Short-term effects of vitamin A and antimalarial treatment on erythropoiesis in severely anemic Zanzibari preschool children\textsuperscript{1–3}

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ABSTRACT

Background: The pathophysiology of anemia in coastal East Africa is complex. Impaired erythropoietin production is one possible mechanism. \textit{Plasmodium falciparum} malaria has been found to blunt erythropoietin production, whereas vitamin A stimulates erythropoietin production in vitro.

Objective: We investigated the 72-h effects of vitamin A and the antimalarial drug sulfadoxine pyramethamine (SP) on erythropoietin production in severely anemic (hemoglobin $\leq 70$ g/L) preschool children in Zanzibar, a region of known vitamin A deficiency. We hypothesized that both treatments would stimulate erythropoietin production directly, within 72 h, before a change in hemoglobin would occur.

Design: One hundred forty-one severely anemic children were identified during the baseline assessment of a morbidity substudy of a community-based micronutrient supplementation trial. All severely anemic children were randomly assigned to receive either vitamin A (100 000 or 200 000 IU depending on age) or SP at baseline; 72 h later they received the opposite treatment plus daily hematinic syrup for 90 d. Erythropoietic and parasitic indicators were assessed at baseline and again after 72 h.

Results: After 72 h, SP reduced the malaria parasite density (by 5029 parasites/μL; $P < 0.001$), CRP concentrations (by 10.6 mg/L; $P = 0.001$), and the proportion of children infected with malaria (by 32.4%; $P < 0.001$). Vitamin A reduced CRP (by 9.6 mg/L; $P = 0.011$), serum ferritin (by 18.1 μg/L; $P = 0.042$), and erythropoietin (by 194.7 mIU/mL; $P = 0.011$) concentrations and increased the reticulocyte production index (by 0.40; $P = 0.041$).

Conclusions: Contrary to our hypothesis, vitamin A significantly decreased erythropoietin concentration. The most important effect of both vitamin A and SP was the rapid reduction of inflammation. Vitamin A also mobilized iron from stores and stimulated the production of new erythrocytes. 


KEY WORDS Anemia, vitamin A, malaria, erythropoiesis, erythropoietin, children, inflammation

INTRODUCTION

Nutritional deficiencies and parasitic infections coexist in coastal East Africa, which results in severe multifactorial anemia that mainly affects young children. In one hospital in a malaria hyperendemic region of Tanzania, severe anemia (hemoglobin $< 80$ g/L) was the stated cause of 20% of infant admissions and 27% of infant deaths.\textsuperscript{1} Elucidating the primary physiologic mechanism of anemia is difficult in this setting because nutritional deficiencies, including those of iron and vitamin A, and parasitic infections, including malaria and helminth infection, often coexist in the same child and potentially cause anemia via multiple physiologic pathways.

Although vitamin A deficiency and malaria infection can each cause anemia by several mechanisms, recent evidence suggests that both conditions may affect erythropoietin production by the kidney. Erythropoietin is the hormone secreted by the kidney in response to hypoxia that stimulates marrow production of red blood cells.\textsuperscript{2} Suppression of erythropoietin production is one mechanism by which \textit{Plasmodium falciparum} malaria has been proposed to cause anemia, although studies have had conflicting results. Several have found a blunted erythropoietin response in malaria patients,\textsuperscript{3–5} while others\textsuperscript{6–8} have found that erythropoietin production was normal or up-regulated in children with \textit{P. falciparum} malaria.

Vitamin A stimulates erythropoietin production in vitro. Okano et al\textsuperscript{9} found that retinoic acid up-regulated erythropoietin production 3-fold in HepG2 cells and further found elevated serum erythropoietin concentrations in rats intragastrically infected with retinoic acid. Jelkmann et al\textsuperscript{10} similarly found that vitamin A, but not vitamin C or vitamin E, increased the rate of erythropoietin production in HepG2 and Hep3B cells.

