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Assessing the Role of Inflammation on Vitamin A Biomarkers in School-age children and Adolescents

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Abstract

Assessing the Role of Inflammation on Vitamin A Biomarkers in School-age children and Adolescents

By Chelsea Cole

OBJECTIVES: Recommended biomarkers for population vitamin A status are also negative acute phase proteins, thus may overestimate the prevalence of vitamin A deficiency (VAD) in settings with inflammation. This analysis expands prior research conducted by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) working group to assess the relation between vitamin A biomarkers and inflammation in school-age children (SAC) and adolescents and investigate the use of the BRINDA inflammation adjustment to account for the role of inflammation.

METHODS: We analyzed nutrition surveys of 12,101 SAC (5-15 years) from six datasets and 7,099 adolescents (10-20 years) from ten datasets from the BRINDA project. We estimated the prevalence of inflammation (α-1-acid-glycoprotein [AGP] >1 g/L and C-reactive protein [CRP] > 5 mg/L) and the prevalence of VAD (using serum retinol < 0.7 μmol/L; retinol binding protein <0.7μmol/L), taking complex survey design into consideration. We calculated survey-weighted rank correlations to examine the relation between serum retinol or RBP and AGP or CRP. We used the BRINDA inflammation adjustment method to adjust serum retinol and RBP using AGP only, CRP only, and both AGP and CRP, and compared them with unadjusted values.

RESULTS: The prevalence of elevated AGP or CRP varied by dataset from 10.7% to 33.0% and 2.1% to 23.4%, respectively. The prevalence of unadjusted VAD ranged from 0.6% to 23.3% in SAC and 0.2% to 17.1% in adolescents. Rank correlations between serum retinol or RBP and AGP or CRP were negative and statistically significant ($p < 0.05$) in all six SAC datasets but inconsistent in adolescents. Compared to the unadjusted value, VAD based on serum retinol was 0.2% to 6.2% lower in five SAC datasets and VAD based on RBP was 8.6% lower in one SAC dataset.

CONCLUSIONS: Inflammation adjustment of vitamin A biomarkers may prevent the overestimation of vitamin A deficiency among SAC. However, results were inconsistent among adolescents and further research is needed to inform decision making.

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CHAPTER 1: Introduction

Introduction

Vitamin A deficiency (VAD) is a globally recognized public health concern, affecting an estimated 5.17% of pre-school age children and 9.75% of pregnant women (WHO, 2009). The regions of the world with the highest prevalence of VAD are Southeast Asia (49.9%) and Africa (44.4%), with southeast Asia accounting for about half of the women and children affected globally (WHO, 2009). Vitamin A plays an essential role in normal functioning of the visual and immune system, cell growth and division, gene expression, and red blood cell formation (Tanumihardjo et al., 2016; West, 2003; WHO, 2009). VAD arises from prolonged periods of inadequate intake of vitamin A, in which the body's liver stores are depleted and no longer able to meet physiological needs. Adequate dietary intake is most important during stages in life with high nutritional demand, such as early childhood and pregnancy (West, 2003; WHO, 2009). VAD is a primary cause of preventable childhood blindness and a major contributor to morbidity and mortality from infections, especially among children and pregnant women in low- and middle-income countries (West, 2003; WHO, 2009). In school-age children (SAC) and adolescents, deficiency of prolonged duration or severity may lead to xerophthalmia, impaired iron uptake, nutritional anemia, impaired tissue growth, abnormal metabolism, and weakened resistance to infection (Tanumihardjo et al., 2016; WHO, 2009).

Indicators of vitamin A status, primarily retinol and retinol binding protein (RBP), are also negative acute phase proteins and are therefore impacted by inflammation (Tomkins, 2003; Tanumihardjo et al., 2016; Suchdev & Young, 2022). Retinol and RBP decrease in concentration during the immune response (Merrill et al.; Raiten et al., 2015), which can lead to overestimates

of VAD at a population level and inaccurate targeting of VAD alleviation programs, especially in settings with substantial inflammation (Tomkins, 2003; Tanumihardjo et al., 2016; Suchdev & Young, 2022). Accurate estimates of VAD prevalence provide vital information to countries and global actors, and they inform public health policy and programs related to VAD prevention and eradication. Population level estimates of VAD prevalence for SAC and adolescents are limited, and it is unknown how inflammation impacts vitamin A status in these populations. The purpose of this research is to assess the relationship between vitamin A biomarkers and inflammation in SAC and adolescents and investigate the need to adjust retinol and RBP for inflammation in SAC and adolescents using the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) regression method (Suchdev et al., 2016; Luo and Addo, 2021). This analysis will expand existing knowledge on the association of inflammation on vitamin A biomarkers into new population groups and provide country-specific prevalence estimates for VAD in SAC and adolescents to help shape future programmatic and policy decisions.

Definition of terms:

School-age children (SAC): individuals age 5-15

Adolescents (ADL): individuals age 10-20

Inflammation: elevated alpha-1-glycoprotein (AGP) (>1 g/L) and/or elevated c-reactive protein (CRP) (>5 mg/L)

Vitamin A deficiency (VAD): retinol or retinol binding protein (RBP) concentrations <0.7 μmol/L

CHAPTER 2: Literature review

Introduction

This literature review will start with an overview of vitamin A, including its physiological functions in humans, the negative health consequences of vitamin A deficiency (VAD), and the populations most impacted by VAD. Then, methods for vitamin A assessment and inflammation-adjustment of vitamin A biomarkers will be discussed. Finally, age- and sex-based differences in VAD prevalence estimates and inflammation-adjustment methods will be reviewed.

Vitamin A

Vitamin A plays an essential role in normal functioning of the visual and immune system, cell growth and division, gene expression, and red blood cell formation (West, 2003). Vitamin A deficiency (VAD) is a globally recognized public health concern, affecting an estimated 5.17% of pre-school age children and 9.75% of pregnant women; however, population level estimates of VAD prevalence in SAC and adolescents are limited (WHO, 2009). The regions of the world with the highest prevalence of VAD are Southeast Asia (49.9%) and Africa (44.4%), with Southeast Asia accounting for about half of the women and children affected globally (WHO, 2009). VAD arises from prolonged periods of inadequate intake of vitamin A, which depletes the body's liver stores until it is no longer able to meet physiological needs. Adequate dietary intake is most important during stages in life with high nutritional demand, such as early childhood and

pregnancy (West, 2003). VAD is a primary cause of preventable childhood blindness and a major contributor to morbidity and mortality from infections, especially among children and pregnant women in low- and middle-income countries (West, 2003). In school-age children (SAC) and adolescents, deficiency of prolonged duration or severity may lead to xerophthalmia, impaired iron uptake, nutritional anemia, impaired tissue growth, abnormal metabolism, and weakened resistance to infection (Tanumihardjo et al., 2016; WHO, 2009). Accurate estimates of the prevalence of VAD among all groups are needed to inform policies and programs related to VAD prevention and eradication.

Assessment of Vitamin A status

Although biopsy of liver tissue is considered the gold standard for vitamin A assessment, it is impractical to measure liver reserves in population studies and other methods are used instead. Vitamin A status can also be assessed using biological, functional, histologic, and biochemical methods (Tanumihardjo et al., 2016). Xerophthalmia is a biological indictor of VAD that results in damage to the visual system ranging from reversible Bitot's spots to irreversible blindness due to cornea scarring (Tanumihardjo et al., 2016). Night blindness and dark adaptation are functional indicators of VAD that can be reversible with treatment (Tanumihardjo et al., 2016). Xerophthalmia and night blindness can be used to obtain population estimates of VAD, whereas dark adaptation is typically used to assess interventions (Tanumihardjo et al., 2016). Conjunctival impression cytology is a histological indicator of VAD that evaluates distortions in the surface of the eye; this method is not commonly used due to its

inability to assess deficiency in children and in populations with trachoma (Tanumihardjo et al., 2016).

Serum retinol and retinol binding protein (RBP) are commonly used biochemical indicators of vitamin A status. Deficiency is determined based on a concentration of retinol and/or RBP <0.70 μmol/l (WHO, 1996). Vitamin A circulates in the blood as retinol attached to the carrier protein, retinol binding protein (RBP) (WHO, 1996; Berry & Noy, 2012). Although retinol has been recommended as the main indicator to define vitamin A status in populations, measurement via high performance liquid chromatography (HPLC) can be expensive and require 100μL sample volume, as well as careful processing and storage to protect from degradation by light and heat (Gorstein et al., 2008). RBP has been recommended as an indirect measure of vitamin A status because it correlates strongly with retinol and may be more feasible in low resource settings since RBP can withstand higher exposures to light and heat and is less expensive to analyze than retinol (Almekinder et al., 2000). Both retinol and RBP are also negative acute phase responders and therefore are impacted by inflammation (Tomkins, 2003; Tanumihardjo et al., 2016; Suchdev & Young, 2022). During states of infection and stress, the body produces CRP and AGP to trigger an initial and sustained immune response, respectively. This acute phase response reduces pathogens' access to nutrients like vitamin A in the bloodstream (Merrill et al., 2017). Therefore, plasma retinol and RBP decrease in concentration during this immune response (Merrill et al., 2017; Raiten et al., 2015), which can lead to overestimates of VAD at a population level, especially in settings with substantial inflammation (Suchdev & Young, 2022; Tomkins, 2003; Tanumihardjo et al., 2016). In many settings, malnutrition and infection coexist, creating a bidirectional cycle in which malnutrition increases risk and severity of infection, and infection worsens nutritional status by depressing appetite,

reducing nutrient absorption, and increasing metabolism and excretion (WHO, 2009; Raiten et al., 2015; Suchdev et al., 2017).

Inflammation Adjustment

Prior research by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project has determined appropriate methods for adjusting several nutritional biomarkers for inflammation (Suchdev et al., 2016). In summary, the BRINDA regression adjustment uses linear regression to adjust retinol and RBP by the concentration of CRP and/or AGP on a continuous scale using internal survey-specific data [termed the Internal Regression Correction (IRC)]. The adjusted retinol or RBP equation is calculated by subtracting the influence of CRP and AGP as follows:

$$\text{Ln}(\text{Retinol}_{\text{adjusted}}) = \ln(\text{Retinol}_{\text{unadjusted}}) - \beta 1 \times [\ln(\text{AGP}_{\text{obs}}) - \ln(\text{AGP}_{\text{ref}})] - \beta 2 \times [\ln(\text{CRP}_{\text{obs}}) - \ln(\text{CRP}_{\text{ref}})]$$

$$\text{Ln}(\text{RBP}_{\text{adjusted}}) = \ln(\text{RBP}_{\text{unadjusted}}) - \beta 1 \times [\ln(\text{AGP}_{\text{obs}}) - \ln(\text{AGP}_{\text{ref}})] - \beta 2 \times [\ln(\text{CRP}_{\text{obs}}) - \ln(\text{CRP}_{\text{ref}})]$$

Depending on available data, CRP and/or AGP can be included in the model. β1 is the survey-specific regression coefficient between AGP and retinol or RBP, β2 is the survey-specific regression coefficient between CRP and retinol or RBP, obs is the observed value, and ref is the reference value set to the 10^{th} percentile of CRP or AGP using internal survey data. In contrast to

BRINDA analyses in PSC and WRA, external reference values for AGP and CRP deciles in SAC & ADL were not established because of the limited amount of surveys. For observations in which ln(AGP) exceeds the logarithm of the internal AGP reference value, the difference between these values is adjusted by linear regression. If ln(AGP) is less than or equal to the logarithm of the internal AGP value, the difference is set to zero so that no adjustment is applied to observations with AGP below the threshold. The same logic applies to CRP. Lastly, the results from the equations above are exponentiated to yield the final BRINDA adjusted micronutrient biomarker values.

Adjusting Vitamin A Biomarkers for Inflammation

A study by Stephensen & Gildengorin (2000) using 1988 to 1984 National Health and Nutrition Examination Survey (NHANES) data (n = 22,346) found that retinol concentrations were significantly lower in subjects with CRP concentrations ≥ 10 mg/L and that retinol concentrations increased significantly with age. Among males under the age of 10, the percentage of subjects with retinol concentrations ≤1.05 μmol/L was 23.6% for those with CRP < 10 mg/L (normal) and 66.7% for those with CRP ≥ 10 mg/L (elevated); this decreased to 5.1% and 20.2% between age 10-19 and to 0.73% and 4.2% age 20 and older. Among females under the age of 10, the percentage of subjects with retinol concentrations ≤1.05 μmol/L was 22.7% for those with CRP < 10 mg/L (normal) and 68.8% for those with CRP ≥ 10 mg/L (elevated); this decreased to 5.4% and 25.0% between age 10-19 and to 2.4% and 6.6% age 20 and older. This study used the cutoff of 1.05 μmol/L for marginal VAD, whereas <0.70 μmol/L is the cutoff for subclinical deficiency.

