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# Relation between Timing of High-Dose Vitamin A Supplementation and Modified-Relative-Dose–Response Values in Children 12–23 Months in Uganda

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## Abstract

**Background:** High-dose vitamin A (VA) supplements (VAS) can temporarily affect VA status. Hence, micronutrient surveys might need to be timed around VAS campaigns to accurately estimate VA deficiency (VAD) prevalence. Little is known about optimal timing of micronutrient surveys when the modified-relative-dose–response (MRDR) is used as a VA indicator.

**Objectives:** We evaluated the association between days since the end of a VAS campaign and MRDR values in children aged 12–23 mo in Uganda.

**Methods:** We pooled data from 2 cross-sectional, population-based surveys in eastern Uganda conducted in 2015−2016 ($n$ = 118 children). We estimated the prevalence of VAD (MRDR ≥0.060). Days since the end of a VAS campaign ("days since VAS") was calculated as the interview date minus the end date of the VAS campaign. The MRDR value was assessed



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The authors' responsibilities were as follows—CMP: designed research, analyzed data, and wrote paper; RF-A, MEDJ, NDF, RDW, and CM: designed research, interpreted data, and revised manuscript; SAT: provided vitamin A-2 and technical support, oversaw MRDR analyses, interpreted data, and revised the manuscript; SN and SH: designed the research, oversaw data collection, interpreted data, and revised the manuscript; JS: analyzed MRDR samples, interpreted data, and revised the manuscript; and all authors: took responsibility for the final content and read and approved the final manuscript.

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using HPLC. We excluded children whose MRDR values were below the limit of detection (<0.007). We used linear regression to evaluate the association between days since VAS and log-transformed MRDR. In adjusted analyses, we controlled for potential confounders. Statistical analyses accounted for the surveys' complex design.

**Results:** The prevalence of VAD was 5.2% (95% CI: 1.1%, 9.3%). Mean days since VAS was 54.1 d (range 39−68 d). Days since VAS was not associated with log-transformed MRDR in unadjusted analyses ($\hat{\beta} = 0.0055$; 95% CI: −0.009, 0.020; $P = 0.45$) or adjusted analyses ($\hat{\beta} = -0.0073$; 95% CI: −0.024, 0.010; $P = 0.39$).

**Conclusions:** MRDR measurement through a nutrition survey began as early as 1.3 mo after the end of a VAS campaign in eastern Uganda. Days since the end of a VAS campaign was not associated with MRDR in Ugandan children aged 12–23 mo. Future studies should consider longitudinal designs and evaluate time since VAS and MRDR in children of different ages and in regions with higher VAD prevalence.

### Keywords

vitamin A; modified-relative-dose–response (MRDR); epidemiology; children; Uganda

## Introduction

The WHO recommends high-dose vitamin A (VA) supplementation (VAS) in areas with VA deficiency (VAD) to reduce morbidity and mortality in children aged 6–59 mo (1). Many countries distribute high-dose VAS during biannual campaigns, which can bundle nutrition interventions, immunizations, and other services. High-dose VAS can cause transient improvements in VA status (2, 3). To obtain accurate estimates of VAD prevalence, it could be necessary to time micronutrient surveys around VAS campaigns; however, this could be challenging because of competing factors influencing survey timing. The literature shows serum retinol concentrations return to preintervention levels 1–3 mo post-VAS; researchers suggest waiting ≥2 mo after VAS to assess serum retinol in population-based surveys (2, 3). Less is known about the optimal timing of nutrition surveys for other indicators of VA status.

