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Original Research

The Effects of One Egg Per Day on Vitamin A Status Among Young Malawian Children: A Secondary Analysis of a Randomized Controlled Trial

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A B S T R A C T

Background: Vitamin A deficiency (VAD) is common in populations with limited dietary diversity and access to vitamin A-rich foods.
Objectives: This analysis aimed to determine the impact of supplementing children's diets with 1 egg/d on the concentration of plasma retinol and RBP and the prevalence of VAD.
Methods: Children age 6–9 mo living in the Mangochi district of Malawi were individually randomly assigned to receive 1 egg/d for 6 mo (n = 331) or continue their usual diet (n = 329) in the Mazira trial (clinicaltrials.gov; NCT03385252). This secondary analysis measured plasma retinol by HPLC and RBP, CRP, and α-1-acid glycoprotein (AGP) by ELISA techniques at enrollment and 6 mo follow-up. Retinol and RBP were adjusted for inflammation, and mean concentrations were compared between groups using linear regression models. In addition, prevalence ratios of VAD (retinol <0.7 μmol/L) were compared between groups using log-binomial or modified Poisson regression models.
Results: After 6 mo of study participation, 489 were assessed for retinol (egg: n = 238; control: n = 251), and 575 (egg: n = 281; control: n = 294) were assessed for RBP. Prevalence of inflammation (CRP >5 mg/L or AGP >1 g/L: 62%) and inflammation-adjusted VAD (7%) at enrollment did not differ between groups. At follow-up, the egg intervention group did not differ from the control in inflammation-adjusted retinol [geometric mean (95% CI); egg: 1.10 μmol/L (1.07, 1.13); control: 1.08 (1.05, 1.12)], RBP [egg: 0.99 μmol/L (0.96, 1.02); control: 0.97 (0.94, 1.00)], or prevalence of VAD [egg: 6%; control: 3%; prevalence ratio: 1.87 (0.83, 4.24)].
Conclusions: Provision of 1 egg/d did not impact VAD, plasma retinol, or RBP among young children in rural Malawi, where the prevalence of VAD was low. *Curr Dev Nutr* 2023;x:xx.
This trial was registered at [clinicaltrials.gov] as [NCT03385252].

Keywords: eggs, retinol, RBP, vitamin A, vitamin A deficiency (VAD), infant and young child feeding, animal-source foods, Malawi, sub-Saharan Africa

Introduction

Vitamin A is an essential nutrient supporting growth, vision, and immunity that can be obtained from dietary supplements or vitamin A-rich foods like animal products, dark orange fruits and vegetables, leafy greens, or fortified foods [1]. Vitamin A deficiency (VAD) puts young children at higher risk for blindness, measles, and diarrhea [2]. Globally, VAD affects 29% of children under 5 y old [3], and in sub-Saharan Africa, nearly half of the children under 5 y old are estimated to have VAD [3]. The government of Malawi and its partners has implemented several ongoing vitamin A interventions, including high-dose vitamin A supplementation, micronutrient powders for home fortification, mandatory fortification of maize and wheat flour, sugar, and oil,

Abbreviations: AGP, α-1-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; EAR, estimated average requirement; RAE, retinol activity equivalent; VAD, vitamin A deficiency.

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and the promotion of biofortified sweet potato and cassava [4–6]. Among Malawian children under 5 y, national micronutrient surveys have shown that VAD declined from 22% in 2009 to 4% in 2015–2016 [6,7]. This suggests that the combination of multiple vitamin A programs carries the promise of controlling VAD in Malawi.

However, children under 2 y old may be at high risk for VAD because of the high nutrient needs to support rapid growth, low dietary intake of breast milk and vitamin A-rich foods, or ineffective coverage of existing vitamin A programs. Young children have a small gastric capacity and need to consume nutrient-dense foods to meet their nutrient requirements [8,9]. Animal-source foods like milk, small fish, and eggs are highly bioavailable, vitamin A-dense foods but are infrequently consumed by infants [10]. For infants consuming predominantly cereal-based diets, breast milk is often the primary source of vitamin A and may be an important contributor to vitamin A adequacy of Malawian children through 23 mo of age, the median duration of breastfeeding in Malawi [10,11]. However, the concentration of vitamin A in breast milk depends on the vitamin A intake of the mother [12], and after 6 mo of age, breast milk is no longer adequate to meet the child's vitamin A requirements. Among breastfed children in Malawi, 24% of children under 2 y old achieved minimum dietary diversity—a proxy for adequate micronutrient density of the diet—and consumption of vitamin A-rich food groups was reported in the following percentage of 24-h dietary recalls: 5% dairy; 12% eggs; 30% meat, fish, or poultry; and 74% vitamin A-rich fruits or vegetables [10].

