Randomized controlled safety and efficacy trial of 2 vitamin A supplementation schedules in Tanzanian infants

Boniface Idindili, Honarati Masanja, Honorathy Urassa, Wilbert Bunini, Paul van Jaarsveld, John J Aponte, Elizeus Kahigwa, Hassan Mshinda, David Ross, and David M Schellenberg

ABSTRACT

Background: Vitamin A supplementation reduces morbidity and mortality in children living in areas endemic for vitamin A deficiency. Routine vitamin A supplementation usually starts only at age 9 mo, but high rates of illness and mortality are seen in the first months of life.

Objective: The objective of the study was to evaluate the safety and efficacy of vitamin A supplementation at the same time as routine vaccination in infants aged 1–3 mo.

Design: We recruited 780 newborn infants and their mothers to a randomized double-blind controlled trial in Ifakara in southern Tanzania. In one group, mothers received 60 000 μg vitamin A palmitate shortly after delivery, and their infants received 7500 μg at the same time as vaccinations given at ≈1, 2, and 3 mo of age. In the other group, mothers received a second 60 000-μg dose when their infant was aged 1 mo, and their infants received 15 000 μg at the same time as the routine vaccinations. VAD was defined as a modified relative dose-response test result of ≥0.060.

Results: High-dose vitamin A supplementation was well tolerated. The relative risk of VAD at 6 mo in the high-dose group compared with the lower dose group was 0.91 (95% CI: 0.76, 1.09; P = 0.32). Serum retinol and incidence of illness did not differ significantly between the 2 groups. Some vitamin A capsules degraded toward the end of the study.

Conclusions: Doubling the doses of vitamin A to mothers and their young infants is safe but unlikely to reduce short-term morbidity or to substantially enhance the biochemical vitamin A status of infants at age 6 mo. The stability of vitamin A capsules merits further investigation.

KEY WORDS Vitamin A, supplementation, safety, efficacy, Tanzania, infants

INTRODUCTION

There is strong evidence that supplementation with vitamin A from the age of 6 mo reduces morbidity and mortality in areas with endemic vitamin A deficiency (VAD) (1, 2). In practice, one of the main opportunities for supplementing this age group is at the time of measles vaccination; this vaccination is usually given as soon as possible after the infant is 9 mo old. However, because a high proportion of all child deaths occur before the age of 9 mo, the effects of earlier vitamin A supplementation were investigated, with conflicting results (3–7). Two trials in Asia gave ≤50 000 IU vitamin A to infants within the first few days of life and found 22–64% reductions in infant mortality (3, 4). A more recent trial involving 9208 mother-infant pairs in a less vitamin A–deficient population in Zimbabwe showed that giving 400 000 IU vitamin A to mothers and 50 000 IU vitamin A to their infants, within 96 h of birth, did not lower infant mortality [hazard ratio: 1.28 (95% CI: 0.83, 1.98)] (5).

A further study explored the effect of supplementation in slightly older infants and made use of the Expanded Program on Immunization (EPI) of the World Health Organization (WHO). EPI routinely delivers 3 doses of diptheria-pertussis-tetanus/oral polio vaccines (DPT/OPV) (sometimes with hepatitis B or Haemophilus influenzae vaccine or both) to millions of infants aged <6 mo living in VAD-endemic settings. The WHO coordinated a randomized, double-blind, placebo-controlled trial in 9424 infants to investigate the effects of supplementation with 25 000 IU vitamin A at the time of these routine vaccinations at 6, 10, and 14 wk of age. This study, conducted in Ghana, India, and Peru, showed that the intervention was safe but also that it produced only a modest and short-lived improvement in vitamin A status when infants were aged 6 mo (serum retinol ≤0.70 μmol/L; 29.9% of supplemented infants and 37.1% of unsupplemented infants; risk difference: −7.2% (95% CI: −14.3%, −0.2%)). The benefits were no longer apparent on biochemical testing when the infants were 9 mo old (8).

Supplementation of mothers has been shown to improve the vitamin A intake of breastfeeding infants. Four studies, all in Asia (9–12), showed that a single vitamin A dose of 200 000 to

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30 000 IU given to mothers within 3 wk of delivery significantly increased their breast-milk retinol concentration for 3–8 mo. Combined supplementation of mothers shortly after delivery and of their young children may help improve children’s vitamin A status. An assessment of vitamin A requirements in infancy suggested that supplementation of women after delivery with 2 doses of 200 000 IU and of infants with 3 doses of 50 000 IU in the first 6 mo of life would be sufficient to prevent VAD in most infants (13). Metabolic studies also suggested that this dosing regimen was very unlikely to be toxic (14).

