Low Dose Daily Iron Supplementation Improves Iron Status and Appetite but Not Anemia, whereas Quarterly Anthelminthic Treatment Improves Growth, Appetite and Anemia in Zanzibari Preschool Children

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ABSTRACT Iron deficiency and helminth infections are two common conditions of children in developing countries. The consequences of helminth infection in young children are not well described, and the efficacy of low dose iron supplementation is not well documented in malaria-endemic settings. A 12-mo randomized, placebo controlled, double-blind trial of 10 mg daily iron and/or mebendazole (500 mg) every 3 mo was conducted in a community-based sample of 459 Zanzibari children age 6–71 mo with hemoglobin > 70 g/L at baseline. The trial was designed to examine treatment effects on growth, anemia and appetite in two age subgroups. Iron did not affect growth retardation, hemoglobin concentration or mild or moderate anemia (hemoglobin < 110 g/L or < 90 g/L, respectively), but iron significantly improved serum ferritin and erythrocyte protoporphyrin. Mebendazole significantly reduced wasting malnutrition, but only in children <30 mo old. The adjusted odds ratios (AORs) for mebendazole in this age group were 0.38 (95% CI: 0.16, 0.90) for weight-for-height less than –1 Z-score and 0.29 (0.09, 0.91) for small arm circumference. In children <24 mo old, mebendazole also reduced moderate anemia (AOR: 0.41, 0.18, 0.94). Both iron and mebendazole improved children’s appetite, according to mothers’ report. In this study, iron’s effect on anemia was limited, likely constrained by infection, inflammation and perhaps other nutrient deficiencies. Mebendazole treatment caused unexpected and significant reductions in wasting malnutrition and anemia in very young children with light infections. We hypothesize that incident helminth infections may stimulate inflammatory immune responses in young children, with deleterious effects on protein metabolism and erythropoiesis. J. Nutr. 134: 348–356, 2004.

KEY WORDS: • anemia • iron • growth • appetite • helminths

Iron deficiency and helminth infections are two of the most common conditions affecting children in less-developed countries. Iron deficiency is estimated to affect about one fifth of the world’s population, and young children are among the most severely affected (1). Evidence is mounting that iron deficiency anemia adversely affects brain development (2,3), with measurable effects on children’s behavior, motor development and cognition (4–6). Severe anemia also contributes to mortality in children (7). For these reasons, the provision of iron-fortified weaning foods or low dose iron supplements is advocated by the WHO and UNICEF (8). However the efficacy of low dose daily iron supplementation according to current international recommendations is not well documented in malaria-endemic settings.

On a global basis the geohelminths, Ascaris lumbricoides, Trichuris trichiura, and the hookworms, are perhaps the most common chronic subclinical infections of childhood, with widespread geographic distribution (9). The health consequences of chronic helminth infections are believed to be proportional to the intensity of infection (10). Therefore, the main programmatic focus of helminth control has been school-age children, who harbor the greatest number of worms. Studies have found that anthelminthic treatment of school-age children has improved iron status, growth and cognition, although study findings were not entirely consistent for any of these outcomes (11). This inconsistency is not surprising, given the diversity of ages, nutritional risk, helminth species and helminth transmission intensities in the populations studied.

The health consequences of helminth infections in preschool-age children have been studied less frequently, because the worm burdens in young children are not as great as in school children and are therefore assumed to be less detrimen-
tal. However, young children are at much greater risk of iron deficiency anemia and growth retardation than their school-age counterparts.

Coastal East Africa is a setting in which helminth infection is intense, and children acquire worms at relatively young ages. Anemia is also prevalent and severe because of iron deficiency, malarial infection and perhaps, helminth infections. In this environment we aimed to achieve the following: 1) measure the efficacy of anthelmintic treatment (mebendazole) every 3 mo and/or low dose daily iron supplementation to improve anemia and growth of preschool children; 2) test whether iron or mebendazole improves appetite of preschool children, based on mother's perception; and 3) compare the effects of iron and mebendazole in younger and older preschool children. Our prior expectation was that mebendazole would confer more benefit on older preschool children because of their greater worm burdens, whereas iron supplementation would benefit both older and younger preschool children. The effects of iron and mebendazole on children's language and motor development were published previously (12).