Previous food-based interventions, lipid-based nutrient supplements containing vitamin A together with other micronutrients and biofortified maize, have not improved the vitamin A status of Malawian and Zambian children [11,13]. Whereas lipid-based nutrient supplements provide vitamin A as retinyl palmitate and biofortified maize provides provitamin A carotenoids, animal-source foods like eggs contain highly bioavailable, preformed retinol. One 50 g USDA commercial egg contains an estimated 75 μg retinol activity equivalent (RAE) (25% RDA for children 1–3 y old) [14], and one 52 g Malawian commercial egg contains 79 μg RAE (26% RDA for children 1–3 y old) [15]. Thus, supplementing the diets of young children with 1 egg/d may improve their vitamin A status.

This study is a secondary analysis of a 6 mo egg intervention trial conducted in Malawi, which evaluates the impact of providing 1 egg/d to young children on vitamin A status. Previously, the population of children in this study location has been shown to have mild to moderate VAD [11]. Children enrolled in this trial are at risk of VAD from low intake of vitamin A-rich foods [15], low vitamin A density in breast milk from inadequate maternal dietary intake of vitamin A [11], and low coverage of vitamin A supplementation programs [10]. This analysis hypothesized that children receiving the egg intervention would have higher plasma retinol and RBP concentrations and lower prevalence of VAD than children in the control group at the 6 mo follow-up.

Methods

Study design, participants, and randomization

The Mazira Project enrolled young children in the Mangochi district of Malawi between February 2018 and July 2018 (clinicaltrials.gov registry NCT03385252). Children were individually randomly assigned to the intervention group that received 1 egg/d or the control group that continued their usual diet for 6 mo. Age-eligible children were identified through child listings and recruited from villages near the Lungwena and Malindi health care centers. Children were eligible for study participation if they enrolled between the ages of 6–9.9 mo, were of singleton birth, and their family intended to remain in the area for the duration of the study. Children were excluded from the study based on the presence of wasting (mid-upper arm circumference <12.5 cm), severe anemia (hemoglobin ≤ 5 g/dL), bipedal edema, egg allergy, recent hospitalization, or other morbidities that may affect growth and development. Children were referred to local health facilities when they screened positive for malaria, wasting, severe anemia, bipedal edema, or other symptoms warranting immediate medical care.

Caregivers of study participants were informed of the study design, research purpose, measures of assessment, rights to withdraw, and incentives to study participation. They were provided with the opportunity to ask staff members questions in groups as well as individually in a private environment prior to enrollment. Caregivers provided written, informed consent by signature or thumbprint to confirm their participation in the study and allow collected data and samples to be used for future research. All procedures were reviewed and approved by the University of California, Davis institutional review board and the University of Malawi College of Medicine research ethics committee.

The study was designed to enroll 662 children based on the ability to detect a 0.25 SD difference between groups in length-for-age z-score with 2-sided hypothesis testing, $\alpha = 0.05$, $\beta = 0.2$, and 20% attrition. After completing initial assessments at the clinic, participants were individually randomly assigned to either the egg intervention or nonintervention group in a 1:1 allocation ratio within blocks of 10. Caregivers randomly selected 1 opaque, sealed envelope and opened the envelope containing a card with a unique code to reveal their group assignment. Participants' group assignment was masked to staff conducting outcome assessments.

Intervention

A full description of the egg intervention and control group has been published elsewhere [16]. Briefly, households in the egg intervention group received 1 egg/d for 6 mo for the study child and an additional 7 eggs/wk for household sharing. Twice per week, eggs were delivered to households, and caregivers were asked to serve the child the egg during the home visits. If the child had already eaten the egg, then caregivers reported the last time the egg was fed to the enrolled child. Children randomly assigned to the control group continued their usual diet for the study duration. They were visited twice per week to maintain the same schedule of staff visits as the egg group. Caregivers in the control group were compensated with nonperishable goods like wash tubs, buckets, and plastic bins during the study and a basket of eggs, other foods, and kitchen goods at the end of the study. After completing the 6 mo follow-up visit, all participants received fabric, sugar, and soap tablets.

Data and sample collection

Anthropometric measurements, cognitive development, and dietary intake were assessed at enrollment and the 6 mo follow-

up. Recumbent length and weight were measured and converted into *z*-scores using the WHO growth standards [17]. At enrollment, demographic information about the household, parents, and index children was collected, including surveys regarding household assets and food security [18]. Vitamin A supplementation was recorded from the child's health passport or by parental report of attending national child health week campaigns during enrollment, 3 mo, and 6 mo follow-up visits.

Venous whole blood was collected in 5 mL lithium heparin tubes at enrollment and the 6 mo follow-up. At the point of care, hemoglobin was assessed using Hemocue Hb 201 devices (HemoCue Inc.), and the presence of malarial antigens was determined using a rapid diagnostic test kit (SD Bioline Malaria Ag P.f/Pan; Abbott Diagnostics). Blood collection tubes were wrapped in foil, placed on ice, and centrifuged at 1040 x g for 15 min at room temperature. Plasma was placed into multiple aliquots as sample volume allowed, filling foil-wrapped cryovials designated for retinol analysis prior to cryovials used for RBP analysis. All aliquots were stored in a −80°C freezer and shipped on dry ice to the laboratories conducting analyses.