We conducted a double-blind, randomized, 2-arm study to compare the safety and efficacy of the previously tested low-dose regimen (25 000 IU at the time of DTP/OPV doses 1, 2, and 3) with those of the suggested high-dose regimen (a second 200 000-IU dose to mothers when their infants were 1 mo old and 50 000 IU to infants at the time of DTP/OPV doses 1, 2, and 3).

SUBJECTS AND METHODS

Setting

The study was conducted in Ifakara, in southern Tanzania, where 56% of infants aged 6 mo–6 y had serum retinol concentrations ≤ 0.70 μmol/L in 1997 (15). The town is served by the St Francis Designated District Hospital (SFDDH) and an adjacent Maternal and Child Health Clinic (MCHC). The MCHC provides routine EPI vaccinations: bacille Calmette-Guerin (BCG) and OPV are given immediately after birth; doses of DPT/OPV are given at age 1, 2, and 3 mo; and measles vaccination is given at age 9 mo. The study area covers a 6-km radius from the SFDDH, which ensures good access to the hospital-based clinical surveillance systems.

Design and intervention

This 2-arm, randomized, double-blind trial was designed to compare the safety and efficacy of 2 vitamin A supplementation regimens. In the low-dose group, mothers received 60 000 μg (200 000 IU) vitamin A (as vitamin A palmitate) at the time of their infant’s BCG vaccination, and their infants received doses of 7500 μg (25 000 IU) at the time of the 3 DPT/OPV vaccinations. In the high-dose group, mothers received an additional dose of 60 000 μg (200 000 IU) when their infant received the first DPT/OPV vaccination, and their infants received 3 doses of 15 000 μg (50 000 IU) at the same time as the 3 DPT/OPV vaccinations. In accordance with national guidelines, all infants received 30 000 μg (100 000 IU) vitamin A at the time of measles vaccination. Vitamin A capsules providing different doses were manufactured by Accucaps Industries (Windsor, Canada) (25 000 and 50 000 IU) and RpScherer (Aprilia, Italy) (200 000 IU), and were supplied to the project by the WHO. All capsules contained a small amount of vitamin E (10 IU in the 25 000-IU and 50 000-IU capsules, 20 IU in the 100 000-IU capsules, and 40 IU in the 200 000-IU capsules). Capsules were transported and stored in lightproof boxes and kept in an air-conditioned room at the trial site.

Subject recruitment and randomization and administration of vitamin A

Recruitment began in April 2002 and lasted until March 2003. Mothers who were resident in the study area and brought their infants to the SFDDH and MCHC for BCG vaccination within 7 d of birth were invited to participate in the trial. Details of the trial were explained, and a series of standardized questions were asked to ensure adequate understanding before seeking written informed consent. Demographic information was documented before the lowest available study number was assigned to mother-infant pairs. Individual randomization was achieved by using a list of study numbers that had been randomly assigned to an intervention arm in blocks of 10, generated by the Data and Safety Monitoring Board.

Mothers were invited to bring their infant to the MCHC for vaccination and vitamin A dosing on a specific date when the infant would be ≈1 mo old. Before each supplementation, project clinical officers examined mothers or their infants (or both), and a standardized morbidity questionnaire was completed. The contents of a vitamin A capsule, labeled only with the participant’s study number, were squeezed into the recipient’s mouth. Mother-infant pairs who had not attended the MCHC within 1 wk of their appointment were visited at home to confirm residence and survival status and, if present, were reminded to attend the study clinic.

All mothers provided written informed consent. The trial received ethical approval from the Ifakara Health Research & Development Centre, the National Medical Research Coordinating Committee of Tanzania, the London School of Hygiene and Tropical Medicine, and the WHO; it was registered on the Current Controlled Trials Register (number ISRCTN 83465214).

Follow-up

The timing of the interventions and follow-up is summarized in Figure 1. Safety was assessed throughout the study by passive case detection with the use of a clinical surveillance system (CSS) and was actively assessed at the participants’ home on each of the 2 d after each supplementation. The primary assessment of efficacy was based on biochemical indicators of vitamin A status, for whose measurement blood samples were collected during cross-sectional surveys when the infants were aged 6 and 9 mo.

