Relation between the response to iron supplementation and sickle cell hemoglobin phenotype in preschool children in western Kenya

Dianne J Terlouw, Meghna R Desai, Kathleen A Wannemuehler, Simon K Kariuki, Christine M Pfeiffer, Piet A Kager, Ya Ping Shi, and Feiko O ter Kuile

ABSTRACT

Background: Iron supplementation has been associated with greater susceptibility to malaria and lower hematologic responses in pregnant Gambian women with sickle cell trait (HbAS) than in similar women with the normal (HbAA) phenotype. It is not known whether a similar interaction exists in children.

Objective: Our aim was to determine the influence of the HbAS phenotype on hematologic responses and malaria after iron supplementation in anemic (hemoglobin: 70–109 g/L) children aged 2–35 mo.

Design: We conducted a double-blind, randomized, placebo-controlled trial (HbAS, n = 115; HbAA, n = 408) of intermittent preventive treatment with sulfadoxine pyrimethamine (IPT-SP) at 4 and 8 wk and daily supervised iron for 12 wk.

Results: The mean difference in hemoglobin concentrations at 12 wk between children assigned iron and placebo iron, after adjustment for the effect of IPT-SP, was 9.1 g/L (95% CI: 6.4, 11.8) and 8.2 g/L (4.0, 12.4) in HbAA and HbAS children, respectively (P for interaction = 0.68). Although malaria parasitemia and clinical malaria occurred more often in HbAS children in the iron group than in those in the placebo iron group, this difference was not significant; incidence rate ratios were 1.23 (95% CI: 0.64, 2.34) and 1.41 (0.39, 5.00), respectively. The corresponding incidence rate ratios in HbAA children in the same groups were 1.07 (95% CI: 0.77, 1.48) and 0.59 (0.35, 1.01), respectively. The corresponding interactions between the effects of iron and hemoglobin phenotype were not significant.

Conclusions: There was no evidence for a clinically relevant modification by the hemoglobin S phenotype of the effects of iron supplementation in the treatment of mild anemia. The benefits of iron supplementation are likely to outweigh possible risks associated with malaria in children with the HbAA or HbAS phenotype.

KEY WORDS Iron supplementation, sickle cell hemoglobin, hemoglobin, malaria, children, Africa

INTRODUCTION

Anemia (hemoglobin <110 g/L) has a prevalence of 50–75% among preschool children in sub-Saharan Africa (1, 2), and it is predominantly caused by iron deficiency and malaria (3, 4). Despite the well-recognized public health burden of anemia and the beneficial effect of iron supplementation on hematologic status (5–7), the use of iron supplementation for treatment of anemia in sub-Saharan Africa remains a topic of debate (5, 6, 8, 9). Whereas iron deficiency causes a number of biochemical abnormalities and impaired cell-mediated immunity with increased susceptibility to infections (10–12), concerns have also been raised that iron therapy exacerbates infections—in particular, malaria (5, 6, 9). A meta-analysis of 13 clinical trials showed that the hematologic benefits of iron supplementation outweighed the resulting significant increase in malaria parasitemia and nonsignificant increase in the risk of clinical malaria (8). The International Nutritional Anemia Consultative Group reaffirmed in 1999 that iron supplementation should be pursued in the context of an integrated strategy for the prevention and treatment of anemia in malaria-endemic areas (6).

A remaining concern is whether the sickle cell trait phenotype, as well as other hemoglobinopathies that offer protection against malaria, modifies the effect of iron supplementation (5, 6, 9). It has been suggested that, in populations with a high prevalence of hemoglobinopathies—depending on the type of hemoglobinopathy and phenotype involved—a potential deleterious effect of iron on malaria might be either masked by the protective effect in

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carriers or aggravated by carriers’ loss of their preexisting protective effect and thus becoming predisposed to malaria (13).