Laboratory analysis

Retinol was measured by HPLC (1260 Infinity II LC; Agilent Technologies) at the University of California, Davis, in pairs of samples collected at enrollment and 6 mo follow-up visits for each child [19]. An internal standard of retinyl acetate (100 ng) in ethanol was added to each analytical sample of 100 μL plasma before extraction in hexane. Hexane was evaporated under nitrogen gas, and the residue was dissolved in methanol. Using a mobile phase of 95:5 methanol to water, the sample passed through a 2.7 μm reverse phase C18 column (InfinityLab Poroshell 120; Agilent Technologies) with a 5 μm guard column (Zorbax Eclipse Plus-C18; Agilent Technologies), and absorbance was measured at 325 nm. Plasma retinol was quantified by comparing the peak area of retinol to retinyl acetate. Each tray of study samples was analyzed along with 3 controls of pooled defibrinated plasma (Utak) with a known retinol concentration based on calibration with a certified NIST 1950 plasma control. Replicate analysis was conducted when the CV of the controls exceeded 5%. The average intraday CV was 2.0%, and the average interday CV was 5.1%.

RBP, CRP, and α-1-acid glycoprotein (AGP) were measured at the VitMin laboratory in Germany by combining sandwich techniques with ELISA methods [20]. Analysis by ELISA was performed on 50–75 μL aliquots from all children who provided a minimum plasma volume of 450 μL at enrollment or 6 mo follow-up. For quality control, the VitMin laboratory selected a subset of 16 samples with RBP ranging from 0.38–1.38 μmol/L for calibration against retinol measured by HPLC, and these retinol measures from study samples were calibrated against CDC and NIST certified values. Pooled plasma samples were analyzed with each tray with the following CV for each index: RBP (3.6%), CRP (5.8%), and AGP (8.1%).

Statistical analysis

A statistical analysis plan was prepared prior to analysis and posted online (https://osf.io/vfrg7). All data cleaning and analysis were performed in Stata version 15 (StataCorp LLC) [21]. All values for retinol and RBP were within the limit of detection. At the endline, 17% of samples had CRP values below the limit of detection. These values were replaced with zeroes for descriptive statistics and converted to 90% of the lower limit of detection for inflammation-adjusted models performed on the log-transformed scale.

Retinol and RBP were adjusted for inflammation on the log-transformed scale using a linear regression approach adapted from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) working group [22,23]. Retinol and RBP were assessed for bivariate associations ($P < 0.1$) with CRP and AGP at enrollment and 6 mo follow-up. Only CRP retained significance ($P < 0.1$) with retinol and RBP in multivariable regression models and was used for inflammation adjustment. When CRP exceeded 0.1 mg/L, the first decile for preschool-age children in the external BRINDA reference group, a linear correction factor specific to the study time point was applied to observed retinol and RBP values. Dichotomous variables were created for VAD. Per WHO recommendations, the primary definition for VAD was retinol <0.7 μmol/L [24], and secondarily, the prevalence of VAD was assessed using an RBP cutoff of <0.7 μmol/L. Retinol and RBP were compared through simple linear regression and by comparing the prevalence estimates of VAD using either biomarker.

Descriptive statistics were calculated for demographic characteristics, vitamin A indices, and inflammation (CRP >5 mg/L or AGP >1 g/L) at enrollment by group assignment. Linear regression models assessed groupwise differences in the mean concentration of plasma retinol and RBP. Prevalence ratios were assessed using modified Poisson models in instances when binomial family models with a logarithmic link function failed to converge. For descriptive purposes, prevalence differences for VAD were assessed through linear probability models with heteroscedasticity-consistent standard errors.

All models controlled for baseline values of the outcome variables, and the primary inference was drawn from models using inflammation-adjusted values. Fully adjusted models included covariates demonstrating significant bivariate associations with the outcome variable ($P < 0.1$) among the following set of a priori identified variables: child age, child sex, maternal education, household asset index, number of children under 5 y in the household, malaria, month of assessment, minutes between blood collection and aliquot storage, and receipt of vitamin A supplementation during the study period. Malaria was included as a potential covariate because malaria may be significantly associated with VAD even after adjusting for inflammation in malaria-endemic areas [25,26]. Vitamin A supplementation was proposed as a covariate because recent receipt of high-dose vitamin A supplements may elevate plasma retinol concentration. Breastfeeding was not included as a covariate because the percentage of breastfeeding children (>98%) lacked variation, and the intake of breast milk was not quantified in this study.