In the present study, we investigated the 72-h effects of vitamin A and the antimalarial sulfadoxine pyramethamine (SP) on erythropoietin production in severely anemic (hemoglobin $\leq 70$ g/L) preschool children living on Pemba Island, Zanzibar, where vitamin A deficiency is severe (RJ Stoltzfus, unpublished observations, 1999) and \textit{P. falciparum} malaria is holoendemic.\textsuperscript{11} All children received vitamin A, SP, iron, and B vitamins, but for the first 72 h, each child was randomly assigned to receive either vitamin A or SP. We believed that the 72-h interval would provide sufficient time to observe changes in erythropoietic indicators that...
were exacted directly by the intervention and not indirectly by a change in hemoglobin (2, 12). In light of repeated findings of blunted erythropoietin production in patients with *P. falciparum* malaria and because of the specific stimulatory effect that vitamin A has on erythropoietin production in vitro, we hypothesized that both treatments would increase erythropoietin production and stimulate erythropoiesis in these severely anemic children.

**SUBJECTS AND METHODS**

**Setting**

Pemba Island, Zanzibar, United Republic of Tanzania is located in the Indian Ocean, just off the Tanzanian coast. Pemba is almost entirely rural, and the economy is sustained largely by the cultivation and exportation of cloves and seaweed. The staple foods of the Pemban diet are rice and cassava, which are usually eaten with a small amount of legumes or green vegetables. Meat and large fish are expensive and are not regularly consumed. Fruit, including mangoes, pineapples, and oranges, are seasonal.

A recent community-based survey found that 38% of children aged 6–71 mo were stunted, and 31.2% were underweight (13). More than 25% of the children in the same survey had a serum retinol concentration <20 μg/dL (RJ Stoltzfus, unpublished observations), which classifies vitamin A deficiency in Pemba as a public health problem (14).

*P. falciparum* malaria is holoendemic to Pemba and is transmitted throughout the year. A peak in malaria parasite density is observed from June to September, following the long rainy season that occurs from April to June (11). The soil-transmitted helminths *Ascaris lumbricoides, Trichuris trichiura*, and hookworm are endemic (15).

**Participants**

Children enrolled in the study were those found to have a hemoglobin concentration ≤ 70 g/L by HemoCue (HemoCue AB, Angelhom Sweden) during the baseline assessment of a morbidity substudy of a larger, ongoing, community-based micronutrient supplementation trial known as the Zanzibar Infant Nutrition Campaign (Figure 1). The substudy assessed morbidity, dietary intake, micronutrient status, and growth. Substudy clinics were selected on the basis of geographic location. Of 2994 children assessed at 10 substudy baseline clinics in the Wete and Mkoani districts between March and September 2002, 225 children aged 4–43 mo were found to be severely anemic. These children did not receive a randomized treatment assignment in
the larger trial but, instead, were enrolled in the severe anemia component (SAC). After an informed consent process conducted in Swahili by a nurse, each mother gave written consent for the child’s participation in the SAC. This study was approved by the institutional review boards of the Johns Hopkins University Bloomberg School of Public Health and the Ministry of Health and Social Welfare in Zanzibar.

Baseline clinic (day 0)

For children enrolled in the SAC, a venous blood sample was drawn into 2 tubes—one was coated with EDTA and the other was uncoated. Whole blood from the EDTA-coated tube was used to prepare a malaria blood film. Axillary temperature was taken, and a temperature of ≥38 °C indicated a fever. Trained staff measured recumbent length (in children aged <24 mo) or standing height (in children aged >24 mo) to the nearest 0.1 cm with a wooden length board (Shorr Productions, Olney, MD). Weight was measured to the nearest 0.1 kg with a digital scale (Seca Scales, Columbia, MD). Both height and weight were measured in duplicate, and mean values were used for anthropometric calculations.

Each child was then randomly assigned to receive orally either vitamin A (6–12 mo: 100 000 IU; >12 mo: 200 000 IU) or SP (500 mg sulfadoxine plus 25 mg pyrimethamine; 6–11 mo: one-half of a tablet; ≥12 mo: three-fourths of a tablet) in accordance with international guidelines (16, 17). Treatment randomization was stratified by sex and age (6–18 mo, 19–36 mo) in blocks of 8. Each mother was instructed to bring her child back to the clinic in 72 h for further assessment and treatment and was given a plastic container to bring a sample of her child’s stool to the next clinic visit.