Assessment of safety

Mothers were encouraged to bring their infant to a CSS location if the infant experienced any illness at any time during follow-up. The CSS was established at SFDDH in 1994 to document all outpatient attendance of study infants and any pediatric admissions, around the clock, every day of the year. Specially trained clinical officers examined the infants and completed a standardized coded morbidity questionnaire including (but not limited to) such factors as the presence or absence of fever, bulging fontanelle, diarrhea, clinical malaria, and anemia. The CSS thus generated detailed clinical data for the assessment of safety and secondary efficacy endpoints. Necessary laboratory tests and appropriate management were provided for free.

Active clinical surveillance was by fieldworkers who visited the mothers and their infants at home for 2 consecutive days after each maternal and infant supplementation. A standard form was completed to document any symptoms or clinical signs of illness. Study participants found to have potentially serious clinical symptoms or signs were asked to visit the hospital for reexamination by a study clinician.
On 17 April 2018

Assessment of efficacy

Vitamin A status was assessed by analysis of capillary blood samples collected from infants during cross-sectional surveys at age 6 and 9 mo. A fieldworker invited mother-infant pairs to attend these surveys, having documented that the infant was alive and still resident in the study area. Those who did not attend were reinvited each week for a total of 4 wk. Breast milk was collected into 50-mL screw-top centrifuge tubes (Falcon; Becton Dickinson, Franklin Lakes, NJ) for subsequent analysis of retinol content. A single oral dose of 3,4-didehydroretinyl acetate (DRA) was administered to mothers (8.8 μmol) or infants (5.3 μmol) (or both) for assessment of the modified relative dose-response (MRDR test) as described previously (16). Finger-prick capillary blood samples were collected into 500-μL microtainer tubes (Becton Dickinson) 3 h after administration of DRA. Height and weight of infants were also recorded by using standard methods (17).

Laboratory procedures

Blood and milk samples were immediately stored on ice in cool boxes and transported to the laboratory within 2 h. Exposure to light was kept to a minimum. In the base laboratory, serum was immediately separated from whole blood, and aliquots of serum and homogenized milk were stored at −20 °C in 2-mL screw-top freezer tubes (Nunc, Roskilde, Denmark). Frozen samples were transported to the Nutritional Intervention Research Unit of the Medical Research Council (Cape Town, South Africa) for analysis by using standard HPLC procedures (18, 19).

Data management and statistical analysis

Data were double-entered, and daily cross-checking routines were used to detect and correct any discrepancies. A compact disk containing the cleaned and locked database files was exchanged for the treatment randomization code, held by the Data and Safety Monitoring Board. Analyses were conducted, in a masked manner, according to a previously- agreed-upon analytic plan using STATA software (version 7; Stata Corp, College Station, TX). EPINUT software (version 6; EPI Info, Atlanta, GA) was used to generate anthropometric indexes. Before the study, a total of 780 infants (aged 6 mo) were estimated to provide 90% power to detect a relative decrease in the prevalence of VAD from 37% to 25%, at a 5% significance level.

The primary definition of VAD was an MRDR value ≥ 0.060 [ie, the ratio of serum 3,4-didehydroretinol to retinol] 3 h after the DRA dose was administered; VAD was evaluated in all randomly assigned infants who had valid laboratory results. As a secondary endpoint, VAD was also defined as a serum retinol concentration ≤ 0.70 μmol/L; it was considered severe at a concentration ≤ 0.35 μmol/L. These and other categorical variables were compared by using the chi-square test. Mean breast milk and serum retinol concentrations were compared by using the t test. Separate analyses evaluated endpoints at 6 and 9 mo of age. Safety was analyzed by comparing the incidence rates of clinical signs of illnesses and diagnoses documented by the CSS during the 28 d after each supplementation and over all time at risk. Time at risk began from the time of recruitment and continued until whichever occurred first: a clinical episode or censoring due to withdrawal, death, or end of follow-up. Poisson regression models with random effects to take into account between-infant heterogeneity were used to compare incidence rates. The proportions of participants with clinical signs of illnesses and symptoms detected actively within 48 h after each supplementation were also compared. The effect of the intervention on anemia and clinical malaria was evaluated by comparing incidence rates between the 2 groups. Anemia was defined as either mild [a packed cell volume (PCV) < 33%], severe (PCV < 25%), or life-threatening (PCV < 15%). Anthropometric indexes were assessed on all randomly assigned infants at ages 6 and 9 mo by comparing the proportion of infants in each group with a weight-for-age z score (WAZ) < −2, height-for-age z score (HAZ) < −2, and weight-for-height z score (WHZ) < −2.