Results from previous BRINDA research have indicated that vitamin A biomarkers should be adjusted for inflammation in PSC but not for WRA (Larson et al., 2017; Larson et al., 2018; Namaste et al., 2020). A study by Larson et al. (2017) aimed to quantify and adjust for the effect of inflammation on VAD prevalence in a nationally representative survey of Liberian children 6 to 35 months of age (n = 1434). Five approaches to adjust RBP for inflammation and estimate VAD prevalence were compared, including 1) ignoring inflammation, 2) excluding individuals with inflammation (AGP > 1 g/L or CRP > 5 mg/L), 3) multiplying each individual's RBP by an internal correction factor, 4) using an external correction factor, and 5) using regression correction. The unadjusted prevalence of VAD was 24.7%, and children with elevated AGP and/or CRP had significantly lower ($p < 0.001$) geometric mean concentrations of RBP (0.79 μmol/L, 95% CI: 0.76, 0.82) than children with non-elevated AGP and/or CRP (0.95 μmol, 95% CI: 0.92, 0.97). When estimating the prevalence of VAD using approaches 2-5, the prevalences were 11.6%, 14.3%, 13.5% and 7.3%, respectively; the prevalence of VAD decreased 10-17 percentage points in comparing inflammation-adjusted to unadjusted models. This study demonstrated that estimates of VAD prevalence based on RBP can be overestimated if RBP is not adjusted for inflammation and excluding individuals with inflammation reduces the sample size and may introduce bias. Using internal or external correction factors categorizes individuals into four groups based on their stage of inflammation; limitations to this approach are that it requires both AGP and CRP, does not account for inflammation at levels outside of these cutoffs, and does not allow for inclusion of confounders or effect modifiers (Raiten et al., 2015; Thurnham, 2015; Young & Suchdev, 2022). The authors state that the regression correction approach may be the most advantageous due to its ability to retain sample size and adjust for

inflammation across the entire range of AGP and CRP values rather than using a cutoff (Larson, 2017).

A multi-study, cross-sectional analysis by Larson et al., (2017) aimed to assess the relationship between RBP concentrations and inflammation and malaria in preschool children (PSC) 6 to 59 months and women of reproductive age (WRA) age 15 to 49 and to investigate adjustment algorithms to account for these effects. Cross-sectional data from 8 PSC surveys (n = 8803) and 4 WRA surveys (n = 4191) from the BRINDA project were used in the analysis. RBP was adjusted for inflammation using several approaches, including 1) excluding individuals with inflammation (AGP > 1 g/L or CRP > 5 mg/L), 2) the application of arithmetic correction factors, and 3) the use of a regression correction approach. The relationship between AGP and CRP deciles and estimated VAD was linear in PSC but not WRA. Compared to unadjusted values, estimated VAD prevalence decreased by a median of 11-18 percentage points in PSC depending on the approach used to adjust for inflammation. Adjusting for malaria had no effect on estimated VAD. The results of this study suggest that the use of regression correction derived from internal data, which accounts for inflammation, is a valid method for adjusting RBP for inflammation in PSC in regions with inflammation. These findings further support that estimates of VAD based on RBP should be adjusted for inflammation in PSC.

A third study conducted by Larson et al. (2018) that included school-age children (SAC) aimed to examine 1) the association between AGP and/or CRP and retinol or RBP and 2) how different inflammation adjustment methods compare with one another. This study was conducted using 20 surveys in PSC age 6 to 59 months (n = 30,343), six surveys in SAC age 5 to 15 years (n = 12,091), and 14 WRA age 15 to 49 years (n = 27,610). Inflammation indices were significantly associated with retinol and RBP in both PSC and SAC but not in WRA. Using the

regression adjustment approach, VAD estimates decreased 22.1 to 6.0 percentage points compared to unadjusted values. This study concluded that VAD in PSC and SAC populations can be overestimated if biomarkers of vitamin A status are not adjusted for inflammation. This study did not explore differences in vitamin A status and the impact of adjusting vitamin A biomarkers for inflammation by sex or in adolescent-specific populations.

A study by Diana et al. (2017) aimed to assess and compare the micronutrient status of a cohort of Indonesian infants (n = 230) at age six, nine, and 12 months using the following methods: 1) ignoring inflammation, 2) arithmetic correction factors with the use of a four-stage inflammation model, and 3) BRINDA regression correction approach. Adjusting for inflammation significantly changed the prevalence estimates of VAD at six, nine, and 12 months ($p<0.001$). Using the regression correction adjustment approach resulted in the greatest change in mean RBP values and VAD prevalence estimates. Mean RBP values increased from 0.91 μmol/L at six months to 0.94 at nine months, to 0.98 at 12 months. This research shows that adjusting vitamin A biomarkers for inflammation is important in young children to avoid overestimating VAD and that vitamin A status improves from age six months to 12 months.

A study by Namaste et al. (2020) aimed to assess the reproducibility of the BRINDA regression approach to adjust iron (ferritin, soluble transferrin receptor (sTfR)) and vitamin A (retinol, RBP) for inflammation (AGP, CRP). This study was conducted using 17 PSC datasets (n = 14,417) and 13 WRA datasets (n = 17,639). A sensitivity analysis was conducted comparing unadjusted and adjusted estimates of iron and vitamin A deficiency using the internal-survey regression approach. This study found that the relationship between inflammation and iron or vitamin A biomarkers was statistically significant except for vitamin A biomarkers in women. Inflammation-adjusted estimates of vitamin A deficiency decreased by a median of 14

percentage points in children compared to unadjusted values. These findings were consistent with previous BRINDA conclusions that biomarkers of vitamin A status should be adjusted for inflammation in PSC but not WRA. This study acknowledged that more research is needed to understand the etiology of heterogeneity in regression coefficients between surveys, but confirmed that the BRINDA regression adjustment approach is a valid method for adjusting vitamin A biomarkers for inflammation across contexts.

The conclusion from these studies is that unadjusted vitamin A biomarkers lead to overestimation of VAD; therefore, measures of vitamin A status (retinol and RBP) should be adjusted for inflammation (AGP and/or CRP) in PSC but not WRA, and that the BRINDA regression adjustment is a valid method for conducting the adjustment. Only one study investigated VAD in SAC and none looked at VAD in adolescents; more research is needed to understand the relationship between vitamin A biomarkers and inflammation in SAC and adolescents and determine if vitamin A biomarkers should be adjusted for inflammation in these populations.

Vitamin A status by age

Vitamin A deficiency affects an estimated 5.17% of pre-school age children and 9.75% of pregnant women (WHO, 2009). Women of reproductive age and children are most vulnerable to developing VAD due to the increased need for vitamin A during pregnancy and as children are growing and developing (West, 2003). Vitamin A is essential during pregnancy for maternal night vision and proper development of the fetal visual system, immune system, skeleton, and organs (Bastos Maia et al., 2019).The recommended daily intake (RDI) of vitamin A increases

from 275 µg for children four to eight years to 420 µg (female) and 445 µg (males) for adolescents age nine to 13 years, to 485 µg (females) and 630 µg (males) for adolescents age 14 to 18. The RDI for adult females and males is 700 µg and 900 µg, respectively (NIH). The RDI for pregnant women is 770 µg and 1,300 µg for breastfeeding women (NIH). However, population estimates of VAD in SAC and adolescents are limited and further research is needed in these populations

A 2016-18 nationally representative survey of SAC and adolescents in India by Reddy et al. (2022) found that serum retinol concentrations were significantly lower in school-age children (1.02 µmol/L, CI 1.01-1.03) than adolescents (1.13 µmol/L, CI: 1.12-1.15) and that the inflammation adjusted prevalence of VAD in SAC and adolescents was 19.3% (CI 1.01-1.03) and 14.4% (CI 13.9-14.9), respectively. Two cross-sectional, nationally representative surveys of Chinese children age six to 13 (n = 14,186) between 2002 and 2012 found that unadjusted retinol was consistently higher in the 10-13 age group than the six to nine age group ($p<0.001$) (Yang et al., 2016). A cross-sectional study of healthy European adolescents (n=1,054) age 12 to 17 found that retinol was significantly higher in older age groups in both males and females ($p < 0.001$) (Breidenassel et al., 2011). The 50th percentile of retinol in males increased from 1.08 µmol/L (310.4 ng/mL) at age 12.5-13.99 to 1.48 µmol/L (423.4 ng/mL) at age 16.0-17.49. The 50th percentile of retinol in females increased from 1.08 µmol/L (309.3 ng/mL) at age 12.5-13.99 to 1.23 µmol/L (351.8 ng/mL) at age 16.0-17.49. However, a prospective and descriptive transverse study of Brazilian SAC (n = 103) age five to 11 found no difference in VAD between ages 5.5 to 7.3 (24.4%), 7.4 to 9.1 (20.0%), and 9.2 to 11 (14.8%) (Custodio et al., 2009).

The Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population (CDC, 2012) found that the geometric mean of retinol was higher as age increased,

from 1.27 µmol/L (36.4 µg/dL) age 6-11 (n = 860), to 1.62 µmol/L (46.5 µg/dL) age 12-19 (n = 1,954), to 1.90 µmol/L (54.3 µg/dL) age 20-39 (n = 1,688), to 2.05 µmol/L (58.7 µg/dL) age 40-59 (n = 1,365), to 2.25µmol/L (64.4 µg/dL) age 60 and older (n = 1,387). Similarly, a global estimate from WHO found that the prevalence of VAD (serum retinol < 0.70 µmol/L) was 33.3% in PSC and only 15.3% in pregnant women (WHO, 2009).

Although it is not known exactly why, there is a consistent finding in the literature that vitamin A status improves with age. One theory about why this may be is due to increased stores of vitamin A in the liver over time, leading to increased circulation of retinol in the blood with increased age (Tanumihardjo et al., 2016). Differences in body fat and metabolism may contribute to differences in vitamin A status between children and adults due to the role vitamin A plays in regulating body fat and metabolism (Cordeiro et al., 2018; Wei et al., 2016). SAC may be more likely to be vitamin A deficient due to an increased need for vitamin A during periods of rapid growth and increased risk of infection at a young age (Zhao et al., 2022). Additionally, sociocultural factors may prohibit infants and young children from consuming animal source foods that are rich in vitamin A (Pachon et al., 2007).

Summary

The conclusion from this body of research is that measures of vitamin A status (retinol and RBP) should be adjusted for inflammation (AGP and/or CRP) to avoid overestimating the prevalence of VAD in PSC but not WRA. While studies have used several inflammation-adjustment methods, the BRINDA regression adjustment is one approach that is advantageous due to its ability to retain sample size and adjust for inflammation across the entire range of AGP

and CRP values rather than using a cutoff. A limited number of initial studies also suggest that vitamin A biomarkers should be adjusted for inflammation in SAC, however, more research is needed in SAC and adolescents to make a comprehensive recommendation on whether to adjust vitamin A biomarkers for inflammation in these populations. Considering inflammation adjustment is currently recommended for PSC but not WRA, more research is needed to further examine the relationship between vitamin A status and inflammation across the lifespan with particular attention to how vitamin A status and inflammation may change in adolescence.

This analysis builds on previous work by 1) assessing the relationship between vitamin A biomarkers and inflammation in SAC and adolescents and 2) investigating the need to adjust retinol and RBP for inflammation in SAC and adolescents using the BRINDA regression method. This analysis will expand existing knowledge on the association of inflammation on vitamin A biomarkers into new population groups and provide survey-specific prevalence estimates for VAD in these populations to help shape future programmatic and policy decisions.

CHAPTER 3: Manuscript

Title: Assessing the Role of Inflammation on Vitamin A Biomarkers in School-age Children and Adolescents: the BRINDA Project

Student contribution to manuscript:
The student conducted all statistical analyses, created all figures, and wrote all parts of this manuscript with assistance from co-authors. Co-authors provided assistance with the analysis, manuscript review, and technical expertise.

Target journal:
American Journal of Clinical Nutrition

Abstract:

OBJECTIVES: Recommended biomarkers for population vitamin A status are also negative acute phase proteins, thus may overestimate the prevalence of vitamin A deficiency (VAD) in settings with inflammation. This analysis expands prior research conducted by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) working group to assess the relation between vitamin A biomarkers and inflammation in school-age children (SAC) and adolescents and investigate the use of the BRINDA inflammation adjustment to account for the role of inflammation.

METHODS: We analyzed nutrition surveys of 12,101 SAC (5-15 years) from six datasets and 7,099 adolescents (10-20 years) from ten datasets from the BRINDA project. We estimated the prevalence of inflammation (α-1-acid-glycoprotein [AGP] >1 g/L and C-reactive protein [CRP] > 5 mg/L) and the prevalence of VAD (using serum retinol < 0.7 µmol/L; retinol binding protein <0.7µmol/L), taking complex survey design into consideration. We calculated survey-weighted rank correlations to examine the relation between serum retinol or RBP and AGP or CRP. We used the BRINDA inflammation adjustment method to adjust serum retinol and RBP using AGP only, CRP only, and both AGP and CRP, and compared them with unadjusted values.

RESULTS: The prevalence of elevated AGP or CRP varied by dataset from 10.7% to 33.0% and 2.1% to 23.4%, respectively. The prevalence of unadjusted VAD ranged from 0.6% to 23.3% in SAC and 0.2% to 17.1% in adolescents. Rank correlations between serum retinol or RBP and AGP or CRP were negative and statistically significant ($p < 0.05$) in all six SAC datasets but inconsistent in adolescents. Compared to the unadjusted value, VAD based on serum retinol was 0.2% to 6.2% lower in five SAC datasets and VAD based on RBP was 8.6% lower in one SAC dataset.

CONCLUSIONS: Inflammation adjustment of vitamin A biomarkers may prevent the overestimation of vitamin A deficiency among SAC. However, results were inconsistent among adolescents and further research is needed to inform decision making.

