 12% of children had received vitamin A supplementation in the past 6 mo, and about half of households (both for children and women) had adequately vitamin A–fortified oil. Inflammation (CRP >5 mg/L and/or AGP >1 g/L) was present in 41% of children and 16% of

TABLE 2 Serum indicators of vitamin A and prevalence of VAD as estimated by each indicator, among Ghanaian children and women[1]

	Children			Women		
Biomarkers of VA	***n*[2]**	**Values**	**Minimum, maximum**	***n***	**Values**	**Minimum, maximum**
MRDR ratio[3]	149	0.03 ± 0.02[4]	0.006, 0.17	158	0.02 ± 0.02	0.003, 0.09
Serum retinol, unadjusted,[5] μmol/L	149	0.80 ± 0.25	0.33, 1.69	159	1.38 ± 0.52	0.46, 3.37
Serum retinol, Thurnham adjustment,[6] μmol/L	147	0.87 ± 0.28	0.34, 1.82	153	1.35 ± 0.51	0.46, 3.25
RBP, unadjusted, μmol/L	157	0.92 ± 0.28	0.38, 1.83	167	1.59 ± 0.65	0.52, 4.00
RBP, Thurnham adjustment,[6] μmol/L	157	1.02 ± 0.31	0.45, 1.94	167	1.55 ± 0.64	0.52, 4.00
RBP, BRINDA adjustment,[7] μmol/L	157	1.11 ± 0.32	0.52, 1.99	—	—	—
Prevalence of VAD, by indicator, frequency (%)						
MRDR[3]	149	10 (7)	—	158	6 (4)	—
Serum retinol, unadjusted[5]	149	59 (40)	—	159	6 (4)	—
Serum retinol, Thurnham adjustment[6]	147	43 (29)	—	153	6 (4)	—
RBP, unadjusted[5]	157	37 (24)	—	167	1 (1)	—
RBP, Thurnham adjustment[6]	157	21 (13)	—	167	1 (1)	—
RBP, BRINDA adjustment[7]	157	11 (7)	—	—	—	—

[1]BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; MRDR, modified-relative dose response; RBP, retinol-binding protein; VAD, vitamin A deficiency.
[2]Some sample sizes reduced due to insufficient serum for analysis.
[3]Cutoff for VAD: ratio of 3,4-didehydroretinol to serum retinol ≥0.060.
[4]Mean ± SD (all such values).
[5]Cutoff for VAD: serum retinol or RBP <0.7 μmol/L.
[6]Using the method of Thurnham and colleagues (5).
[7]Adjusted using the BRINDA regression approach.

women. Table 2 describes serum indicators of vitamin A and prevalence of VAD among the subjects. Due to insufficient serum for analysis, 17 children and 25 women were missing serum retinol and MRDR values and 11 women and 9 children were missing RBP values (Figure 1).

In children, the unweighted prevalence of VAD among indicators ranged from a low of 7% using the MRDR test to a high of 40% using unadjusted serum retinol concentrations. Adjustment of RBP and serum retinol concentrations for inflammation reduced VAD prevalence estimates for these 2 indicators, but still categorized them differently with