Quality control

Standardized quality-control procedures were used to ensure consistency in data collection and assessment of adverse events. Previously coded forms with labels bearing identification information were used for all routine contacts. Training in the assessment of clinical signs of illness and monitoring of clinical officers and fieldworkers was continued throughout the trial. Field activities were supervised through regular accompanied and repeated interviews. Vitamin A capsules for quality control were transported at regular intervals to the Institute of Nutritional Research (University of Oslo, Oslo, Norway). These analyses confirmed that the 200 000- and 100 000-IU capsules contained a...
satisfactory amount of retinyl palmitate throughout the trial period. The 25 000-IU capsules also had ≥80% potency up to July 2003, when the last dose of this type of capsule had already been given. The 50 000-IU capsules that were tested were also fully potent up to and through February 2003. However, between February and July 2003, the vitamin A content of the 50 000-IU capsules declined markedly, so that the 33 capsules that were analyzed between August 2003 and January 2004 contained only 1800–23 993 IU (mean: 15 861 IU), or 32% of the expected amount. Taking into consideration the results of similar analyses from a sister trial in Kintampo, Ghana, the investigators, the WHO, and the trial’s Data and Safety Monitoring Board reached a consensus, before the code was broken, that the 50 000-IU capsules in Ifakara degraded rapidly after May 2003. Hence, secondary statistical analyses were performed by excluding the 5% of infants who received doses after 31 May 2003, and tertiary analyses were performed after exclusion of the 30% of infants who received doses after 31 January 2003. In addition, assessments of both efficacy and safety were evaluated by taking into consideration the time elapsed since the date on which recruitment started, by using the likelihood ratio test comparing a model with the effect of the vitamin A with a model in which the cohort had been split into tertiles by date of recruitment, and by looking for an interaction between the date of recruitment tertile and efficacy.

RESULTS

Recruitment of 780 mother-infant pairs took from April 2002 to March 2003, and follow-up was completed by the end of 2003. Completeness of follow-up (Figure 2) and baseline characteristics (Table 1) did not differ significantly between the 2 groups. Infants who did not complete follow-up were older at the time of their first dose, less likely to be exclusively breastfed, and more likely to use ITNs, and they had a slightly lower mean serum retinol than did infants who completed follow-up (data not shown). However, none of the baseline comparators differed significantly between the 2 arms for infants who completed follow-up and contributed to the following analyses.

The incidence of all clinical signs of illnesses, symptoms, and diagnoses detected by the CSS did not differ significantly between the 2 groups. Active case detection documented a significantly (P = 0.01) lower prevalence of fever after the first dose of vitamin A in infants in the high-dose arm ([147 of 308 (48%)] than in the infants in the low-dose arm [183 of 318 (58%); risk ratio: 0.83 (95% CI: 0.71, 0.96)]. No similar pattern was found after other doses, and the prevalence of other signs and symptoms (eg, fits, jaundice, and chest indrawing) was similar after all doses (data not shown).

The proportion of infants with inadequate vitamin A liver stores (MRDR ≥ 0.060) at 6 mo was 43% in the high-dose arm and 47% in the low-dose arm (Table 3). VAD of different severities (defined according to serum retinol and mean serum retinol concentrations) also did not differ significantly between the 2 groups. The incidence of malaria and anemia during follow-up, the cross-sectional prevalence of anemia and malaria, and the anthropometric indexes did not differ significantly between the 2 groups (Table 4). The secondary and tertiary analyses excluding data from infants who received vitamin A supplements after May 31, 2003 and January 31, 2003, respectively, did not significantly change these results (data not shown).

DISCUSSION

This double-blind randomized controlled trial has shown that supplementation of mothers with a second dose of 200 000 IU vitamin A and of their infants with 50 000 IU at the time of routine vaccinations at 1, 2, and 3 mo of age was safe but not more efficacious than a lower-dose regimen. Neither biochemical markers of vitamin A status nor mean breast-milk retinol concentrations differed significantly between infants in the 2 study arms at 6 and 9 mo of age. Clinical endpoints detected passively and actively did not suggest any consistent clinical benefits of the high-dose regimen.