The effect of sickle cell trait on the response to iron supplementation was as part of a placebo-controlled trial of daily oral iron supplementation in multigravid women in The Gambia. In contrast to the observed beneficial effect of iron on hemoglobin concentrations and birth weight in women with the normal phenotype (HbAA), iron supplementation in women with the sickle cell trait (HbAS) resulted in lower hemoglobin concentrations and birth weights. HbAS women who were assigned to the iron group were also at an increased risk of placental malaria, whereas HbAA women were not (14). To our knowledge, no other studies have addressed this potential interaction between iron supplementation and the hemoglobin S (HbS) phenotype.

In Asembo Bay, an area of intense perennial malaria transmission on the shores of Lake Victoria in western Kenya, the sickle cell trait offers significant protection from severe malaria morbidity and mortality in children aged 2–16 mo (15). We previously conducted a randomized, placebo-controlled, treatment study among anemic preschool children in this area to compare the efficacy of therapy with 12 wk of daily supervised iron supplementation with or without intermittent (at 4 and 8 wk) preventive treatment (IPT) with sulfadoxine pyrimethamine (SP; IPT with SP, IPT-SP) in improving hemoglobin concentrations. The effect of the treatments on the risk of malaria was also assessed. The methods and results of that study were reported in detail elsewhere (16). Iron supplementation alone was associated with marked hemoglobin improvements, without increased risk of malaria. The IPT-SP approximately halved the incidence of malaria parasitemia, and the combination of IPT-SP and iron supplementation was most effective in the treatment of mild anemia. A secondary objective of this trial was to determine the risks and benefits of iron supplementation on the sickle cell phenotype. The results of this subanalysis are presented here.

SUBJECTS AND METHODS

Study population

This study was conducted between April 1999 and November 2000 in 15 villages in the Asembo Bay area, Bondo district, northeast of Lake Victoria in Nyanza Province in western Kenya. The study site was previously described in detail (17, 18). Briefly, the population is ethnically homogeneous; >95% are members of the Luo tribe. Malaria transmission is intense and perennial (19), but recent area-wide deployment of insecticide-treated bednets (ITNs) substantially reduced the transmission pressure (20–22). All study participants in this anemia treatment study were living in households using ITNs that were routinely treated with insecticide every 6 mo. Despite the high prevalence of anemia, most local clinics in this area lack standardized guidelines for the use of iron supplementation in the treatment of anemia or prevention of anemia (16). Clinic-based surveillance showed that iron supplementation was not routinely given to children with mild or moderate anemia and was prescribed for only 12% of the children <5 y of age with clinically diagnosed severe anemia, whereas all children received presumptive antimalarial treatment (23).

The study was approved by the institutional ethical review boards of the Kenya Medical Research Institute (Nairobi), the Centers for Disease Control and Prevention (Atlanta), and the Academic Medical Center at the University of Amsterdam. Written informed consent was obtained from the parent or guardian of each individual participant.

Study design

The study design and subject recruitment were described in detail elsewhere (16). In brief, the study was a double-blind, randomized placebo-controlled anemia treatment trial with 2 × 2 factorial design. All resident children from the 15 villages aged 2–36 mo for whom consent was obtained were screened. Children were eligible for enrollment if they had mild anemia (hemoglobin concentration 70–109 g/L); were aparasitemic or had parasite counts < 20 000/mm3; had received no iron supplementation, SP treatment, or blood transfusion within the last 2 wk; and did not have the HbSS phenotype. Children with the HbSS phenotype were referred to a local pediatrician for further counseling and management free of charge. Children were assigned sequentially (by MRD) to 1 of 4 treatment groups, by using balanced block randomization (8 children per block) and a random number listing generated independently before the study (by FOK). On enrollment all children were given a single presumptive treatment dose of SP. The subsequent 4 study treatment regimens included IPT-SP at 4 and 8 wk plus daily oral iron for 12 wk, placebo (for SP) at 4 and 8 wk plus daily iron, SP at 4 and 8 wk plus daily placebo (for iron), and placebo SP at 4 and 8 wk plus daily placebo iron. The iron (40 mg ferrous sulfate/mL, 27.5% elemental iron, syrup) was dosed according to body weight, and the target dose was 3 mg/kg (24). All children were visited daily at their homes by our study staff, who supervised the administration of all iron doses. The parents or guardians received instructions in the local language with regard to expected side effects, safety issues, and the correct dose of iron supplementation to be delivered. All iron bottles were labeled with subject identifiers and dosing instructions.