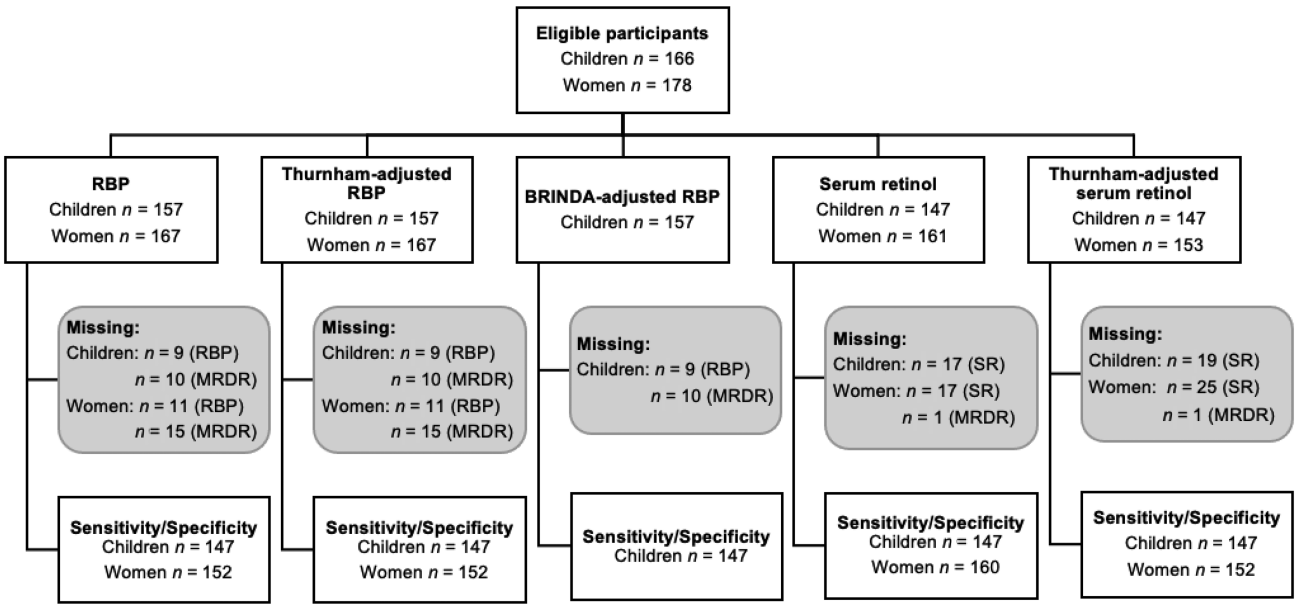

FIGURE 1 Flow diagram of participants through the study. The most common reason for missing an index or reference test was insufficient serum available. BRINDA, Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia; MRDR, modified-relative dose response; RBP, retinol-binding protein; SR, serum retinol.

regard to public health relevance according to WHO criteria (28): "severe" for unadjusted and adjusted serum retinol and unadjusted RBP, "moderate" to "mild" for adjusted RBP, and "mild" when using MRDR results. In these groups, the BRINDA adjustment for RBP resulted in the closest VAD estimates as compared with MRDR. Among the women, the prevalence of VAD was very low, ranging from 1% using RBP to 4% (all other biomarkers), with no changes after adjustment for inflammation using serum retinol or RBP concentrations.

The relationships between the reference MRDR values and the unadjusted serum retinol or RBP concentrations among children and women are shown in **Figure 2**. The majority of subjects were true negatives—that is, cases where both MRDR and serum retinol or RBP found no VAD. The children (Figure 2A) also had many false positives, where serum retinol or RBP found deficiency but MRDR did not. After adjusting RBP using the BRINDA method, the values shifted higher, resulting in a decrease in false positives as well as true positives (Figure 2B). The women (Figure 2C) had more false negatives, where serum retinol and RBP found no VAD but MRDR did (1 woman had VAD according to RBP; however, she did not have MRDR data).

Results of the sensitivity and specificity analysis among the children are shown in **Table 3**. Using MRDR as the reference test, we found highly variable sensitivity and specificity results for VAD prevalence estimated using unadjusted and inflammation-adjusted serum retinol and RBP. Sensitivity (95% CI), the percentage of children with VAD who were correctly identified as having VAD, ranged from 22.2% (2.81%, 60.0%; Thurnham- and BRINDA-adjusted RBP) to 80.0% (44.4%, 97.5%; unadjusted serum retinol). Confidence intervals for sensitivity estimates were quite large due to the low number of true VAD cases. Specificity, the percentage of children without VAD who were correctly identified as not having VAD, ranged from 63.3% (54.7%, 71.3%; unadjusted serum retinol) to 93.5% (88.0%, 97.0%; BRINDA-adjusted RBP). Overall, as inflammation adjustment improved specificity, sensitivity decreased. Results of the sensitivity and specificity analysis among women showed low sensitivity and high specificity (Table 3). Sensitivities of unadjusted and inflammation-adjusted serum retinol and RBP were 0% (0%, 45.9%) due to the nonexistence of true positive cases of VAD in this group [6 cases of VAD identified by MRDR were classified as sufficient (false negative) by all test measures]. Specificity of all 4 test measures was high (>96%), with slightly higher values among RBP measures compared with serum retinol measures due to decreased false positives.

