In Ivorian school-age children, infection with hookworm does not reduce dietary iron absorption or systemic iron utilization, whereas afebrile *Plasmodium falciparum* infection reduces iron absorption by half1–4

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**ABSTRACT**

**Background:** In sub-Saharan Africa, parasitic diseases and low bioavailable iron intake are major causes of anemia. Anemia results from inflammation, iron recycling and decreasing dietary iron absorption. Hookworm, *Plasmodium*, and *Schistosoma* infections contribute to anemia, but their influence on dietary iron absorption and recycling is unknown.

**Objective:** The objective was to measure inflammation biomarkers, hepcidin, iron absorption, and utilization pre- and posttreatment in children with afebrile malaria, hookworm, and *Schistosoma haematobium* infection.

**Design:** Ivorian children aged 11–17 y with afebrile *Plasmodium falciparum* (n = 17), hookworm (n = 16), or *S. haematobium* infection (n = 8) consumed a syrup containing 3 mg 57Fe as ferrous sulfate and received an intravenous infusion of 50 μg 58Fe as ferrous citrate. Children were treated for their respective infection, and the iron studies were repeated 4 wk later. Iron and inflammation biomarkers and hepcidin were measured.

**Results:** Geometric mean iron absorptions in the afebrile malaria and hookworm groups were 12.9% and 32.2% (P < 0.001) before treatment and 23.6% and 30.0% (P = 0.113) after treatment, respectively. Treatment of afebrile malaria reduced iron absorption (P < 0.001) and serum hepcidin (P = 0.004) and improved iron absorption (P = 0.003). Treatment of hookworm infection neither affected inflammation biomarkers nor altered iron absorption. Similarly, there was a lack of treatment effects in the *S. haematobium*-infected group; however, the small sample size limits conclusions. Geometric mean iron utilization ranged between 79.1% and 88.0% in the afebrile malaria and hookworm groups with no significant differences pre- and posttreatment.

**Conclusions:** In school-age children, hookworm infection does not produce inflammation or increase serum hepcidin, and it does not influence iron metabolism or the etiology of anemia in parasitic infections. This trial was registered at clinicaltrials.gov as NCT01163877.

**Keywords** Côte d’Ivoire, afebrile *Plasmodium*, hookworm, *Schistosoma haematobium*, inflammation, hepcidin, stable isotopes, iron, absorption, anemia

**INTRODUCTION**

Anemia affects about 1.7 billion people worldwide, mostly women and children in developing countries. The etiology of anemia is multifactorial; major contributing factors are iron deficiency, infection, other micronutrient deficiencies, and inherited hemoglobinopathies (1, 2). In sub-Saharan Africa, infectious diseases combined with iron deficiency are likely the major causes of anemia (3). Indeed, parasitic diseases are common and may result in chronic inflammation and blood loss. Although blood loss causes anemia through iron depletion, inflammation causes anemia by blocking iron recycling from senescent red blood cells and by reducing iron absorption (4). Inflammatory cytokines, such as IL-6, induce hepcidin production in the liver (5), reducing iron efflux from enterocytes, hepatocytes, and macrophages. As a result, less iron is available for erythropoiesis (6). Hepcidin-mediated inhibition of dietary iron absorption may limit the efficacy of iron supplementation and food fortification (7, 8). A deeper understanding of iron

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metabolism during parasitic infections could lead to improved strategies to control anemia in endemic areas.

*Plasmodium falciparum*, hookworm, and *Schistosoma* are common infections in Côte d’Ivoire (9–11). Asymptomatic *Plasmodium* parasitemia causes low-grade inflammation and inhibits dietary iron absorption but not iron utilization in young women (12). Febrile malaria decreases iron bioavailability in children (13). The effects of hookworm and *Schistosoma* infections on iron metabolism are poorly understood. Hookworm infection causes intestinal blood loss (14), the intensity of hookworm infection is negatively associated with hemoglobin concentration (15), and anthelmintic treatment modestly decreases the prevalence of anemia (16). *Schistosoma haematobium* infection causes iron losses through hematuria (17–19), and a meta-analysis found that treatment of *S. haematobium* significantly improves hemoglobin and reduces anemia (20). However, whether these infections incite intense local inflammation or a low-grade systemic inflammatory response that could increase hepcidin and affect iron absorption or utilization is unclear.

It has been estimated that the prevalence of iron deficiency anemia in school-age children in Côte d’Ivoire is 25% (21). The aim of the present study was to investigate iron absorption and utilization in Ivorian children aged 11–17 y before and after treatment of afebrile *P. falciparum*, hookworm, and *S. haematobium* infections by using stable iron isotope techniques (22). Our hypothesis was that all 3 infections would incite inflammation and increase circulating hepcidin and thereby reduce iron absorption.