SUBJECTS AND METHODS

Study sample and randomization. The study was carried out in Kengeja village, Pemba Island, Zanzibar, The United Republic of Tanzania. Kengeja was selected because it is a relatively large rural village, accessible by road, and with access to a spacious primary health care center that became the base of our field activities. Pemba Island is densely populated and mostly rural, with subsistence farming as the main economic activity. Plasmodium falciparum malaria is holoendemic, as are the helminths, A. lumbricoides, T trichiura, Ankylostoma duodenale, and Necator americanus. Nearly all children are breast-fed, but complementary feeding typically begins at a few months of age. By 2 y of age, children are weaned onto the family diet, which consists mainly of rice and cassava, eaten with vegetables and small amounts of fish or meat.

We estimated that 68 children per group would be sufficient to detect a 3 g/L difference in hemoglobin response in two age subgroups, assuming an SD of 9 g/L, α = 0.05 and β = 0.10. Using a factorial design, both interventions could be evaluated with a total sample of 136 children. We multiplied this number by 1.5 to allow for analysis of treatment interactions, doubled this estimate to examine two age groups separately, added 20% for loss to follow-up, and added 20% again, expecting that one fifth of the sample would have to be excluded and treated for severe anemia. Thus, we planned to screen 640 children for enrollment.

A community census was conducted in June 1996 to determine the number of age-eligible children. Based on parental report of age, 684 children 6–59 mo of age in 451 households were identified. Allocation to iron or placebo was carried out by household rather than by child, so that mothers would not be responsible for administering different bottles of supplement to different children within the household. Households were grouped into three strata: those with children <36 mo, those with children ≥ 36 mo, and those with children in both age subgroups. Within these strata, households were randomly assigned to iron or placebo, in blocks of four. Children were randomly allocated to mebendazole or placebo, stratified by iron allocation and household, in blocks of four.

In September 1996, 614 children were assessed and were administered randomized treatments (Fig. 1). Of those children, 76 were lost to follow-up before the 12-mo assessment; thus 538 completed the 12-mo trial. The children lost to follow-up and those who completed the trial were similar on a wide of variety of characteristics, including anthropometry, appetite and hemoglobin. All children with severe anemia (hemoglobin < 70 g/L) at the baseline assessment were treated with a therapeutic course of oral iron plus mebendazole and were excluded from these analyses. This was the sole exclusion criterion.

Consent to participate in the study was first obtained from community leaders and political and health officials. Informed consent was obtained from all parents of children who participated in the study. The study was approved by the Zanzibar Health Research Council, the Committee on Human Research of The Johns Hopkins University School of Public Health and the ethical committee of the WHO.

Interventions. The iron supplement was a liquid preparation containing 20 g/L iron as ferrous sulfate or an identical placebo (ALPharma, Baltimore, MD) in 50-mL opaque bottles with child-proof dropper caps. The placebo was matched in color, packaging and flavor. Each bottle was labeled with one of six batch numbers assigned by ALPharma, three corresponding to iron and three to placebo. At the baseline clinic, mothers were trained how to give a 0.5-mL dose (10 mg iron) to the study child each day. For the 12-mo period between the baseline and follow-up clinics, local staff visited mothers once weekly. At these weekly visits, the worker asked mothers how many days in the past week she gave the supplement to the child, and attempted to address any compliance problems using a problemsolving algorithm. She also replaced nearly empty bottles with full bottles as needed. Mothers who reported giving the supplement fewer than 5 d in a week were reported to the supervisor, who visited the household to further motivate compliance. The potency of the supplement was monitored by the manufacturer, and was found to be 80% potent after 12 mo of storage at room temperature.

In spite of child-proof packaging, two children consumed a large portion of a bottle of iron supplement. These children were monitored closely for toxic sequela, and none were found. They are included in these analyses. After these events, the amount of iron supplement in each bottle was reduced to 25 mL, thereby reducing the total amount of elemental iron in one bottle from 1000 to 500 mg.