Discussion

This study examined the sensitivity and specificity of serum retinol and RBP, with and without adjusting for inflammation, to identify cases of VAD among Ghanaian children and women of reproductive age using MRDR as the reference test. We found a low prevalence of VAD identified by any indicator among women; however, among children, estimates of VAD prevalence varied widely by indicator and the inflammation-adjustment procedure used. The VAD prevalence estimated using MRDR would be classified as a mild public health significance, which might be considered a limitation in the analysis. However, this information suggests that the population is mostly meeting their vitamin A needs, which may include intake from intervention sources such as high-dose supplementation and oil fortification. A recent analysis of MRDR data from Ugandan children suggested that survey timing should consider time since high-dose supplementation; however, a relation between vitamin A status and days since high-dose supplementation was not noted (33).

Comparing the prevalence of VAD among children with other studies, one in Ghana found baseline MRDR values for infants in intervention and control groups at 0.032 ± 0.018 (6.9% VAD) and 0.031 ± 0.018 (11.8% VAD), respectively (34), which is quite similar to our mean values, and which are also consistent with values found among well-nourished American children residing in the United States (10) and in Ugandan children (35). Previous work in Indonesian children has shown generally higher MRDR values (indicating lower TLR and higher prevalence of VAD). An intervention study among infants and preschool children (0.6–6.6 y) found baseline MRDR values of 0.054 ± 0.038 to 0.065 ± 0.059 (31–40% with VAD) and serum retinol VAD prevalence of 38–59% among the study groups (20). Two groups of preschool-aged children (0.7–6.5 y) had MRDR values indicating 12% and 48% VAD (19). A mean MRDR value of 0.10 ± 0.06 (72.3% VAD) and serum retinol VAD prevalence of 58.2% were found in a cohort of infants (8). This last study in Indonesia is an interesting example of a population with a high prevalence of both VAD and inflammation (CRP and AGP were elevated in 15% and 20% of infants, respectively). In this case, if serum retinol were to be adjusted for inflammation, it might result in an even lower estimate of VAD, further reducing its accuracy instead of improving it as it did in our population. Comparing our results in women with others, 2 studies in postpartum Ghanaian women found mean MRDR values of 0.048 ± 0.037 (21.5% VAD) (21) and 0.09 ± 0.05 (51% VAD) (36), respectively, which are higher than our current findings. It is interesting to note that serum retinol did not differ between the women in these 2 studies [i.e., 1.4 ± 0.5 (21) and 1.5 ± 0.6 μmol/L (36)], even though there was a much higher prevalence of VAD in the latter group. This example best illustrates the homeostatic control of serum retinol concentrations over a wide range of liver vitamin A reserves because serum retinol was maintained while liver reserves were more depleted in the second group. A study of nonpregnant Indonesian women found a mean MRDR value of 0.034 ± 0.015 (7% VAD), just slightly higher than the women in our study (37). Nonpregnant Ugandan women had a lower adequate median MRDR value of 0.014 (95% CI: 0.009, 0.019) (35).

Few studies have examined the sensitivity and specificity of serum retinol and/or RBP to determine VAD. Our results in children are consistent with a previous analysis, which found that adjusted serum retinol had better specificity than unadjusted serum retinol (83% vs. 97%) among Zambian children, using TLRs determined by retinol isotope dilution as the reference test for VAD (24). Our unadjusted findings are similar to another study that examined the accuracy of (unadjusted) serum retinol and RBP compared with the relative dose-response test among children with liver disease and found sensitivity and specificity for serum retinol of 90% and 78% and 40% and 91% for RBP (38). This evidence indicates that serum retinol and RBP can have varying levels of sensitivity and specificity to determine VAD according to factors unrelated to vitamin A status. Among the children in this study population, the inflammation adjustments made to serum retinol and RBP values, which increased specificity and decreased sensitivity, resulted in an improvement in the percentage of cases correctly classified as vitamin A deficient and sufficient. Mathematically, this is due to the decrease in