72-h Clinic (day 3)

When the severely anemic children returned to the clinic after 72 h, another venous blood sample was drawn according to the same protocol that was used at the baseline clinic, and another malaria blood film was prepared. Stool samples were also collected. Children who received vitamin A at baseline received SP, and children who received SP at baseline received vitamin A. A first dose of hematinic syrup (Tonoferon, East India Pharmaceutical Works, Ltd, Calcutta, India) was given orally to each child. Thereafter, the syrup was to be administered daily by the mother for 90 d. Each daily dose contained 25 mg Fe as ferrous sulfate, 100 μg folic acid, and 1 μg vitamin B-12 (16).

Laboratory assessments

Blood and stool samples were placed in a cooler and brought back to the Public Health Laboratory within 4 h. Erythrocyte protoporphyrin was measured in whole blood from the EDTA-coated tubes with the use of a fluorometer (Aviv Biomedical, Lakewood, NJ) and for manual counting of reticulocytes according to the method of Sigma Diagnostics (18).

Malaria blood films prepared in the field were fixed with ethanol and stained with Giemsa (19). The number of malaria parasites per 200 leukocytes was counted on thick films. If no parasites were detected, the microscopist continued until 500 leukocytes were counted. Malaria parasite densities were calculated assuming 8000 leukocytes/μL blood (20).

From the tubes of blood not coated with EDTA, serum was collected, divided, and frozen up to 4 mo at −20 °C until analyzed. Serum concentrations of erythropoietin (R&D Systems, Minneapolis, MN), transferrin receptor (Ramco Laboratories Inc, Stafford, TX), serum ferritin (Ramco Laboratories Inc), and C-reactive protein (CRP; Alpha Diagnostic International, San Antonio, TX) were measured by enzyme-linked immunosorbent assay. During the assay, the identity of each child and the day on which the sample was collected (day 0 or day 3) were concealed. The average CV for the erythropoietin assay was 4.2% (range: 0.93–7.7%), for the TIR assay was 4.4% (range: 0.68–17.1%), for the serum ferritin assay was 6.2% (range: 1.3–22.2%), and for the CRP assay was 8.7% (range: 0.1–19.4%). Stool samples were refrigerated overnight and were analyzed for helminth eggs the following morning with the Kato-Katz technique (19).

Statistical analysis

To accomplish our primary aim of evaluating the 72-h effects of vitamin A and SP on erythropoietin and other hematologic indicators, we compared day 0 and day 3 indicator values within each treatment group with the use of a paired t test. In this paired analysis, all variables were analyzed in their natural units. A 95% CI was calculated around the mean 72-h change for each indicator. A CI that did not include zero represented a statistically significant within-group change.

In a secondary analysis, we used multivariate regression to compare the mean 72-h difference for each indicator between the 2 treatment groups. For this between-group comparison, the mean 72-h difference for each indicator was modeled as the dependent variable, with the baseline value of the indicator and treatment group (vitamin A or SP) as independent variables. A 95% CI was calculated for each adjusted group difference. A CI that did not include zero indicated that the mean 72-h change for a given indicator was statistically significantly different between the 2 treatment groups.

For all analyses, reticulocyte counts were used to calculate a reticulocyte production index for each child as follows: (number of reticulocyte/erythrocytes) × (hematocrit/normal hematocrit) × 100. This index adjusts the reticulocyte count for the degree of anemia (21). A value of 31.5 was considered to be the normal hematocrit value (10.5 g/dL × 3) and was used to normalize the reticulocyte counts in our sample.

For the anthropometric calculations, we defined stunted as a height-for-age z score less than −2 and wasted as a weight-for-height z score as less than −2. Height-for-age and weight-for-height z scores were calculated by using ANTHRO version 3.0 (Centers for Disease Control and Prevention, Atlanta, GA). All other analyses were performed with the use of STATA 6 statistical software (Stata Corp, College Station, TX).

RESULTS

Baseline characteristics

Of the 225 children found to be severely anemic, 141 had sufficient serum on both day 0 and day 3 for a complete paired analysis (Figure 1). None of the characteristics were significantly different between the 2 treatment groups at baseline (Table 1).