FUNDING SOURCES: Bill and Melinda Gates Foundation, Centers for Disease Control and Prevention, Eunice Kennedy Shriver National Institute of Child Health and Human Development, HarvestPlus, United States Agency for International Development

KEYWORDS: Vitamin A, Vitamin A deficiency, Retinol, Retinol Binding Protein, Inflammation, School-age children, Adolescents, BRINDA

Introduction

Vitamin A deficiency (VAD) is a globally recognized public health concern, affecting an estimated 5.17% of pre-school age children and 9.75% of pregnant women (WHO, 2009). Vitamin A plays an essential role in normal functioning of the visual and immune system, cell growth and division, gene expression, and red blood cell formation (West, 2003). VAD arises from prolonged periods of inadequate intake of vitamin A, in which the body's liver stores are depleted and no longer able to meet physiological needs. Adequate dietary intake is most important during stages in life with high nutritional demand, such as early childhood and pregnancy (West, 2003). VAD is a primary cause of preventable childhood blindness and a major contributor to morbidity and mortality from infections, especially among children and pregnant women in low- and middle-income countries (West, 2003). In school-age children (SAC) and adolescents, deficiency of prolonged duration or severity may lead to xerophthalmia, impaired iron uptake, nutritional anemia, impaired tissue growth, abnormal metabolism, and weakened resistance to infection (WHO, 2009); however, population level estimates of VAD prevalence for SAC and adolescents are limited.

In many settings, malnutrition and infection coexist, creating a bidirectional cycle in which malnutrition increases risk and severity of infection, and infection worsens nutritional status by depressing appetite, reducing nutrient absorption, and increasing metabolism and excretion (WHO, 2009; Raiten et al., 2015; Suchdev et al., 2017). During states of infection and stress, the body produces CRP and AGP to trigger an initial and sustained immune response, respectively. This acute phase response reduces pathogens' access to nutrients like vitamin A in the bloodstream (Merrill et al., 2017). Therefore, plasma retinol and RBP decrease in

concentration during this immune response (Merrill et al., 2017; Raiten et al. 2015), which can lead to overestimates of VAD at a population level, especially in settings with substantial inflammation (Tomkins, 2003; Tanumihardjo et al., 2016; Young & Suchdev, 2022).

Prior research by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project has determined appropriate methods for adjusting several nutritional biomarkers for inflammation (Suchdev et al., 2016) and developed recommendations to adjust vitamin A biomarkers for inflammation in PSC and SAC but not for WRA (Larson et al., 2017; Larson et al., 2018; Namaste et al., 2020). This analysis builds on previous BRINDA work by 1) assessing the relation between vitamin A biomarkers and inflammation in SAC and adolescents and 2) investigating the need to adjust retinol and RBP for inflammation in SAC and adolescents using the BRINDA regression method. This analysis will expand existing knowledge on the association of inflammation on vitamin A biomarkers into new population groups and provide survey-specific prevalence estimates for VAD in SAC and adolescent populations to help shape future programmatic and policy decisions.

Methods

Study setting and data source

All data used for this analysis came from cross-sectional nutrition surveys that were previously obtained and harmonized by the BRINDA project (www.brinda-nutrition.org). A description of the BRINDA project has been reported previously (Namaste et al., 2017). The BRINDA protocol was reviewed by the Institutional Review Board of Emory University and was

deemed to be non-human-subjects research. To be included in the analysis, datasets had to 1) include SAC (age 5-15 years) or adolescents (10-20 years), 2) measure at least one biomarker of vitamin A (serum retinol or RBP) and at least one biomarker of inflammation (AGP or CRP), and 3) have a sample size greater than 100 (**Supplementary figure 1**). A detailed summary of the surveys used in the analysis is available in **Supplementary table 1**.

Lab methods

Venous or capillary blood was collected from SAC and adolescents. Retinol (serum or plasma) was measured using High Performance Liquid Chromatography (HPLC) or Reversed-Phase HPLC. RBP (serum or plasma) was measured by sandwich enzyme linked immunosorbent assay (ELISA) techniques. AGP and CRP were also analyzed by sandwich ELISA or nephelometry. Details of lab methods for all inflammation and vitamin A biomarker assessments can be found in **Supplementary table 2**. There was limited information on laboratories' quality assurance and cold chain storage procedures.

Statistical analysis

Cutoffs for the biomarkers were consistent with previous BRINDA publications and WHO recommendations according to the following definitions: VAD (retinol or RBP concentrations <0.7 μmol/L), elevated AGP (>1 g/L), and elevated CRP (>5 mg/L) (WHO, 2011; Larson et al., 2017; Thurnham et al. 2010). All analyses were conducted in R version 4.2.1 and included cluster,

strata, and biomarker-specific sampling weights to account for complex survey design effects when applicable.

Median (IQR) was calculated for age, inflammation status, and vitamin A status for each dataset; the prevalence of elevated AGP and CRP were estimated. Rank correlations were calculated to determine the relationship between biomarkers of inflammation (AGP and CRP) and vitamin A. Unweighted internal deciles for inflammation biomarkers were calculated for each dataset, and pooled geometric means of retinol and RBP were calculated for each decile. In addition to pooled estimates, the relationship between inflammation deciles and micronutrients were reviewed for each dataset separately. Using the BRINDA R package (Luo and Addo, 2021), we adjusted retinol and RBP for inflammation by all available combinations of inflammation biomarkers (AGP only, CRP only, and both AGP and CRP) using linear regression when inflammation indices exceeded the first internal decile. Inflammation-adjusted estimates of VAD prevalence were obtained and compared with unadjusted values.

A sensitivity analysis was conducted to investigate the role of sex by comparing the results with a subset of females only as well as to explore any possible influence from menarche by comparing results of SAC females younger than 12 years with SAC females older than 12 years.

Results

After applying this inclusion criteria, six SAC datasets (n = 12,101) and 10 adolescent datasets (n = 7,099) from the BRINDA project were used in the analysis. In SAC, two datasets had both AGP and CRP values and four had CRP values only. In SAC, prevalence of elevated AGP ranged from 15.3% in Bangladesh to 31.8% in Malawi; elevated CRP ranged from 4.2% in

Bangladesh to 15.9% in Malawi (**Table 1**). In adolescents, six datasets had both AGP and CRP values and four had CRP values only. In adolescents, prevalence of elevated AGP ranged from 10.7% in Bangladesh to 33.0% in Papua New Guinea; elevated CRP ranged from 2.1% in Bangladesh to 23.4% in Cote D'Ivoire (Table 1). In SAC, five datasets had retinol values and one dataset had RBP values. Median retinol values ranged from 0.9 in Bangladesh and Ecuador to 1.3 µmol/L in U.S.; the median RBP value was 1.0 in Malawi (**Table 2)**. In adolescents, five datasets had retinol values and five datasets had RBP values. Median retinol values ranged from 0.9 µmol/L in Bangladesh to 1.5 µmol/L in U.S. & Vietnam; median RBP values ranged from 1.1 in Malawi to 1.6 µmol/L in Papua New Guinea (Table 2).

Rank correlations between inflammation and vitamin A biomarkers for each dataset in SAC ranged from -0.32 to -0.10 and were all statistically significant (**Table 3**). Results were inconsistent in the direction and degree of significance of associations in adolescents; seven were negative and significant (-0.24 to -0.12), one was positive and significant (0.13), and eight were null (-0.16 to 0.12) (Table 3).

In pooled analyses shown in **Figure 1,** there was a clear negative trend between vitamin A and inflammation indices in SAC, especially after the eighth decile of AGP and CRP; the overall trend for adolescents was unclear. This was also the case when looking at the trend between vitamin A and inflammation indices by dataset; all SAC datasets had a negative trend between vitamin A and inflammation indices, while the trend for adolescents was mixed with some positive and some negative (**Supplemental Figure 2 & 3**).

Few SAC and adolescent datasets measured both AGP and CRP in addition to retinol or RBP, which limited the number of datasets available to compare VAD prevalence estimates from several inflammation-adjusted methods to unadjusted VAD estimates. In SAC, unadjusted VAD

prevalence ranged from 0.59% in the U.S. to 23.31% in Bangladesh based on retinol and was 12.88% in Malawi based on RBP (**Figure 2**). In adolescents, unadjusted VAD prevalence ranged from 0.18% in U.S. to 17.14% in Bangladesh based on retinol and from 0.00% in Azerbaijan, Cote D'Ivoire, and Papua New Guinea to 3.87% in Malawi based on RBP (**Figure 3).** Comparison of inflammation-adjustment methods for vitamin A was limited to one retinol dataset for SAC and adolescents (Bangladesh 2012), one RBP dataset for SAC (Malawi 2016), and five RBP datasets for adolescents that measured both AGP and CRP. In SAC, all inflammation-adjustment methods reduced the prevalence of VAD (<1 to 8.58 percentage points) compared to unadjusted models. When estimating VAD based on retinol in Bangladeshi SAC, adjusting for only AGP resulted in the greatest reduction in estimated VAD (6.22 percentage points), followed by adjusting for both AGP and CRP (4.24 percentage points) and then CRP only (4.03 percentage points). Estimates for VAD based on RBP in Malawian SAC had the greatest reduction when adjusting for both AGP and CRP (8.58 percentage points), followed by CRP only (8.06 percentage points), and then AGP only (6.47 percentage points).

In contrast to SAC, inflammation-adjustment had inconsistent effects on prevalence of VAD in ADL. Based on retinol, estimates of inflammation-adjusted VAD were lower in four datasets (0.01 to 1.2 percentage points), showed no change in two datasets, and increased in one dataset (0.47 percentage points). Based on RBP, estimates of inflammation-adjusted VAD decreased in five datasets (0.42 to 2.94 percentage points) and showed no change in one dataset. In Cote D' Ivoire (2007) and Papua New Guinea (2005), the VAD prevalence was <1% and models failed to converge. Three datasets had both AGP and CRP values; in Liberia (2011), adjusting for CRP only resulted in the greatest reduction in estimated VAD (0.73 percentage points), followed by AGP and CRP (0.42 percentage points), and AGP only (no change). In Malawi (2016),

adjusting for both AGP and CRP resulted in the greatest reduction in estimated VAD (2.94 percentage points), followed by AGP only (2.57 percentage points), and CRP only (2.53 percentage points). In Azerbaijan, there was no change in estimated VAD when adjusting for AGP only, CRP only, and both AGP and CRP.

Prevalence of elevated AGP and / or CRP and median retinol or RBP values for females only (n = 10,932) were similar to the original analysis with females and males included (**Supplementary table 3 & 4**). There was still a negative trend between vitamin A and inflammation indices when males were not included (**Supplementary figure 4).** The percentage point reduction in VAD prevalence from inflammation adjustment methods was similar in female only models (range 0 to 7.79) to both sexes (range 0 to 8.58) (**Supplementary figures 5 & 6**).

Prevalence of elevated AGP and / or CRP and median retinol or RBP values for females < 12 (n = 4,908) and > 12 (n = 1,090) were similar to the original analysis between SAC and adolescents (**Supplementary table 5 & 6**). The negative trend between vitamin A and inflammation indices in female SAC < 12 was similar to the trend in the original SAC analysis and the trend in female SAC > 12 was similar to the adolescent trend in the original analysis (**Supplementary figure 7**). The percentage point reduction in VAD prevalence from inflammation adjustment methods was similar in female only models (range 0 to 13.3) to both sexes (range 0 to 8.58) (**Supplementary figures 8 & 9**).

Discussion

Our study found significant correlations between inflammation and vitamin A indicators as well as reductions in prevalence estimates of VAD in SAC but not adolescents. In all six SAC

datasets, retinol and RBP concentrations were significantly negatively correlated with both AGP and CRP, and adjusting for inflammation reduced VAD prevalence estimates by 0.2 to 8.58 (median 4.13) percentage points. In adolescents, the relationship between vitamin A and inflammation biomarkers were inconsistent in direction, and thus inflammation-adjusted VAD prevalence estimates did not consistently reduce VAD prevalence estimates.

Our finding that the association of vitamin A and inflammation biomarkers in SAC but not adolescents is consistent with previous studies in older children and other age groups. Prior BRINDA studies in preschool-age children (PSC) and SAC have shown correlation between inflammation and vitamin A biomarkers and reduced prevalence estimates of inflammation-adjusted VAD (Larson, 2017; Larson, 2018; Namaste 2020), which is similar to our findings in SAC. Larson (2018) utilized a similar SAC dataset, but adolescents were not included in the analysis and no sensitivity analyses exploring differences by sex or age were conducted. Reductions in inflammation-adjusted VAD prevalence estimates were larger in PSC (median 13.1 to 16.4 percentage points) than what was observed for SAC in Larson's (2018) study (median 6.9 to 8.8 percentage points) and the present analysis of SAC (3.9 to 8.1 percentage points). Prior studies in WRA did not show significant correlation between vitamin A and inflammation or consistent reductions in prevalence estimates of inflammation-adjusted VAD (Larson, 2018; Namaste 2020), which was similar to our findings in adolescents.

In PSC, adjusting for both AGP and CRP consistently resulted in the greatest reduction in estimated VAD prevalence; however, this trend was inconsistent among SAC and adolescents. In the one SAC retinol dataset (Bangladesh) that converged with both AGP and CRP, adjusting for AGP only resulted in the greatest reduction in VAD prevalence, followed by both AGP and CRP and then CRP only. In the one SAC RBP dataset (Malawi), adjusting for both AGP and CRP

resulted in the greatest reduction in VAD prevalence, followed by CRP only and then AGP only. In the one adolescent retinol dataset (Bangladesh), adjusting for CRP only resulted in the greatest reduction in VAD prevalence, adjusting by AGP only resulted in no change, and adjusting for both AGP and CRP resulted in a slight increase in estimated VAD prevalence. There were three adolescent RBP datasets; in Azerbaijan there was no change in estimated VAD prevalence when adjusting for both AGP and CRP, AGP only, or CRP only. In Liberia, adjusting for CRP only resulted in the greatest reduction in VAD prevalence, followed by both AGP and CRP; there was no change in estimated VAD when adjusting for AGP only. In Malawi, adjusting for both AGP and CRP resulted in the greatest reduction in VAD prevalence, followed by AGP only and then CRP only. Because inflammation is theorized to only result in transient changes in plasma vitamin A, this would most directly support a CRP adjustment model, as CRP levels rise more rapidly than AGP; however, country-level prevalence estimates of elevated AGP and CRP and VAD may impact which adjustment method results in the largest reduction in VAD (Louw et al., 1992). Although the prevalence of inflammation in adolescents was higher than SAC, differences in VAD prevalence estimates from various inflammation-adjustment methods were difficult to detect given the overall low prevalence of VAD among adolescents. The greater prevalence of VAD among SAC gave us more power to detect a difference using different adjustment methods.