Follow-up

In addition to the daily visits by staff to administer the iron or iron placebo, each child was visited at home every 2 wk, at which time a standardized morbidity questionnaire was completed and the axillary temperature recorded. At every other visit (ie, every 4 wk), a finger-prick or heel-prick blood sample (250–500 μL) was taken to determine hemoglobin concentration and the presence of malaria parasites. Subjects had access to free outpatient and inpatient care in the local hospital and 3 dedicated health facilities. Details of all clinic visits were monitored by using continuous, passive malaria case detection. Children with uncomplicated symptomatic malaria (axillary temperature ≥ 37.5 °C with any malaria parasitemia) and those without fever but with high-density parasitemia (> 5000/mm3) detected at follow-up visits or through passive surveillance were treated with supervised oral quinine (10 mg/kg 3 times per d for 7 d). Children diagnosed during active or passive follow-up visits with severe malaria, severe anemia (hemoglobin concentrations < 50 g/L), or other severe disease requiring hospitalization were referred to the local hospital for further management.

Laboratory methods

An ACT 10 Coulter Counter (Coulter Company, Miami) was used to determine the hemoglobin and mean corpuscular volume.
The CV for hemoglobin was \( \leq 2.0\% \) (range: 12.0–18.0 g/dL). The CV for the MCV analyses was \( \leq 3.0\% \) (range: 80–100 fL). Slides were Giemsa stained, and plasmodium parasites were counted until a simultaneous count of 300 leukocytes was reached. Slides were considered negative if no asexual parasites were found in 200 high-power ocular fields of the thick smear. Parasite densities were expressed per microliter on the basis of individual white blood cell counts determined with the use of a Coulter Counter. For subjects from whom white blood cell counts were not available, the average of all white blood cell counts during the intervention period \((10.9 \times 10^3)\) was used. Hemoglobin phenotype was assessed in fresh blood samples by hemoglobin electrophoresis of a red blood cell hemolysate on cellulose acetate plates (Helena Laboratories, Beaumont, TX). Serum samples were stored at \(-70^\circ\mathrm{C}\), subsequently transported on dry ice to the Centers for Disease Control and Prevention laboratories in Atlanta, and kept in liquid nitrogen until further assays of serum transferrin receptor (sTfR) concentrations. The \( sTfR \) concentrations on enrollment and at 12 wk were determined 10–15 mo after sample collection for the first 154 children enrolled in the study \((16)\) by using a commercially available enzyme immunoassay (Ramco Laboratories Inc, Stafford, TX). The CV for the normal control (range: 4.29–7.42%) was 14%. All \( sTfR \) assays were determined in duplicate, and all assays with a CV of \( >15\% \) were repeated.

Definitions

Malaria parasitemia was defined as the presence of asexual blood-stage malaria parasites of any plasmodium species and density that were detectable in a thick blood smear by using microscopy. Clinical malaria was defined as an axillary temperature \( \geq 37.5^\circ\mathrm{C}\) in the presence of any malaria parasitemia \((25)\). Adequate hematologic recovery was defined as the absence of anemia (ie, hemoglobin concentration \( \geq 110\ \text{g/L} \)) by 12 wk after enrollment. Microcythemia was defined as an MCV below an age-specific cutoff: \( < 70\ \text{fL} \) for subjects aged 0–5 mo, \( < 73\ \text{fL} \) for those aged 6–11 mo, and \( < 75\ \text{fL} \) for those aged \( \geq 12\ \text{mo} \) \((26)\).

Statistical methods

All statistical analyses were conducted with the use of SAS (Statistical Application Software Institute, version 8.0, Cary, NC) on a per-protocol basis. The effect on mean hemoglobin concentrations, mean MCVs, and geometric mean parasite densities (GMPDs) was analyzed by using a linear model with repeated measures. Reported \( P \) values and CIs were adjusted for within-subject correlation. Missing data were assumed to be missing at random. Poisson regression models were used to estimate the incidence rate ratio (IRR) of malaria parasitemia and clinical malaria between the iron and placebo iron groups. Adjusted hazard ratios obtained from Cox proportional-hazards models were used to compare the rate of adequate hematologic recovery between groups. Geometric mean \( sTfR \) concentrations were analyzed by using analysis of variance.

