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Association between vitamin A status, inflammations, and infections in children 36–59 months of age in rural Burkina Faso: A cross - sectional study

Olivier O. Sombié[1,2*], Augustin N. Zeba[1], Jérome W. Somé[3], Adama Kazienga[4], Jean F. Bationo[5], Christopher Davis[6], Sherry A. Tanumihardjo[6], Stefaan De Henauw[2] and Souheila Abbeddou[2]

Abstract

Background Assessing vitamin A (VA) status using retinol and retinol-binding protein (RBP) in the presence of infection and inflammation remains challenging, as both markers prove to be unreliable during such physiological disturbances.

Objective This study aimed to assess the association between common infections and inflammation and VA status of children in rural Burkina Faso.

Methods Two community-based cross-sectional studies were conducted in the villages of Sourkoudougou and Banakeledaga, in Southwestern Burkina Faso, one during the dry season (November 2016– January 2017) and the second during the rainy season (August– September 2017). In total, 115 children, 36–59 months of age, were included. The ^{13}C-retinol isotope dilution test (RID) was used to assess total body VA stores (TBS) and VA total liver reserves (TLR). Malaria infection and intestinal parasites were evaluated at enrollment. Serum C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) were measured. Univariable and multivariable linear regressions were used to test the associations between VA status and infection and inflammation status.

Results No VA deficiency (TLR ≤ 0.1 µmol/g liver) was detected using RID method. Geometric means (95% confidence interval) of TBS and TLR were respectively 473 (412; 543) µmol and 0.86 (0.75; 0.99) µmol/g liver. One-third of study participants were found to have hypervitaminosis A (TLR > 1.0 µmol/g liver). Elevated CRP (> 5.0 mg/L) and AGP (> 1.0 g/L) were respectively detected in 1.9% and 28.6% of children. Positive malaria was diagnosed in 5 children. Intestinal parasites were found in one out of two (47.6%) participants, and other morbidities were detected in 2 participants. In a multivariable adjusted regression, significant positive weak associations were found between Log TLR and CRP concentrations ($N = 79$, $\beta = 0.058$, $p = 0.004$) and between Log TBS and CRP concentrations ($N = 79$, $\beta = 0.054$, $p = 0.005$).

*Correspondence:
Olivier O. Sombié
ouahaminolivier.sombie@ugent.be; sombieolivier@yahoo.fr

Full list of author information is available at the end of the article

Conclusion Study children were apparently healthy with high prevalence of asymptomatic intestinal parasites and chronic inflammation. TLR and TBS were positively associated with the acute phase protein CRP warranting further investigation.

Trial registration The study was registered retrospectively (22 March 2018) as a clinical trial with the Pan African Clinical Trials Registry (Cochrane South Africa; PACTR201803002999356).

Keywords Vitamin A, Total liver reserves, Infections, Inflammation, Children

Introduction

Vitamin A (VA) plays an important role in supporting sight, maintaining the functioning of the immune system of individuals, and in children's growth and development [1]. Vitamin A deficiency (VAD) can lead to anemia, weakened resistance to infections, blindness, and death [2, 3]. Severe VAD remains a serious public health problem particularly in vulnerable populations of children and pregnant women in low- and middle-income countries (LMICs) [4].

Dietary VA in the form of retinol and provitamin A carotenoids is first metabolized in the intestine, absorbed in the form of retinyl esters, transported in the lipid core of chylomicron, and stored, in its majority (90%), in the liver during adequate VA status [5]. Vitamin A (95% all-trans-retinol) is transported principally by plasma retinol-binding protein (RBP) to the tissues. Plasma RBP exists in a ratio of 1:1 with plasma retinol [6]. Plasma (or serum) RBP concentration is a preferred biomarker of VA status in field surveys where storage and laboratory capacities are limited. During an inflammatory process, acute phase proteins (APPs) are induced and regulated by cytokines. C-reactive protein (CRP) and α-1-acid glycoprotein (AGP), two biomarkers of inflammation commonly measured, are enhanced by the cytokine's Interleukin-6, Interleukin-1, and Tumor Necrosis Factor [7]. The rise in CRP concentrations occurs 4–6 h post-infection and corresponds to an acute response to the inflammatory stimulus with a much slower and longer response by AGP [8–10]. The acute phase response has a transient negative influence on retinol and RBP mainly because RBP is a negative APP [4, 9–11]. Evidence is strong that both retinol and RBP are poor indicators of VA status during inflammation. Additionally, the ratio retinol: RBP of 1:1 may be affected by VA status, inflammation, and nutritional status [11–13]. Previous studies found that RBP and retinol concentrations potentially overestimated the prevalence of VAD in populations with high levels of inflammation, making the assessment of VAD difficult when infections are common [11, 12, 14–16]. Adjusting for inflammation indicators improves the accuracy of VAD prevalence estimates based on plasma or serum retinol or RBP concentrations [17]. Wessells and coauthors suggested that asymptomatic malaria affects VA indicators, hence the importance of adjusting for it [18].

It is evident that severe or recurrent infections can lead to the development of VAD, at least in subjects who have low-to-marginal intakes of VA [11]. Furthermore, infections and high concentrations of APP are highly associated during VAD [19, 20]. VAD results in increased macrophage-mediated inflammation through the enhanced production of Interleukin 12 (IL-12) and Interferon gamma (IFN-γ) cytokines and the reduced phagocytic capacity of macrophages resulting in exacerbated inflammation [21–23].