The modified-relative-dose–response (MRDR) is a qualitative measure of vitamin A liver stores (4). During VA depletion, *apo*-retinol-binding protein (RBP) accumulates in the liver (5). After a challenge dose of 3,4-didehydroretinyl acetate, 3,4-didehydroretinol (DR) binds to the accumulated RBP quickly after dosage and is circulated into plasma. The molar ratio of DR to retinol (DR:R) is measured by HPLC in a single blood sample taken 4–6 h after dose administration (4). DR:R is less affected by inflammation than serum retinol concentration, which can be more related to the ratio than absolute changes in a single analyte (6, 7). The MRDR test is not affected by weight-for-age in children (8). Considering the minimal invasiveness of the MRDR test requiring a small blood sample, it is well suited to studies in children and vulnerable women (8, 9). Because serum retinol is homeostatically controlled, it might not change after VA intervention; hence, it is important to include other indicators of VA status, such as MRDR, that are useful for identifying changes in VA status in an intervention evaluation context (4).

A kinetic modeling study suggests that VA liver stores return to preintervention levels within ~3 mo of high-dose VAS (10). However, the association between time since high-dose VAS and MRDR has not been extensively explored in population-based surveys. Our objective was to evaluate the association between days since the end of a VAS campaign and MRDR values in Ugandan children 12–23 mo old.

## Methods

We used data from baseline (June/July 2015) and endline (June/July 2016) cross-sectional, population-based surveys for an integrated infant and young child feeding (IYCF)/ micronutrient powder (MNP) intervention targeting children 6–23 mo in eastern Uganda. Each survey used multistage cluster sampling and was representative of children 12–23 mo in rural areas of Amuria (intervention district; completely rural) and Soroti (nonintervention district; only rural clusters were included). Additional information is published elsewhere (11–13). Caregivers in the intervention district received enhanced IYCF education behavior change sessions and 30 MNP sachets—each of which contained 400 $\mu$g retinol—every 2 mo for ≤12 mo. Children in the nonintervention district received the IYCF standard of care (no MNP).

During interviews, caregivers were shown a sample high-dose VA capsule and asked whether their child received VAS (100,000 IU for children 6 to <12 mo; 200,000 IU for children 12–23 mo) during the most recent biannual Child Health Plus Day, which occurred April 1–30 in 2015 (baseline survey) and 2016 (endline survey). A total of 98 children 12–23 mo (49 per district; 1–2 per cluster) were recruited for MRDR testing in each survey. One child was randomly selected from each of 38 clusters per district. Next, 11 clusters were randomly selected, and a second child was randomly selected from each of these 11 clusters in each district. There was no replacement of recruited participants for any reason (11, 12).

For MRDR testing, children consumed a small dose of 3,4-didehydroretinyl acetate (vitamin A-2) mixed with olive oil (4). A venous blood sample was collected 4–6 h after vitamin A-2 administration. Enumerators asked participants to avoid consuming VA-rich foods and beverages for 2 h before vitamin A-2 administration. Participants were given water and non–VA-containing food while they stayed at the blood collection location. Blood samples were processed and stored frozen on the day of collection. Cold chain was maintained while frozen plasma samples were shipped to the University of Wisconsin-Madison Department of Nutritional Sciences for analysis. Plasma retinol and 3,4-didehydroretinol were analyzed using HPLC. Quality control procedures were followed (11, 12).

## Statistical analysis

We pooled the Uganda baseline and endline surveys based on similar VA status [VAD prevalence was low in both surveys, and the intervention did not affect VA status, iron status, or anemia (13)]. The exposure variable, days since the end of the VAS campaign (“days since VAS”), was calculated as date of blood collection minus the end date of the VAS campaign (April 30 in 2015 and 2016). We used the campaign end date to calculate days since VAS due to lack of precise data on date of VAS administration per child.

We used a natural log transformation to transform MRDR. Normality of log-transformed MRDR was assessed using statistical goodness-of-fit tests and by visually inspecting probability plots and histograms of log(MRDR) values. We performed a linear regression of log(MRDR) on days since VAS. In a multivariable analysis, we adjusted for potential confounders, including child's age, inflammation biomarkers (C-reactive protein and $\alpha$-1-acid glycoprotein concentrations), consumption of provitamin A–rich fruits and vegetables the day preceding the survey, breastfed at the time of the survey, reported distance to health center, MNP consumption in prior 14 d, receipt of VAS from a source other than the campaign in the past 3 mo, positive malaria test, district, and survey year.