Missing values were imputed using linear regression models for 20% of children missing RBP and CRP at enrollment and 13% of children missing CRP at the 6 mo follow-up. Missing RBP at enrollment was imputed using the strongest predictive variables available: first using baseline retinol and date of HPLC analysis ($n = 25$), then baseline hemoglobin ($n = 31$), and lastly, ownership of goats ($n = 60$). Missing CRP at enrollment was imputed using the presence of malarial antigens and hemoglobin concentration, and CRP at the 6 mo follow-up was imputed using the presence of

malarial antigens and maternal education. All variables in the imputation models demonstrated a bivariate association with RBP or CRP and retained significance ($P < 0.1$) in multivariable models. The demographic characteristics of participants with missing measures were compared to children with complete measures. As a robustness sensitivity analysis, models were rerun with inverse probability of censoring-weighting to match the demographical characteristics of the original sample and compared the results from this model to those from the principal models.

Results

A total of 660 children were enrolled and randomly assigned to the egg intervention group ($n = 331$) or the control group ($n = 329$). Participant and household characteristics were generally balanced between groups, though the egg group had a higher percentage of children who reported consuming an animal-source food (fish, meat, egg, or milk) during the 24-h recall period prior to enrollment (40%) compared to the control group (31%). Study participants were typically from households with 6 members and experienced moderate to severe food insecurity. Most of the children's mothers had not completed primary education and were unable to read or write. Children were enrolled at an average age of 7 mo; all except 1 child were breastfeeding, and 3 children received high-dose vitamin A capsules prior to enrollment (Table 1). By the 6 mo follow-up, 45% of children had received a high-dose vitamin A supplement since enrollment, with no differences between the egg and control groups (data not shown). Among children with measured indices at enrollment, 62% had 1 or more elevated markers of inflammation. Median plasma retinol was 0.89 μmol/L, and the prevalence of VAD (retinol <0.7 μmol/L) was 21%. After adjusting for inflammation, the geometric mean for plasma retinol was 1.05 μmol/L, and the prevalence of VAD decreased to 7% (Table 1).

This analysis includes 575 children with RBP at the 6 mo follow-up (egg group: $n = 281$; control group: $n = 294$) and 489 children with matching retinol samples from enrollment and 6 mo follow-up time points (egg group: $n = 238$; control group $n = 251$; Figure 1). The number of missing values at the 6 mo follow-up did not differ between the egg and control groups for RBP (15% compared with 11%) or retinol (28% compared with 24%). Participants with missing retinol or RBP data tended to have enrolled earlier in the study and resided closer to the Lungwena

TABLE 1
Enrollment characteristics of children in the Mazira Project, Malawi, 2018–2019, by intervention group[1]

Characteristic	Egg		Control	
	n	Value	*n*	Value
Maternal				
Maternal age, y	329	25.9 ± 6.7	325	26.1 ± 6.8
Maternal primary education,[2] %	331	24	329	16
Maternal literacy, %	322	50	321	42
Household				
Number of children under 5 y	319	1.7 ± 0.8	319	1.7 ± 0.8
Number of household members	321	5.8 ± 2.6	320	6.0 ± 2.7
Moderate or severe food insecurity,[3] %	331	75	329	81
Child				
Child age, mo	331	7.4 ± 1.2	329	7.3 ± 1.2
Female, %	331	48	329	48
Breastfeeding, %	330	100	329	100
Consumed animal-source food in past 24 h, %	330	40	329	31
Received high-dose vitamin A capsule, %	331	0	329	1
Prevalence of stunting (LAZ <−2), %	331	13	329	14
Prevalence of underweight (WAZ <−2), %	331	7	329	9
Prevalence of wasting (WLZ <−2), %	331	1	329	1
Inflammation				
CRP >5 mg/L,[4] %	265	34	260	36
AGP >1 g/L,[4] %	265	60	260	61
Hemoglobin, g/dL	292	10.5 (9.5, 11.5)	290	10.6 (9.3, 11.5)
Anemia (hemoglobin <11 g/dL), %	292	60	290	61
Plasma retinol,[5] μmol/L	236	0.88 (0.73, 1.10)	251	0.89 (0.73, 1.08)
Plasma RBP,[4] μmol/L	265	0.84 (0.70, 1.01)	260	0.80 (0.68, 1.01)
Vitamin A deficiency (retinol[5] <0.7 μmol/L), %	236	20	251	22
Inflammation-adjusted				
Plasma retinol,[5] μmol/L	236	1.06 (0.89, 1.26)	251	1.03 (0.86, 1.25)
Plasma RBP,[4] μmol/L	265	0.96 (0.83, 1.13)	260	0.94 (0.81, 1.13)
Vitamin A deficiency (retinol[5] <0.7 μmol/L), %	236	7	251	8

AGP, α-1-acid glycoprotein; LAZ, length-for-age z-score; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score.
[1] Values are %, mean ± SD, or median (P25, P75).
[2] Percent completed primary or greater.
[3] Food insecurity assessed using the Household Food Insecurity Access Scale [18].
[4] Reasons for missing AGP, CRP, and RBP measures: 4% refused consent, 8% incomplete blood draws, and 9% insufficient plasma volume.
[5] Reasons for missing retinol measures: 4% refused consent, 8% incomplete blood draws, 5% insufficient plasma volume, and 9% excluded from analysis because a matching sample at 6 mo follow-up was not obtained.