Infections, inflammation, and VAD are prevalent in low income settings including Africa [24]. In Burkina Faso, VAD is still a major nutritional concern with varying prevalence from rural to urban settings [25]. In the presence of common infections and the high prevalence of VAD and inflammation, and the bidirectional relationship between VAD and inflammation or the immune response, it becomes difficult to assess VA status using serum concentrations of retinol and RBP. Liver biopsy and autopsy samples are considered to be the reference methods to assess VA status. Retinol isotope dilution (RID) has been promoted as a sensitive method in assessing VA status throughout a large spectrum from depletion to toxicity [26]. This study aims at assessing the association between inflammation and infections and VA status using the ^{13}C-RID test in Burkinabe children 36–59 months of age.

Materials and methods

Study area

The study was conducted in the villages of Sourkoudougou and Banakeledaga. Both villages are located in the health district of Dandé, which is located in the "Hauts-Bassins region" at 30 km from the main city of Bobo-Dioulasso. The climate is Soudano-Sahelian and characterized by two seasons, a dry season lasting between October– May and a rainy season between June and September. The region receives 800 to 1200 mm of irregular rainfall. The rainy season is characterized by a high prevalence of common infections including malaria and diarrhea. Agriculture remains the dominant economic activity throughout the health district of Dandé. Staple crops, including maize, rice and sweet potatoes,

are grown during the rainy season. A variety of irrigation-based garden vegetables and fruits are cultivated using water from a river that crosses the two villages. The district has poor sanitation facilities. The situation is exacerbated during the rainy season, which makes the river and many small swamps a breeding ground for mosquitoes, the vector of malaria and other infectious diseases. As a result, malaria and diarrhea are the main causes for medical consultation and hospitalization among children under five years of age [27]. Furthermore, malnutrition is a significant public health concern, primarily affecting children under the age of five in this setting [27].

Study population

The study population consisted of 115 preschool children, 36–59 months of age from both sexes, with an ascertained birthdate, living in the two villages and not suffering from a severe or chronic illness. Children were not included if they were severely malnourished (weight-for-height z-score [WHZ] below − 3 standard deviations [SD], according to the 2006 World Health Organization (WHO) Child Growth Standards) [28]; had fever (fever was defined as an uncorrected axillary temperature above 37.5 °C) or reportedly had fever in the previous 24 h; or were suffering from serious illnesses requiring hospitalization such as severe anemia (hemoglobin concentrations < 7.0 g/dL), convulsion, coma, incoercible vomiting, severe dehydration, or severe respiratory illness.

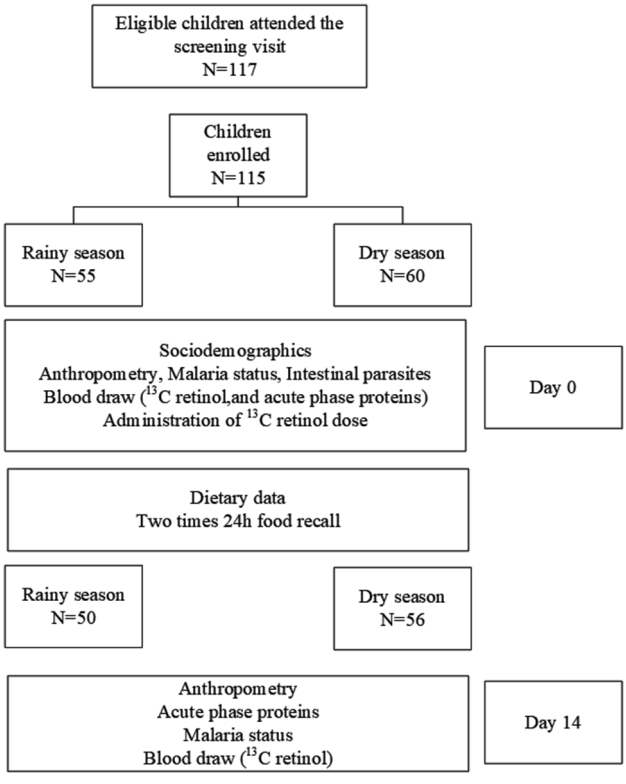

Fig. 1 Schematic diagram of the study design, the 13 C-retinol isotope dilution test protocol and the data collection timeline

Sample size

This study is a secondary analysis of country-specific data from Burkina Faso, derived from a larger regional African study that recruited a minimum of 50 children per site [29, 30]. The sample size estimation was based on literature recommending a sample size of 30 subjects per treatment group for isotope studies on supplementation [26, 31]. For the current study, we enrolled 55 children during the rainy season and 60 during the dry season to account for a 10% dropout rate over the 15-day protocol period and an additional 10% dropout rate over the one-month period following high-dose VA supplementation (part of a separate sub-study not within the scope of this study).

Study design and duration

Eligible children were enrolled in two community-based cross-sectional surveys during the dry season ($N = 60$, November 2016 to January 2017) and the rainy season ($N = 55$, August to September 2017) (Fig. 1).

Socioeconomic, dietary and anthropometric data collection

Demographic and socio-economic data were collected using a semi-structured questionnaire. The birth date of the child was extracted directly from the birth certificate that the parents were asked to bring along during enrollment. Additional data included maternal age and education, parental occupation, and number of members in the household including children under 5 years old. Socioeconomic status was assessed by household characteristics and possession of a set of assets including building materials, radio, television, telephone, refrigerator, bicycle, and motorcycle. Dietary intake and information on breastmilk intake were collected using an adapted 24-h recall questionnaire, on two days within the two weeks of enrollment, one on weekday and the second on a weekend. Briefly, the mother or the caregiver who accompanied the child was asked to list all the foods and drinks that the child consumed the day before the interview, from the time they got up until the next day [32, 33]. After obtaining the list of the foods during the first step, the mother or caregiver was probed for detailed description of each food, then the quantity consumed and the detailed description of the recipe including the ingredients if the food was a mixed dish.