Anthelmintic treatment consisted of 500 mg mebendazole as orange-flavored chewable tablets, or identical placebo tablets (Pharmamed, Zejtun, Malta). These pills were packaged in 6 bottles with unique treatment codes, 3 corresponding to mebendazole and 3 to placebo. The first treatment dose was given at the baseline clinic; thereafter study staff made home visits to all children at 3-mo intervals to administer anthelmintic treatments. For children too young to chew, the tablet was crushed, suspended in water, and given by spoon.

At the conclusion of the trial, severely anemic children were treated with therapeutic oral iron, and all children were provided with mebendazole.

Assessment methods. All of the following assessments were carried out at baseline and at the 12-mo follow-up. Parents were asked to bring a small sample of their child's feces to the village clinic, and were given a container for this purpose. Fecal samples were stored in Pemba at −70° C until analysis. Ferritin was assayed using a fluorescence-linked immunoassay (DELFIA System by Wallac, Turku, Finland) and erythrocyte protoporphyrin using a hematofluorometer (Aviv Biomedical, Lakewood, NY). The remaining blood was centrifuged at 1000 × g for 20 min at room temperature and serum was collected. Thick blood films were fixed with ethanol and stained with Giemsa, and malaria parasites were counted using leucocytes. The microscope counted fields containing >200 leucocytes. If <10 parasites were seen, counting continued up to 500 leucocytes. Parasite densities were calculated by assuming 8 × 10⁹ leucocytes/L blood (14). Sera were stored in Pemba at −10°C for up to 3 mo, then transported in liquid nitrogen to Baltimore, MD where they were stored at −70°C until analysis. Ferritin was assayed using a fluorescence-linked immunoassay (DELFIA System by Wallac, Gaithersburg, MD). The mean CV for this assay was 3% (range: 6.0–7.0%).

Weight was measured to the nearest 0.1 kg using a digital scale (Seca, Columbia, MD). Supine length was measured in triplicate to the nearest 0.1 cm using a wooden length board (Shorr Productions, Olney, MD), and the mean value was used for analysis. Mid-upper arm circumference was measured in triplicate and the mean value was...
used for analysis. Age was ascertained by examination of birth certificate or another official document, and was frequently found to differ from the age reported by the parents in the census. Therefore the actual age range of the children was 6–71 mo rather than the 6–59 mo age range selected from the census.

Appetite was assessed by maternal report, a method shown to be valid in Lima, Peru (15). Mothers were asked in the local language, “Lately, how has your child’s appetite been?” The answer was rated on a 5-point scale from very bad to very good. At the follow-up visit, 84% of mothers answered “very good.” Therefore the other four categories were combined to create the variable “poor appetite.” Mothers were asked to recall all foods and drink given to the child in the past 24 h including breast milk, and this was used to classify current breast-feeding status. Mothers were also asked if their child had illness symptoms in the past week (i.e., fever, cough, diarrhea, dysentery, rapid or difficult breathing), using standard and specific terms in Swahili.

**Data analysis.** The effects of mebendazole on wasting malnutrition differed by age and therefore the results are displayed in two age groups, <30 mo and ≥30 mo. The age cut-off of 30 mo was chosen because it most strongly differentiated preintervention relationships between iron status and parasitic infections (16), and it also differentiated mebendazole treatment effects on the outcomes reported here. Although 30 mo was nearly the median age in the entire baseline sample, it was below the median age of the sample reported in this paper because at baseline, severe anemia was much more common in the younger age group (16) and those children are excluded from analysis. Treatment effects on hemoglobin, serum ferritin and erythrocyte protoporphyrin, severe anemia and poor appetite are reported without age stratification because they were similar in the two age groups. Data are presented with children grouped by the two factors in this factorial trial. That is, children who received iron vs. no iron, and children who received mebendazole vs. no mebendazole. This is appropriate because we found no treatment interaction between iron and mebendazole on any outcome. Thus, the results of interest are the main effects of the interventions. However, we present the primary outcomes (i.e., wasting, stunting, anemia and poor appetite) for each treatment cell (i.e., double placebo, iron only, and so on), thus making potential interactions apparent.