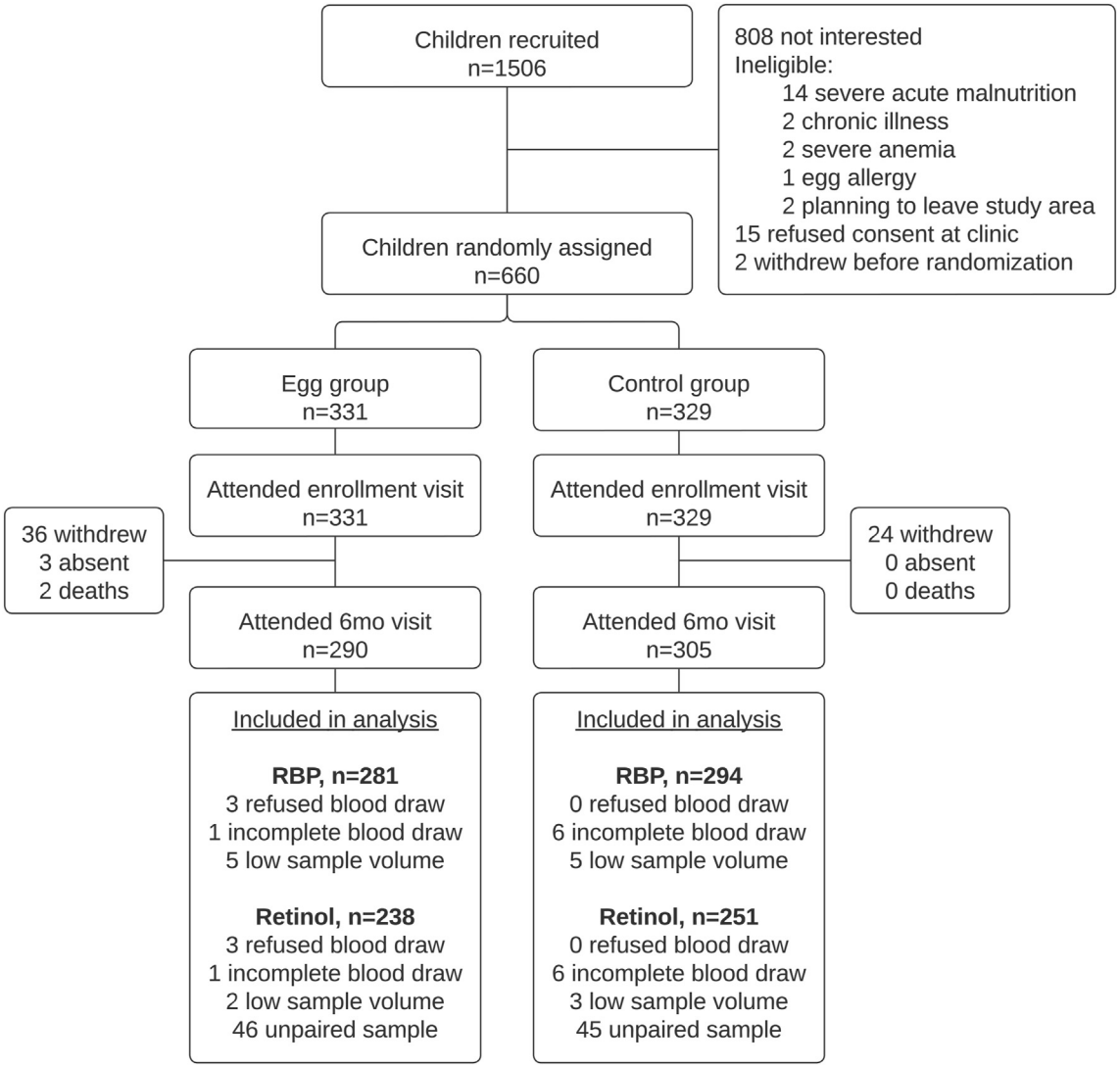

FIGURE 1. Participant flow diagram for the vitamin A analyses of the Mazira Project, Malawi, 2018–2019. Retinol samples at 6 mo follow-up were analyzed if the child also provided a matching sample from the enrollment visit.

health center (Supplemental Table 1). Children with missing RBP data also tended to be from households with less maternal education, literacy, and employment in the service industry. These children also lived in households with more food insecurity and in homes constructed with lower-quality building materials such as mud, straw thatching, or unburnt bricks (Supplemental Table 2).