Height was measured to the nearest 0.1 cm with a portable length board (Seca 213, Hamburg, Germany). Weight was measured to the nearest 0.1 kg using an electronic balance (Seca 899, Hamburg, Germany). Weight

and height were measured in duplicate according to standard procedures. A third measurement was done if the difference in measurements of weight was greater than 0.1 kg and of height of more than 0.5 cm. The mean value of the two closest values was used in the analysis.

Blood sample collection, stable isotope administration and procedure

To estimate total body VA stores (TBS) and VA total liver reserves (TLR), the ^{13}C-RID test was used. For that, on day 0 (baseline), 6 mL venous blood were drawn in plain tubes from included participants by trained nurses. Afterwards, a prepared dose of 1.0 µmol $^{13}C_2$-retinyl acetate was administered orally to the child, followed immediately by the oral administration of 1 ml of non-VA fortified vegetable oil to improve the absorption of the stable isotope. Collected blood samples were stored in a cool box (+4˚C) and transported the same day to the laboratories of the Institut de Recherche en Sciences de la Santé (IRSS, Bobo-Dioulasso). On day 14, children who completed the protocol on day 0 provided another sample of 6 mL venous blood in plain tubes. Collected blood samples were stored and transported as above.

Blood samples were centrifuged at 3000 rpm for 10 min with a Universal 320R centrifuge (Hettich Zentrifugen, D-78532 Tuttlingen) on the same day of their reception. Volumes of 1.0 to 1.5 mL of serum were transferred to brown 2 mL cryotubes (Eppendorf, Hamburg, Germany) under yellow light to protect VA indicators against photodegradation. Samples were stored at −80 °C until shipped on dry ice to the laboratories of UW-Madison, for ^{13}C analysis and to the VitMin Lab, Willstaett, Germany for analysis of inflammatory biomarkers.

Hemoglobin concentration, malaria, intestinal parasites and inflammation indicators

On day 0, the remaining blood drops from the venous blood draw were used to measure hemoglobin (Hb) concentration with a point-of-care HemoCue (Hb-301, Ängelholm, Sweden). Rapid diagnosis test (Malaria-Ag-Pf/Pan™) and blood smear microscopy were used to assess malaria status [34]. On a sample of 30 gram collected stool, a direct examination on a slide after preparation was performed by qualified field workers to search for the presence of intestinal parasites on site. The test was positive if at least one parasite was identified. Serum concentrations of CRP and AGP were simultaneously measured in VitMin Lab using a sandwich ELISA technique [35]. The coefficients of variation (CVs) for CRP and AGP were, respectively, 3.61% and 5.84%.

Analysis of ^{13}C

Method of extraction, purification and analysis of ^{13}C using gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) was previously described [36, 37]. Briefly to 0.3 to 1.0 mL serum, 1 ml ethanol was added to precipitate proteins. C23 β-apo-carotenol was used as an internal standard. Retinol was extracted 3 times with hexanes. Hexane layers were pooled, dried under nitrogen, resuspended in methanol (100 mL), frozen at -80˚C for 5 min, centrifuged at 1380 x g briefly, and injected into HPLC system 1 for quantification and purification. The retinol fraction was collected, dried under nitrogen, and resuspended in 100 µL methanol for injection into system 2, dried in a Thermo Savant Speed-Vacuum centrifuge (Thermo Scientific, Waltham, MA), reconstituted in 10 µl hexanes, and 1.5 µl was injected into a GC/C/IRMS. The determination of TBS of VA was detailed previously [36, 37]. After sample's injection simulating on-column injection with the PTV injector, the δ ^{13}C-retinol values were determined from serum samples by GC/C/IRMS in duplicate. Synthetic retinol, prepared by quick retinyl acetate saponification (Sigma-Aldrich, St. Louis, MO), was purified twice similarly to serum retinol and used as an external standard. Atom percentage (At %) was directly calculated (Isodat version 2.0; Thermo Scientific) in reference to carbon dioxide, which was calibrated against a sucrose standard (National Institute of Standards and Technology, 8542).

Assessment of VA total body store (TBS) and total liver reserve (TLR)

TBS (μmol) were calculated as previously described [37, 38] using the following equation:

$$\text{TBS} = a \times \frac{1}{TTR} \times (\text{factors for absorption and storage})$$

Where a is the amount of ^{13}C retinyl acetate in the dose administered (1.0 μmol); TTR is the tracer-to-tracee ratio or the fraction of ^{13}C to ^{12}C in the dose which was 0.11, that accounts for natural abundance. The following assumptions were used [38] in calculating TBS of VA: a 90% absorption rate of the dose, a specific activity of 0.8 because dietary access to vitamin A was not restricted in the 14 days between the two blood samples, and a half-life of 136 days.

TLRs (µmol/g) were calculated using the formula:

$$TLR = \left(\frac{\text{TBS}}{BW(kg) \times liver\ fraction\ of\ BW}\right) x\ 1000\ x\ fraction\ of\ TBS\ in\ liver$$

Where BW is body weight (kg), liver fraction of BW was estimated as 3% in preschool children, and 80% of TBS were assumed to be in the liver storage pool of children with adequate to hypervitaminotic VA status [37, 38]. Hypervitaminosis A was defined as an elevated concentration of VA (> 1.0 µmol/g liver) and VA deficiency as TLR < 0.1 µmol/g liver [39].