Helminth and malaria infection densities are reported as geometric means because of their highly skewed distributions. Children with no parasites are included in these geometric means by setting their values (for helminth eggs/g feces or malaria parasites/µL blood) equal to 1 before making the logarithmic transformation.

Treatment effects on primary outcomes are reported with and without adjustment for baseline values and other important covariates, using the generalized estimating equation approach (17) to account for the intrahousehold clustering introduced by household-level randomization of the iron intervention. Anthropometric Z-scores were computed using Anthro version 3.0 (CDC, Atlanta, GA). We defined underweight as weight-for-age Z-score less than −2, stunting as height-for-age Z-score less than −2, and wasting as weight-for-age less than −2. Wasting was too rare to analyze as an

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**FIGURE 1** Trial profile. R, randomized.
Most characteristics of the children were similar across the treatment groups (Tables 1–4), with height-for-age in the younger age group as an exception. Stunting was more common in children who received iron compared with no iron, and more common in children who received mebendazole compared with no mebendazole (Table 1). This was driven by a high rate of earlier stunting in the children assigned to the iron + mebendazole group. The rates of stunting in children <30 mo old in the four treatment cells were: double placebo, 14/55 (29%); iron only, 14/43 (33%, P = 0.44 compared with double placebo); mebendazole only 9/33 (27%, P = 0.85); iron + mebendazole, 24/49 (49%, P < 0.02).

Compliance. Mothers and children generally liked the iron supplement. According to mothers’ weekly report, >90% of children received at least 70% of the intended doses. The serum ferritin concentrations at the end of the trial confirm that children actually consumed the iron supplement (Table 2). Serum ferritin, which is both an iron storage protein and an acute phase protein, increases with iron consumption or with recent illness. However, at the 12-mo assessment when blood was collected for serum ferritin and other assays, reported rates of recent illness were not higher in the iron-supplemented children. For example, fever in the past week was reported in 27% (63/232) of children who received iron and 37% (85/227) of children who did not (P = 0.09). The mebendazole was also accepted well by mothers and children; >92% of children received at least 3 of the 4 intended doses during the 12-mo study period.

Parasitic infections. Helminth infections were prevalent, and increased strikingly with age (Table 3). Mebendazole was highly effective in controlling A. lumbricoides, somewhat less effective against T. trichiura and least effective against hookworms, especially in older children. The relative effectiveness of a single dose of mebendazole on the three geohelminths in this trial was consistent with other reports in the literature (21,22).

Malaria infection is strongly associated with anaemia in young children in this sample (16). Malaria infection was very common, but its prevalence and intensity were similar across treatment groups, both before and after treatment (data not shown).

### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fe treatment</th>
<th>&lt;30 mo</th>
<th>≥30 mo</th>
<th>MEB treatment</th>
<th>&lt;30 mo</th>
<th>≥30 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mo</td>
<td>No Fe</td>
<td>18.2 ± 6.7</td>
<td>47.0 ± 10.7</td>
<td>No MEB</td>
<td>19.3 ± 6.8</td>
<td>47.1 ± 10.6</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>19.6 ± 7.3</td>
<td>45.9 ± 10.2</td>
<td>MEB</td>
<td>18.6 ± 7.3</td>
<td>45.7 ± 10.2</td>
</tr>
<tr>
<td>Male</td>
<td>No Fe</td>
<td>41/90 (46)</td>
<td>78/137 (57)</td>
<td>No MEB</td>
<td>44/99 (44)</td>
<td>78/140 (56)</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>41/94 (44)</td>
<td>79/138 (57)</td>
<td>MEB</td>
<td>38/85 (45)</td>
<td>79/135 (59)</td>
</tr>
<tr>
<td>WHZ &lt; -1</td>
<td>No Fe</td>
<td>32/88 (36)</td>
<td>45/137 (33)</td>
<td>No MEB</td>
<td>38/98 (39)</td>
<td>42/139 (30)</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>34/93 (37)</td>
<td>41/137 (30)</td>
<td>MEB</td>
<td>28/83 (34)</td>
<td>44/135 (33)</td>
</tr>
<tr>
<td>HAZ &lt; -2</td>
<td>No Fe</td>
<td>23/88 (26)</td>
<td>55/134 (41)</td>
<td>No MEB</td>
<td>28/98 (29)</td>
<td>65/137 (47)</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>38/94 (40)</td>
<td>56/134 (42)</td>
<td>MEB</td>
<td>33/82 (40)</td>
<td>46/131 (35)</td>
</tr>
<tr>
<td>Arm circumference</td>
<td>No Fe</td>
<td>20/90 (22)</td>
<td>15/137 (11)</td>
<td>No MEB</td>
<td>23/99 (23)</td>
<td>17/140 (12)</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>18/94 (19)</td>
<td>19/138 (14)</td>
<td>MEB</td>
<td>15/85 (18)</td>
<td>17/135 (13)</td>
</tr>
<tr>
<td>Poor arm circumference &lt; 5th centile</td>
<td>No Fe</td>
<td>72/90 (80)</td>
<td>89/137 (65)</td>
<td>No MEB</td>
<td>81/98 (83)</td>
<td>93/140 (66)</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>73/93 (78)</td>
<td>92/138 (66)</td>
<td>MEB</td>
<td>64/85 (75)</td>
<td>88/135 (65)</td>
</tr>
</tbody>
</table>

1 Groups are by main effect, i.e., Fe group includes children allocated to Fe only and Fe + MEB; No Fe group includes children allocated to double placebo and MEB only, and so on.
2 Values are means ± SD. Asterisks indicate different from associated No Fe or No MEB group. * P < 0.10; ** P < 0.05, by χ² test.
3 Values are n/group n (%).
4 Abbreviations: HAZ, height-for-age Z-score; WHZ, weight-for-height Z-score.
Hemoglobin and iron status indicators at baseline and 12 mo, by treatment with iron (Fe) and mebendazole (MEB) in a community-based sample of 459 Zanzibari children age 6–71 mo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>352</td>
<td>No Fe 91 ± 12 (227)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No MEB 91 ± 12 (239)</td>
</tr>
<tr>
<td>Serum ferritin, mg/L</td>
<td></td>
<td>No Fe 30.2 (12.2, 74.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No MEB 29.5 (12.6, 68.9)</td>
</tr>
<tr>
<td>Erythrocyte protoporphyrin, μmol/mol heme</td>
<td>No Fe 162 ± 90 (227)</td>
<td>Fe 162 ± 98 (231)</td>
</tr>
</tbody>
</table>