We evaluated interactions between days since VAS and district, year, and VAS receipt from another source. In addition, we estimated VAD prevalence [MRDR ≥0.060 (4)]. We used SAS 9.4 (SAS Institute Inc.) for all analyses; $P$ values <0.05 were considered statistically significant. Except for scatter plots and goodness-of-fit tests for normality, all analyses accounted for complex survey design. A small number of very ill or deceased children were excluded from the larger baseline and endline surveys completely (not shown) (11, 12). A total of 21 children selected for MRDR testing declined to participate. We excluded children whose MRDR values were below the limit of detection (<0.007), those who did not receive VAS, those with insufficient blood sample volume, and those with missing covariate or exposure data. For these reasons, 78 of 196 children 12–23 mo selected for MRDR testing were excluded, leaving 118 children in the analysis (Figure 1).

Surveys received ethical approval by the School of Biomedical Sciences Higher Degrees, Research and Ethics Committee, College of Health Sciences, Makerere University, and Uganda National Council for Science and Technology. Caregivers provided written and oral consent for themselves and their child(ren).

## Results

Background characteristics of children in the sample are shown in Table 1. The mean time between the end of the VAS campaign and the MRDR test was 54.1 d (range 39−68 d). The prevalence of VAD was 5.2% (95% CI: 1.1%, 9.3%). Of 31 children who did not receive VAS (and were thus excluded from the analytic sample) but who had MRDR values above the limit of detection, $n = 3$ were VA deficient (data not shown). The mean MRDR value was 0.029, and the mean log-transformed MRDR value was −3.76 (Table 1). Log-transformed MRDR was normally distributed according to goodness-of-fit tests, as well as probability plots and histograms of log(MRDR) values (not shown). Figure 2 presents a scatter plot of log-transformed MRDR by days since VAS.

Days since VAS was not associated with log-transformed MRDR in unadjusted analyses ($\hat{\beta} = 0.0055$; 95% CI: −0.009, 0.020; $P = 0.45$). After adjusting for confounders, the association remained nonsignificant ($\hat{\beta} = -0.0073$; 95% CI: −0.024, 0.010; $P = 0.39$) (Table 1). This adjusted estimate equates to a −0.7% (95% CI: −2.4%, 1.0%) change in MRDR values for a 1-d change in days since VAS. There was no interaction between days since VAS and other variables.

## Discussion

This study found that days since the end of a VAS campaign was not associated with VA status, as assessed by MRDR, in children 12–23 mo in 2 rural districts of eastern Uganda. Although serum retinol concentrations are homeostatically controlled, the response to a challenge dose is dependent on VA status. Because MRDR is not homeostatically controlled and we adjusted for potential confounders, we would have expected to see a difference in MRDR values by time since VAS if a true difference existed (4). The mean time elapsed between the end of the VAS campaign and MRDR testing was almost 2 mo (54.1 d), with a range of 1.3–2.2 mo. Due to a lack of precise data on date of VAS administration, we used the end of the VAS campaign (April 30) to calculate days since VAS. For most children, the true time interval between VAS administration and MRDR testing was likely longer than estimated (e.g., a half-month longer on average, or ~1.8–2.7 mo total). Identifying the shortest waiting period to collect survey data after VAS campaigns is important, because many factors influence survey timing, including the timing of other surveys in the country, other objectives of the survey, duration of data collection, seasonality, religious holidays, elections, start of new interventions, insecurity, public health crises, and funding cycles. Notably, some of these factors are outside of the control of those planning the survey.

The VAD prevalence was low in our study, which is likely due to multiple overlapping micronutrient interventions. In addition to mass VAS campaigns for young children, Uganda also industrially fortifies vegetable cooking oil and cereal flours with preformed retinyl palmitate A. However, VA supplementation of breastfeeding women was likely rare in eastern rural Uganda. Notably, our findings might not be generalizable to regions with higher VAD prevalence.