Data processing and statistical analysis

Z-scores of height-for-age (HAZ), weight-for- height (WHZ) and weight-for-age (WAZ) were calculated using the STATA macros of the WHO Child Growth Standards [28]. Stunting, wasting, and underweight were defined as HAZ, WHZ, and WAZ < -2 SDs, respectively.

From the 24-h dietary recall data, dietary diversity score (DDS) and VA-rich food intake were constructed using the Food and Agriculture Organization (FAO) DDS with nine foods groups [40] and calculated using the consumption or not of these food groups ranging between 0 and 9. The nine food groups included: (1) Starchy foods (cereals, roots and tubers), (2) Dark green leafy vegetables, (3) VA rich fruits and vegetables, (4) Other fruits and vegetables, (5) Organ meat, (6) Meat and fish, (7) Eggs, (8) Legumes, nuts and seeds, and (9) Dairy products (milk, yogurt and cheese). Additionally, VA-rich foods were summarized as VA animal source food (preformed VA) and VA-rich plant source food (pro–VA carotenoids). The scores of VA animal source food and VA-rich plant source food ranged from 0 to 3 both [40].

Inflammation status was defined as elevated concentration of CRP (> 5.0 mg/L) and AGP (> 1.0 g/L) [17]. Anemia was defined in children with Hb concentrations < 11.0 g/dL [41].

Household asset index was constructed based on ownership of a set of assets (radio, television, telephone, refrigerator, bicycle, or motorcycle) and building materials using multiple correspondence analysis [42].

Statistical analyses were carried out using Stata software, version 15.1 (Stata Corp, TX, USA). The baseline assessments were performed by descriptive statistics [means (± standard deviation), geometric mean (90% confident interval), median (interquartile range) and proportions]. Variables that were not normally distributed (CRP, AGP, TBS and TLR) were logarithm-transformed, which were back transformed for reporting of descriptive results. As part of a quality control check, TLR values exceeding 15.0 µmol/g liver were excluded due to their implausibility. In our dataset, one data point (TLR = 27.289 µmol/g liver) was dropped.

The associations between VA status of the child and their infection status and inflammation indicators were tested using the univariate linear model, including only the dependent variables (TBS and TLR) and the independent variables (CRP and AGP concentrations, malaria status and intestinal parasite test). Another model was built including the four indicators of infection and inflammation. In a multivariate linear regression, preselected variables (covariates) were included in the model that could explain the changes in the dependent variables. They were selected based on literature and included child's sex, age, Hb concentration, nutritional status (WHZ), DDS, and the scores of VA-rich plant source food and VA animal source food, season, family size, mother's educational status, the occupation of household head, and household wealth index. Included co-variables were tested for collinearity and those causing the variance inflation factor (VIF) > 10 were dropped. For each model, the residue normality was tested by visual inspection of qnorm, and the equality of variances was assessed by estat hottest. $P < 0.05$ was considered significant. TLR and TBS were log transformed to achieve a normal distribution for these variables, ensuring the best-fitted statistical model.

In a secondary analysis, we tested how well VA status (TBS and TLR) can categorize the children based on their infection and inflammation statuses. Differences between TBS and TLR were assessed according to infection and inflammation statuses using Student t-test. Additionally, categories of infection and inflammation statuses were tested against TLR categories (high and normal) using Chi-Square test.

Results

Socio demographic characteristics of the study children, their parents and households

The study enrolled 115 children in the two cross-sectional surveys, 53 (46.1%) were female, and were aged (mean ± SD) 48.2 (± 6.8) months. The majority of children (85.1%) had normal Hb concentrations (Hb > 11.0 g/dL), 17.4% were suffering from moderate stunting (-3 SD < HAZ< -2 SD), and 7.8% were moderately wasted (-3 SD < WAZ< -2 SD). Infections were common in this study population mainly because of intestinal parasites which affected 47.6% of the children. While 5% of children tested positive for malaria. Amoeba in several forms were the most prevalent parasites and found (cyst and parasite) in 43 participants (53.1%), *Giardia intestinalis* in 14 participants (17.3%), and *Trichomonas intestinalis* in 5 participants (6.2%). Almost one-third of children had elevated AGP (28.6%) and only 2 children (1.9%) had high CRP. Most of children's fathers (94.8%) were farmers and their mothers (79.1%) did not have any formal school education. The mean size of the family was 6.4 persons. Table 1 summarizes the main characteristics of study participants, their parents and their households.

Vitamin A total body store (TBS) and total liver store (TLR)

Children in this study sample had a geometric mean (95% Confidence Interval, 95% CI) TBS of 473 (412; 543) µmol and TLR of 0.86 (0.75; 0.99) µmol/g liver. TBS and TLR were significantly higher during the rainy season ($p < 0.01$). During the rainy season, the geometric mean (95% CI) of TBS and TLR were 586 (475; 724) µmol and 1.10 (0.89; 1.36) µmol/g liver, respectively. During the dry season, geometric mean (95% CI) TBS and TLR concentrations were respectively, 388 (328; 460) µmol and 0.69

Table 1 Baseline characteristics of participating children 36–59 months of age ($N = 115$) in rural Burkina Faso