| Table 3 |

Helminth infections at baseline and 12-mo follow-up, by mebendazole (MEB) treatment and age group in a community-based sample of 459 Zanzibari children age 6–71 mo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment group</th>
<th>&lt;30 mo</th>
<th>≥30 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris lumbricoides (positive)</td>
<td>No MEB 33/96 (34)</td>
<td>42/94 (45)</td>
<td>69/133 (52)</td>
</tr>
<tr>
<td></td>
<td>MEB 22/80 (28)</td>
<td>19/82 (23)</td>
<td>68/136 (50)</td>
</tr>
<tr>
<td></td>
<td>RR (CI)</td>
<td>0.52 (0.33, 0.82)</td>
<td>0.56 (0.42, 0.76)</td>
</tr>
<tr>
<td>Ascaris lumbricoides (eggs/g feces)</td>
<td>No MEB 14 (7, 32)</td>
<td>78 (28, 78)</td>
<td>56 (27, 114)</td>
</tr>
<tr>
<td></td>
<td>MEB 9 (4, 21)</td>
<td>7 (3, 16)</td>
<td>69 (33, 143)</td>
</tr>
<tr>
<td></td>
<td>RR (CI)</td>
<td>0.78 (0.60, 1.02)</td>
<td>0.80 (0.69, 0.94)</td>
</tr>
<tr>
<td>Trichuris trichiura (positive)</td>
<td>No MEB 48/96 (50)</td>
<td>60/94 (64)</td>
<td>118/136 (87)</td>
</tr>
<tr>
<td></td>
<td>MEB 36/80 (45)</td>
<td>41/82 (50)</td>
<td>115/133 (86)</td>
</tr>
<tr>
<td></td>
<td>RR (CI)</td>
<td>0.74 (0.56, 0.99)</td>
<td>0.99 (0.86, 1.15)</td>
</tr>
<tr>
<td>Trichuris trichiura (eggs/g feces)</td>
<td>No MEB 20 (11, 40)</td>
<td>191 (82, 442)</td>
<td>227 (139, 330)</td>
</tr>
<tr>
<td></td>
<td>MEB 13 (7, 24)</td>
<td>48 (20, 115)</td>
<td>213 (139, 330)</td>
</tr>
<tr>
<td></td>
<td>RR (CI)</td>
<td>0.74 (0.56, 0.99)</td>
<td>0.99 (0.86, 1.15)</td>
</tr>
<tr>
<td>Hookworms (positive)</td>
<td>No MEB 34/96 (35)</td>
<td>57/94 (61)</td>
<td>92/136 (68)</td>
</tr>
<tr>
<td></td>
<td>MEB 21/80 (28)</td>
<td>37/82 (45)</td>
<td>94/133 (71)</td>
</tr>
<tr>
<td></td>
<td>RR (CI)</td>
<td>0.74 (0.56, 0.99)</td>
<td>0.99 (0.86, 1.15)</td>
</tr>
<tr>
<td>Hookworms (eggs/g feces)</td>
<td>No MEB 7 (4, 12)</td>
<td>118 (52, 265)</td>
<td>57 (34, 94)</td>
</tr>
<tr>
<td></td>
<td>MEB 4 (2, 7)</td>
<td>31 (13, 74)</td>
<td>76 (45, 128)</td>
</tr>
</tbody>
</table>

1 The no MEB group includes double placebo group and Fe only group; MEB group includes MEB only and MEB + Fe groups.
2 Values are positive n/group n (%).
3 Risk ratio (95% CI).
4 Values are geometric means (95% CI) of infected and uninfected children combined; sample size per group corresponds to rows above.

Anthropometry and appetite outcomes. Iron had no significant effects on mild wasting or stunting malnutrition in either age group after adjusting for baseline anthropometric status (Table 4). Mebendazole significantly decreased mild wasting malnutrition in the younger age group only. This was evident in both indicators of thinness. In children <30 mo old, mebendazole decreased mild wasting malnutrition by 62% (95% CI: 10, 84) and prevalence of small arm circumference by 71% (95% CI: 9, 81).

The adjusted odds ratios (AORs) for stunting in children <30 mo old were >1, indicating that there were more stunted children in the treated groups, although the CI included 1 after adjustment for baseline characteristics (Table 4). The pattern of stunting in the four treatment cells was nearly identical to the pattern at baseline (see baseline characteristics, above), with similar rates in the double placebo, iron only and mebendazole only groups, and a higher rate in the iron + mebendazole group. When an iron by mebendazole treatment interaction term was entered in this model, the iron and mebendazole AOR in the younger age group were close to 1 [iron AOR: 0.92, 95% CI: 0.25, 3.36; mebendazole AOR: 1.29, 95% CI: 0.32, 5.15], and the treatment interaction term was positive but not significant (1.66, 95% CI: 0.26, 10.60).

We also explored further the mebendazole treatment effects on mild wasting by age because the effect was highly protective in the younger children, but appeared to be adverse in the older age group. This possible adverse effect of mebendazole was limited to children ≥ 48 mo old, in whom the AOR was 2.88 (95% CI: 0.82, 10.14). In children 24–48 mo old, the AOR was 1.06 (0.47, 2.38).
Both interventions reduced poor appetite in the children, by ~40%, according to mothers’ report. This effect was consistent across age groups; thus the combined results are presented in Table 5.