These results are not what had been expected (13), and it is important to consider all possible explanations. The degradation of vitamin A capsules, which was most marked in the 50 000-IU capsules, is a prime concern. However, the analyses excluding infants who may have received lower amounts of vitamin A than intended reassured us that the deterioration of the capsules was not the reason for the lack of efficacy of the intervention. This finding shows the importance of the quality-control assessments that were included in this clinical trial and emphasizes the importance of such checks both in future clinical trials and in routine supplementation programs.

Another possibility is that the measures of VAD used in this study do not accurately reflect the vitamin A status of the very young African infants involved. Young infants are subject to major physiologic changes and a high incidence of infectious disease. The integrity of serum retinol as a measure of VAD may be compromised, for example, by elevated acute phase proteins resulting from infections, which could lead to an underestimate of serum retinol concentration (20) and a reduction of the specificity of serum retinol as a marker of VAD. This reduced specificity would tend to downwardly bias the efficacy estimates based on this measure. However, MRDR tests were used as the primary outcome measure, and they are not affected by subclinical inflammation (21, 22). We collected blood samples for MRDR evaluation 3 h after DRA dosing. It is now clear that samples should be collected ≥4 h after supplementation because earlier sampling reduces the sensitivity of the test. Nevertheless, we found a high prevalence of VAD and, because the test specificity would be relatively high, we conclude that the timing of MRDR blood sample collection is unlikely to explain the lack of efficacy in this study. The gold standard measure of vitamin A status requires the taking of liver tissue for biopsy, which is too invasive an investigation for a trial such as this. The MRDR test is the most appropriate indicator of vitamin A status, and it has been shown to work well in older populations when the test is tightly controlled and care taken with its interpretation. However, its use in field settings involving young African infants merits further investigation.

The study was not placebo controlled because it was not considered ethical to randomly assign some infants to placebo when the low-dose regimen had been shown in a previous trial to have a marginal beneficial effect. Hence, our efficacy estimate may be lower than it would be had this been a placebo-controlled trial. Nevertheless, the prevalence of VAD in the 2 arms was high, and it did not differ significantly between the groups: it seems unlikely that we missed a substantial effect of the high-dose.
FIGURE 2. Trial profile. CSV-1, cross-sectional survey conducted at 6 mo of age; CSV-2, cross-sectional survey conducted at 9 mo of age.
regimen. It also seems unlikely that losses to follow-up biased our efficacy estimate, because there were no differences in baseline characteristics between the groups of infants completing follow-up.

The study benefited from the availability of a CSS, which generated detailed clinical safety and efficacy information. The data generated by this passive case detection system was qualitatively different from that produced by the active case detection used to identify any immediate, short-term side effects of the supplementation. The CSS documented episodes of illness that were severe enough for a child to be brought to the clinic, and it covered the whole 9 mo of follow up for each infant rather

### Table 1
Baseline characteristics of study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>High-dose (n = 390)</th>
<th>Low-dose (n = 390)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male [n (%)]</td>
<td>215 (55)</td>
<td>197 (51)</td>
<td>0.202</td>
</tr>
<tr>
<td>Exclusive breastfeeding at age 1 mo [n (%)]</td>
<td>337 (86)</td>
<td>338 (87)</td>
<td>0.922</td>
</tr>
<tr>
<td>VAD (MRDR ≥ 0.06) before child’s first dose [n (%)]</td>
<td>255 (64)</td>
<td>238 (62)</td>
<td>0.448</td>
</tr>
<tr>
<td>Maternal education [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>61 (16)</td>
<td>71 (18)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>314 (81)</td>
<td>301 (77)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>15 (4)</td>
<td>18 (5)</td>
<td>0.522</td>
</tr>
<tr>
<td>Distance from child’s home to hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st tertile, &lt;0.4 km</td>
<td>112 (34)</td>
<td>124 (37)</td>
<td></td>
</tr>
<tr>
<td>2nd tertile, 0.4–1.0 km</td>
<td>112 (34)</td>
<td>107 (32)</td>
<td></td>
</tr>
<tr>
<td>3rd tertile, &gt;1.0–5.1 km</td>
<td>108 (33)</td>
<td>100 (30)</td>
<td>0.602</td>
</tr>
<tr>
<td>Insecticide-treated net use [n (%)]</td>
<td>334 (86)</td>
<td>337 (87)</td>
<td>0.762</td>
</tr>
<tr>
<td>Age at dose 1 (mo)</td>
<td>1.41 ± 0.06</td>
<td>1.38 ± 0.09</td>
<td>0.684</td>
</tr>
<tr>
<td>Mean birth weight (kg)</td>
<td>2.94 ± 0.46</td>
<td>2.90 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Serum retinol concentration before child’s first dose (μmol/L)</td>
<td>0.67 ± 0.19</td>
<td>0.67 ± 0.18</td>
<td>0.764</td>
</tr>
<tr>
<td>Breast milk retinol after delivery (μmol/L)</td>
<td>4.34 ± 3.01</td>
<td>4.60 ± 3.52</td>
<td>0.344</td>
</tr>
</tbody>
</table>