Before therapy began, baseline erythropoietin concentrations were well above the normal range of 8–18 mU/mL (22), and TIR concentrations were above the normal upper-limit cutoff of 11 mg/L (23); these findings indicated that erythropoiesis was activated (Table 1). Erythrocyte protoporphyrin was also highly elevated at baseline, well above 90 μmol/mol heme—the upper limit of the normal range (23). CRP concentrations in both groups
CRP, reduce all indicators of malaria infection. Malaria parasite density: vitamin A group (data not shown).

9.1% for weight-for-height score, 57; for reticulocyte production index, 47; for fever, 38°C; and 4.2% for hookworm for the combined group (data not shown).

TABLE 1
Baseline erythropoietic, iron-status, parasitic, and anthropometric indicators by treatment group

<table>
<thead>
<tr>
<th>Indicator</th>
<th>SP (n = 69)</th>
<th>Vitamin A (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (mo)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>17.7 (15.3, 20.2)</td>
<td>16.4 (14.3, 18.6)</td>
</tr>
<tr>
<td>Erythropoietin (mIU/mL)</td>
<td>64.6 (63.1, 66.0)</td>
<td>63.1 (61.5, 64.7)</td>
</tr>
<tr>
<td>Transferrin receptor (mg/L)</td>
<td>21.1 (19.0, 23.2)</td>
<td>19.3 (17.6, 21.0)</td>
</tr>
<tr>
<td>Reticulocyte production index</td>
<td>2.1 (1.4, 2.7)</td>
<td>1.5 (1.2, 1.8)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>21.8 (15.4, 28.3)</td>
<td>23.6 (17.0, 30.1)</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>127.5 (85.0, 170.0)</td>
<td>127.1 (82.5, 171.6)</td>
</tr>
<tr>
<td>Erythrocyte protoporphyrin (µmol/mol heme)</td>
<td>285.5 (253.2, 317.8)</td>
<td>293.7 (251.4, 336.1)</td>
</tr>
<tr>
<td>Malaria parasite density (parasites/µL)</td>
<td>58.28 (3544, 8112)</td>
<td>4737 (3204, 6269)</td>
</tr>
<tr>
<td>Malaria positive (%)</td>
<td>82.4 (73.1, 91.6)</td>
<td>79.2 (69.6, 88.8)</td>
</tr>
<tr>
<td>Malaria infection, ≥5000 parasites/µL (%)</td>
<td>33.8 (22.3, 45.4)</td>
<td>33.3 (22.2, 44.5)</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>26.1 (15.5, 36.7)</td>
<td>15.7 (7.0, 24.5)</td>
</tr>
<tr>
<td>Height-for-age z score</td>
<td>−1.56 ± 1.45⁵</td>
<td>−1.91 ± 1.22</td>
</tr>
<tr>
<td>Weight-for-height z score</td>
<td>−0.64 ± 0.92</td>
<td>−0.66 ± 0.92</td>
</tr>
</tbody>
</table>

¹ SP, sulfadoxine pyramethamine; CRP, C-reactive protein.
² For hemoglobin, n = 68; for reticulocyte production index, n = 39; for CRP, n = 57; for erythrocyte protoporphyrin, n = 61; for malaria indexes, n = 68; for anthropometric measures, n = 54.
³ For hemoglobin, n = 71; for reticulocyte production index, n = 47; for CRP, n = 57; for erythrocyte protoporphyrin, n = 66; for fever, n = 70; for anthropometric measures, n = 60.
⁴ Means; 95% CIs in parentheses (all such values).
⁵ Baseline axillary temperature ≥ 38°C
⁶ x ± SD (all such values).

were more than twice the normal upper limit of 10 mg/L (24). Malaria was prevalent and relatively severe; ≈80% of the children had malaria, ≈33% of whom had ≥5000 parasites/µL. Nearly half (47.4%; 95% CI: 38.1, 56.7) of the children were stunted, and 5.3% (95% CI: 1.1, 9.4) of the children were wasted. The prevalence of helminth infection was low: 2.4% for Ascaris, 9.1% for Trichuris, and 4.2% for hookworm for the combined treatment groups (data not shown).