**SUBJECTS AND METHODS**

**Ethical considerations**

The study was approved by the ethics committees of ETH Zurich (reference no. EK 2009-N-19), Basel (EKBB; reference no. 252/09), and Côte d’Ivoire (reference no. 1086 MSHP/CNER). Village authorities, school directors and teachers, participants, and parents/legal guardians of children were informed about the purpose, procedures, and potential risks and benefits of the study. Written informed consent was obtained from participants and their parents/legal guardians. All participating children received treatment with a 2-wk delay, and this issue had been approved by the ethics committees. Regarding malaria, only children with asymptomatic *P. falciparum* infection were included. Those children who developed clinical malaria were immediately treated, following national guidelines. Children with a hemoglobin concentration <8 g/dL were excluded and referred for treatment. Only villages in close proximity to the hospital in Taabo were selected for the screening to ensure timely and high-quality medical supervision.

**Study area and sample size calculation**

The study was carried out in the Taabo region of south-central Côte d’Ivoire. Malaria transmission is perennial, with seasonal peaks during the long rainy season from April to July and during a short rainy season from September to October. Hookworm and *Schistosoma* infections are coendemic in the Taabo area (23–26).

We based our sample size calculation on previous iron isotope studies from our laboratory (12, 27). In brief, assuming an 80% power to detect a 40% difference in iron absorption in subjects before and after intervention, an SD of the log-transformed data of 0.228, and a type I error rate of 5%, we needed to include 18 children in each infection group. Allowing for a dropout of 10%, we aimed to recruit 20 children in each group.

**Recruitment and screening methods**

For recruitment, we applied the following inclusion criteria: 1) age 11–17 y, 2) body weight >30 kg, 3) no chronic medical conditions as judged by the study physician, 4) no intake of vitamin/mineral supplements from at least 2 wk before the study until the last blood collection, and 5) hemoglobin >8.0 g/dL. For females, we applied 2 additional criteria: 1) no menstruation between stable iron isotope administration and venipuncture 2 wk later and 2) not pregnant (confirmed by pregnancy test). Within each study group, additional inclusion criteria were 1) infection with the parasite of interest (as detailed below) and 2) no other apparent coinfections. Other inclusion criteria were as follows: axillary temperature <37.5°C and *P. falciparum* parasitemia >1500 parasites/μL of blood for the afebrile malaria group, >1000 eggs/1 g of stool (28) for the hookworm group, and >25 eggs/10 mL of urine (28) for the *S. haematobium* group.

We screened 1554 subjects aged 11–17 y in the primary and secondary schools of Taabo Cité and in primary schools of 5 neighboring villages: Kötissou, N’Dénou, Sahoua, Taabo Village, and Zoukoussi (26). We enrolled 20 subjects singly infected with *P. falciparum*. Because we were unable to recruit the desired 20 subjects with a single infection of either hookworm or *S. haematobium*, 5 subjects coinfected at screening with *P. falciparum* were included in the *S. haematobium* group, and 4 subjects coinfected at screening with *P. falciparum* were included in the hookworm group. These subjects were treated for malaria with artesunate-amodiaquine (Maphar, Sanofi-Aventis) at least 4 wk prior to inclusion and were determined to be free of *Plasmodium* before the first stable iron isotope administration. Because of interruptions in the screening process caused by the political unrest in Côte d’Ivoire from October 2010 through April 2011 (29), we were able to enroll only 16 children into the hookworm group and 8 children into the *S. haematobium* group.

We measured hemoglobin concentration on a finger-prick blood sample by using a HemoCue 301 (HemoCue AB). *P. falciparum* infection was also determined on a finger-prick blood sample by using a rapid diagnostic test (ICT ML01 malaria Pf kit; ICT Diagnostics). In addition, we prepared thick and thin blood films on microscope slides that were stained with Giemsa and examined quantitatively for *Plasmodium* species (30). We collected spot urine and stool samples in separate plastic containers. For helminth diagnosis, we prepared duplicate Kato-Katz thick smears of each stool sample, and these we examined under a microscope by an experienced laboratory technician for hookworm and *Schistosoma mansoni* eggs (31). We determined *S. haematobium* infection with the urine filtration method (32).

**Iron absorption and utilization**

We measured erythrocyte incorporation of $^{57}$Fe from an orally administered syrup and erythrocyte incorporation of $^{58}$Fe from an intravenous dose for each subject. The erythrocyte incorporation
of iron from the intravenous dose represents the utilization of absorbed iron, and we used this together with the erythrocyte incorporation of the orally administered iron to calculate iron absorption (33, 34). The stable iron isotopes (elemental $^{57}$Fe, 97.82% isotopic enrichment and $^{58}$Fe, 99.49% isotopic enrichment) were purchased from Chemgas. We prepared $^{57}$FeSO$_4$ from isotopically enriched $^{57}$Fe by dissolution in 0.1 mol H$_2$SO$_4$/L. The solution was kept refrigerated (5°C) in Teflon containers. Iron citrate, enriched with $^{58}$Fe, was prepared for intravenous infusion from elemental $^{58}$Fe (35). The solution (1 g) was divided in ampoules containing 50 μg iron, sterilized, and checked for pyrogens and sterility at the pharmacy of the University Hospital Zurich.