1 VAD, vitamin A deficiency; MRDR, modified relative dose response.
2 Chi-square test.
3 ± SD (all such values).
4 t Test.

### Table 2
Incidence of signs and symptoms detected by clinical surveillance system from recruitment to end of time at risk

<table>
<thead>
<tr>
<th>Outcome</th>
<th>High dose</th>
<th>Low dose</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Episodes</td>
<td>PYAR</td>
<td>Rate²</td>
</tr>
<tr>
<td>Fever</td>
<td>739</td>
<td>197.8</td>
<td>3.74</td>
</tr>
<tr>
<td>Runny nose</td>
<td>451</td>
<td>208.4</td>
<td>2.16</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>272</td>
<td>222.6</td>
<td>1.22</td>
</tr>
<tr>
<td>Vomiting</td>
<td>120</td>
<td>234.0</td>
<td>0.51</td>
</tr>
<tr>
<td>Coughing</td>
<td>741</td>
<td>186.4</td>
<td>3.98</td>
</tr>
<tr>
<td>Fast breathing</td>
<td>28</td>
<td>240.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Convulsion</td>
<td>2</td>
<td>242.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Pallor</td>
<td>13</td>
<td>241.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Jaundice</td>
<td>0</td>
<td>242.7</td>
<td>0</td>
</tr>
<tr>
<td>Nasal flaring</td>
<td>76</td>
<td>237.1</td>
<td>0.32</td>
</tr>
<tr>
<td>Chest indrawing</td>
<td>164</td>
<td>230.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Pus in ears</td>
<td>19</td>
<td>241.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Bulging fontanelle</td>
<td>0</td>
<td>242.7</td>
<td>0</td>
</tr>
<tr>
<td>Wheezing</td>
<td>230</td>
<td>225.2</td>
<td>1.02</td>
</tr>
<tr>
<td>Stiff neck</td>
<td>5</td>
<td>242.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>8</td>
<td>242.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>95</td>
<td>235.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Oedema</td>
<td>6</td>
<td>242.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Oral candidiasis</td>
<td>1</td>
<td>242.7</td>
<td>0</td>
</tr>
<tr>
<td>Chest cracks</td>
<td>363</td>
<td>215.5</td>
<td>1.68</td>
</tr>
</tbody>
</table>

1 PYAR, person-years at risk; RR, relative risk.
2 Number of episodes per person-year at risk.
than just the 2 d after supplementation. The fact that there was no increase in either the short-term side effects or passively detected illnesses, including bulging fontanelle, provides reassurance that the high-dose regimen was as safe as the low-dose regimen. However, the lack of any decrease in incidence of illnesses detected through the CSS suggests that the high-dose regimen did not confer any clinically important absolute effect on morbidity.

The best approach to improving the vitamin A status of young infants remains unclear. It may be that even higher doses of vitamin A given to infants at the time of routine vaccinations, to mothers of young children, or both would have an effect. Alternatively, given the success of immunization campaigns to increase vitamin A supplementation coverage, it may be useful to assess the effects of these on vitamin A status and to consider supplementing infants aged \(<6\) mo in such campaigns. Another