72-h changes: SP
As we expected, hemoglobin did not change significantly over 72 h in the SP group (Table 2). However, SP did significantly reduce all indicators of malaria infection. Malaria parasite density decreased by >5000 parasites/µL, the proportion of malaria-positive children decreased from >80% to 50%, and the proportion of children with malaria infection of ≥5000 parasites/µL decreased nearly 30%. SP also significantly reduced CRP concentrations by >10 mg/L. Although erythropoietin concentrations decreased slightly in children who received SP at baseline, the reduction was not statistically significant.

72-h Changes: vitamin A
As we predicted, hemoglobin did not change significantly over 72 h in the vitamin A group (Table 2). Although vitamin A did not significantly change any indicator of malaria infection, it did significantly decrease CRP and serum ferritin concentrations. Contrary to our hypothesis, vitamin A significantly decreased erythropoietin concentrations by nearly 200 mIU/mL.

Vitamin A also significantly increased the reticulocyte production index, which indicated the production of new red blood cells in children who received vitamin A.

With the exception of the malaria indexes, there were no statistically significant 72-h differences between the 2 treatment groups for any indicator (Table 2). There was also no significant interaction of treatment and malarial status for any of the indicators measured (data not shown).

DISCUSSION
We investigated the 72-h effects of vitamin A and the antimalarial drug SP on erythropoietin production in severely anemic (hemoglobin ≤ 70 g/L) Zanzibari preschool children. We hypothesized that both treatments would increase erythropoietin production and stimulate erythropoiesis. However, after 72 h, serum erythropoietin was lower in both treatment groups, and this reduction was statistically significant in those children who received vitamin A. The most striking effect of both treatments was the rapid reduction in inflammation. Vitamin A also mobilized stored iron and did so without a significant reduction in the proportion of children with malaria or malaria parasite density.

Effects of SP
In 72 h, SP significantly decreased the mean malaria parasite density and the proportion of children with malaria, which indicated that this antimalarial drug is still effective in a region where the early failure rate of chloroquine treatment was found in one study to be 34.6% (25). The concurrent significant reduction in CRP concentration by SP suggests that malaria was an important contributor to the severe inflammation present in these children. CRP is a positive acute phase response protein that is produced by liver hepatocytes in response to cytokines such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and IL-1 (26, 27). Concentrations of these proinflammatory cytokines, particularly TNF-α, have been found to be elevated in children with P. falciparum infection (7, 28) and have been implicated in causing the dyserthropoiesis and erythrophagocytosis observed in the bone marrow of these children (29, 30). Both TNF-α and IL-1β have also been found to inhibit erythropoietin production in vitro (31, 32).

In contrast with the findings of several studies (3–5) that found blunted erythropoietin production in patients with P. falciparum malaria, we found no evidence that the malaria-induced inflammation present in the children in our study was associated with blunted erythropoietin production because the reduction in malaria parasite titers and CRP caused by SP resulted in a slightly, but not statistically significantly, lower mean erythropoietin concentration.

Our finding of a trend toward lower erythropoietin with parasite clearance is in contrast with the finding of Nussenblatt et al (7), who found that mean erythropoietin increased slightly between baseline and day 3 in hospitalized, P. falciparum–infected children aged 12–48 mo living in a malaria-endemic region of Uganda who were initially treated with chloroquine sulfate. Although the malaria parasite density of the Ugandan children was similar to that of the children in our study at baseline, the mean CRP of the Ugandan children at baseline was more than twice that of the children in our study (44.8 compared with 21.8 mg/L). TNF-α concentrations were also elevated, perhaps indicating the presence of more severe inflammation in these acutely ill children.
Vitamin A effects

Vitamin A rapidly and significantly reduced inflammation, on the basis of CRP concentrations, in 72 h. Vitamin A deficiency in mice has been shown to be associated with increased production of transcripts for the proinflammatory cytokines IL-12 and γ-interferon and with the down-regulated production of transcripts for antiinflammatory cytokines IL-4 and IL-10 (33). Recent in vitro evidence further suggests that vitamin A may reduce the inflammation caused by malaria infection. Human monocytes co-treated with *P. falciparum* malaria and 9-cis-retinoic acid secreted significantly less TNF-α than did control monocytes (34). The authors proposed that this in vitro evidence provides a physiologic explanation for the recent finding by Shankar et al (35) that vitamin A significantly reduced the number of *P. falciparum* febrile episodes by 30% in young children in Papua New Guinea.