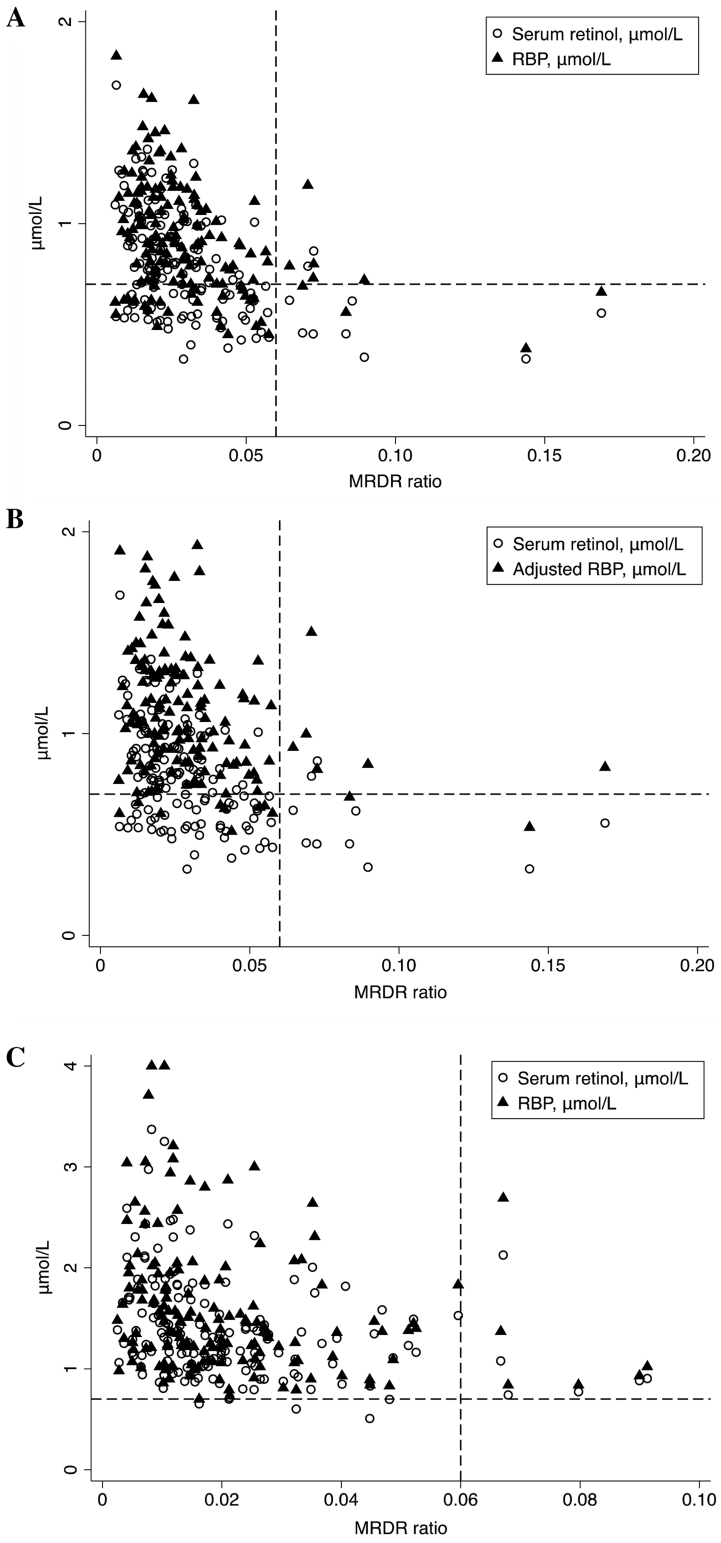

FIGURE 2 (A) Serum retinol and RBP concentrations compared with the MRDR ratio in Ghanaian children ($n = 149$). (B) Serum retinol and RBP (regression-adjusted for inflammation) concentrations compared with the MRDR ratio in Ghanaian children ($n = 147$). (C) Serum retinol and RBP concentration compared with the MRDR ratio in Ghanaian women ($n = 153$). Horizontal dashed lines represent the cutoff for VAD defined by serum retinol and RBP concentration at <0.7 µmol/L; vertical dashed lines represent the cutoff for VAD defined by an MRDR ratio of ≥0.060. Using MRDR as the reference test for VAD, data points in the upper left quadrant are true negatives, points in the lower left quadrant are false positives, points in the lower right quadrant are true positives, and points in the upper right quadrant are false negatives. MRDR, modified-relative dose response; RBP, retinol-binding protein; VAD, vitamin A deficiency.

TABLE 3 Sensitivity and specificity of serum retinol and RBP, unadjusted and adjusted for inflammation, to determine VAD using MRDR as the reference test among Ghanaian children ($n = 147$) and women ($n = 152$)[1]