We first measured iron absorption and utilization while the subjects were infected; we then treated the infection and reassessed iron absorption and utilization in subjects free of infection (Figure 1). On designated day 1, the subjects arrived at the hospital after an overnight fast, and we measured anthropometric data (body weight to the nearest 0.5 kg; height to the nearest 0.5 cm) and axillary temperature (to the nearest 0.1°C). The subjects completed a questionnaire relating to previous infections and treatments (recall period: 4 wk), and girls were tested for pregnancy with a rapid test (TestPack +hCG Urine; Alere GmbH). Subsequently, we obtained a first venous blood sample (7 mL). The subjects then consumed the test drink (200 mL syrup consisting of 40 g locally produced orange-flavored syrup concentrate, free of vitamin C, made up to 200 mg with water) containing 3 mg $^{57}$Fe in the form of FeSO$_4$. One hour later, we added 1 mL of a 22-mmol/L sodium citrate solution containing 50 μg $^{58}$Fe as iron citrate to a 100-mL saline solution (B. Braun Medical AG) and infused this solution intravenously over 20 min (35–37). We based the rate of intravenous administration of iron on the estimated 2-μg/min plasma appearance of iron normally absorbed from the gastrointestinal tract (37). There was no intake of food and fluids for 3 h after the test drink; we then gave all subjects a standardized local meal of rice with a vegetable and fish sauce. Fourteen days later (day 15), we collected a second venous blood sample, and subjects began treatment for their respective infection.

We treated subjects in the afebrile malaria group with artesunate-amodiaquine (Maphar; Sanofi-Aventis) over 3 d (day 15 evening until day 18 morning), according to national guidelines. After treatment, subjects continued a prophylactic treatment with sulfadoxine-pyrimethamine (Fansidar; Roche Pharma), given twice directly after treatment (day 19) and 2 wk later (day 33). In addition, children received anthelmintic treatment (day 19) against hookworm (albendazole, 400 mg; GlaxoSmithKline) and Schistosoma infection (praziquantel, 40 mg/kg; Bayer). We treated subjects in the hookworm group with albendazole (day 15) and also gave sulfadoxine-pyrimethamine as prophylaxis against malaria (days 15 and 29) and praziquantel against Schistosoma infection (day 15). We treated subjects in the S. haematobium group with praziquantel (day 15) and also gave prophylaxis with sulfadoxine-pyrimethamine against malaria (days 15 and 29) and albendazole against hookworm (day 15).

To confirm the absence of infection, we collected a finger-prick blood sample and stool and urine samples that were analyzed on day 28. On day 29, we collected the third venous blood sample (this served as the new baseline blood sample) and administered the test drink labeled with 3 mg $^{57}$Fe as FeSO$_4$ and the $^{58}$Fe as iron citrate infusion, as before. We then collected venous blood samples after 14 d (day 43) (Figure 1).

**Laboratory analysis**

From each participant, we collected 4 venous blood samples (on days 1, 15, 29, and 43) that were subjected to isotopic
composition, iron status indicators, hemoglobin, and inflammation biomarkers. Blood samples were drawn into 10-mL heparin-coated tubes (Becton Dickinson AG). We measured hemoglobin in whole blood on the day of collection by using a HemoCue 301 (HemoCue AB). We separated plasma and froze it at −20°C pending further analysis. We analyzed α1-acid glycoprotein (AGP), 5 C-reactive protein (CRP), plasma ferritin (PF), and soluble transferrin receptor (TIR) concentrations with a simple sandwich ELISA technique described elsewhere (38). We measured IL-6 concentrations by using an IMMULITE 2000 (Siemens Healthcare Diagnostics AG), with a lower limit of detection of 2 pg/mL. We measured hepcidin by a combination of weak cation-exchange chromatography and time-of-flight mass spectrometry as described elsewhere, with a lower limit of detection of 0.5 nmol/L (39). We considered CRP >5 mg/L or AGP concentrations >1 g/L elevated. Iron deficiency was defined as TIR >8.3 mg/L because of the known effects of inflammation during infection on PF.