Retinol and RBP were strongly correlated with each other ($r = 0.9$) and provided good internal agreement. However, RBP values measured by VitMin laboratories were lower than retinol values measured at the University of California, Davis: the RBP equivalent of 0.7 μmol/L retinol was 0.68 μmol/L at enrollment and 0.66 μmol/L at follow-up (Supplemental Figure 1). The distribution of retinol by treatment group at enrollment and 6 mo follow-up is shown in Supplemental Figure 2.

After 6 mo of study participation, 51% of children had ≥1 elevated marker of inflammation with no differences in group assignment (data not shown). The unadjusted prevalence of VAD was 21%, which decreased to 5% after adjusting for inflammation. The egg and control groups did not differ in the mean concentration of retinol (1.09 μmol/L) or RBP (0.98 μmol/L), nor did the prevalence of VAD and low RBP differ (Table 2). Inflammation-adjusted estimates of VAD were similar for both indicators: retinol <0.7 μmol/L (5%) and RBP <0.7 μmol/L (8%). Results did not differ between the principal models and sensitivity analysis models using inverse probability censoring-weighting (data not shown).

Discussion

In regions historically impacted by VAD, vitamin A adequacy may be attained for infants and young children through the promotion of vitamin A-rich animal-source foods, fruits, and vegetables in dietary guidelines or food-based interventions, such as the daily provision of eggs. In this study, mean retinol concentration and prevalence of VAD did not differ between children receiving the 1 egg/d intervention and control groups after 6 mo; however, the prevalence of VAD was low in this cohort. As previously reported, the egg intervention group had a higher usual dietary intake of vitamin A than the control group; however, both groups had a low proportion of children with

Table 2
Differences in means, prevalence ratios, and prevalence difference of vitamin A indices between treatment groups after 6mo of participation in the Mazira Project, Malawi, 2018-2019

Retinol and RBP	*N*		Geometric mean (95% CI)		Geometric mean ratio (95% CI)
	Egg	Control	Egg	Control	
Plasma retinol, μmol/L					
Minimally adjusted[1]	238	251	0.90 (0.87, 0.94)	0.89 (0.86, 0.92)	1.01 (0.96, 1.06)
Inflammation-adjusted[1,2]	238	251	1.10 (1.07, 1.13)	1.08 (1.05, 1.12)	1.02 (0.97, 1.06)
Fully adjusted[1,2,3]	238	251	1.10 (1.07, 1.14)	1.08 (1.05, 1.11)	1.02 (0.98, 1.06)
Plasma RBP, μmol/L					
Minimally adjusted[1]	281	294	0.81 (0.79, 0.84)	0.80 (0.77, 0.82)	1.02 (0.98, 1.07)
Inflammation-adjusted[1,2]	281	294	0.99 (0.96, 1.02)	0.97 (0.94, 1.00)	1.02 (0.97, 1.06)
Fully adjusted[1,2,3]	281	294	0.99 (0.96, 1.02)	0.97 (0.94, 1.00)	1.02 (0.98, 1.06)

Vitamin A deficiency	*N*		Prevalence		Prevalence ratio (95% CI)	Prevalence difference (95% CI)
	Egg	Control	Egg	Control		
Retinol < 0.7μmol/L, %						
Minimally adjusted[1]	238	251	21.2	20.1	1.06 (0.76, 1.49)	1.34 (-5.64, 8.33)
Inflammation-adjusted[1,2]	238	251	6.2	3.3	1.87 (0.83, 4.24)	3.36 (-0.37, 7.09)
Fully adjusted[1,2,3]	238	251	6.1	3.4	1.80 (0.79, 4.08)	3.50 (-0.23, 7.23)
RBP < 0.7μmol/L, %						
Minimally adjusted[1]	281	294	26.7	33.2	0.80 (0.63, 1.03)	-6.31 (-13.52, 0.90)
Inflammation-adjusted[1,2]	281	294	8.2	8.3	0.99 (0.56, 1.75)	0.11 (-4.71, 4.94)
Fully adjusted[1,2,3]	281	294	8.5	8.1	1.05 (0.59, 1.85)	-0.02 (-4.74, 4.70)

RBP = retinol binding protein
[1] adjusted for continuous baseline measures
[2] inflammation-adjusted using methods adapted from the BRINDA (Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia) approach
[3] adjusted for covariates selected based on a bivariate association ($p > 0.1$) with the outcome among the following list: child sex, maternal education, number of children under 5yrs in the household, malaria, month of assessment, and minutes between blood collection and completion of aliquoting

inadequate intake of vitamin A (egg: 9%; control: 15%) at the 6 mo follow-up [15]. Therefore, the lack of intervention effect may be attributable to the low risk of deficiency because plasma retinol is not sensitive to changes in dietary intake of vitamin A when liver stores are adequate.