Anemia outcomes. The prevalence of mild anemia (<110 g/L) remained high (66–87%) at the end of the intervention period in all study groups, and there were no treatment effects on this outcome in either age group. Similarly, there were no treatment effects on mean hemoglobin concentration, despite large and significant improvements in serum ferritin and erythrocyte protoporphyrin (Table 2). The effect of iron supplementation in children <30 mo old was potentially biologically important (mean ± SD for iron vs. no iron, 99 ± 16 vs. 95 ± 19), but this finding was not significant (P = 0.113).

Neither iron nor mebendazole significantly reduced moderate anemia in either age group (Table 5). However the prevalence in mebendazole-treated children was ~30% lower than in children who did not receive mebendazole. We explored this effect further by dividing the sample into three age groups. The protective effect of mebendazole occurred exclusively in children <24 mo old and was significant in this age group (Fig. 2). To determine whether this effect was explained by improved iron status, we examined mebendazole’s effects on within-child changes in serum ferritin in the three age subgroups. A strong age modification was apparent, but in children <24 mo old, mebendazole treatment decreased the serum ferritin increments.

By the end of the trial, most children had aged out of the highest risk period for severe anemia [i.e., under 24 mo (16)], and therefore the prevalence of severe anemia at the end of the trial was low, 5.0% in children who received both placebos (Table 5). The protective effect of iron was large, i.e., a two-thirds reduction, and marginally significant (P = 0.066, Table 5). Mebendazole had no protective effect on severe anemia.

### DISCUSSION

The main finding of this trial was that in children who were <30 mo old at baseline, mebendazole significantly reduced...
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![Graph A](image)

**FIGURE 2** Prevalence of moderate anemia postsupplementation (panel A) and within-child change in serum ferritin concentration pre- to postsupplementation (panel B) by mebendazole treatment and age. Error bars are SEM (n = 41–106). Moderate anemia outcome was adjusted for age, baseline hemoglobin, malaria parasite density and recent fever. Significance levels of mebendazole effects are P = 0.035 in <24 mo, P ≥ 0.40 in other age groups. Serum ferritin outcome was adjusted for age, baseline hemoglobin, baseline serum ferritin, recent fever, and malaria parasite density. Significance levels are P = 0.057 in <24 mo, P ≥ 0.24 in other age groups.

two indices of wasting malnutrition by > 60%. Specifically, low weight-for-height (< −1 Z-score) was reduced by 62% (95% CI: 10, 84) and small arm circumference was reduced by 71% (9, 91). Furthermore, in children <24 mo old at baseline, mebendazole significantly reduced moderate anemia (AOR: 0.41, 95% CI: 0.18, 0.94). Both iron and mebendazole improved children’s appetite, according to mother’s reports.

Iron supplementation did not affect mild or moderate anemia or growth retardation, but reduced the prevalence of severe anemia at the final assessment by 66% (95% CI: −2, 89, P = 0.066). This large effect was marginally significant because of the small number of severely anemic children at the end of the trial, but is consistent with the trial conducted by Menendez et al. (23) in Ifakara, Tanzania. In that trial, low dose daily iron was provided to infants from 2 to 6 mo of age, and it reduced the incidence of severe anemia (hemoglobin < 80 g/L) in the second half of infancy by 29%. Although we treated and excluded severely anemic children at baseline, and the incidence of severe anemia was not a primary outcome of this study, our result is consistent with the conclusion that low dose daily iron supplementation substantially prevents severe anemia in young children exposed to intense transmission of *P. falciparum* malaria.

At the same time, iron supplementation was remarkably ineffective in preventing mild-to-moderate anemia, despite the fact that iron status improved. Although a lack of hemoglobin response to iron supplementation is sometimes taken as evidence that iron deficiency does not exist, we did not draw this conclusion from our data, for three reasons. First, the evidence from multiple iron status indicators measured in these children at baseline (16) confirmed severe iron deficiency. Second, iron treatment had a large preventive effect on severe anemia, and it is implausible that only the severely anemic children were iron deficient. Third, in the same sample of children, iron treatment significantly improved motor and language development (12), which can be explained only by a correction of iron deficiency severe enough to limit the biological systems involved in learning and behavior. Thus, we conclude that other anemia-causing factors such as malaria, vitamin deficiencies, ineffective erythropoiesis due to inflammatory processes, or hemoglobinopathies imposed a ceiling on the children’s hematologic response to iron.