### TABLE 3
Prevalence of vitamin A deficiency and mean serum retinol concentration at age 6 and 9 mo

<table>
<thead>
<tr>
<th>Outcome</th>
<th>High dose</th>
<th>Low dose</th>
<th>Relative risk (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Age 6 mo</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRDR ≥ 0.06</td>
<td>43 (125/293)²</td>
<td>47 (132/282)</td>
<td>0.91 (0.76, 1.09)</td>
<td>0.32²</td>
</tr>
<tr>
<td>Serum retinol ≤ 0.70 µmol/L</td>
<td>36 (111/306)</td>
<td>41 (121/296)</td>
<td>0.89 (0.73, 1.09)</td>
<td>0.25²</td>
</tr>
<tr>
<td>Serum retinol ≤ 0.35 µmol/L</td>
<td>1.0 (3/306)</td>
<td>2.4 (7/296)</td>
<td>0.41 (0.11, 1.59)</td>
<td>0.18³</td>
</tr>
<tr>
<td>Serum retinol (µmol/L)</td>
<td>0.79 ± 0.24</td>
<td>0.81 ± 0.25</td>
<td>—</td>
<td>0.16³</td>
</tr>
<tr>
<td>Breast milk retinol (µmol/L)</td>
<td>1.82 ± 1.09</td>
<td>1.88 ± 1.09</td>
<td>—</td>
<td>0.49³</td>
</tr>
<tr>
<td><em>Age 9 mo</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRDR ≥ 0.06</td>
<td>41 (113/278)</td>
<td>40 (108/269)</td>
<td>1.01 (0.83, 1.24)</td>
<td>0.91³</td>
</tr>
<tr>
<td>Serum retinol ≤ 0.70 µmol/L</td>
<td>29 (85/292)</td>
<td>32 (90/286)</td>
<td>0.93 (0.72, 1.18)</td>
<td>0.54³</td>
</tr>
<tr>
<td>Serum retinol ≤ 0.35 µmol/L</td>
<td>1.7 (5/292)</td>
<td>1.8 (5/286)</td>
<td>0.98 (0.29, 3.35)</td>
<td>0.97³</td>
</tr>
<tr>
<td>Serum retinol (µmol/L)</td>
<td>0.86 ± 0.32</td>
<td>0.90 ± 0.36</td>
<td>—</td>
<td>0.16³</td>
</tr>
<tr>
<td>Breast milk retinol (µmol/L)</td>
<td>1.86 ± 1.19</td>
<td>1.95 ± 0.97</td>
<td>—</td>
<td>0.33³</td>
</tr>
</tbody>
</table>

¹ MRDR, modified relative dose response.
² %; n/N in parentheses (all such values). The differences in denominators reflect the number of samples in which no estimate of 3,4-didehydroretinol was possible for technical reasons.
³ Chi-square test.
⁴ \(\bar{x} \pm SD\) (all such values).
⁵ \(t\) Test.

### TABLE 4
Anthropometric, malaria, and anemia indexes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>High dose</th>
<th>Low dose</th>
<th>Relative risk (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence at age 6 mo [n/N (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAZ &lt; -2</td>
<td>12/305 (3.9)</td>
<td>15/289 (5.2)</td>
<td>0.76 (0.36, 1.59)</td>
<td>0.46²</td>
</tr>
<tr>
<td>HAZ &lt; -2</td>
<td>29/309 (9.4)</td>
<td>18/297 (6.1)</td>
<td>1.55 (0.88, 2.73)</td>
<td>0.13²</td>
</tr>
<tr>
<td>WHZ &lt; -2</td>
<td>3/304 (1.0)</td>
<td>7/289 (2.4)</td>
<td>0.41 (0.11, 1.56)</td>
<td>0.17²</td>
</tr>
<tr>
<td>Moderate anemia, PCV &lt; 33%</td>
<td>125/311 (40.2)</td>
<td>121/304 (39.8)</td>
<td>1.01 (0.83, 1.23)</td>
<td>0.92²</td>
</tr>
<tr>
<td>Severe anemia, PCV &lt; 25%</td>
<td>3/311 (1.0)</td>
<td>3/304 (1.0)</td>
<td>0.98 (0.20, 4.81)</td>
<td>0.98²</td>
</tr>
<tr>
<td>Prevalence at age 9 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAZ &lt; -2</td>
<td>38/287 (13.2)</td>
<td>43/272 (15.8)</td>
<td>0.84 (0.56, 1.25)</td>
<td>0.39²</td>
</tr>
<tr>
<td>HAZ &lt; -2</td>
<td>39/297 (13.1)</td>
<td>31/279 (11.1)</td>
<td>1.18 (0.76, 1.84)</td>
<td>0.46²</td>
</tr>
<tr>
<td>WHZ &lt; -2</td>
<td>12/288 (4.2)</td>
<td>12/271 (4.4)</td>
<td>0.94 (0.43, 2.06)</td>
<td>0.88³</td>
</tr>
<tr>
<td>Mild anemia, PCV &lt; 33%</td>
<td>87/298 (29.2)</td>
<td>89/288 (30.9)</td>
<td>0.94 (0.74, 1.21)</td>
<td>0.65³</td>
</tr>
<tr>
<td>Severe anemia, PCV &lt; 25%</td>
<td>5/298 (1.7)</td>
<td>5/288 (1.7)</td>
<td>0.97 (0.28, 3.30)</td>
<td>0.96²</td>
</tr>
<tr>
<td>Incidence during all time at risk after recruitment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First malaria episode</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events/PYAR</td>
<td>46/240.4</td>
<td>37/242.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Incidence</td>
<td>0.19</td>
<td>0.15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Incidence rate ratio (95% CI)</td>
<td>1.25 (0.81, 1.93)</td>
<td>—</td>
<td>—</td>
<td>0.309</td>
</tr>
<tr>
<td>First mild anemia episode</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events/PYAR</td>
<td>50/241.2</td>
<td>45/241.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Incidence</td>
<td>0.23</td>
<td>0.19</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Incidence rate ratio (95% CI)</td>
<td>1.11 (0.74, 1.66)</td>
<td>—</td>
<td>—</td>
<td>0.605</td>
</tr>
</tbody>
</table>