Several factors may contribute to a low prevalence of VAD in this study population, including dietary intake of vitamin A from breast milk, fortification of staple foods such as flour, sugar, and oil, and coverage of high-dose vitamin A supplements. At enrollment, the unadjusted prevalence of VAD was 21% and decreased to 7% after adjusting for inflammation using the BRINDA regression approach with CRP. The usual intake of vitamin A from the combination of breast milk and solid foods at enrollment was 353 μg RAE/d, and the prevalence of nutrient intake inadequacy was 94%, based on 500 μg RAE/d for adequate intake for 7–12 mo because an estimated average requirement (EAR) has not been established for this age group [15]. Breast milk, dark leafy green vegetables, and fish were commonly reported in the 24-h recalls completed at enrollment [27]. After 6 mo of study participation, the usual intake of vitamin A in the control group (335 μg RAE/d) was similar to baseline intake; however, the prevalence of inadequate intake was lowered to 15%, reflecting the lower EAR for 1–3-y-old children of 210 μg RAE/d [15]. Though the availability of vitamin A-fortified flour, sugar, and oil in children's homes were not assessed in this study, the 2015–2016 Malawian Micronutrient Survey indicated that 69% of households with preschool-age children had both oil and sugar available with a mean concentration of 31 μg RAE/teaspoon of oil and 24 μg RAE/teaspoon of sugar [7]. Industrial fortification may improve the vitamin A intake of young children by direct consumption of fortified foods or indirectly through the consumption of breast milk that has a higher vitamin A concentration because of maternal intake of fortified foods [28–30]. Malawian children 6–59 mo of age may also receive high-dose vitamin A supplements biannually. Though only 3 children in the present study received vitamin A supplementation prior to enrollment, 45% reported receiving high-dose vitamin A supplementation during the 6 mo study period from health centers or biannual campaigns occurring in June 2018 in Malindi and December 2018 in Lungwena. This could have contributed to the low prevalence of VAD at the 6 mo follow-up visit and further reduced the possibility of an impact of the egg intervention on vitamin A status.

The prevalence of VAD among young children in Malawi has been assessed by multiple studies within the last 10 y, providing consensus of a low prevalence of VAD that poses a mild public health problem [VAD: 2%–9%; WHO (24)]. The Malawian micronutrient survey was conducted in 2015–2016 and reported VAD of 4% among preschool children using the RBP equivalent (<0.46 μmol/L) to a retinol cutoff of <0.7 μmol/L, without adjusting for inflammation. Because these retinol and RBP values had a poor linear relationship, the prevalence of VAD was later reassessed using the cutoff of <0.7 μmol/L RBP, yielding a 24% prevalence of VAD (unadjusted) and 10% prevalence after adjusting for inflammation using the BRINDA method [31]. In 2011–2012, a small quantity lipid-based nutrient supplement trial (iLiNS-DYAD [32]) that provided 800 μg RAE/d to pregnant and postpartum women and 400 μg RAE/d to children from 6–18 mo of age was conducted in the same region of Malawi as the present study. The overall prevalence of VAD (<0.7 μmol/L) among children in the iLiNS-DYAD (6 mo of age: unadjusted

22%; inflammation-adjusted 10%) in 2011 [11] was similar to VAD among children in the Mazira Project (enrollment at 6–9 mo: unadjusted 21%; inflammation-adjusted 7%) in 2018. However, the overall geometric mean for inflammation-adjusted plasma retinol concentrations of children in the Mazira Project [1.05 μmol/L at enrollment (6–9 mo of age); 1.09 μmol/L at the 6 mo follow-up (12–15 mo of age)] was higher than children in the iLiNS-DYAD (0.97 μmol/L at 6 mo of age; 1.00 μmol/L at 18 mo of age).

The results in the present study are comparable to observations in a similar trial providing 1 egg/d to children 6–9 mo of age in Ecuador: mean retinol concentrations did not differ between the egg (656.35 ng/mL or 2.30 μmol/L) and control (643.79 ng/mL or 2.25 μmol/L) groups after 6 mo [33]. However, in that trial, no children were vitamin A deficient (retinol <0.7 μmol/L), and only 3% of children had retinol <1.05 μmol/L at baseline. Compared with children in the study in Ecuador, children in the Mazira Project study had greater potential to benefit from the egg intervention based on the historically higher prevalence of VAD and higher retinol concentration of the eggs in this trial (150 μg RAE/100 g) compared with the Ecuadorian trial (107 μg RAE/100 g) [33,34]. Additionally, the present study had greater power to detect an effect because of a larger sample size enrolled ($n = 660$) and analyzed ($n = 489$) for retinol than the Ecuadorian trial (enrolled: $n = 169$; analyzed: $n = 139$). Despite the large sample size and potential for high response to the intervention among vitamin A deficient children, the Mazira Project in Malawi also did not observe a difference in mean plasma retinol concentration between the egg intervention and control groups [33,34].