Other trials of iron supplementation to children ≥ 6 mo old in malaria endemic parts of sub-Saharan Africa had greater effects on hemoglobin than did our trial. Trials in the Gambia (24) and Ethiopia (25) used daily iron doses at least three times greater than what we did, and both observed significant increases in mean hemoglobin. A recent trial in Kenya (26) administered twice weekly doses [6 mg/(kg·wk)] of iron to children 6–36 mo old, approximately the weekly dose in our trial, although our supplement was given in smaller daily doses (10 mg/d). They reported a 12.5 g/L significant increase in hemoglobin in the iron supplemented group compared with the placebo group. All three of these trials were carried out in sites with less intense malaria transmission than Zanzibar. We found a 4 g/L increase in hemoglobin concentration in the younger age group, which was not significant. Although we achieved our estimated required sample size in each age subgroup, our actual statistical power was only 67% because the variation in hemoglobin response within children was larger than we had anticipated (16 vs. 9 g/L). Nonetheless, the hemoglobin response to iron supplementation in this study was lower than expected and, from a public health standpoint, disappointing.

The low daily iron dose that we used was equal to the U.S. recommended daily allowance (10 mg/d) (27), and similar to the WHO-recommended dose of 12.5 mg/d for children <24 mo old (8). However, the WHO recommends a higher dose (20–30 mg/d) for children 2–5 y of age, and it is possible that a higher iron dose would have yielded more benefit in Pemban children. Our dose was intentionally kept at dietary levels
children during the same period are acquiring first time helminth infections, which we speculate may induce proinflammatory mediators that are detrimental to protein metabolism (33,34), appetite (35,36) and erythropoiesis (37,38). This hypothesis is consistent with the observation that mebendazole decreased increments of serum ferritin (an acute phase protein) with the same differential age response. However, it is also possible that mebendazole acted directly on the intestinal microflora or intestinal immune function through pathways that do not involve worms.

Our assessment of appetite yielded interesting findings, because mothers reported greater improvements in children’s appetites after treatment with iron or mebendazole. Because this study was randomized and double-blind, we conclude that some real change occurred in mother-child feeding interactions due to the interventions. However, our appetite question was not validated against actual intakes, and it is clear from the distribution of responses that mothers did not fully appreciate the severity of the anemia or other experiences interviewing Pemban mothers, we found that they are averse to reporting that anything is “bad” about their children. Future studies of appetite in this setting will require development of more informative methods.

Several other randomized trials of anthelminthic treatment in preschool children were reported. These trials employed a variety of drugs and treatment schedules, included children of various ages with different degrees of nutritional risk, and had divergent results. Studies in Tanzania (39), Myanmar (40) and India (41) reported benefits to growth, studies in Bangladesh (42,43), Ethiopia (44), Guatemala (45) and Brazil (46) reported no benefit to growth, and one study in Zaire (47) reported poorer growth in children treated for worms. It seems likely that there are certain conditions in which anthelminthic treatment can improve growth of children, but further research is required to elucidate those conditions. It is noteworthy that nearly all other randomized trials were conducted in populations affected mainly by ascariasis. It is possible that the worm species being treated affects the growth benefit from treatment, and that the large benefit to young children in our study was due to the presence of Trichuris or hookworm infection. It is also remarkable that all four of the positive trials (including ours) reported benefits in ponderal growth, but only one reported a benefit to linear growth (40). In our study, the youngest children benefited exclusively from mebendazole, and many other trials did not include children <2 y old. Indeed, if we had not designed this study to look for differing effects by age subgroup, we would have missed the important benefits to toddlers.

In summary, we observed unexpected benefits of anthelminthic treatment to very young African children. Iron supplementation programs currently targeted to school-age children should be expanded to include young children.

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LITERATURE CITED