¹ WAZ, weight-for-age \(z\) score; HAZ, height-for-age \(z\) score; WHZ, weight-for-height \(z\) score; PCV, packed cell volume; PYAR, person-years at risk.
² Chi-square test.
approach would be to further evaluate the effects of early neo-
natal supplementation.

The high-dose supplementation regimen we evaluated has
already been recommended by the International Vitamin A Con-
sultative Group [IVACG (23)]. Although not previously tested in
a clinical trial, the recommendation was based on theoretical
calculations of its likely effect on infants’ vitamin A status, the
potential for reductions in infant morbidity and mortality, and a
low probability of any important toxic side effects. Our results do
not support this current IVACG recommendation. Instead, our
findings highlight the difficulties inherent in balancing the ur-
gency of reducing high infant mortality rates by implementing
interventions that seem likely to be beneficial against the time
taken to conduct appropriate studies to gain empirical evidence of
their efficacy. However, the EPI offers unparalleled opportu-
nities to deliver health interventions to infants—the age group of
children who suffer the highest mortality rates—and it is essential
that the safety and efficacy of any potential intervention are tested in
carefully conducted clinical trials before implementation.

In conclusion, higher-dose vitamin A supplementation was
well tolerated by mothers and infants, but it failed to improve the
vitamin A status or the incidence of illness episodes in trial par-
ticipants. The stability of the vitamin A capsules is of great
concern and draws attention to the need for quality-assurance
checks of such capsules in clinical trials and routine supplemen-
tation programs. Specific validation of the biochemical indica-
tors of vitamin A status may be useful in very young infants. This
study highlights the potential dangers of basing policy recom-
mandations on theoretical calculations and extrapolation from
existing empirical data.

We are grateful to the infants and families of study participants, to the staff
of the St Francis Designated District Hospital and the Ifakara Maternal and
Child Health Clinic, and to Eldrich Harmse (Nutritional Intervention Re-
search Unit, Medical Research Council, South Africa) for performing the
modified relative dose-response laboratory analyses. We thank the trial’s
Data & Safety Monitoring Board for their monitoring of the safety data.

The authors’ responsibilities were as follows—DR, EK, and DS: study
design; BI and EK: coordinating field work; HM and JJA: statistical analysis;
WB: operated the clinical surveillance system used to capture safety data; Hu
and PviJ: coordinated laboratory activities; HM: assured the availability of
institutional resources in Tanzania and assisted with interpretation of data
and data dissemination to Tanzanian partners; DR: provided support on
technical issues and field activities; DMS: coordinated the research team; and
WB: operated the clinical surveillance system used to capture safety data; HU
and PviJ: coordinated laboratory activities; HM: assured the availability of
institutional resources in Tanzania and assisted with interpretation of data
and data dissemination to Tanzanian partners; DR: provided support on
technical issues and field activities; DMS: coordinated the research team; and
WB and PviJ: wrote the manuscript, with editing and critiques from all authors.
None of the authors had a personal or financial conflict of interest.

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