	Vitamin A status per indicator[2]	Vitamin A status per MRDR test[3]		Sensitivity,[4] %	Specificity, %	Correctly classified, %
		Deficient	Sufficient			
Children						
Serum retinol	Deficient	8	51	80.0 (44.4, 97.5)	63.3 (54.7, 71.3)	64.4
	Sufficient	2	88			
Thurnham-adjusted serum retinol[5]	Deficient	7	36	77.8 (40.0, 97.2)	73.9 (65.8, 81.0)	74.2
	Sufficient	2	102			
RBP	Deficient	4	29	44.4 (13.7, 78.8)	79.0 (71.2, 85.5)	76.9
	Sufficient	5	109			
Thurnham-adjusted RBP[5]	Deficient	2	18	22.2 (2.81, 60.0)	87.0 (80.2, 92.1)	83.0
	Sufficient	7	120			
BRINDA-adjusted RBP[6]	Deficient	2	9	22.2 (2.81, 60.0)	93.5 (88.0, 97.0)	89.1
	Sufficient	7	129			
Women						
Serum retinol	Deficient	0	5	0 (0, 45.9)	96.7 (92.5, 98.9)	93.0
	Sufficient	6	147			
Thurnham-adjusted serum retinol[5]	Deficient	0	5	0 (0, 45.9)	96.6 (92.2, 98.9)	92.8
	Sufficient	6	141			
RBP	Deficient	0	0	0 (0, 45.9)	100 (97.5, 100)	96.1
	Sufficient	6	146			
Thurnham-adjusted RBP[5]	Deficient	0	0	0 (0, 45.9)	100 (97.5, 100)	96.1
	Sufficient	6	146			

[1] BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; MRDR, modified relative dose-response; RBP, retinol-binding protein; VAD, vitamin A deficiency.
[2] Cutoffs for VAD: serum retinol <0.7 μmol/L; RBP <0.7 μmol/L.
[3] Cutoff for VAD: MRDR test ratio of 3,4-didehydroretinol to serum retinol ≥0.060.
[4] Values in parentheses are 95% CIs; intervals starting with 0 or ending in 1 are 1-sided, 97.5% CIs.
[5] Using the method of Thurnham and colleagues (5).
[6] Adjusted using the BRINDA regression approach (30).

false positives/increase in true negatives. Reducing false positives was beneficial to the test's accuracy because the population had low rates of VAD. However, if the population had high rates of VAD and the same effect of inflammation adjustment were seen, it could reduce the percentage of those correctly classified, because a potential decrease in sensitivity would result in fewer true positives and more false negatives.

Thus, adjustments for inflammation can improve specificity but at the expense of sensitivity—that is, they are not improving the test per se. For example, in a population that is mostly vitamin A sufficient but has high levels of inflammation, both serum retinol and RBP would be suppressed and it would be beneficial to adjust for inflammation to improve specificity, which would help reduce the false-positive VAD cases. The children in our study appear to be this type of population, so the VAD estimates become more accurate with adjustment for inflammation as the specificity increases. However, in situations where there is a high prevalence of VAD in addition to inflammation, this adjustment could potentially mask the deficiency—that is, lower sensitivity would mean missing true-positive VAD cases. The women in our study, on the other hand, appear to have low VAD and relatively low inflammation; therefore, the adjustments to serum retinol have little effect and the VAD estimates are similar among the indicators. Thus, the decision to rely on adjusted serum retinol and/or RBP to estimate VAD may vary based on the prevalence of VAD and inflammation status of the population being surveilled, but it would not be possible to know this without first having more reliable data with which to compare the estimates from these indicators.

Despite the WHO recommendation that serum retinol and its surrogate, RBP, cannot stand alone, the assessment of VAD in the field using the combination of serum retinol or RBP and MRDR has been limited, likely due to the lack of widely available technical support, limited availability of 3,4-didehydroretinol, and the scarcity of laboratories that can measure the serum MRDR value. Arguments for the use of serum retinol and RBP cite their relative ease to collect and analyze compared with more accurate but more complex and costly techniques requiring more blood samples, such as retinol isotope dilution. Albeit less widely available, the MRDR test is similar in technical difficulty as measuring serum retinol by HPLC (1). If a study's researchers prefer RBP or serum retinol due to cost and logistical reasons, formative research prior to the study's implementation can be conducted to assess the underlying prevalence of inflammation. Alternatively, the MRDR assessment for VAD risk can be conducted in other population-based surveys—as done in Ghana—to give context around the accuracy and interpretation of RBP in a variety of populations. While total replacement of serum retinol or RBP by MRDR may not be feasible in large-scale surveys, in part due to the 4–6-h wait period, data generated through this work clearly indicate that the MRDR test should be considered as a complement to serum retinol/RBP, especially in the case of high inflammation and potentially high or unknown levels of VAD. In fact, none of the above-investigated biomarkers is suitable to detect hypervitaminosis A, a risk in populations exposed to multiple sources of preformed vitamin A (39–42) (e.g., high-dose supplementation, fortification) and may have negative implications in bone metabolism and growth (43–46). This is

a concern because potential hypervitaminosis A may be ignored if only screening for VAD is conducted (and especially if those indicators are inflating the true VAD prevalence); other indicators have been recommended for evaluation of potential hypervitaminosis in at-risk populations (39, 41). Given the growing evidence surrounding the risks of hypervitaminosis A, greater efforts to accurately target truly at-risk populations and individuals to the extent possible are needed.