In the present study, VAD prevalence was higher in SAC than adolescents. This is consistent with prior literature documenting that vitamin A stores tends to increase with age (Zhao et al., 2022; Reddy et al., 2022; Burke et al., 2018; Yang et al., 2016; CDC, 2012; Breidenassel et al., 2011; Custodio et al., 2009; WHO, 2009). Although it is unclear why we see these underlying associations between vitamin A and inflammation in PSC and SAC, but not adolescents or WRA, there are several factors that could be contributing to the difference. One theory about why this

may be is due to increased stores of vitamin A in the liver over time, leading to adequate concentrations of plasma retinol in older children and adults (Tanumihardjo et al., 2016). Differences in body fat and metabolism may contribute to differences in vitamin A status between children and adults due to the role vitamin A plays in regulating body fat and metabolism (Cordeiro et al., 2018; Wei et al., 2016). SAC have an increased need for vitamin A during periods of rapid growth and may have an increased risk of infection at a young age (Zhao et al., 2022). Additionally, sociocultural factors may prohibit infants and young children from consuming animal source foods that are rich in vitamin A (Pachon et al., 2007). Since vitamin A supplementation is currently recommended for children age six months to five years (WHO, 2009), young SAC may have recently aged out of receiving vitamin A supplements and may not yet be consuming enough vitamin A in their diets; thus, they are left in a coverage gap and vulnerable to developing VAD. It is possible that older SAC and adolescents may have adequate vitamin A intake from consuming a greater amount and variety of vitamin A-rich foods than PSC. Additionally, SAC and adolescents with similar dietary intakes to adults may benefit from consumption of vitamin A-fortified foods. Thus, fortification of foods and coverage of vitamin A programs are important factors of consideration in interpreting differences in VAD prevalence between countries (UNICEF, 2023).

WHO guidelines are used to determine the severity of VAD within a country (WHO, 2011). Unadjusted prevalence of low serum retinol (<0.70 μmol) between two and nine percent is considered a mild public health problem, 10 to 19% is considered a moderate public health problem, and 20% or more is considered a severe public health problem. For SAC in our study, the public health significance of VAD dropped from severe to moderate in Bangladesh, from moderate to mild in Malawi, and from mild to no public health problem in Mexico after adjusting for inflammation. However, these WHO categories are based on unadjusted VAD, and it is unclear

how inflammation-adjusted values should be used with these cutoffs. These cutoffs and the corresponding degree of public health significance by population group help countries determine the type and scale of vitamin A programs that are implemented to reach a specific target population suffering from VAD.

This analysis builds on previous BRINDA work by investigating the need to adjust vitamin A biomarkers for inflammation in SAC and adolescents, which had not been investigated in adolescent populations before (Larson et al., 2017; Larson et al., 2018; Namaste et al., 2020). This is a multi-country analysis that utilized a diverse set of data from high-, middle-, and low-income countries, making the results generalizable to a broader population of SAC and adolescents than single country analyses. The results of this analysis can be used by other researchers when determining when to adjust vitamin A biomarkers for inflammation in SAC and adolescent populations. Adjusting vitamin A biomarkers for inflammation will provide more accurate VAD prevalence estimates that can then be used to inform programmatic and policy decisions.

The conclusions for this analysis were limited by the number of available datasets with measures of vitamin A biomarkers and both inflammation indices. Low prevalence of VAD (RBP <0.7) in three adolescent datasets further reduced the number of countries in which inflammation-adjustment methods could be conducted. Accordingly, the conclusions for this analysis were based on only six SAC datasets and eight adolescent datasets. The available data was insufficient to draw definitive conclusions from comparing approaches of inflammation adjustment (AGP, CRP, or both) in both SAC and adolescents. Other factors that may impact inflammation and/or vitamin A status, such as coverage of vitamin A programs, malaria, and recent illness, were not examined due to limited data availability; additional data would allow for a more robust analysis of inflammation-adjusted prevalence estimates. The lack of a vitamin A biomarker that is not

influenced by inflammation and is feasible to collect at the population level limits our ability to compare the inflammation-adjusted values from the BRINDA regression to a gold-standard. Additionally, different lab methods used to analyze vitamin A biomarkers could lead to systematic differences in values obtained.

It has been noted that CRP values outside the limit of detection (LOD) may impact the inflammation adjustment of iron biomarkers, and this paper did not conduct a sensitivity analysis to determine if this is also true for vitamin A biomarkers (Gosdsin et al., 2022). Future work should explore the impact of CRP values outside the LOD on inflammation-adjusted vitamin A biomarkers. SAC (5-15 years) and adolescents (10-20) are age groups defined by WHO; however, these age groups overlap with each other, and the adolescent age range also overlaps with the age range for WRA (15-49). The current study conducted a sensitivity analysis looking at results from females younger than 12 to females older than 12 and found no meaningful difference from the original analysis using the predefined age groups, but future analyses in adolescent populations could explore different analysis approaches utilizing smaller age categories to further examine age trends within the adolescent age range.

In many settings, inflammation and malnutrition coexist and create a bidirectional malnutrition-infection cycle in which undernutrition increases risk and severity of inflammation and infection, and inflammation worsens nutritional status by decreasing food intake and reducing micronutrient absorption (Raiten et al., 2015; Suchdev et al., 2017). Adjusting vitamin A biomarkers for inflammation improves the accuracy of prevalence estimates, which provide vital information to countries and global actors and inform public health policy and programs related to VAD prevention and eradication in vulnerable populations. Ignoring inflammation may result in an overestimation of VAD prevalence and inaccurate targeting of VAD alleviation programs. Our

findings indicate that biomarkers of vitamin A status should be adjusted for inflammation in SAC. No recommendation for adjustment can be made for adolescents at this time. There is a clear need to measure biomarkers of inflammation (AGP and CRP) when assessing vitamin A status, especially among SAC, to further examine different adjustment methods and close the current data gap. Additionally, more research is needed in adolescent populations to better understand how the relationship between vitamin A status and inflammation changes during this time period between childhood and adulthood before a recommendation can be made concerning the need for inflammation adjustment of vitamin A biomarkers in adolescents.

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Tables and Figures

Table 1: Age and inflammation status in school-age children and adolescents: BRINDA project*

	Age		AGP, g/L			CRP, mg/L		
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**Elevated AGP, %(95% CI)**	**n**	**Median (IQR)**	**Elevated CRP, %(95% CI)**
School-age children								
Bangladesh(2012)	1273	9(7, 11)	1272	0.7(0.6, 0.9)	15.3(12.2, 18.7)	1272	0.3(0.3, 0.7)	4.2(2.3, 6.9)
Ecuador(2012)	3281	8(6, 9)	_	_	_	3281	1.9(1.9, 2.2)	7.4(5.7, 9.4)
Malawi(2016)	758	9(7, 12)	758	0.8(0.6, 1.1)	31.8(27.2, 36.5)	758	0.8(0.3, 2.2)	15.9(12.2, 20.2)
Mexico(2012)	3144	9(7, 10)	_	_	_	3144	0.5(0.2, 1.5)	7.7(6.4, 9.0)
UK(2014)	556	10(7, 12)	_	_	_	556	1.4(0.8, 2.0)	4.6(2.6, 7.3)
US(2006)	3089	11(8, 13)	_	_	_	3089	0.3(0.1, 1.1)	6.5(5.2, 8.0)
Adolescents								
Azerbaijan(2013)	363	17(16, 19)	363	0.8(0.7, 0.9)	20.5(16.0, 25.6)	363	0.4(0.2, 1.1)	5.1(2.9, 8.1)
Bangladesh(2012)	802	12(11, 14)	801	0.7(0.6, 0.8)	10.7(7.9, 14.1)	801	0.3(0.3, 0.7)	2.1(1.1, 3.6)
Cote D'Ivoire(2007)	110	17(16, 18)	110	0.8(0.7, 1.0)	26.5(19.6, 34.1)	110	1.7(0.5, 4.9)	23.4(15.9, 32.2)
Liberia(2011)	378	18(17, 19)	378	0.8(0.6, 0.9)	11.9(8.4, 16.2)	378	0.7(0.4, 2.2)	12.9(9.4, 17.2)
Malawi(2016)	514	14(12, 16)	514	0.7(0.5, 0.9)	17.4(13.8, 21.5)	514	0.6(0.2, 1.9)	10.4(7.5, 13.9)
Mexico(2012)	937	11(10, 12)	_	_	_	937	0.5(0.2, 1.7)	8.3(6.2, 10.9)
Papua New Guinea(2005)	133	17(16, 18)	133	0.9(0.7, 1.1)	33.0(24.0, 42.9)	133	0.6(0.1, 1.5)	8.8(3.6, 17.1)
UK(2014)	521	14(12, 16)	_	_	_	521	1.5(0.8, 2.1)	5.9(3.7, 8.9)
US(2006)	3156	14(12, 16)	_	_	_	3156	0.4(0.1, 1.4)	9.0(7.7, 10.4)
Vietnam(2010)	185	17(16, 18)	_	_	_	185	0.3(0.2, 0.7)	4.9(2.4, 8.6)

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; IQR, Interquartile Range; Elevated AGP defined as AGP >1 g/L; Elevated CRP defined as CRP >5 mg/L.

Table 2: Serum retinol and RBP in school-age children and adolescents: BRINDA project*

	Serum retinol, µmol/L		RBP, µmol/L	
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**
School-age Children				
Bangladesh(2012)	1273	0.9(0.7, 1.0)	-	-
Ecuador(2012)	3281	0.9(0.8, 1.1)	-	-
Malawi(2016)	-	-	758	1.0(0.8, 1.2)
Mexico(2012)	3144	1.2(1.0, 1.3)	-	-
UK(2014)	556	1.2(1.0, 1.4)	-	-
US(2006)	3089	1.3(1.2, 1.6)	-	-
Adolescents				
Azerbaijan(2013)	-	-	363	1.4(1.2, 1.6)
Bangladesh(2012)	802	0.9(0.8, 1.1)	-	-
Cote D'Ivoire(2007)	-	-	110	1.4(1.2, 1.7)
Liberia(2011)	-	-	378	1.2(1.0, 1.5)
Malawi(2016)	-	-	514	1.1(0.9, 1.4)
Mexico(2012)	937	1.2(1.1, 1.4)	-	-
Papua New Guinea(2005)	-	-	133	1.6(1.2, 1.9)
UK(2014)	521	1.3(1.1, 1.6)	-	-
US(2006)	3156	1.5(1.3, 1.7)	-	-
Vietnam(2010)	185	1.5(1.3, 1.7)	-	-

* BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; IQR, Interquartile Range; RBP, Retinol Binding Protein.

Table 3: Rank correlation coefficients between serum retinol, RBP, AGP, and CRP concentrations in school-age children and adolescents: BRINDA project*

	Retinol				RBP			
	AGP * retinol		CRP * retinol		AGP * RBP		CRP * RBP	
Dataset(year)	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**
School-age Children								
Bangladesh(2012)	1272	-0.13*	1272	-0.18*	-	-	-	-
Ecuador(2012)	-	-	3281	-0.27*	-	-	-	-
Malawi(2016)	-	-	-	-	758	-0.29*	758	-0.32*
Mexico(2012)	-	-	3144	-0.12*	-	-	-	-
UK(2014)	-	-	556	-0.16*	-	-	-	-
US(2006)	-	-	3089	-0.10*	-	-	-	-
Adolescents								
Azerbaijan(2013)	-	-	-	-	363	0.13*	363	0.12
Bangladesh(2012)	801	-0.07	801	-0.15*	-	-	-	-
Cote D'Ivoire(2007)	-	-	-	-	110	-0.22*	110	-0.24*
Liberia(2011)	-	-	-	-	378	0.02	378	-0.12*
Malawi(2016)	-	-	-	-	514	-0.17*	514	-0.24*
Mexico(2012)	-	-	937	-0.14*	-	-	-	-
Papua New Guinea(2005)	-	-	-	-	133	0.10	133	-0.16
UK(2014)	-	-	521	-0.03	-	-	-	-
US(2006)	-	-	3156	0.01	-	-	-	-
Vietnam(2010)	-	-	185	-0.03	-	-	-	-

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; RBP, Retinol Binding Protein.