In addition to reducing CRP, vitamin A also significantly reduced serum ferritin, an iron storage and positive acute phase response protein whose concentration increases during inflammation regardless of true iron status (36). This decrease in serum ferritin may reflect both reduced inflammation and the mobilization of iron from storage to functional compartments. The significant increase in the reticulocyte production index observed in children who received vitamin A provides functional evidence for such a mobilization of iron and subsequent incorporation of iron into new erythrocytes.

Mobilization of stored iron may also provide an explanation for the observed significant decrease in serum erythropoietin concentrations following supplementation with vitamin A. In both in vitro and in vivo studies, Kling et al (37) found that cellular iron deprivation stimulated erythropoietin production. Using Hep3B cells, Kling et al found that chelation with deferoxamine and blockade of transferrin receptor–mediated iron uptake stimulated erythropoietin production, whereas administration of hemin impaired erythropoietin production. Similarly, in healthy volunteers, high-dose treatment with deferoxamine coupled to hydroxethyl starch significantly increased erythropoietin concentrations (37).

Furthermore, in a study in Uganda, Totin et al (38) found that the log erythropoietin/hemoglobin regression curve was significantly

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**TABLE 2**

72-h Changes in erythropoietic, iron-status, and parasitic indicators in the 2 treatment groups

<table>
<thead>
<tr>
<th>Indicator and group</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Within-group mean change</th>
<th>Adjusted between-group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemoglobin (g/L)</strong></td>
<td></td>
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<tr>
<td>SP (n = 68)</td>
<td>64.6</td>
<td>65.3</td>
<td>0.72 (−1.3, 2.7)</td>
<td>0.50 (−3.6, 2.6)</td>
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<tr>
<td>Vitamin A (n = 71)</td>
<td>63.1</td>
<td>63.7</td>
<td>0.59 (−1.8, 2.9)</td>
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</tr>
<tr>
<td><strong>Malaria parasite density (parasites/μL)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SP (n = 68)</td>
<td>5828</td>
<td>800</td>
<td>−5029 (−7385, −2672)</td>
<td>−2502 (−1142, −3862)</td>
</tr>
<tr>
<td>Vitamin A (n = 72)</td>
<td>4737</td>
<td>3238</td>
<td>−1498 (−3235, 238)</td>
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<tr>
<td><strong>Malaria positive (%)</strong></td>
<td></td>
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<tr>
<td>SP (n = 68)</td>
<td>82.4</td>
<td>50.0</td>
<td>−32.4 (−46.5, −18.2)</td>
<td>−25.6 (−41.1, −10.0)</td>
</tr>
<tr>
<td>Vitamin A (n = 72)</td>
<td>79.2</td>
<td>75.0</td>
<td>−4.2 (−16.9, 8.6)</td>
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<tr>
<td><strong>Malaria density ≥5000 parasites/μL (%)</strong></td>
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<td></td>
</tr>
<tr>
<td>SP (n = 68)</td>
<td>33.8</td>
<td>5.9</td>
<td>−27.9 (−40.4, −15.5)</td>
<td>−17.8 (−29.2, −6.4)</td>
</tr>
<tr>
<td>Vitamin A (n = 72)</td>
<td>33.3</td>
<td>23.6</td>
<td>−9.7 (−22.3, 2.8)</td>
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<tr>
<td><strong>CRP (mg/L)</strong></td>
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<tr>
<td>SP (n = 57)</td>
<td>21.8</td>
<td>11.2</td>
<td>−10.6 (−16.7, −4.4)</td>
<td>−2.6 (−25.7, 7.5)</td>
</tr>
<tr>
<td>Vitamin A (n = 57)</td>
<td>23.6</td>
<td>13.9</td>
<td>−9.6 (−17.0, −2.3)</td>
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<tr>
<td><strong>Serum ferritin (μg/L)</strong></td>
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<tr>
<td>SP (n = 69)</td>
<td>127.5</td>
<td>116.1</td>
<td>−11.5 (−48.2, −25.2)</td>
<td>6.8 (−42.2, 28.7)</td>
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<tr>
<td>Vitamin A (n = 72)</td>
<td>127.1</td>
<td>109.0</td>
<td>−18.1 (−35.5, −0.67)</td>
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<tr>
<td><strong>Erythropoietin (mIU/mL)</strong></td>
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<tr>
<td>SP (n = 69)</td>
<td>498.0</td>
<td>441.1</td>
<td>−56.9 (−215.7, 101.9)</td>
<td>77.9 (−247.6, 91.8)</td>
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<td>Vitamin A (n = 72)</td>
<td>634.3</td>
<td>439.6</td>
<td>−194.7 (−343.9, −45.5)</td>
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<tr>
<td><strong>Transferrin receptor (mg/L)</strong></td>
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<tr>
<td>SP (n = 69)</td>
<td>21.1</td>
<td>22.7</td>
<td>1.7 (−0.8, 4.2)</td>
<td>1.6 (−4.4, 1.2)</td>
</tr>
<tr>
<td>Vitamin A (n = 72)</td>
<td>19.3</td>
<td>19.7</td>
<td>0.41 (−1.0, 1.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Reticulocyte production index</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SP (n = 39)</td>
<td>2.1</td>
<td>2.2</td>
<td>0.11 (−0.44, 0.67)</td>
<td>−0.05 (−0.6, 0.5)</td>
</tr>
<tr>
<td>Vitamin A (n = 47)</td>
<td>1.5</td>
<td>1.9</td>
<td>0.40 (0.02, 0.78)</td>
<td></td>
</tr>
<tr>
<td><strong>Erythropoietin protoporphyrin (μmol/mol heme)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP (n = 61)</td>
<td>285.5</td>
<td>263.1</td>
<td>−22.4 (−55.5, 10.7)</td>
<td>−16.0 (−16.5, 48.6)</td>
</tr>
<tr>
<td>Vitamin A (n = 66)</td>
<td>293.7</td>
<td>286.1</td>
<td>−7.6 (−18.7, 3.5)</td>
<td></td>
</tr>
</tbody>
</table>