Parameters		Study population[1]
Child age (months)		48.9 ± 6.9
Sex	Female	53 (46.1)
Mother's formal education	None	91 (79.1)
	Primary	17 (14.8)
	Secondary	5 (4.4)
Father's occupation	None	2 (1.7)
	Farmer	109 (94.8)
	trader	1 (0.9)
	Other	3 (2.6)
Family size		6.4 ± 3.0
Number of children < 5 years in household	1 child	70 (60.9)
	2 children	43 (37.4)
	> 2 children	2 (1.8)
Hemoglobin concentration (g/dL)		12.2 ± 1.4
Anemia	Non-anemic	97 (85.1)
	Mildly anemic	17 (14.9)
Anthropometric indices	HAZ	-1.21 ± 1.08
	WHZ	-0.09 ± 0.92
	WAZ	-0.78 ± 0.80
Malaria status	Positive malaria smear ($n = 112$)	5 (4.5)
	Positive RDT	6 (5.2)
Acute phase proteins	CRP[3]	0.44 ± 5.71
	High CRP (> 5.0 mg/L)	2 (1. 9)
	AGP[3]	0.89 ± 0.52
	High AGP (> 1.0 g/L)	30 (28.6)
Intestinal parasites	Positive ($n = 105$)	50 (47.6)
Morbidity[2]		2 (1.7)
Breastfeeding	Yes	113 (98.2)
Dietary diversity score		4.1 ± 1.1
VA rich plant source score		1.0 ± 0.5
VA rich animal source food score		0.1 ± 0.4
Household economic status	wealth index	0.10 (-0.57–0.58)

HAZ, height-for-age z-score; WHZ, weight-for-height z-score; WAZ, weight-for-age z-score; RDT, rapid diagnostic test; CRP, C-reactive protein; AGP, alpha (1)-acid glycoprotein

[1]Values presented are means ± SD, n (%), and median (Q25- Q75)

[2]Presence of clinical illness including cough, runny nose, and reported vomiting

[3]Values were first log-transformed for analysis

(0.59; 0.81) µmol/g liver for TLR. One-third (29 children, 32%) of study participants were found to have hypervitaminosis A (TLR > 1.0 µmol/g liver). None of the children were VA deficient (TLR < 0.1µmol/g liver).

Univariate linear regression analysis of the association between VA biomarkers and infection statuses and inflammation indicators

Univariate linear regressions assessing the associations between VA biomarkers (TLR and TBS) and inflammation indicators, as well as infection markers, have found no significant associations, except for associations of TLR ($N = 90$, $\beta = 0.032$, $p = 0.041$) and TBS ($N = 90$, $\beta = 0.033$, $p = 0.036$) with CRP concentration.

Assessing the differences in Log TLR and Log TBS based on the presence of acute and chronic inflammations, positive blood smear test for malaria, and the presence of intestinal parasites showed no significant differences in either indicator (Fig. 2). Similarly, when testing categories of infection and inflammation statuses against TLR categories (high vs. normal) using a Chi-Square test (Fig. 3), no significant differences were observed. In other words, the high TLR values did not show a distinct pattern concerning infection and inflammation statuses.

Multivariate linear regression analysis of the association between VA status biomarkers and infection status and inflammation indicators

In a multivariable model that included the four indicators of infection and inflammation, adjusted for season, child's age and sex, mother education, family size, wealth index, DDS, VA rich food (preformed VA and pro VA) consumption score, no significant associations were found, except for a weak but significant association between log-transformed TLR and CRP concentrations ($N = 79$, $\beta = 0.058$, $p = 0.004$), and between log-transformed TBS and CRP concentrations ($N = 79$, $\beta = 0.054$, $p = 0.005$) (Table 2). The association was stronger with season, as log-transformed TLR and TBS were significantly higher during the rainy season. In contrast, the associations with wealth index showed no clear pattern, although a significantly association was observed in participants from the third quartile.

Discussion

The study sample had an overall high prevalence of hypervitaminosis A and none of the pre-school children was VA deficient as assessed by the ^{13}C-RID test. Malaria was uncommon but the prevalence of intestinal parasites was high in this study sample. In this context, biomarkers of VA status including TLR and TBS were found to be significantly associated with acute inflammation indicator (CRP).

The lack of VAD is in contrast with the situation in 2008 [43] and 2014 [44], where similar research was conducted in school children in the same area and reported high prevalence (46.1% and 46.0% of children respectively) of VAD using serum retinol (< 0.7 µmol/L). Additionally, our study sample showed high prevalence of VAD, with 30.9% assessed by serum retinol (< 0.7 µmol/L) and 33.3% by RBP (< 0.7 µmol/L) [45]. Firstly, VAD is more common in pre-school children [46], yet national surveys rely on serum retinol and RBP as biomarkers for VA assessment.