Figure 1: Geometric means of retinol and RBP and inflammation deciles in school-age children and adolescents: BRINDA project*

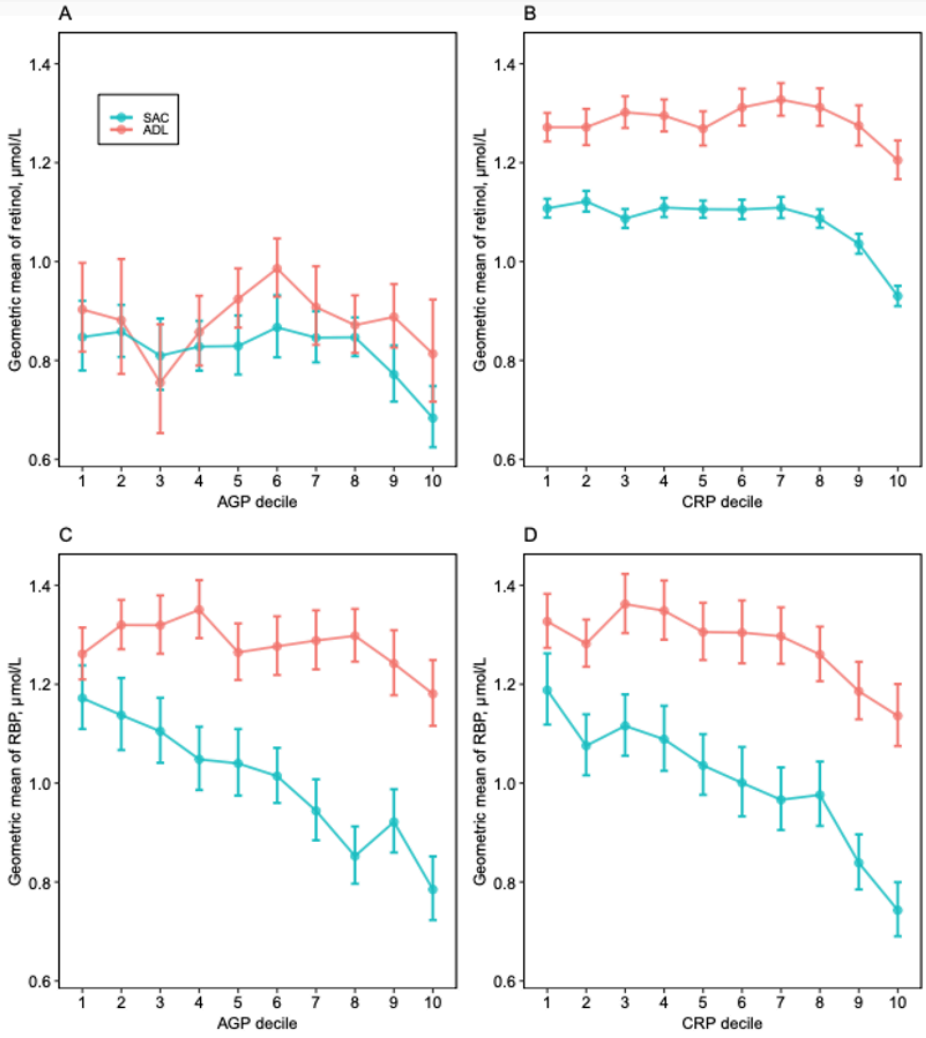

Figure 1: Geometric means of serum retinol by (A) AGP deciles and (B) CRP deciles in School-age Children and Adolescents; geometric means of RBP by (C) AGP deciles and (D) CRP deciles in School-age Children and Adolescents: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; RBP, Retinol Binding Protein; Error bars represent 95% confidence interval.

Figure 2: Estimated prevalence of vitamin A deficiency using serum retinol in school-age children and adolescents: BRINDA project*

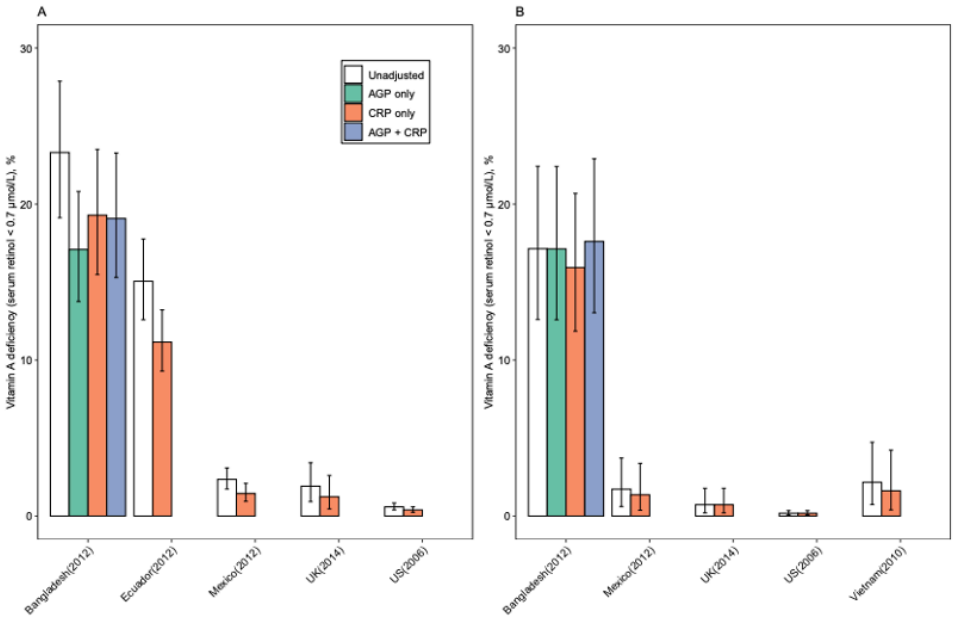

Figure 2: Estimated prevalence of Vitamin A deficiency using serum retinol < 0.7 µmol/L in A) School-age Children and B) Adolescents with the use of different BRINDA adjustment approaches: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.

Figure 3: Estimated prevalence of vitamin A deficiency using RBP in school-age children and adolescents: BRINDA project*

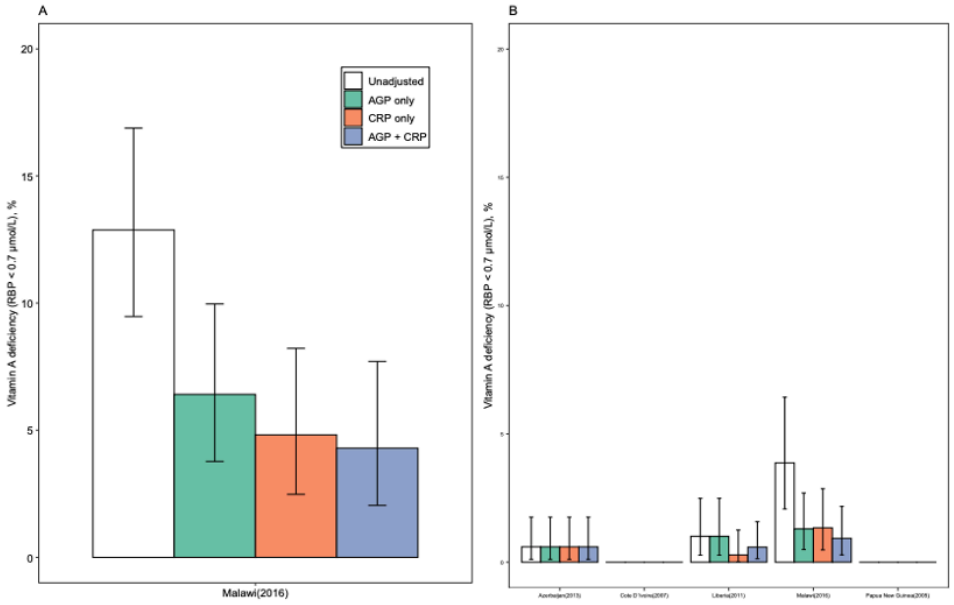

Figure 3: Estimated prevalence of Vitamin A deficiency using RBP < 0.7 µmol/L in A) School-age Children and B) Adolescents with the use of different BRINDA adjustment approaches: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; RBP, Retinol Binding Protein.

CHAPTER 4: Discussion

Our study found significant correlations between inflammation and vitamin A indices as well as reductions in prevalence estimates of VAD in SAC but not adolescents. In all six SAC datasets, retinol and RBP concentrations were significantly negatively correlated with both AGP and CRP and adjusting for inflammation reduced VAD prevalence estimates by 0.2 to 8.58 (median 4.13) percentage points. In adolescents, the relationship between vitamin A and inflammation biomarkers were inconsistent in direction, and thus did not consistently reduce VAD prevalence estimates.

These findings are consistent with findings from previous studies in older children and other age groups. Prior BRINDA studies in PSC and SAC have shown correlation between

inflammation and vitamin A biomarkers and inflammation adjustment led to reduced prevalence estimates of VAD (Larson, 2017; Larson, 2018; Namaste 2020), which is similar to our findings in SAC. Larson (2018) utilized a similar SAC dataset, but adolescents were not included in the analysis and no sensitivity analyses exploring differences by sex or age were conducted. Reductions in the VAD prevalence estimates were larger in PSC (range 6 to 22.1 percentage points) than what was observed for SAC in Larson's (2018) study (range 0.20 to 8.80 percentage points) and this study (range 0.20 to 8.58 percentage points). Prior studies in WRA did not show significant correlation between vitamin A and inflammation or consistent reductions in prevalence estimates of inflammation-adjusted VAD (Larson, 2018; Namaste 2020), which was similar to our findings in adolescents. In PSC, adjusting for both AGP and CRP consistently resulted in the largest reduction in estimated VAD prevalence; however, this trend was inconsistent among SAC and adolescents. Among the six datasets with both AGP and CRP, adjusting for both AGP and CRP resulted in the largest reduction in VAD prevalence in two datasets, adjusting for AGP only resulted in the largest reduction in VAD prevalence in one dataset, adjusting for CRP only resulted in the largest reduction in VAD prevalence in two datasets, and there was one dataset with no change when adjusting for inflammation. The overall prevalence of VAD was low among adolescents, making it difficult to detect differences in VAD prevalence using different adjustment methods. The greater prevalence of VAD among SAC gave us more power to detect a difference using different adjustment methods.

In the present study, VAD prevalence was higher in SAC and inflammation was higher in adolescents. This is consistent with prior literature documenting that vitamin A status tends to increase with age (Zhao et al., 2022; Reddy et al., 2022; Burke et al., 2018; Yang et al., 2016; CDC, 2012; Breidenassel et al., 2011; Custodio et al., 2009; WHO, 2009). Although it is unclear

why we see these underlying associations between vitamin A and inflammation in PSC and SAC, but not adolescents or WRA, there are several factors that could be contributing to the difference. One theory about why this may be is due to increased stores of vitamin A in the liver over time, leading to increased circulation of retinol in the blood with increased age (Tanumihardjo et al., 2016). Differences in body fat and metabolism may contribute to differences in vitamin A status between children and adults due to the role vitamin A plays in regulating body fat and metabolism (Cordeiro et al., 2018; Wei et al., 2016). Furthermore, the source of inflammation may differ between children and adults (infection vs. obesity), contributing to differences in the vitamin A inflammation relationship between children and women (Larson et al., 2017; Larson et al., 2018; Namaste et al., 2020). Although the RDI increases with age, it is possible that adolescents are consuming more food overall and therefore sufficient quantities of Vitamin A. SAC have an increased need for vitamin A during periods of rapid growth and may have an increased risk of infection at a young age (Zhao et al., 2022). Vitamin A supplementation is currently recommended for children age six months to five years (WHO, 2009); SAC have recently aged out of receiving vitamin A supplements and may not yet be consuming enough vitamin A fortified foods to benefit; thus, they are left in a coverage gap and vulnerable to developing VAD. Fortification of foods with vitamin A and/or coverage of vitamin A programs may account for differences between countries (UNICEF, 2023).

Study comparison table

Study	Population	Vitamin A biomarker(s)	Correlation coefficients	Prevalence difference for inflammation-adjusted value (median percentage points)	Recommended Inflammation Adjustment
Larson 2017	PSC	RBP	RBP/AGP: -0.18 to -0.32 RBP/CRP: -0.26 to -0.37	Reduced 17.8 percentage points	Adjust for inflammation
	WRA	RBP	RBP/AGP: close to 0 RBP/CRP: -0.02 to -0.13	Not conducted	Adjust for inflammation
Larson 2018	PSC	RBP	RBP/AGP: -0.45 to 0.64 RBP/CRP: -0.48 to 0.09	Reduced 16.4 percentage points	Adjust for inflammation
		Retinol	Retinol/AGP: -0.31 to -0.01 Retinol/CRP: -0.37 to -0.17	Reduced 13.1 percentage points	
	SAC	RBP	RBP/AGP: -0.37 RBP/CRP: -0.39	Reduced 8.8 percentage points	Adjust for inflammation
		Retinol	Retinol/AGP: -0.14 Retinol/CRP: -0.23 to -0.09	Reduced 6.9 percentage points	
	WRA	RBP	RBP/AGP: -0.05 to 0.69 RBP/CRP: -0.17 to 0.22	Not conducted	Don't adjust
		Retinol	Retinol/AGP: -0.16 to 0.05 Retinol/CRP: -0.08 to 0.11		
Namaste 2020	PSC	RBP/retinol	RBP or retinol / AGP: -0.32 to 0.48 RBP or retinol / CRP: -0.37 to 0.08	Reduced by median of 13.5 percentage points	Adjust for inflammation
	WRA		RBP or retinol / AGP: -0.07 to 0.50	Not conducted	Don't adjust

			RBP or retinol / CRP: -0.17 to 0.12		
Current study	SAC	RBP	RBP/AGP: -0.29 RBP/CRP: -0.32	Reduced by 4.13 percentage points	Adjust for inflammation
		Retinol	Retinol/AGP: -0.13 Retinol/CRP: -0.27 to -0.10		
	Adolescents	RBP	RBP/AGP: -0.22 to 0.10 RBP/CRP: -0.24 to 0.12	Reduced by 0.628 percentage points	Don't adjust
		Retinol	Retinol/AGP: -0.07 Retinol/CRP: -0.15 to 0.01		

WHO Guidelines are used to determine the severity of VAD within a country (WHO, 2011). Unadjusted prevalence of low serum retinol (<0.70 μmol) between two and nine percent is considered a mild public health problem, 10 to 19% is considered a moderate public health problem, and 20% or more is considered a severe public health problem. In our study, the public health significance of VAD in SAC dropped from severe to moderate in Bangladesh, from moderate to mild in Malawi, and from mild to no public health problem in Mexico after adjusting for inflammation. However, these WHO categories are based on unadjusted VAD, and it is unclear how inflammation-adjusted values should be used with these cutoffs. These cutoffs and the corresponding degree of public health significance by population group help countries determine the type and scale of vitamin A programs that are implemented to reach a specific target population suffering from VAD.