This study has limitations. The analysis was cross-sectional, limiting our ability to make conclusions about changes over time within individuals. We only evaluated time since VAS within a specific range (1.3–2.2 mo). Results could differ for time intervals <1.3 or >2.2 mo. Another study estimated that VA liver stores returned to preintervention levels by 3 mo after high-dose VAS (10); however, we could not evaluate the full 0–3 mo time range in this study. On a practical level, if surveys waited to start data collection until 3 mo after the end of biannual campaigns, then each year (depending on campaign duration) that would generally leave only 2 opportunities of ~2–3 mo each to collect the survey data. In many cases, this limited time frame would be a large challenge to overcome, considering the many factors that influence survey timing and duration. April 30 was used as the end date of the VAS campaign. However, this campaign could have extended into early May for a small number of children. In addition, VAS consumption was not directly observed and could be subject to recall bias. Due to exclusion criteria, 40% of the original sample was excluded. Although children should not have consumed foods or beverages containing VA between vitamin A-2 administration and blood draw, some children might have breastfed during this time. Although precise information on estimated daily VA intake was not available in our survey, we adjusted for consumption of VA-rich fruits and vegetables the day before the survey, MNP consumption in the last 14 d, breastfed at the time of the survey (yes/no), and receipt of VAS from another source besides the campaign in the past 3 mo (however, these indicators are subject to recall bias). Notably, the mean MRDR value was similar in the 118

children (analytic sample) that received VAS and the 31 children with MRDR values above the limit of detection but who reported they did not receive VAS (excluded), acknowledging the limitations of the small ($n$ = 31) sample size.

Our study has several strengths. We collected high-quality laboratory data on MRDR (4) and adjusted for many potential confounders, including malaria infection. Unlike retinol, MRDR is not thought to be as influenced by inflammation resulting from infection (6, 7). Still, to be conservative we adjusted for inflammation biomarkers (C-reactive protein and $\alpha$-1-acid glycoprotein) in our multivariable analyses. To our knowledge, our study is one of the first to examine the association between time since VAS and MRDR values. Future studies should consider longitudinal designs with accurate assessment of high-dose VAS intake and evaluate this issue in children of different ages and in regions with higher VAD prevalence.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.

## Abbreviations used:

| | |
|---|---|
| **DR** | 3,4-didehydroretinol |
| **DR:R** | molar ratio of 3,4-didehydroretinol to retinol |
| **IYCF** | infant and young child feeding |
| **MNP** | micronutrient powder |
| **MRDR** | modified-relative-dose–response |
| **R** | retinol |
| **RBP** | retinol-binding protein |
| **VA** | vitamin A |
| **VAD** | vitamin A deficiency |
| **VAS** | vitamin A supplementation |