A limitation to this study was the low rate of true VAD cases among both children (10) and women (6), which makes sensitivity calculations more volatile (hence the large confidence intervals). Future studies in populations with low VAD prevalence could use larger sample sizes to address this issue, as well as investigate the accuracy of serum retinol and RBP in a variety of contexts of both high and low VAD and inflammation. However, the finding of such low VAD rates in this population argues precisely for the use of a more accurate indicator (or greater confidence in an inflammation-adjusted measure) to assess VAD in the first place. Both young children and women of reproductive age are higher risk groups for VAD, yet in this study population, that risk was found to be low, perhaps due to the success of vitamin A programs, due to dietary changes, or a combination. As mentioned above, as VAD decreases in a population exposed to multiple overlapping VA interventions it may also be important to screen for hypervitaminosis.

This study contributes to evidence that serum retinol and RBP are not consistently accurate indicators of VAD. Adjusting for the presence of inflammation improved specificity but sacrificed sensitivity among the children in this study, which could have different impacts on the validity of VAD estimates depending on the underlying inflammation and VAD prevalence of the population under surveillance. Especially in populations with high inflammation, we recommend that MRDR analysis should be used in place of or in conjunction with serum retinol and/or RBP in estimating VAD prevalence when possible. To render the MRDR test more available, capacity-building efforts are warranted. Furthermore, if vitamin A programs overlap, more sensitive measures of high status need to be undertaken to cover the hypervitaminotic range of TLRs, such as retinol isotope dilution or retinyl ester concentrations.

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Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending the agreement of the requesting institution to the terms and conditions of formalized data transfer agreements of University of Wisconsin and University of Ghana-Legon/GroundWork.