Even after adjusting for inflammation, VAD prevalence remained high in some countries; there are several methods commonly used to reduce VAD prevalence within a country. Vitamin A

supplementation (VAS) targeting at children six to 59 months has been successful in reducing VAD in children in some countries, however low coverage of VAS in other countries remains a challenge (Imad et al., 2017). Agriculture based interventions that aim to increase the availability and consumption of vitamin A rich foods, such as orange flesh sweet potatoes and maize, have be implemented; however, the acceptance and popularity of these foods remains low in some areas (Low et al., 2017). Food fortification is another strategy used to increase vitamin A intake that also has had limited success due to the popularity and acceptance of the foods being fortified, such as corn (Siwela et al., 2020). VAD remains high among populations with low socioeconomic status and education; policies that aim to lift individuals and communities out of poverty and increase access to high quality education are also needed to reduce the prevalence of VAD globally (Zhao, 2022).

Key Findings:

- Prevalence of elevated AGP or CRP was higher among adolescents (median 10.55) than SAC (7.55)
- VAD prevalence was higher among SAC (median 5.62) than adolescents (median 1.32)
- There was a consistent negative relationship between vitamin A biomarkers and inflammation in SAC; the trend was inconsistent in adolescents
- Adjusting for inflammation reduced estimated VAD prevalence in SAC (median 4.1 percentage points)
- Inflammation adjustment had inconsistent effects on the prevalence of VAD in adolescents

This study has several strengths. This analysis builds on previous BRINDA work by investigating the need to adjust vitamin A biomarkers for inflammation in SAC and adolescents, which had not been investigated in adolescent populations before (Larson et al., 2017; Larson et al., 2018; Namaste et al., 2020). This is a multi-country analysis that utilized a diverse set of data from high-, middle-, and low-income countries, making the results generalizable to a broader

population of SAC and adolescents than single country analyses. The results of this analysis can be used by other researchers when determining when to adjust vitamin A biomarkers for inflammation in SAC and adolescent populations. Adjusting vitamin A biomarkers for inflammation will provide more accurate VAD prevalence estimates that can then be used to inform programmatic and policy decisions.

The conclusions for this analysis were limited by the number of available datasets with measures of vitamin A biomarkers and both inflammation indices. Low prevalence of VAD (RBP <0.7) in three adolescent datasets further reduced the number of countries in which adjustment could be conducted. Accordingly, the conclusions for this analysis were based on only six SAC datasets and eight adolescent datasets. The available data was insufficient to draw definitive conclusions from comparing approaches of inflammation adjustment (AGP, CRP, or both) in both SAC and adolescents. Other factors that may impact inflammation and/or vitamin A status, such as coverage of vitamin A programs, malaria, and recent illness, were not examined due to limited data availability; additional data would allow for a more robust analysis. The lack of a vitamin A biomarker that is not influenced by inflammation and is feasible to collect at the population level limits our ability to compare the inflammation-adjusted values from the BRINDA regression to a gold-standard. Additionally, different lab methods used to analyze vitamin A biomarkers could lead to systematic differences in values obtained.

It has been noted that CRP values outside the limit of detection (LOD) may impact the inflammation adjustment of iron biomarkers, and this paper did not conduct a sensitivity analysis to determine if this is also true for vitamin A biomarkers (Gosdsin et al., 2022). Future work should explore the impact of CRP values outside the LOD on inflammation adjustment of vitamin A biomarkers. SAC (5-15 years) and adolescents (10-20) are age groups defined by WHO; however,

these age groups overlap and the adolescent age range overlaps with the age range for WRA (15-49). The current study conducted a sensitivity analysis looking at results from females younger than 12 to females older than 12 and found no meaningful difference from the original analysis using the predefined age groups, but future analyses in adolescent populations could explore different analysis approaches utilizing smaller age categories to further examine age trends within the adolescent age range. For example, an overall analysis could be conducted with all individuals age 5-19 and additional analyses could be conducted in smaller age groups (5-9, 10-14, 15-19) with tests for interactions by age group. Additionally, future analyses including more datasets that have at least one vitamin A biomarker and both AGP and CRP values in SAC and adolescent populations would allow us to further elucidate the relationship between vitamin A and inflammation in SAC and adolescents, obtain VAD prevalence estimates for SAC and adolescents in more countries, and better compare the approaches to inflammation adjustment. Longitudinal studies that collect measures of inflammation and vitamin A status from early childhood through age 40 would be beneficial to understand how inflammation and vitamin A status change with age.

Key Research Gaps & Recommendations for Next Steps

- More data in SAC and adolescent populations with measures of vitamin A and inflammation status is needed
- Future analyses should be conducted using smaller age categories and exploring interactions by age group
- Future research could focus on investigating how and why vitamin A status tends to increase with age

In many settings, inflammation and malnutrition coexist and create a bidirectional malnutrition-infection cycle in which undernutrition increases risk and severity of inflammation and infection, and inflammation worsens nutritional status by decreasing food intake and reducing micronutrient absorption (Raiten et al., 2015; Suchdev et al., 2017). Adjusting vitamin A

biomarkers for inflammation improves the accuracy of prevalence estimates, which is of particular importance in vulnerable populations. Accurate estimates of VAD prevalence provide vital information to countries and global actors and inform public health policy and programs related to VAD prevention and eradication. Ignoring inflammation may result in an overestimation of VAD prevalence, and inaccurate targeting of VAD alleviation programs. Our findings indicate that biomarkers of vitamin A status should be adjusted for inflammation in SAC. No recommendation for adjustment can be made for adolescents at this time. There is a clear need to measure biomarkers of inflammation (AGP and CRP) when assessing vitamin A status, especially among SAC, to further examine different adjustment methods and close the current data gap. Additionally, more research is needed in adolescent populations to better understand how the relationship between vitamin A status and inflammation changes during this time period between childhood and adulthood and be able to make a recommendation of whether to adjust vitamin A biomarkers for inflammation in this population or not.

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Appendices

Supplementary table 1. List of datasets used in the analysis

Country (year)	Survey name	Retinol	RBP	Data source
Azerbaijan (2013)	Azerbaijan Nutrition Survey (AzNS)		ADL	UNICEF; Ministry of Health of the Republic of Azerbaijan. Azerbaijan Nutrition Survey (AzNS); 2013.
Bangladesh (2012)	National Micronutrient Survey 2012	SAC, ADL		Institute of Public Health Nutrition (Bangladesh); UNICEF; iicddr,b; GAIN. National Micronutrient Survey 2011-12, Final Report. Dhaka, Bangladesh; 2012.
Cote D'Ivoire (2007)	Cote D'Ivoire multiple indicator cluster survey 2006		ADL	Rohner, F., Northrop-Clewes, C., Tschannen, A. B., Bosso, P. E., Kouassi-Gohou, V., Erhardt, J. G., Bui, M., Zimmermann, M. B., & Mascie-Taylor, C. N. (2014). Prevalence and public health relevance of micronutrient deficiencies and undernutrition in pre-school children and women of reproductive age in Côte d'Ivoire, West Africa. *Public Health Nutrition*, *17*(9), 2016–2028.
Ecuador (2012)	Ecuador's National Health and Nutrition Survey	SAC		Freire, W. B.; Belmont, P.; Lopez-Cevallos, D. F.; Waters, W. F. Ecuador's National Health and Nutrition Survey: Objectives, Design, and Methods. Annals of Epidemiology 2015, 25 (11), 877–878.
UK (2014)	National Diet and Nutrition Survey Results	SAC, ADL		Public Health England; Food Standards Agency. National Diet and Nutrition Survey Results from Years 5 and 6 (Combined) of the Rolling Programme (2012/2013 - 2013/2014); 2016.
Liberia (2011)	Liberia National Micronutrient Survey 2011		ADL	UNICEF; Liberia Institute for Statistics and Geo-information Services (LISGIS); Government of Liberia. Liberia National Micronutrient Survey 2011; 2011.
Malawi (2016)	Malawi Demographic and Health Survey 2015-2016		SAC, ADL	National Statistics Office (NSO); ICF. Malawi Demographic and Health Survey 2015-2016; 2016.
Mexico (2012)	Encuesta Nacional de Salud y Nutrición 2012. Resultados Nacionales	SAC, ADL		Gutiérrez, J. P.; Rivera-Dommarco, J.; Shamah-Levy, T.; Villalpando-Hernández, S.; Franco, A.; Cuevas-Nasu, L.; Romero-Martínez, M.; Hernández-Ávila, M. Encuesta Nacional de Salud y Nutrición 2012. Resultados Nacionales [National Health and Nutrition Survey 2012. National Results]; 2012.
Papua New Guinea (2005)	Papua New Guinea National Nutrition Survey 2005		ADL	Department of Health Papua New Guinea; UNICEF; University of Papua New Guinea; CDC. Papua New Guinea National Nutrition Survey 2005; 2005.
US (2006)	National Health and Nutrition Examination Survey 2003-2006	SAC, ADL		CDC. Second National Report on Biochemical Indicators of Diet and Nutrition in the US Population; 2012.
Vietnam (2010)	2010 micronutrient status survey	ADL		Laillou, A.; Van Pham, T.; Tran, N. T.; Le, H. T.; Wieringa, F.; Rohner, F.; Fortin, S.; Bach Le, M.; Tran, D. T.; Moench-Pfanner, R.; Berger, J. Micronutrient Deficits Are Still Public Health Issues among Women and Young Children in Vietnam. PLoS ONE 2012, 7 (4).

Supplementary table 2. Lab methods for retinol and RBP by dataset

	Biomarker			
Dataset	AGP	CRP	Retinol	RBP
Azerbaijan (2013)	Sandwich ELISA	Sandwich ELISA	-	Sandwich ELISA
Bangladesh (2012)	Sandwich ELISA	Sandwich ELISA	HPLC	-
Cote D'Ivoire (2007)	Sandwich ELISA	Sandwich ELISA	-	Sandwich ELISA
Ecuador (2012)	-	Nephelometry	HPLC	-
Liberia (2011)	Sandwich ELISA	Sandwich ELISA		Sandwich ELISA
Malawi (2016)	Sandwich ELISA	Sandwich ELISA	HPLC	Sandwich ELISA
Mexico (2012)	-	Nephelometry	HPLC	-
Papa New Guinea (2005)	Sandwich ELISA	Sandwich ELISA	-	Sandwich ELISA
UK (2014)	-	PETIA	HPLC	-
US (2006)	-	Nephelometry	Reversed-phase HPLC	-
Vietnam (2010)	-	ELISA	Reversed-phase HPLC	-

ELISA, Enzyme-linked immunosorbent assay; HPLC, High performance liquid chromatography

Supplementary table 3: Age and inflammation status in female school-age children and adolescents: BRINDA project*

	Age		AGP, g/L			CRP, mg/L		
Dataset(year)	n	Median (IQR)	n	Median (IQR)	Elevated AGP, % (95% CI)	n	Median (IQR)	Elevated CRP, % (95% CI)
School-age children								
Bangladesh(2012)	655	9(7, 11)	654	0.7(0.6, 0.9)	17.2(12.8, 22.4)	654	0.3(0.3, 0.9)	5.3(3.0, 8.5)
Ecuador(2012)	1613	8(6, 9)	_	_	_	1613	1.9(1.9, 2.5)	8.4(5.7, 11.7)
UK(2014)	259	9(7, 12)	_	_	_	259	1.4(0.8, 2.1)	4.9(2.2, 9.1)
Malawi(2016)	372	9(7, 12)	372	0.8(0.6, 1.1)	30.1(24.4, 36.1)	372	0.7(0.2, 2.0)	14.0(9.8, 19.0)
Mexico(2012)	1536	9(7, 10)	_	_	_	1536	0.6(0.3, 1.7)	7.1(5.5, 8.9)
US(2006)	1563	11(9, 13)	_	_	_	1563	0.3(0.1, 1.1)	5.8(4.2, 7.6)
Adolescents								
Azerbaijan(2013)	363	17(16, 19)	363	0.8(0.7, 0.9)	20.5(16.0, 25.6)	363	0.4(0.2, 1.1)	5.1(2.9, 8.1)
Bangladesh(2012)	497	13(11, 16)	496	0.7(0.6, 0.9)	12.8(8.5, 18.1)	496	0.3(0.3, 0.8)	1.7(0.7, 3.4)
Cote D'Ivoire(2007)	110	17(16, 18)	110	0.8(0.7, 1.0)	26.5(19.6, 34.1)	110	1.7(0.5, 4.9)	23.4(15.9, 32.2)
UK(2014)	343	15(13, 17)	_	_	_	343	1.5(0.8, 2.0)	6.3(3.6, 10.0)
Liberia(2011)	378	18(17, 19)	378	0.8(0.6, 0.9)	11.9(8.4, 16.2)	378	0.7(0.4, 2.2)	12.9(9.4, 17.2)
Malawi(2016)	330	15(12, 17)	330	0.6(0.5, 0.9)	14.9(10.7, 19.8)	330	0.5(0.2, 1.6)	9.7(6.3, 14.1)
Mexico(2012)	441	11(10, 12)	_	_	_	441	0.5(0.2, 1.6)	7.8(5.0, 11.4)
Papua New Guinea(2005)	133	17(16, 18)	133	0.9(0.7, 1.1)	33.0(24.0, 42.9)	133	0.6(0.1, 1.5)	8.8(3.6, 17.1)
US(2006)	2154	15(13, 17)	_	_	_	2154	0.4(0.1, 1.6)	9.6(8.2, 11.1)
Vietnam(2010)	185	17(16, 18)	_	_	_	185	0.3(0.2, 0.7)	4.9(2.4, 8.6)

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; IQR, Interquartile Range; Elevated AGP defined as AGP >1 g/L; Elevated CRP defined as CRP >5 mg/L.