The presence of a significant interaction between HbS phenotype and iron (or any other two- and three-factor interactions tested) was assessed in each model by using the \(-2 \log \text{likelihood ratio test} \). Covariates that were determined to be significantly associated with the outcome but that did not cause confounding or effect modification or have a marked effect on the precision of the point estimates associated with treatment group were omitted from the final model. Models were controlled for IPT, age, baseline hemoglobin (centered at the overall mean of \( 94.7\ \text{g/L} \)), and baseline presence of parasitemia. Age was categorized as being less and more than 12 mo.

RESULTS

A total of 753 children were screened between April and November 1999; it was initially determined that 554 children fulfilled the enrollment criteria, and they were randomly assigned to 1 of the 4 treatment groups \((16)\). The hemoglobin phenotype was successfully determined in 531 children, 8 of whom were excluded from enrollment because hemoglobin electrophoresis showed that they had the HbSS phenotype. In all, 523 children with known HbS phenotype were enrolled \((\text{Figure 1})\). This group involved 505 households; 18 households contributed 2 siblings.

Of these 523 children, 22% carried the sickle cell trait, and 55.6% and 89.7% of the children were \(< 1\) and \(< 2\ y\) old, respectively. Hemoglobin and \( sTfR \) did not differ significantly among the 4 exposure groups, but mean MCVs were lower in both HbAS groups \((\text{Table 1})\). Parasitemia prevalence was not different, but HbAS children had lower GMPDs than did HbAA children \((P = 0.009)\). Nearly all \((95.5\%)\) of the 133 malaria infections identified at baseline were caused by \( P.\ falciparum \), and the rest were due either to mixed infections of \( P.\ falciparum \) with either \( P.\ malariae \) or \( P.\ ovale \) \((3.8\%)\) or to monoinfection with \( P.\ ovale \) \((0.8\%)\). The average daily dose of iron, based on the enrollment weight of the child, was \( 3.8\ \text{mg/kg} \) \((\text{range: 2.8–5.0 mg/kg})\).

Forty children \((8\%)\) did not complete the 12-wk follow-up period: 7 died, 1 was removed when the caretaker withdrew consent, and 32 moved or left the area for an indefinite period of time. These children were equally divided among the study groups \((\text{Figure 1})\). Baseline characteristics of children lost to follow-up did not differ significantly from those of the 483 children who were successfully followed for 12 wk.

Hematologic effects of iron supplementation

There was no evidence of interaction among hemoglobin phenotype, iron supplementation, and IPT-SP with respect to the effect on hemoglobin concentrations at 12 wk \((\text{three-factor interaction}: P = 0.16)\). Similarly, no evidence was found for two-factor interactions between iron and IPT-SP \((P = 0.74)\), hemoglobin phenotype and IPT-SP \((P = 0.48)\), or hemoglobin phenotype and age \((P = 0.97)\) with respect to hemoglobin concentrations. The effects of iron on mean hemoglobin concentrations and adequate hematologic recovery by 12 wk did not differ significantly between HbAA and HbAS children, in the 2 IPT-SP groups \((\text{data not shown})\) or in models that assessed the effect of iron exclusively after adjustment for the effect of IPT-SP \((\text{Table 2})\). The main effect of HbS phenotype on mean hemoglobin concentrations was not significant \((P = 0.32)\); mean hemoglobin concentrations at 12 wk were \( 106\ \text{g/L} \) \((95\%\ CI: 104, 107)\) and \( 104\ \text{g/L} \) \((95\%\ CI: 102, 107)\) in HbAA and HbAS children, respectively. The effects of iron on MCV or on \( sTfR \) concentrations did not differ significantly between HbAA and HbAS children \((\text{Table 2})\).
Effects of iron supplementation on malaria