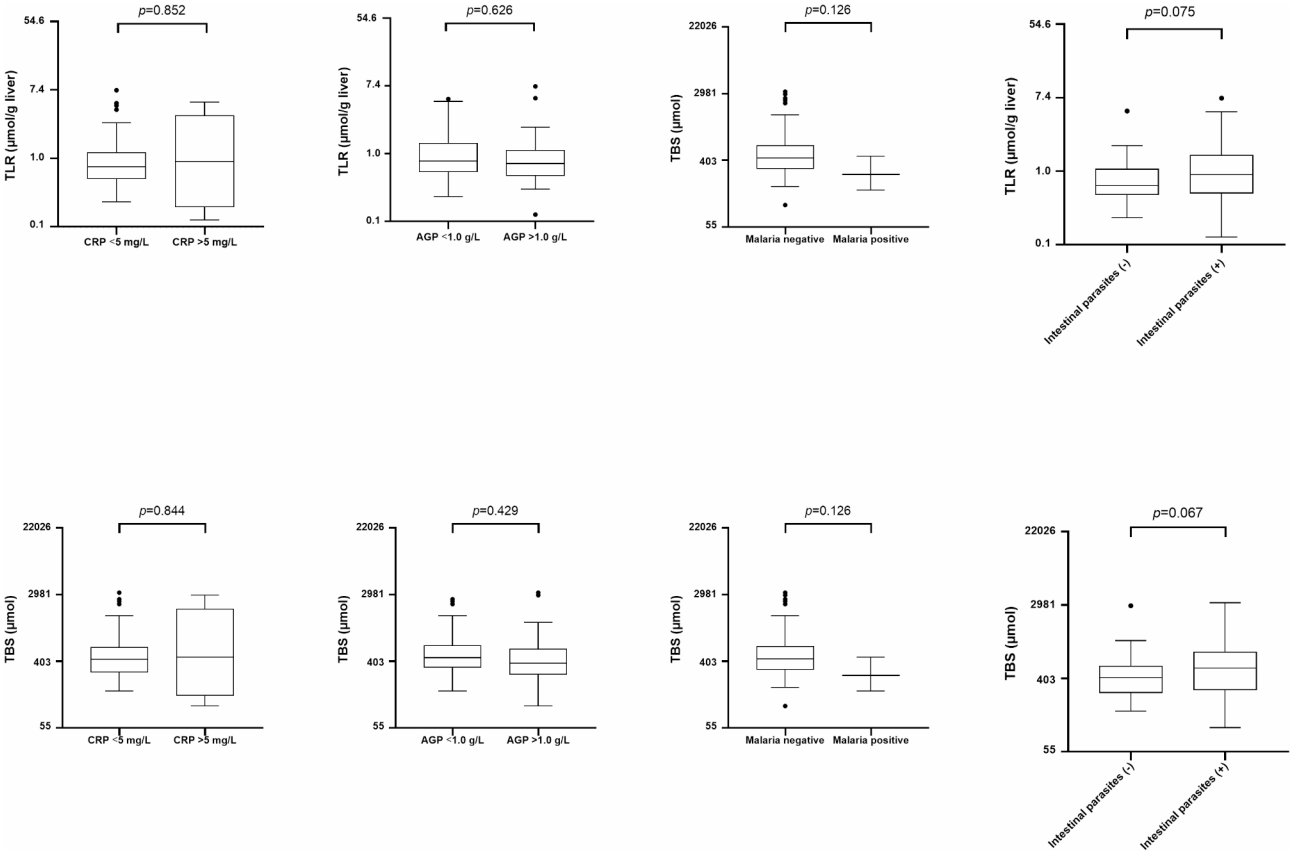

Fig. 2 Distribution of total body vitamin A stores (TBS) and vitamin A total liver reserves (TLR) by season, inflammation status and infections

Secondly, previous studies used serum retinol to assess VA status, which is considered a poor indicator, particularly in the presence of infections and inflammations [47], despite adjusting for inflammation [45]. Finally, this difference could be the results of national interventions to prevent VAD. Concomitantly, national campaigns of VA supplementation and seasonal malaria prevention took place, respectively, 5 and 3 months before the present study implementation. Other countries are also reporting higher prevalence of hypervitaminosis based on TLR. In Zambia, half of the VA supplemented children (5–7 years old) have been diagnosed hypervitaminotic A (>1.0 µmol/g liver) [37]. Around 71.6% of South African children (aged 36–60 months) exposed to VA supplementation and fortification had elevated TLR one month after VA supplementation with 1 dose of 200,000 IU VA [48]. The current study was conducted among a relatively healthy population. Additionally, Burkina Faso has engaged in several national programs for the prevention of VAD including fortification of vegetable oil with VA, promotion of VA-rich food consumption and bi-annual supplementation with high-dose VA [49]. Our results suggest that the current multiple VA interventions should be monitored using reliable methods such as the RID to prevent the risk of VA toxicity.

The present study was conducted in an endemic malaria area where infections and inflammation are prevalent. Unexpectedly, the majority of the children tested negative for malaria parasites, were non-anemic, and had marginal levels of acute inflammation (CRP), regardless of the season. Previously, it has been reported that infections such as malaria and diarrhea were prevalent during the rainy season [50].

Indicators of VA status, TLR and TBS were found to be positively and significantly associated with serum CRP concentrations. RID parameters potentially affected by inflammation include the absorption level of the tracer [51]. In contrast to our findings, Rubin et al. detailed in their review how methods used to assess VA status, including the RID, may be affected by inflammation. Their analysis, based on a study including Zambian children, showed that the distribution curve of calculated VA reserves (micromoles per gram of liver) differed between children with and without elevated CRP levels (> 5 mg/L). Children with elevated CRP had a slightly lower distribution curve of calculated VA reserves [52], which could be attributed to reduced food intake, including vitamin A-rich foods, decreased vitamin A absorption, or increased vitamin A losses [53]. However, these results should be interpreted with cautions for several reasons.