1 All values are means; 95% CIs in parentheses. The sample size varied because of insufficient blood samples. SP, sulfadoxine pyrimethamine.
2 72-h difference (day 3 − day 0). CIs that do not contain 0 indicate a statistically significant within-group change.
3 Adjusted difference in mean 72-h change between treatment groups (mean change in SP group − mean change in vitamin A group). The differences were adjusted for the baseline value of each indicator. CI intervals that do not include 0 indicate a statistically significant between-group change.
4 P < 0.05. CIs do not contain 0.
steeper in iron-deficient children than in non-iron-deficient children, which indicated that the erythropoietin response is up-regulated during iron deficiency. These studies perhaps help explain the observed reduction in erythropoietin in our study after treatment with vitamin A. If vitamin A indeed mobilized stored iron, more iron would become available for erythropoiesis, which would lower erythropoietin concentrations.

Such inferences with regard to the direct effects of vitamin A and SP on the indicators we measured, however, are somewhat limited by the ethically dictated omission of a placebo or control group. Our inclusion of only children with extremely low hemoglobin concentrations means that some regression to the mean was likely. However, the short time interval between the 2 measurements (72 h) makes substantial regression to the mean less likely. Indeed, the lack of a significant change in many indicators from day 0 to day 3 (eg, hemoglobin, transferrin receptor, and malaria indexes in the vitamin A group) and the finding that different indicators changed within each treatment group increase the plausibility that the significant changes that we observed were in fact due to the treatments themselves.

In conclusion, in a setting where malnutrition is severe and malaria is holoendemic, treatment of severely anemic children with vitamin A was associated with rapid changes indicative of reduced inflammation, iron mobilization, and stimulated erythropoiesis. Antimalarial treatment reduced circulating malaria parasites and inflammation but did not cause significant changes in other measures. Contrary to our hypothesis, neither vitamin A nor SP stimulated erythropoietin production. Rather, vitamin A significantly reduced erythropoietin, perhaps by increasing cellular iron availability after the observed reduction in inflammation. More research on the role of vitamin A in the treatment of severe anemia is needed, including an investigation of whether the beneficial short-term effects that we observed are sustained.

REFERENCES