Supplementary table 4: Serum retinol and RBP in female school-age children and adolescents: BRINDA project*

	Serum retinol, µmol/L		RBP, µmol/L	
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**
School-age Children				
Bangladesh(2012)	655	0.9(0.7, 1.0)	-	-
Ecuador(2012)	1613	0.9(0.8, 1.1)	-	-
Malawi(2016)	-	-	372	1.0(0.8, 1.2)
Mexico(2012)	1536	1.2(1.0, 1.3)	-	-
UK(2014)	259	1.2(1.0, 1.4)	-	-
US(2006)	1563	1.3(1.2, 1.5)	-	-
Adolescents				
Azerbaijan(2013)	-	-	363	1.4(1.2, 1.6)
Bangladesh(2012)	497	0.9(0.8, 1.1)	-	-
Cote D'Ivoire(2007)	-	-	110	1.4(1.2, 1.7)
Liberia(2011)	-	-	378	1.2(1.0, 1.5)
Malawi(2016)	-	-	330	1.2(0.9, 1.5)
Mexico(2012)	441	1.2(1.1, 1.5)	-	-
Papua New Guinea(2005)	-	-	133	1.6(1.2, 1.9)
UK(2014)	343	1.4(1.1, 1.6)	-	-
US(2006)	2154	1.5(1.3, 1.7)	-	-
Vietnam(2010)	185	1.5(1.3, 1.7)	-	-

* BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; IQR, Interquartile Range; RBP, Retinol Binding Protein.

Supplementary table 5: Rank correlation coefficients between serum retinol, RBP, AGP, and CRP concentrations in female school-age children and adolescents: BRINDA project*

	AGP * retinol		CRP * retinol		AGP * RBP		CRP * RBP	
Dataset(year)	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**
School-age Children								
Bangladesh(2012)	1272	-0.13*	1272	-0.18*	-	-	-	-
Ecuador(2012)	-	-	3281	-0.27*	-	-	-	-
Malawi(2016)	-	-	-	-	758	-0.29*	758	-0.32*
Mexico(2012)	-	-	3144	-0.12*	-	-	-	-
UK(2014)	-	-	556	-0.16*	-	-	-	-
US(2006)	-	-	3089	-0.10*	-	-	-	-
Adolescents								
Azerbaijan(2013)	-	-	-	-	363	0.13*	363	0.12
Bangladesh(2012)	801	-0.07	801	-0.15*	-	-	-	-
Cote D'Ivoire(2007)	-	-	-	-	110	-0.22*	110	-0.24*
Liberia(2011)	-	-	-	-	378	0.02	378	-0.12*
Malawi(2016)	-	-	-	-	514	-0.17*	514	-0.24*
Mexico(2012)	-	-	937	-0.14*	-	-	-	-
Papua New Guinea(2005)	-	-	-	-	133	0.10	133	-0.16
UK(2014)	-	-	521	-0.03	-	-	-	-
US(2006)	-	-	3156	0.01	-	-	-	-
Vietnam(2010)	-	-	185	-0.03	-	-	-	-

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.

Supplementary table 6: Age and inflammation status in female school-age children by age group: BRINDA project*

	Age		AGP, g/L			CRP, mg/L		
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**Elevated AGP, % (95% CI)**	**n**	**Median (IQR)**	**Elevated CRP, % (95% CI)**
Age < 12								
Bangladesh(2012)	492	8(7, 10)	492	0.7(0.6, 0.9)	17.7(12.4, 24.0)	492	0.3(0.3, 0.9)	6.2(3.5, 9.9)
Ecuador(2012)	1613	8(6, 9)	_	_	_	1613	1.9(1.9, 2.5)	8.4(5.7, 11.7)
UK(2014)	154	8(6, 9)	_	_	_	154	1.4(1.0, 2.2)	5.2(1.7, 11.4)
Malawi(2016)	282	8(6, 10)	282	0.8(0.6, 1.2)	35.7(29.2, 42.7)	282	0.8(0.2, 2.1)	14.8(10.4, 20.1)
Mexico(2012)	1532	9(7, 10)	_	_	_	1532	0.6(0.3, 1.7)	7.1(5.5, 8.9)
US(2006)	835	9(8, 11)	_	_	_	835	0.4(0.1, 1.2)	5.4(3.4, 7.9)
Age > 12								
Bangladesh(2012)	163	13(12, 13)	162	0.7(0.5, 0.8)	15.2(8.2, 24.8)	162	0.3(0.3, 0.4)	1.5(0.3, 4.3)
UK(2014)	105	13(12, 14)	_	_	_	105	1.4(0.8, 2.0)	4.2(1.2, 10.1)
Malawi(2016)	90	14(13, 14)	90	0.6(0.5, 0.9)	12.7(5.3, 23.9)	90	0.5(0.2, 1.4)	11.5(4.0, 23.9)
Mexico(2012)	4	12(12, 12)	_	_	_	4	1.3(1.3, 1.3)	3.1(NA, 39.1)
US(2006)	728	14(13, 14)	_	_	_	728	0.3(0.1, 0.9)	6.4(4.2, 9.3)

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; IQR, Interquartile Range; Elevated AGP defined as AGP >1 g/L; Elevated CRP defined as CRP >5 mg/L.

Supplementary table 7: Serum retinol and RBP in female school-age children and adolescents: BRINDA project*

	Serum retinol, µmol/L		RBP, µmol/L	
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**
Age < 12				
Bangladesh(2012)	492	0.8(0.7, 1.0)	-	-
Ecuador(2012)	1613	0.9(0.8, 1.1)	-	-
Malawi(2016)	-	-	282	0.9(0.8, 1.1)
Mexico(2012)	1532	1.2(1.0, 1.3)	-	-
UK(2014)	154	1.1(0.9, 1.3)	-	-
US(2006)	835	1.3(1.1, 1.5)	-	-
Age > 12				
Bangladesh(2012)	163	1.0(0.9, 1.2)	-	-
Malawi(2016)	-	-	90	1.1(0.9, 1.3)
Mexico(2012)	4	1.3(1.3, 1.7)	-	-
UK(2014)	105	1.3(1.1, 1.5)	-	-
US(2006)	728	1.5(1.3, 1.6)	-	-

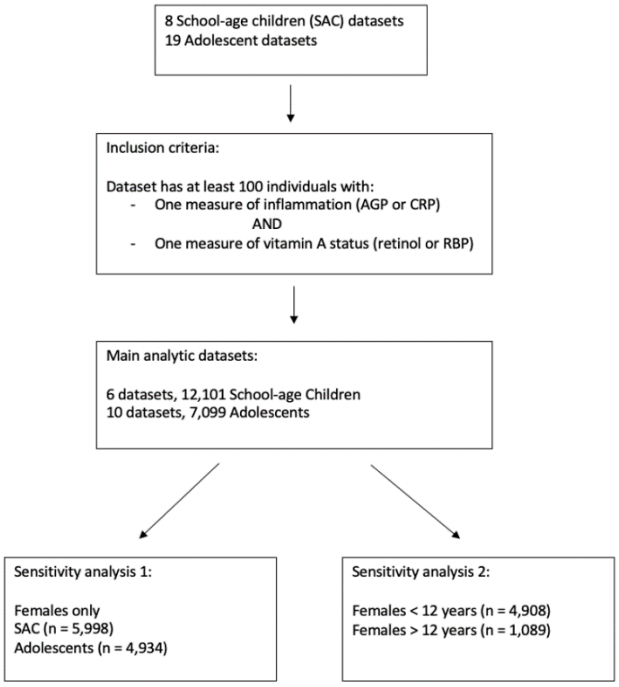
* BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; IQR, Interquartile Range; RBP, Retinol Binding Protein.

Supplementary table 8: Rank correlation coefficients between serum retinol, RBP, AGP, and CRP concentrations in female school-age children and adolescents: BRINDA project*

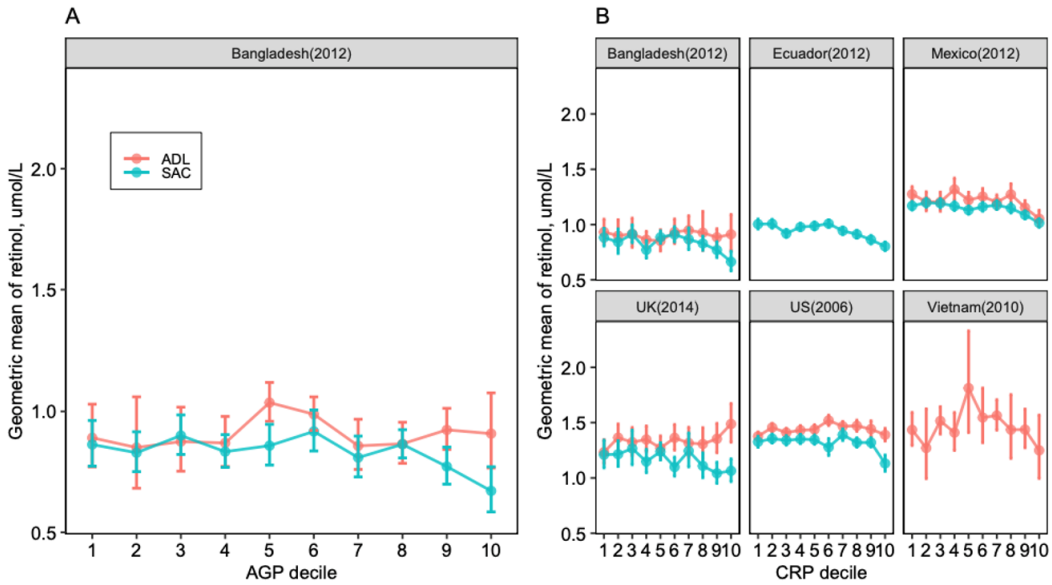
	AGP * retinol		CRP * retinol		AGP * RBP		CRP * RBP	
Dataset(year)	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**
Age < 12								
Bangladesh(2012)	492	-0.17*	492	-0.18	-	-	-	-
Ecuador(2012)	-	-	1613	-0.28*	-	-	-	-
Malawi(2016)	-	-	-	-	282	-0.26*	282	-0.22*
Mexico(2012)	-	-	1532	-0.17*	-	-	-	-
UK(2014)	-	-	154	-0.22*	-	-	-	-
US(2006)	-	-	835	-0.18*	-	-	-	-
Age > 12								
Bangladesh(2012)	162	0.00	162	-0.21	-	-	-	-
Malawi(2016)	-	-	-	-	90	-0.24	90	-0.32*
Mexico(2012)	-	-	4	-0.45	-	-	-	-
UK(2014)	-	-	105	-0.22*	-	-	-	-
US(2006)	-	-	728	-0.05	-	-	-	-

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.

Supplementary figure 1. Dataset inclusion flowchart

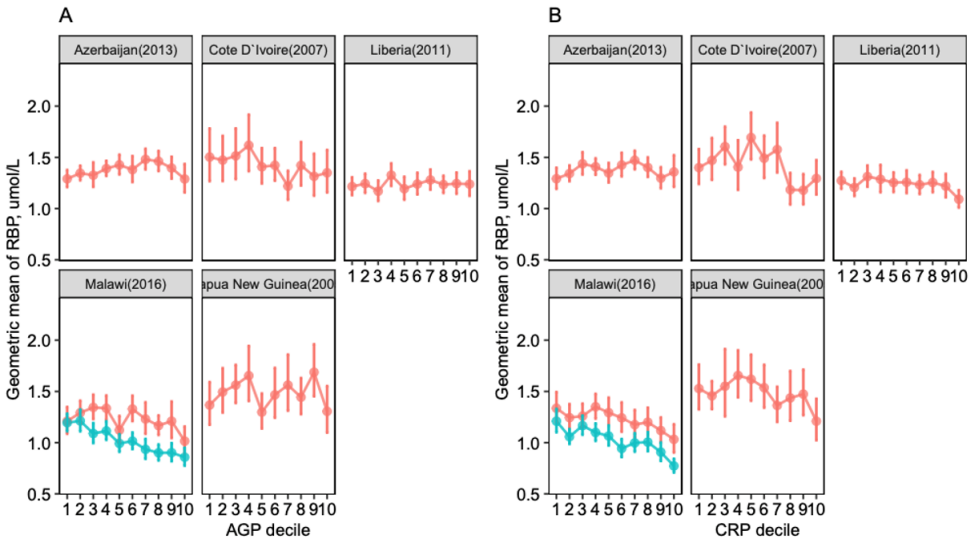


Supplementary figure 2: Geometric means of retinol and inflammation deciles in school-age children and adolescents by dataset: BRINDA project*



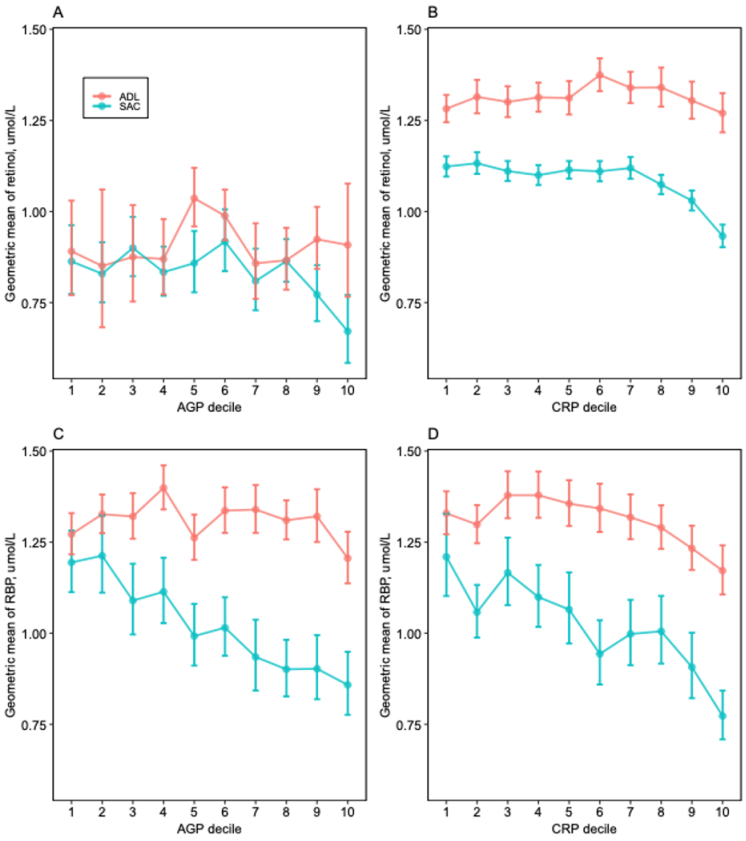
Geometric means of serum retinol in each dataset by (A) AGP deciles and (B) CRP deciles; AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-Reactive Protein.