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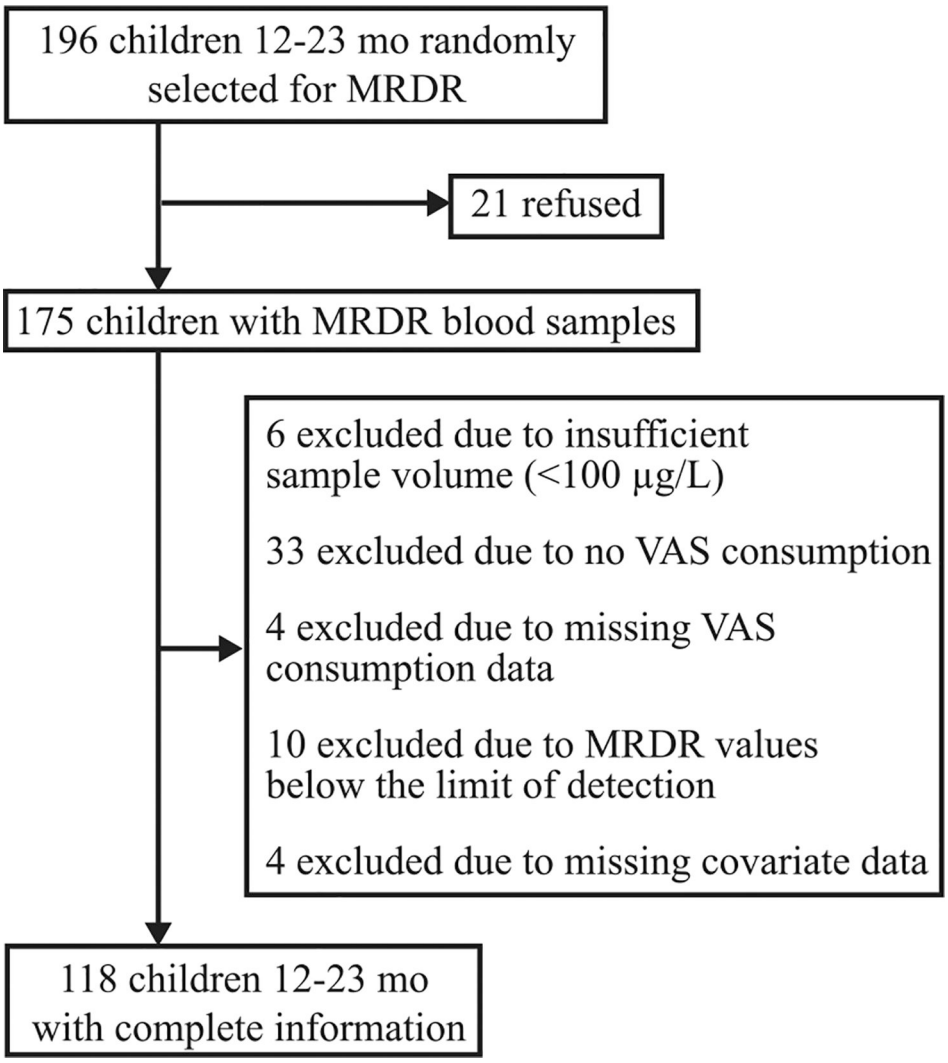


**FIGURE 1.**

Study exclusion criteria, Uganda pooled baseline and endline surveys, 2015−2016. Of 196 children aged 12–23 mo randomly selected for MRDR testing, 21 refused. An additional 57 children were excluded from our analytic sample for various reasons. The most common reason for exclusion was that children did not receive VAS at the most recent Child Health Day ($n$ = 33). A total of 118 children 12–23 mo remained in our analytic sample after applying exclusion criteria. MRDR, modified-relative-dose–response; VAS, vitamin A supplementation.