References

1. Tanumihardjo SA, Russell RM, Stephensen CB, Gannon BM, Craft NE, Haskell MJ, Lietz G, Schulze K, Raiten DJ. Biomarkers of Nutrition for Development (BOND)—vitamin A review. J Nutr 2016;146:1816S–48S.
2. Tanumihardjo SA. Vitamin A: Biomarkers of Nutrition for Development. Am J Clin Nutr 2011;94:658S–65S.
3. Gannon BM, Tanumihardjo SA. Comparisons among equations used for retinol isotope dilution in the assessment of total body stores and total liver reserves. J Nutr 2015;145:847–54.
4. Muto Y, Smith JE, Milch PO, Goodman DS. Regulation of retinol-binding protein metabolism by vitamin A status in the rat. J Biol Chem 1972;247:2542–50.
5. Thurnham DI, McCabe GP, Northrop-Clewes CA, Nestel P. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: meta-analysis. Lancet North Am Ed 2003;362:2052–8.
6. Filteau SM, Morris SS, Abbott RA, Tomkins AM, Kirkwood BR, Arthur P, Ross DA, Gyapong JO, Raynes JG. Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. Am J Clin Nutr 1993;58:192–7.
7. Bresnahan KA, Chileshe J, Arscott S, Nuss E, Surles R, Masi C, Kafwembe E, Tanumihardjo SA. The acute phase response affected traditional measures of micronutrient status in rural Zambian children during a randomized, controlled feeding trial. J Nutr 2014;144:972–8.
8. Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal. Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. J Nutr 2002;132:3061–6.
9. Tanumihardjo SA, Muhilal YY, Permaesih D, Sulaiman Z, Karyadi D, Olson JA. Vitamin A status in preschool-age Indonesian children as assessed by the modified relative-dose-response assay. Am J Clin Nutr 1990;52:1068–72.
10. Tanumihardjo SA, Koellner PG, Olson JA. The modified relative-dose-response assay as an indicator of vitamin A status in a population of well-nourished American children. Am J Clin Nutr 1990;52:1064–7.
11. Valentine AR, Tanumihardjo SA. Adjustments to the modified relative dose response (MRDR) test for assessment of vitamin A status minimize the blood volume used in piglets. J Nutr 2004;134:1186–92.
12. Valentine AR, Tanumihardjo SA. One-time vitamin A supplementation of lactating sows enhances hepatic retinol in their offspring independent of dose size. Am J Clin Nutr 2005;81:427–33.
13. Surles RL, Hutson PR, Valentine AR, Mills JP, Tanumihardjo SA. 3, 4-Didehydroretinol kinetics differ during lactation in sows on a retinol depletion regimen and the serum:milk 3, 4-didehydroretinol:retinol ratios are correlated. J Nutr 2011;141:554–9.
14. Tanumihardjo SA, Olson JA. A modified relative dose-response assay employing 3,4-didehydroretinol (vitamin A_2) in rats. J Nutr 1988;118:598–603.
15. Tanumihardjo SA, Furr HC, Erdman JW, Olson JA. Use of the modified relative dose response (MRDR) assay in rats and its application to humans for the measurement of vitamin A status. Eur J Clin Nutr 1990;44:219–24.
16. Surles RL, Li J, Tanumihardjo SA. The modified-relative-dose-response values in serum and milk are positively correlated over time in lactating sows with adequate vitamin A status. J Nutr 2006;136:939–45.
17. Amédée-Manesme O, Anderson D, Olson JA. Relation of the relative dose response to liver concentrations of vitamin A in generally well-nourished surgical patients. Am J Clin Nutr 1984;39:898–902.
18. Amedee-Manesme O, Mourey MS, Hanck A, Therasse J. Vitamin A relative dose response test: validation by intravenous injection in children with liver disease. Am J Clin Nutr 1987;46:286–9.
19. Tanumihardjo SA, Permaesih D, Dahro AM, Rustan E, Muhilal, Karyadi D, Olson JA. Comparison of vitamin A status assessment techniques in children from two Indonesian villages. Am J Clin Nutr 1994;60:136–41.
20. Tanumihardjo SA, Permaesih D, Muherdiyantiningsih, Rustan E, Rusmil K, Fatah AC, Wilbur S, Muhilal, Karyadi D, Olson JA. Vitamin A status of Indonesian children infected with *Ascaris lumbricoides* after dosing with vitamin A supplements and albendazole. J Nutr 1996;126:451–7.