Supplementary figure 3: Geometric means of RBP and inflammation deciles in school-age children and adolescents by dataset: BRINDA project*



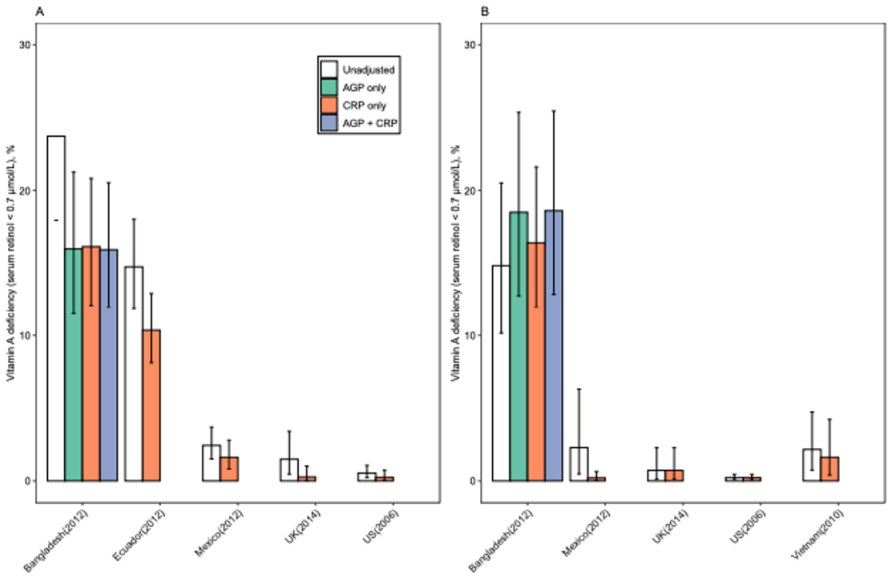
Geometric means of serum retinol in each dataset by (A) AGP deciles and (B) CRP deciles; AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-Reactive Protein; RBP, Retinol Binding Protein.

Supplementary figure 4: Geometric means of retinol and inflammation deciles in female school-age children and adolescents: BRINDA project*



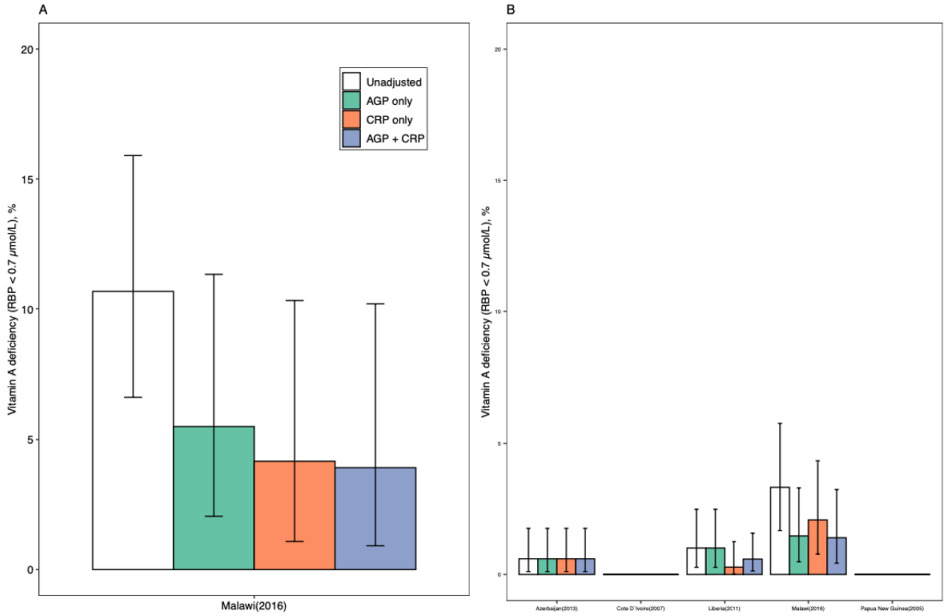
Geometric means of serum retinol by (A) AGP deciles and (B) CRP deciles in female school-age children and adolescents; geometric means of RBP by (C) AGP deciles and (D) CRP deciles in female school-age children and adolescents: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; RBP, Retinol Binding Protein; Error bars represent 95% confidence interval.

Supplementary figure 5: Estimated prevalence of vitamin A deficiency using retinol in female school-age children and adolescents: BRINDA project*



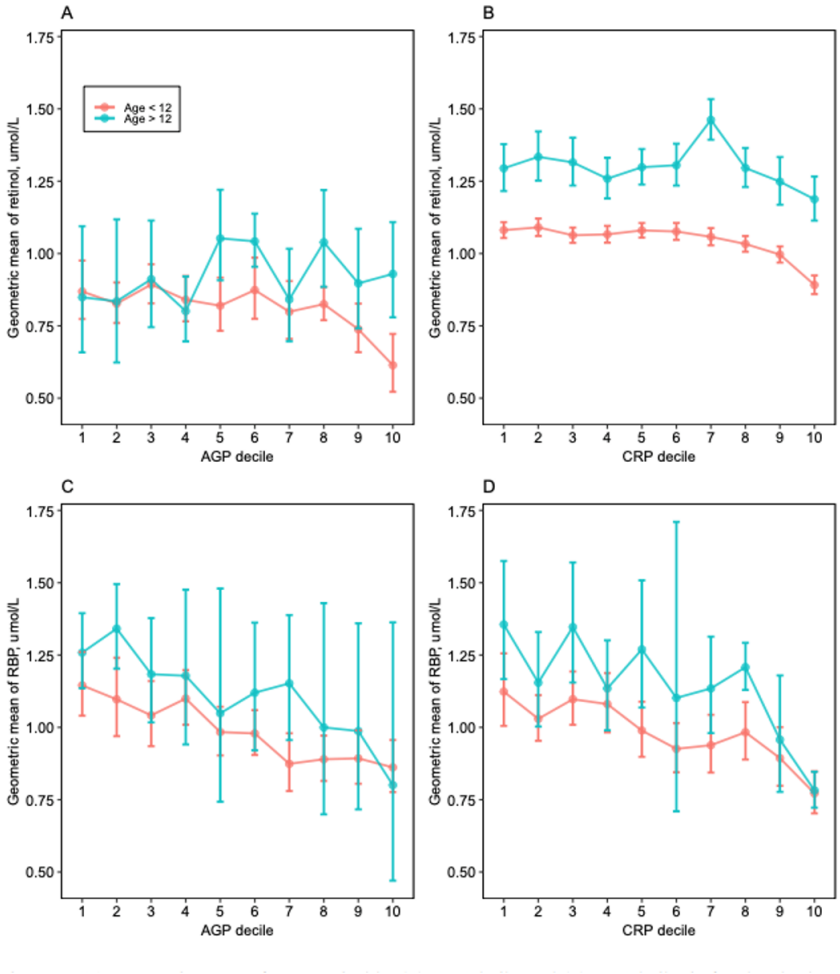
Estimated prevalence of vitamin A deficiency using serum retinol < 0.7 µmol/L in female (A) school-age children and (B) adolescents with the use of different adjustment approaches: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.

Supplementary figure 6: Estimated prevalence of vitamin A deficiency using RBP in female school-age children and adolescents: BRINDA project*



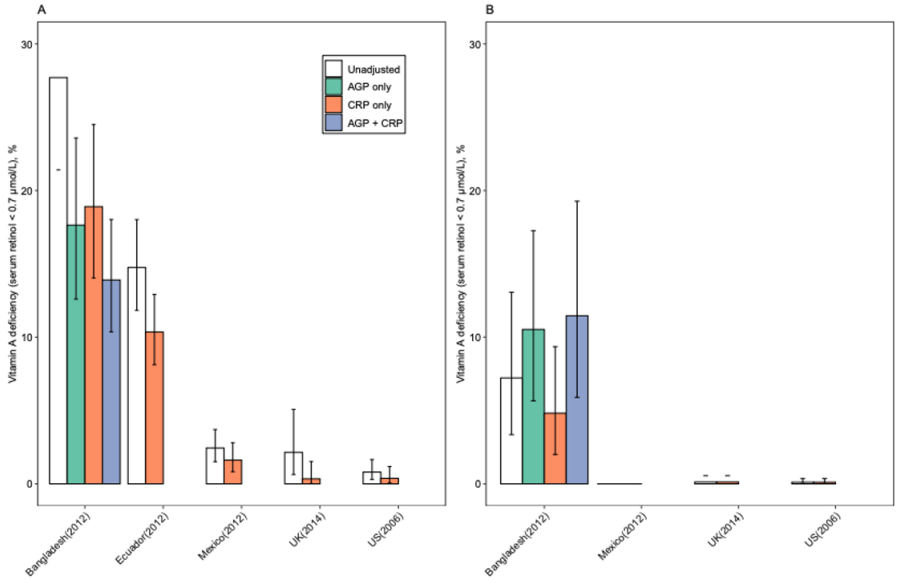
Estimated prevalence of vitamin A deficiency using RBP < 0.7 µmol/L in female (A) school-age children and (B) adolescents with the use of different adjustment approaches: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; RBP, Retinol Binding Protein.

Supplementary figure 7: Geometric means of retinol and inflammation deciles in female school-age children by age group: BRINDA project*



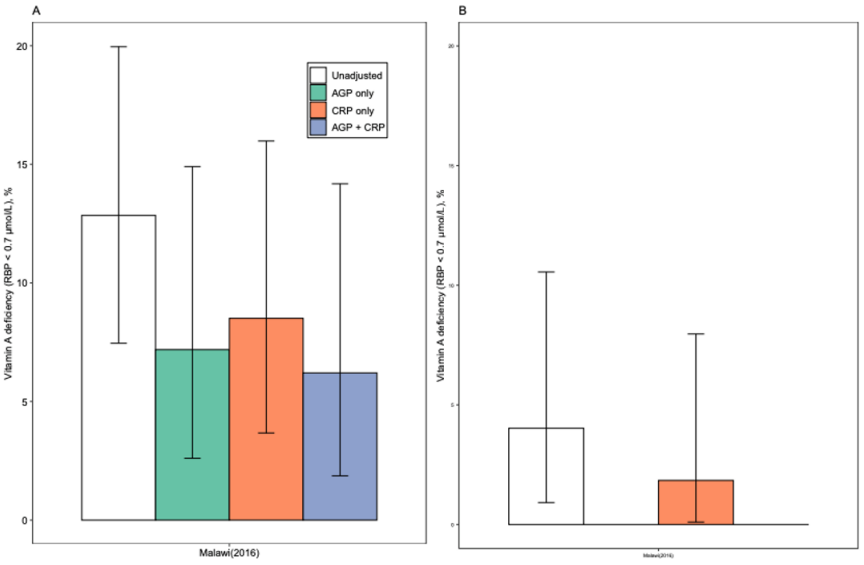
Geometric means of serum retinol by (A) AGP deciles and (B) CRP deciles in female school-age children by age group; geometric means of RBP by (C) AGP deciles and (D) CRP deciles in female school-age children by age group: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; RBP, Retinol Binding Protein; Error bars represent 95% confidence interval.

Supplementary figure 8: Estimated prevalence of vitamin A deficiency using retinol in female school-age children by age group: BRINDA project*



Estimated prevalence of vitamin A deficiency using serum retinol < 0.7 µmol/L in female school-age children (A) age < 12 and (B) Age > 12 with the use of different adjustment approaches: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.

Supplementary figure 9: Estimated prevalence of vitamin A deficiency using retinol in female school-age children by age group: BRINDA project*



Estimated prevalence of vitamin A deficiency using serum RBP < 0.7 µmol/L in female school-age children (A) age < 12 and (B) Age > 12 with the use of different adjustment approaches: BRINDA project. AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.