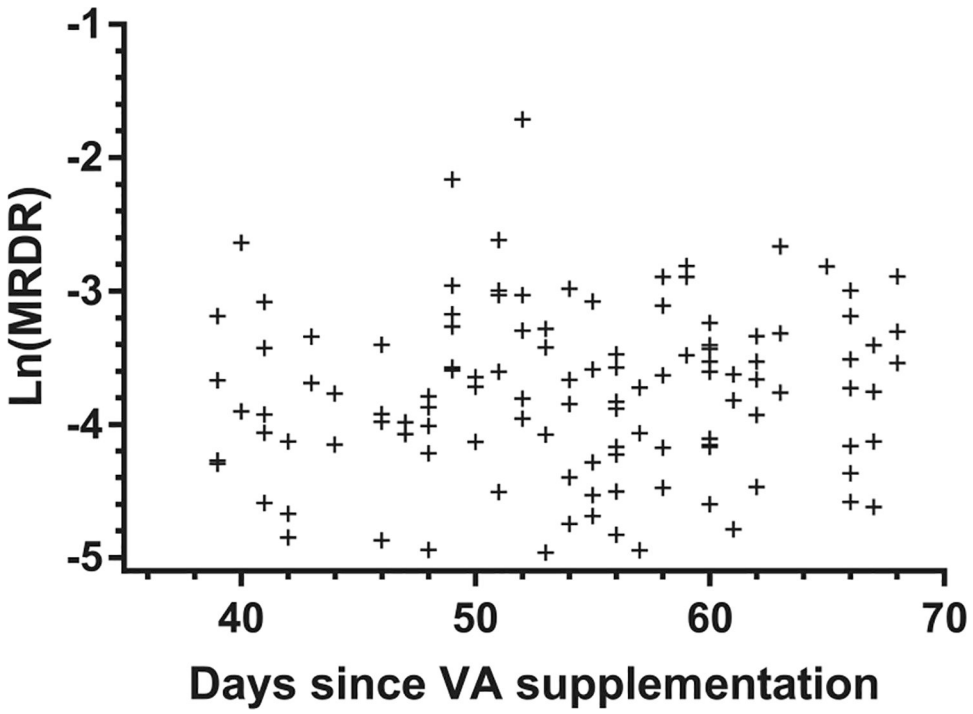


**FIGURE 2.**

Scatter plot showing log-transformed MRDR values (on the *y*-axis) by days since the end of a VAS campaign (on the *x*-axis) in children aged 12–23 mo in Amuria and Soroti Districts, eastern Uganda, 2015–2016. MRDR, modified-relative-dose–response; VA, vitamin A.

## TABLE 1

Background characteristics and relation between days since the end of a mass VAS campaign and VA status in children aged 12–23 mo in Amuria and Soroti Districts, Uganda (2015−2016)[1]

| Characteristic | Mean or % (95% CI) |
|---|---|
| Mean age, mo | 18.1 (17.4, 18.8) |
| Male | 51.8 (42.2, 61.3) |
| Breastfed at time of survey | 61.3 (52.5, 70.1) |
| Consumed VA-rich fruits and vegetables the day before the survey | 69.7 (61.0, 78.3) |
| Received VAS from another source in the 3 mo prior to the survey, in addition to VAS received during the campaign | 22.3 (14.9, 29.7) |
| Mean days since the end of the VAS campaign and the MRDR test | 54.1 (52.5, 55.8) |
| Positive for malaria via rapid test kit | 53.2 (44.2, 62.1) |
| VA deficient[2] | 5.2 (1.1, 9.3) |
| Mean plasma retinol concentration, $\mu$mol/L | 0.89 (0.83, 0.95) |
| Mean of MRDR values[3] | 0.029 (0.024, 0.033) |
| Mean of natural log-transformed MRDR values | − 3.76 (−3.87, −3.64) |
| $\hat{\beta}$ for unadjusted change in log(MRDR) for 1-d increase in time since end of VAS campaign | 0.0055 (−0.009, 0.020) |
| $\hat{\beta}$ for adjusted change in log(MRDR) for 1-d increase in time since end of VAS campaign[4] | − 0.0073 (−0.024, 0.010) |
| Adjusted % change in MRDR for 1-d increase in time since end of VAS campaign[5] | − 0.7 (−2.4, 1.0) |

[1] Prevalences are weighted. SEs account for complex sampling. MRDR, modified-relative-dose–response; VA, vitamin A; VAS, vitamin A supplementation.

[2] MRDR ≥0.060 indicates deficiency (4).

[3] Mean (95% CI) of MRDR values was 0.028 (0.021, 0.035) in 31 children with MRDR values above the limit of detection but who did not consume VAS so were not part of the analytic sample.

[4] Findings were adjusted for age in months, C-reactive protein concentration, $\alpha$-1-acid glycoprotein concentration, vitamin A–rich fruit and vegetable consumption the day prior to the survey (yes/no), reported distance to health center in minutes, micronutrient powder consumption in last 14 d (yes/no), currently breastfed at time of survey (yes/no), receipt of VAS from another source besides the campaign in the past 3 mo (yes/no), positive for malaria via rapid test kit (yes/no), district [Amuria (intervention district)/Soroti (nonintervention district)], and year (2015/2016).

[5] Parameter estimates and 95% confidence limits for log(MRDR) were exponentiated and converted to % change.