Other dietary intervention trials have similarly observed a lack of biological response in plasma retinol and reduced power to detect an intervention effect from enrolling a study population with a lower prevalence of VAD than expected. In Zambia, 4–8-y-old children were provided with ~50% of the RDA of vitamin A RAE from maize meal biofortified with 15–20 μg β-carotene/g for 6 d/wk for 6 mo [35]. The biofortified maize intervention group had a 0.14 μmol/L higher mean β-carotene concentration compared to the white maize meal control (<2 μg β-carotene/g), but mean serum retinol (0.99 μmol/L) and VAD (17%; retinol < 0.7 μmol/L) did not differ between study arms after 6 mo. Although a lack of bioconversion from β-carotene to retinol may partially explain the lack of effect of the biofortified maize intervention on serum retinol concentrations, bioconversion is not a concern for the egg intervention because the vitamin A found in Mazira Project eggs was predominantly preformed retinol and the concentration of β-carotene was below the limit of detection [34]. However, mean baseline retinol concentrations were within normal ranges for both the maize and egg intervention studies, and this may explain the lack of intervention effects on serum or plasma retinol concentrations.

This secondary analysis has several limitations. First, there is missing data on vitamin A for 13% of children, primarily because of the blood draw refusals or insufficient sample volume. Though plasma aliquots for assessment of retinol were prepared before aliquots for RBP, fewer children were assessed for retinol because of the exclusion of those who did not provide a blood sample at enrollment ($n = 91$). Children at enrollment had more missing blood draws because of the difficulty in locating the small veins of 6–9-mo-old children and higher refusal rate among caregivers [36]. This missing data could have contributed to selection bias. To mitigate the impact of this analysis, an inverse probability of censoring-weighted analyses was conducted. Missing data could also reduce the power to detect differences in groups. However, this study retained adequate power to detect small differences in vitamin A status because of the large number of enrolled children.

Second, plasma retinol is homeostatically regulated over a broad range of liver reserves and may not be sensitive to the effect of dietary interventions when the prevalence of VAD in the studied population is low. Measuring the change in liver stores through MRDR or isotope dilution techniques would provide a more sensitive assessment of vitamin A status for an intervention providing eggs for daily consumption over 6 mo. However, these techniques for vitamin A assessment require the administration of a vitamin A tracer prior to conducting the blood draw, which was not included as part of the initial design for primary study objectives. Thus, this secondary analysis was limited to plasma retinol and RBP with the available plasma samples.

Lastly, both the amount and concentration of vitamin A in breast milk, which is influenced by maternal dietary intake, may contribute to liver stores of vitamin A in young, breastfed children. Thus, controlling for vitamin A content from breast milk or maternal diet could refine the estimate of the impact of the egg intervention on measures of children's vitamin A status. However, quantitative data collection on breast milk intake or maternal diet was not included as part of the study design.

The strengths of this study are its design as a randomized controlled trial and strong quality controls. First, the study had high adherence to the assigned intervention [15,16,27,36–38]. Through 24-h dietary recalls conducted at follow-up visits, consumption of eggs was higher among children in the egg group (3 mo: 85%; 6 mo: 71%) than the control (3 mo: 7%; 6 mo: 7%) [16,27]. Additionally, analysis of plasma metabolites indicated that biomarkers in the choline pathway were elevated in the egg group compared to the control group, which suggests that egg intake was higher in the egg group than control, although this does not provide a direct measure of the number of eggs consumed [38]. Second, vitamin A was assessed by retinol and RBP. Laboratory analysis of plasma retinol and RBP had good reliability, with the average intraday CV of 2.0% for retinol and 3.6% for RBP. Lastly, an analysis of differences between groups was conducted using 3 scenarios: controlling for baseline measures only, adding an adjustment for inflammation, and adding additional variables for demographics and laboratory analyses. No differences were observed through any of these models.

In conclusion, providing 1 egg/d in a population with a low prevalence of VAD did not impact vitamin A status. Results may differ in populations with a higher risk of VAD. The efficacy of using eggs to improve vitamin A status among at-risk populations would benefit from assessment by MRDR or isotope dilution instead of plasma concentrations of retinol and RBP. However, when plasma retinol and RBP concentrations are viewed together with prior studies in this community and national trends in vitamin A status, this study provides further evidence that the vitamin A situation in Malawi has been improving over time. Nevertheless, deficiencies in other micronutrients remain common, and there is a continued need for interventions focused on improving dietary quality and micronutrient adequacy of young children's diets.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cdnut.2023.100053.

Author contribution

The authors' responsibilities were as follows–CPS, BLC, LLI, CKL, KMM, and ERW: designed the research; CPS, KMM, BLC, CDA, and ERW: conducted the research; ERW, MJH, and CDA: analyzed the data or performed statistical analysis; ERW and CPS: wrote the article; ERW and CPS: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

Data Availability

Data described in the manuscript, code book, and analytic code will be made publicly and freely available without restriction at https://osf.io/vfrg7.

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Author disclosures

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