Malaria smear results were available from 473 children on a monthly basis; 214 smears from 143 of these children were found to be positive. Of the children who were parasitemic at baseline, 41.4% were parasitemic at the first 4-wk follow-up, despite the treatment dose of SP received by all children at baseline. Of the children who were aparasitemic at baseline, 8.7% had become parasitemic at their first 4-wk follow-up. These prevalences were similar in all groups. As was observed for hematologic outcomes, none of the three- and two-factor interaction terms examined above were found to be significant (Table 3). Over the entire 12-wk treatment period, the IRRs of malaria parasitemia among the children receiving iron and those receiving placebo iron were 1.07 (95% CI: 0.77, 1.48) in the HbAA children and 1.23 (95% CI: 0.64, 2.34) in the HbAS children (Table 3). These IRRs did not differ significantly between HbAA and HbAS children, as indicated by the two-factor interaction term P value of 0.70, in the overall model or in each IPT-SP group (IPT-SP placebo, P = 0.48; IPT-SP, P = 0.76) (Table 3). No significant difference (P = 0.40) was found in incidence rates of malaria parasitemia between HbAA and HbAS children, after adjustment for the effect of iron and IPT-SP, over the 12-wk treatment period (IRR: 0.85; 95% CI: 0.60, 1.23).

During this 12-wk treatment period, only 73 episodes of clinical malaria were observed in 64 children. The IRRs for clinical malaria among the iron groups and the placebo iron groups were 0.59 (95% CI: 0.35, 1.01) in the HbAA children and 1.41 (95% CI: 0.39, 5.00) in the HbAS children. This difference between the HbAA and HbAS children was not significant (P = 0.20), though the number of observed events was small (Table 3). GMPDs were higher in HbAS children receiving iron than in those receiving placebo iron, but the difference was not significant (P = 0.57). Among HbAA children, the densities were significantly (P = 0.03) lower in the iron group than in the placebo iron group. Similarly, the difference between the effects of iron on GMPDs in HbAS and HbAA children did not differ significantly (P for interaction = 0.14; Table 3).

DISCUSSION

The primary objective of this randomized, placebo-controlled trial was to determine the risks and benefits of 12 wk of daily iron supplementation and administration at 4 and 8 wk of SP in the treatment of mild-to-moderate anemia in children aged 2–35 mo living in an area of perennial malaria transmission with widespread use of bednets (16). This trial also provided an opportunity to evaluate the potential interaction between the effects of hemoglobin phenotype and iron supplementation on hematologic responses and malaria. In contrast with previous observations by others in multigravid pregnant women in The Gambia (14), we found in the present study no indication that the effect of iron supplementation on hemoglobin, MCV, or sTFR was dependent on the hemoglobin phenotype. Children with the HbAS phenotype benefited as much from iron supplementation as did children with the HbAA phenotype.

The previous study in The Gambia, which was conducted in an area with low to intermediate, highly seasonal malaria transmission, indicated a possible increased risk of placental malaria in HbAS women supplemented with iron (P = 0.06; 14). The current study was conducted in an area of Kenya with perennial and previously intense transmission, but it should be noted that the widespread use of ITNs (> 70% of households regularly deployed bednets) is estimated to have reduced areawide malaria transmission by ≥90% to an annual transmission rate not much higher than that observed in The Gambia. We did not find that HbAS children assigned to the iron supplementation group had a
significantly greater risk of malaria parasitemia at the 4-wk follow-up visits than did children receiving placebo iron (IRR: 1.23; 95% CI: 0.64, 2.34). However, the risk estimate was similar to the slightly but significantly greater risk reported in the meta-analysis of 13 previous iron supplementation trials (IRR: 1.17; 95% CI: 1.08, 1.25; 8). In addition, there was no significant effect

### TABLE 1

Baseline characteristics of 523 enrolled children by hemoglobin S phenotype and iron supplementation group