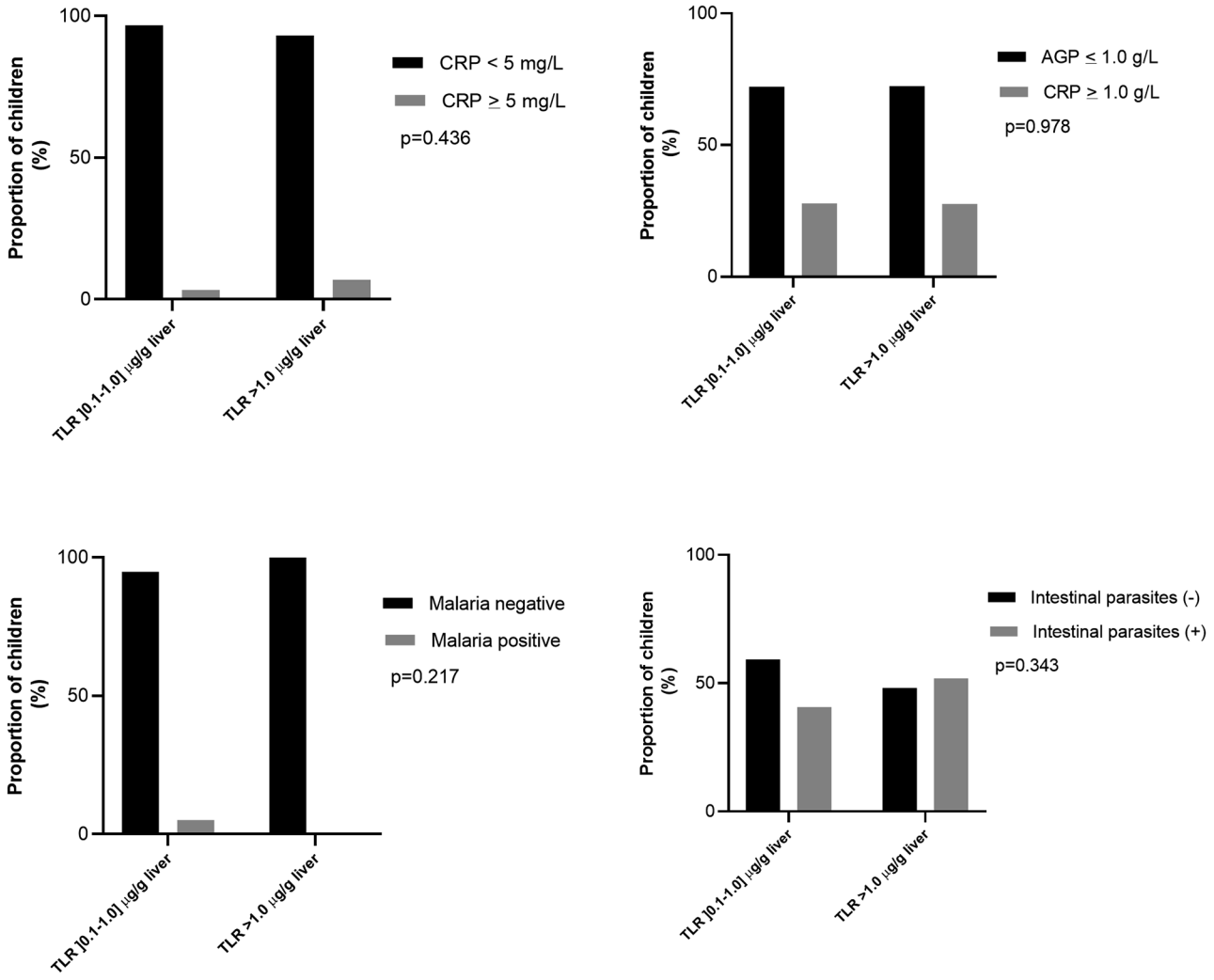

Fig. 3 Distribution of inflammation and infections categories by vitamin A total liver reserves (TLR)

First, the observed associations were weak. Additionally, the sample size for children with high CRP values was limited, with only four participants in this category, only two of whom were included in the analysis. This constraint also limited our ability to examine interaction effects between inflammation and infections on vitamin A status. Second, the study used a cross-sectional design, which limits any interpretation regarding causality. Finally, subsequent analyses revealed no significant differences between TLR and TBS across CRP categories.

Our results showed no relationship between TLR and TBS statues and intestinal parasites. In contrast, a previous study found an association between VA status, assessed by the modified relative dose response test, and *Ascaris lumbricoides* in Indonesian children aged 0.6 to 6 years [54]. This association could be explained by two mechanisms. First, enteric infections can decrease the absorption of nutrients including preformed VA and pro-VA carotenoids [52]. In addition, enteric infections damage the intestinal epithelium and decrease the expression of brush-border enzymes such as lactase, as shown in a piglet model of neonatal diarrhea [52, 55]. Even if the mechanisms of this absorptive defect are unclear, previous studies documented that the impaired absorption contributed to an increased risk of VAD in children with low dietary intakes [11]. Secondly, the presence of intestinal parasites can negatively affect the absorption of $^{13}C_2$-retinyl acetate, which was used in the estimation of TBS and TLR. If dose absorption is negatively impacted that would lead to a false higher dilution of tracer-to-tracee ratio (equation of TBS calculation), and consequently to an overestimation of TBS in children who are infected [38].