21. Tchum SK, Tanumihardjo SA, Newton S, de Benoist B, Owusu-Agyei S, Arthur FK, Tetteh A. Evaluation of vitamin A supplementation regimens in Ghanaian postpartum mothers with the use of the modified-relative-dose-response test. Am J Clin Nutr 2006;84:1344–9.
22. Manorama R, Sarita M, Rukmini C. Red palm oil for combating vitamin A deficiency. Asia Pac J Clin Nutr 1997;6:56–9.
23. Tanumihardjo SA, Muherdiyantiningsih, Permaesih D, Komala, Muhilal, Karyadi D, Olson JA. Daily supplements of vitamin A (8.4 μmol, 8000 IU) improve the vitamin A status of lactating Indonesian women. Am J Clin Nutr 1996;63:32–5.
24. Suri DJ, Tanumihardjo JP, Gannon BM, Pinkaew S, Kaliwile C, Chileshe J, Tanumihardjo SA. Serum retinol concentrations demonstrate high specificity after correcting for inflammation but questionable sensitivity compared with liver stores calculated from isotope dilution in determining vitamin A deficiency in Thai and Zambian children. Am J Clin Nutr 2015;102: 1259–65.
25. Wegmüller R, Bentil H, Wirth JP, Petry N, Tanumihardjo SA, Allen L, Williams TN, Selenje L, Mahama A, Amoaful E, et al. Anemia, micronutrient deficiencies, malaria, hemoglobinopathies and malnutrition in young children and non-pregnant women in Ghana: findings from a national survey. PLoS One 2020;15(1):e0228258.
26. University of Ghana; GroundWork; University of Wisconsin-Madison; KEMRI-Wellcome Trust; UNICEF. Ghana Micronutrient Survey 2017. Accra (Ghana): 2017.
27. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr 2004;134:3127–32.
28. World Health Organization. Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations. Geneva (Switzerland): WHO; 2011.
29. World Health Organization. Global prevalence of vitamin A deficiency in populations at risk 1995–2005. Geneva (Switzerland): WHO; 2009.
30. Namaste SM, Aaron GJ, Varadhan R, Peerson JM, Suchdev PS; BRINDA Working Group. Methodologic approach for the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2017;106(Suppl 1):333S–47S.
31. Larson LM, Namaste SM, Williams AM, Engle-Stone R, Addo OY, Suchdev PS, Wirth JP, Temple V, Serdula M, Northrop-Clewes CA. Adjusting retinol-binding protein concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2017;106(Suppl 1):390S–401S.
32. Trevethan R. Sensitivity, specificity, and predictive values: foundations, pliabilities, and pitfalls in research and practice. Front Public Heal 2017;5:307.
33. Pickens CM, Flores-Ayala R, Ford ND, Whitehead RD, Jr, Tanumihardjo SA, Ngalombi S, Mapango C, Sheftel J, Jefferds MED. Relation between timing of high-dose vitamin A supplementation and modified-relative-dose-response values in children 12–23 months in Uganda. J Nutr 2021;151(4):1025–8.
34. Newton S, Owusu-Agyei S, Asante KP, Amoaful E, Mahama E, Tchum SK, Ali M, Adjei K, Davis CR, Tanumihardjo SA. Vitamin A status and body pool size of infants before and after consuming fortified home-based complementary foods. Arch Public Heal 2016;74:10.
35. Whitehead RD, Jr, Ford ND, Mapango C, Ruth LF, Zhang M, Schleicher RL, Ngalombi S, Halati S, Ahimbisibwe M, Lubowa A, et al. Retinol-binding protein, retinol, and modified-relative-dose response in Ugandan children 12–23 months and their non-pregnant caregivers. Exp Biol Med 2021;246(8):906–15.
36. Tchum S, Newton S, Tanumihardjo S, Fareed K, Tetteh A, Agyei S. Evaluation of a green leafy vegetable intervention in Ghanaian postpartum mothers. African J Food Agric Nutr Dev 2009;9(6):1294–308.
37. Tanumihardjo SA, Muherdiyantiningsih, Permaesih D, Dahro AM, Muhilal, Karyadi D, Olson JA. Assessment of the vitamin A status in lactating and nonlactating, nonpregnant Indonesian women by use of the modified-relative-dose-response (MRDR) test. Am J Clin Nutr 1994;60:142–7.
38. Feranchak AP, Gralla J, King R, Ramirez RO, Corkill M, Narkewicz MR, Sokol RJ. Comparison of indices of vitamin A status in children with chronic liver disease. Hepatology 2005;42:782–92.
39. van Stuijvenberg ME, Dhansay MA, Nel J, Suri D, Grahn M, Davis CR, Tanumihardjo SA. South African preschool children habitually consuming sheep liver and exposed to vitamin A supplementation and fortification have hypervitaminotic A liver stores: a cohort study. Am J Clin Nutr 2019;110:91–101.
40. Olsen K, Suri DJ, Davis C, Sheftel J, Nishimoto K, Yamaoka Y, Toya Y, Welham NV, Tanumihardjo SA. Serum retinyl esters are positively correlated with analyzed total liver vitamin A reserves collected from US adults at time of death. Am J Clin Nutr 2018;108:997–1005.
41. Mondloch S, Gannon BM, Davis CR, Chileshe J, Kaliwile C, Masi C, Rios-Avila L, Gregory JF, III, Tanumihardjo SA. High provitamin A carotenoid serum concentrations, elevated retinyl esters, and saturated retinol-binding protein in Zambian preschool children are consistent with the presence of high liver vitamin A stores. Am J Clin Nutr 2015;102:497–504.
42. Tanumihardjo SA, Mokhtar N, Haskell MJ, Brown KH. Assessing the safety of vitamin A delivered through large-scale intervention programs. Food Nutr Bull 2016;37(2 Suppl):S63–74.
43. Penniston KL, Tanumihardjo SA. The acute and chronic toxic effects of vitamin A. Am J Clin Nutr 2006;83:191–201.
44. Pease CN. Focal retardation and arrestment of growth of bones due to vitamin A intoxication. JAMA 1962;182:980–5.
45. Binkley N, Krueger D. Hypervitaminosis A and bone. Nutr Rev 2000;58:138–44.
46. Tanumihardjo SA, Gannon BM, Kaliwile C, Chileshe J, Binkley NC. Restricting vitamin A intake increases bone formation in Zambian children with high liver stores of vitamin. Arch Osteoporos 2019;14:4–9.