<table>
<thead>
<tr>
<th></th>
<th>HbAA</th>
<th>HbAS</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n&lt;sup&gt;2&lt;/sup&gt;</td>
<td>n&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Placebo</td>
</tr>
<tr>
<td>Male [n (%)]</td>
<td>203/205</td>
<td>60/55</td>
<td>31 (51.7)</td>
</tr>
<tr>
<td>Age (mo)</td>
<td>203/205</td>
<td>60/55</td>
<td>11.7 ± 2.2</td>
</tr>
<tr>
<td>SES ranked</td>
<td>193/195</td>
<td>58/52</td>
<td>50.7 (27.0–76.0)</td>
</tr>
<tr>
<td>Weight-for-age z score</td>
<td>198/194</td>
<td>59/53</td>
<td>−0.37 ± 1.37</td>
</tr>
<tr>
<td>Height-for-age z score</td>
<td>172/173</td>
<td>51/48</td>
<td>−1.10 ± 1.36</td>
</tr>
<tr>
<td>Weight-for-height z score</td>
<td>167/170</td>
<td>50/48</td>
<td>0.48 ± 1.42</td>
</tr>
<tr>
<td>MUAC-for-age z score</td>
<td>119/122</td>
<td>40/38</td>
<td>−0.78 ± 1.08</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>203/205</td>
<td>60/55</td>
<td>95.2 ± 10.2</td>
</tr>
<tr>
<td>Hemoglobin &lt; 80 g/L [n (%)]</td>
<td>203/205</td>
<td>60/55</td>
<td>20 (9.9)</td>
</tr>
<tr>
<td>Any parasitemia [n (%)]</td>
<td>200/199</td>
<td>59/55</td>
<td>46 (23.0)</td>
</tr>
<tr>
<td>GMMD&lt;sup&gt;6&lt;/sup&gt;</td>
<td>46/55</td>
<td>19/13</td>
<td>2962 (1628, 5389)</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>112/173</td>
<td>47/43</td>
<td>75.5 ± 7.2</td>
</tr>
<tr>
<td>Microcythemia [n (%)]</td>
<td>152/147</td>
<td>47/43</td>
<td>91.4 (61.8)</td>
</tr>
<tr>
<td>sTfR (µg/mL)</td>
<td>64/56</td>
<td>15/19</td>
<td>7.7 (2.4–12.7)</td>
</tr>
<tr>
<td>sTfR &lt; 11.2 µg/mL [n (%)]</td>
<td>64/56</td>
<td>15/19</td>
<td>22 (34.4)</td>
</tr>
</tbody>
</table>

<sup>1</sup> HbAA, normal hemoglobin phenotype; HbAS, sickle cell trait; SES, socioeconomic status; GMMD, geometric mean parasite densities; MCV, mean corpuscular volume; MUAC, midupper arm circumference; sTfR, serum transferrin receptor.

<sup>2</sup> Numbers in the iron/placebo iron groups.

<sup>3</sup> Comparison of the iron group with the placebo iron group in the HbAA children.

<sup>4</sup> Comparison of the iron group with the placebo iron group in the HbAS children.

<sup>5</sup> The main effect of phenotype (comparing the pooled HbAS groups and the pooled HbAA groups).

<sup>6</sup> ± SD.

<sup>7</sup> Median; interquartile range in parentheses.

<sup>8</sup> Includes positive smears only.

<sup>9</sup> ±; 95% CI in parentheses.

<sup>10</sup> Fisher’s exact test.

### TABLE 2

Hematologic response by 12 wk to iron or placebo iron supplementation

<table>
<thead>
<tr>
<th></th>
<th>HbAA</th>
<th>HbAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n&lt;sub&gt;iron/place&lt;/sub&gt;</td>
<td>n&lt;sub&gt;iron/place&lt;/sub&gt;</td>
</tr>
<tr>
<td>Hemoglobin ≥ 110 g/L [%]</td>
<td>187/185</td>
<td>56/51</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>187/185</td>
<td>56/51</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>168/167</td>
<td>56/51</td>
</tr>
<tr>
<td>sTfR (µg/mL)</td>
<td>52/42</td>
<td>11/17</td>
</tr>
</tbody>
</table>

<sup>1</sup> HbAA, normal hemoglobin phenotype; HbAS, sickle cell trait; MCV, mean corpuscular volume; sTfR, serum transferrin receptor; IPT-SP, intermittent preventive treatment with sulfadoxine pyrimethamine.