A key limitation of this study is the potential lack of statistical power to detect associations between inflammation, infections, and VA status, given that the sample size was primarily designed for seasonal difference rather than secondary analyses. Additionally, the low number

Table 2 Associations between vitamin A total liver reserves (TLR) and total body vitamin A stores (TBS) and inflammation and infections in children 36–59 months of age in rural Burkina Faso

Parameters	Log TLR		Log TBS	
	β	*P*-value	β	*P*-value
Malaria: Positive vs. negative (ref)	-0.076	0.849	-0.098	0.803
CRP (mg/L)	**0.058**	**0.004**	**0.054**	**0.005**
AGP (g/L)	-0.248	0.121	-0.283	0.075
Intestinal parasites: Positive vs. negative (ref)	0.143	0.431	0.163	0.364
Child's age (days)	-0.0003	0.432	0.000	0.952
Child's sex: female vs. male (ref)	-0.049	0.781	-0.077	0.657
Weight-for-height z-score (SD)	-0.152	0.119	-0.074	0.438
Hemoglobin concentration (g/dL)	0.048	0.456	0.062	0.331
Season: rainy vs. dry (ref)	0.629	0.006	0.624	0.006
Mother's formal education (Reference non formal education)				
Primary school	-0.340	0.163	-0.397	0.101
Secondary school	-0.382	0.284	-0.344	0.329
Other	0.195	0.775	0.086	0.899
Father's occupation (reference none)				
Farmer	0.611	0.368	0.653	0.331
Trader	1.230	0.255	1.370	0.201
Other	0.556	0.498	0.587	0.469
Family size	0.037	0.310	0.050	0.164
Household wealth index (reference first quartile)				
Category 1 (second quartile)	0.202	0.369	0.224	0.316
Category 2 (third quartile)	0.411	0.067	0.459	0.040
Category 3 (fourth quartile)	0.173	0.485	0.223	0.364
Dietary diversity score	0.012	0.888	-0.003	0.976
VA rich plant source score	-0.064	0.746	-0.030	0.876
VA rich animal source food score	-0.083	0.720	-0.068	0.766

VA, Vitamin A; CRP, C-reactive protein; AGP, alpha (1)-acid glycoprotein; TLR, vitamin A total liver reserves; TBS, total body vitamin A stores

of children with asymptomatic malaria or acute inflammation (as indicated by CRP levels) may further limit the findings. Nevertheless, an important strength of this study is the use of the RID test to determine VA status. Unfortunately, none of the children in the study had VAD, and a third of them had hypervitaminosis. To address the research question effectively, it appears essential to include children with VAD in the study for the results to be valid. Future research should consider this inclusion to provide a more comprehensive understanding of VA status in the context of prevalent inflammation and infections.

Conclusion

In a VA sufficient study sample where common infections were prevalent, TLR and TBS were positively associated with the acute phase protein CRP. Further research should be conducted to answer the primary question of this study with oversampling of participants with asymptomatic malaria infection and with VAD. Our study revealed high prevalence of hypervitaminosis in this study sample. This finding highlights the necessity for increased caution in monitoring interventions aimed at addressing VAD. Moving forward, it is important to focus on implementing sustainable and cost-effective interventions targeted at preventing VAD while minimizing the risk of adverse effects associated with excess VA intake.

Abbreviations

AGP	Alpha-1-acid glycoprotein
APPs	Acute phase proteins
At %	Atom percentage
CRP	C-reactive protein
CVs	Coefficients of variation
DDS	Dietary diversity score
FAO	Food and Agriculture Organization
GC/C/IRMS	Gas chromatography combustion isotope ratio mass spectrometry
HAZ	Height-for-age z-score
Hb	Hemoglobin
IAEA	International Atomic Energy Agency
IFN-γ	Interferon gamma
IL-12	Interleukin 12
IRSS	Institut de Recherche en Sciences de la Santé
LMICs	Low-and middle-income countries
RBP	Retinol-binding protein
RID	Retinol isotope dilution
SD	Standard deviation
TBS	Total body vitamin A stores
TLR	Total liver reserves
VA	Vitamin A
VAD	Vitamin A deficiency
VIF	Variance inflation factor
WAZ	Weight-for-age z-score
WHO	World Health Organization
WHZ	Weight-for-height z-score

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Author contributions

ANZ, OOS and ST were responsible for the design of the study. OOS, JFB, CD and MG conducted the research. ANZ, JWS, ST, SDH and SA supervised data collection. OOS, AK and SA completed the statistical analyses and OOS drafted the manuscript. SA and JWS contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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conclusions in this report are those of the authors and do not necessarily reflect IAEA positions or policies. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical consideration
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 Institutional Review Boards of the Institut de Recherche en Sciences de la Santé (IRSS, Bobo-Dioulasso) and the Hauts-Bassins Health Department, respectively, under the reference numbers N/Ref A11- 2016/CEIRES and N°2016- EE0202/MS/RHBS/DRS. Before enrolling the child in the study, caregivers provided written informed consents for participation of their child in the study and for collection of biological samples. Children who tested positive for malaria using blood smear were treated with a combination of artemether and lumefantrine over 3 days. Children who were diagnosed with intestinal parasites were treated with 400 mg albendazole per day, for 3 days. Children with moderate or mild anemia were referred to an outpatient facility at the health center of Sourkoudougou.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Centre National de Recherche Scientifique et Technologique, Institut de Recherche en Sciences de la Santé/Direction Régionale de l'Ouest, Unité Nutrition et Maladies Métaboliques, Bobo– Dioulasso, Burkina Faso
[2]Department of Public Health and Primary Care, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium
[3]Centre National de Recherche Scientifique et Technologique, Institut de Recherche en Sciences de la Santé, Unité Nutrition et Maladies Métaboliques, Ouagadougou, Burkina Faso
[4]Department of Translational Physiology, Infectiology and Public Health, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium
[5]African Union Development Agency, New Partnership for Africa's development, Midrand, South Africa
[6]Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, US

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