<sup>2</sup> Iron/placebo iron groups.

<sup>3</sup> P for the interaction term assessing whether the effect of iron supplementation on hemoglobin, MCV, or sTfR is dependent on hemoglobin phenotype.

<sup>4</sup> Cox proportional hazards analysis of adequate hematologic recovery during the 12-wk intervention, adjusted for age, enrollment hemoglobin concentration, IPT-SP, and presence of parasitemia.

<sup>5</sup> Least-square means at week 12 obtained from a linear model with repeated measures, adjusted for age, enrollment hemoglobin (centered at the overall mean of 94.7 g/L), IPT-SP, and presence of parasitemia.

<sup>6</sup> Least-squares means at 12 wk obtained from a linear model with repeated measures adjusted for age, enrollment MCV, IPT-SP, and presence of parasitemia.

<sup>7</sup> Difference in geometric means is expressed as a ratio. sTfR concentrations were determined in the first 154 children enrolled. Least-squares geometric means at 12 wk were obtained from a linear model, adjusted for IPT-SP and baseline sTfR.
TABLE 3
Crude incidence per 1000 child-months of malaria parasitemia and clinical malaria and geometric mean parasite densities (GMPDs) during 12 wk of iron or placebo supplementation

<table>
<thead>
<tr>
<th></th>
<th>HbAA</th>
<th>HbAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n²</td>
<td>Iron Placebo IRR (95% CI)</td>
</tr>
<tr>
<td>Any parasitemia</td>
<td>184/182</td>
<td>157 (68/434)</td>
</tr>
<tr>
<td>No IPT-SP</td>
<td>87/93</td>
<td>173 (36/208)</td>
</tr>
<tr>
<td>IPT-SP</td>
<td>97/89</td>
<td>142 (32/226)</td>
</tr>
</tbody>
</table>

Any clinical malaria | 186/186 | 49 (21/426) | 0.59 | 0.35 | 1.01 | — | 50/51 | 50 (6/120) | 3.34 | 0.39 | 3.05 |
| No IPT-SP      | 88/95                 | 54 (11/203)          | 0.53 | 0.26 | 1.08 | — | 29/25 | 82 (6/73) | 1.91 | 0.50 | 3.46 |
| IPT-SP         | 98/91                 | 45 (10/223)          | 0.68 | 0.31 | 1.50 | — | 21/26 | 0 (0/47) | 4.43 | 3.06 | — |

GMPD⁶ | 80/94 | 1664 (1035, 2673) | — | 0.03 | 23/24 | 2842 (1157, 6985) | 1986 (827, 4769) | — | 0.57 | 0.14 |
| No IPT-SP | 41/56 | 1292 (347, 4675) | — | 0.24 | 16/12 | 2176 (771, 6141) | 2040 (626, 6649) | — | 0.93 | 0.53 |
| IPT-SP | 39/38 | 1067 (508, 2241) | 0.58 | 0.33 | 1.07 | — | 70/72 | 1835 (849, 7578) | 0.25 | 0.08 |

¹ HbAA, normal hemoglobin phenotype; HbAS, sickle cell trait; IRR, incidence rate ratio; IPT-SP, intermittent preventive treatment with sulfadoxine pyrimethamine. Incidence rate expressed as number events per 1000 child-months (1 person month = 28 d).
² Iron/placebo groups.
³ P for the interaction term assessing whether the effect of iron supplementation on malaria indexes is dependent on hemoglobin phenotype. The presence of other two- and three-factor interactions was assessed by using the −2 log likelihood ratio test for each of the outcomes presented. None of these interaction terms were found to be significant in the model for any parasitemia (HbS phenotype and iron and IPT-SP, P = 0.53; HbS phenotype and IPT-SP, P = 0.84; iron and IPT-SP, P = 0.56), any clinical malaria (model with HbS phenotype and iron and IPT-SP interaction term would not converge; HbS phenotype and IPT-SP, P = 0.45; iron and IPT-SP, P = 0.62), or GMPD (HbS phenotype and iron and IPT-SP, P = 0.26; HbS phenotype and IPT-SP, P = 0.15; iron and IPT-SP, P = 0.90).
⁴ Estimated incidence rate ratios (IRRs) were obtained from Poisson regression models, adjusted for age, presence of parasitemia at enrollment, and IPT-SP.
⁵ Incidence rate; the no. of events/the contributed person-time (in mo) in parentheses.
⁶ The GMDPs of all positive smears recorded during the 12-wk intervention (a child could contribute several positive smears). Least-squares geometric means were obtained from a linear model with repeated measures, adjusted for age and IPT-SP.
⁷ x; 95% CI in parentheses.

The results of this study suggest that HbAS children receiving iron supplementation experienced higher GMPDs and a greater incidence of clinical malaria than did HbAS children not receiving iron. The number of clinical malaria attacks in the HbAS children, however, was very small, and further studies would clearly be needed to verify our observation. Nevertheless, even if the fourfold greater risk of clinical malaria associated with iron that was observed in HbAS children who were not protected by intermittent SP were also found in larger studies, the incidence in these children would still be similar to or lower than that observed among HbAA children without iron supplementation, regardless of IPT-SP status. Similarly, GMPDs in HbAS children supplemented with iron never exceeded those in HbAA children assigned to the placebo iron group. Thus, this possible interaction between the effects of iron and HbS phenotype on malaria is unlikely to outweigh the substantial health benefits associated with the improvement in hemoglobin concentrations achieved with iron supplementation.

The studied population is representative of mildly and moderately anemic young children from this area of Kenya. Most (78.4%) of the children were 2–18 mo old, which is the age group likely to be at high risk of iron deficiency and most vulnerable to the potential adverse effects of malaria in this area (15, 27). This is also the age range during which sickle cell trait carriers are at a significantly lower risk of all-cause mortality, clinical malaria, and severe malarial anemia than are children without the trait (15). The hemoglobin phenotype was determined for 95.8% of the enrolled children, and loss to follow-up by 3 mo was <10%. Potential confounders of the association between iron supplementation and malaria were equally distributed between groups, and parasite prevalence at baseline was controlled for, which makes it less likely that residual bias could explain our findings. Iron intake was measured through observation of the administration of daily doses by study staff. The daily dose of iron was relatively high (x: 3.8 mg · kg⁻¹ · d⁻¹) and exceeded the 2.2 mg · kg⁻¹ · d⁻¹ dose above which iron supplementation has been associated with a small increase in the risk of malaria (5, 8). Thus, these results are likely to be representative of the effect of iron supplementation in the treatment of mild to moderate anemia in this age group in areas with similar, moderate malaria transmission (due to the widespread use of ITNs).

Menendez et al (14) hypothesized that iron may interfere with the genotype-specific nonimmunologic mechanisms that protect against malaria, causing pregnant women with sickle cell trait to be more susceptible to malaria and thus anemia. Our study does not provide conclusive evidence to support or refute this hypothesis, but it differs from the study of Menendez et al in that the HbAS children studied benefited from iron supplementation, whereas the pregnant Gambian women with HbAS whom they studied did not. More studies may be required to determine whether the risk of malaria associated with iron supplementation is indeed modified by the HbS phenotype in pregnant women or in children as well as in those not protected by ITNs. This determination may not require prospective studies because retrospective analysis of available data from random-
ized controlled trials of iron supplementation may provide further insight.

Young children are an important target group for the control of iron deficiency anemia in sub-Saharan Africa, where 10–30% of the population carries the sickle cell trait (28). The present study, conducted in an area with widespread use of ITNs, found that iron supplementation of children with mild anemia was efficacious in increasing hemoglobin concentrations regardless of a child’s HbS phenotype. Available evidence suggests that the benefits of iron supplementation in the treatment of anemia are likely to outweigh any adverse effects caused by an increased risk of malaria in children with either normal hemoglobin or the sickle cell trait.

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