For the measurement of the stable iron isotopes, we first mineralized the whole blood by microwave digestion. We separated iron by anion-exchange chromatography, followed by a solvent-solvent extraction step into diethylether (33). We measured the iron isotopic composition by using negative thermal ionization–mass spectrometry on a magnetic sector field mass spectrometer (Finnigan MAT 262; Thermo Finnigan), equipped with a multicollector system for detection of simultaneous iron beams (33). We quantified circulating iron for each subject from the estimated blood volume based on height and weight and from the hemoglobin concentration (40). We calculated fractional incorporation of oral and intravenous labels according to isotopic dilution principles (33). The absorption of the oral iron was calculated by dividing the percentage of erythrocyte incorporation of the oral dose by the fractional erythrocyte incorporation of the intravenous dose.

Statistical analysis

Data were entered twice into Microsoft Excel 2010 (Microsoft Corporation) and checked by using EpiInfo version 3.4.1 (CDC). The statistical approach was a per-protocol analysis. We analyzed fractional iron absorption, iron utilization (erythrocyte incorporation of intravenous dose), iron status parameters, and inflammation biomarkers with STATA version 11.1 (StataCorp). For within-group comparison, we log-transformed absorption and utilization values, tested the differences between days 1 and 29 for normal distribution, and applied a paired test for testing the null hypothesis (differences between days 1 and 29 equal to zero). For between-group comparisons, we tested the absorption and utilization values with the nonparametric Kruskal-Wallis test because normal distribution between groups was difficult to confirm due to low subject numbers, which also limited the accuracy of an ANOVA analysis. If the Kruskal-Wallis test was significant, we compared the values pairwise with Wilcoxon’s rank-sum test (post hoc) by using Bonferroni-adjusted P values.

We tested blood parameters with the Shapiro-Wilk test for normality and prepared Q-Q plots. We present normally distributed data as means with corresponding SDs. Data that are not normally distributed are presented as medians with 25th and 75th percentiles. We tested significance between means of normally distributed data within subjects with a 1-factor ANOVA. We log-transformed data that were not normally distributed and tested again for normality. When data were normally distributed after log-transformation, we used a 1-factor ANOVA to test for significant differences between means. If data were still not normally distributed after log-transformation, we applied the nonparametric Friedman test for matched data and used the Wilcoxon sign rank test as post hoc for pairwise comparison with Bonferroni-adjusted P values.

To explore associations between fractional iron absorption, iron utilization, iron status, and inflammation, we fitted linear regression models with fractional iron absorption, iron utilization, AGP, CRP, PF, TIR, and P. falciparum parasitemia as the dependent variables. If the measured parameters were under the detectable limit of the diagnostic tests, we used the value of the detection limit for calculations (i.e., 2.0 pg/mL for IL-6 and 0.5 nmol/L for hepcidin).

RESULTS

Subjects

In the afebrile P. falciparum group, 17 of 20 subjects completed the study and were considered for statistical analysis. There were no dropouts in the hookworm and S. haematobium groups, and hence, 16 and 8 subjects, respectively, completed the absorption studies. The means ± SDs of age and sex ratio of the children in the P. falciparum, hookworm, and S. haematobium groups were 14.2 ± 1.3 y, 6 females/11 males; 13.7 ± 1.8 y, 4 females/12 males; and 13.4 ± 2.1 y, 3 females/5 males, respectively.

Treatment of infection

In the afebrile P. falciparum group, the median parasitemia was 2160 and 272 parasites/μL of blood on days 1 and 15 (Table 1). After treatment, all children showed negative blood films at days 29 and 43, with the exception of 2 children on day 43 who had very low P. falciparum parasitemia (32 and 160 parasites/μL of blood). In the hookworm group, the median fecal egg count was 1896 and 1812 eggs/1 g of stool on days 1 and 15. After treatment, there were no hookworm eggs detected in any children. In the S. haematobium group, the median infection intensity was 49.5 and 36.5 eggs/10 mL of urine on days 1 and 15. After treatment, there were no S. haematobium eggs detected in any children, with the exception of one child who had 8 eggs/10 mL of urine at day 43.

Inflammation, iron indexes, and hepcidin

Comparing among the 3 groups at baseline (day 1), the following differences were significant: 1) CRP and PF were higher in the P. falciparum group than in the hookworm group (P < 0.001 for both) and the S. haematobium group (P = 0.037, P = 0.008), and 2) hepcidin was higher in the P. falciparum group than in the hookworm group (P = 0.012).

5 Abbreviations used: AGP, α1-acid glycoprotein; CRP, C-reactive protein; PF, plasma ferritin; TIR, soluble transferrin receptor.
TABLE 1
Iron and inflammation indicators in Ivorian children aged 11–17 y before and at 2 time points after treatment of the respective single infection with Plasmodium falciparum, hookworm, and Schistosoma haematobium1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 29</td>
</tr>
<tr>
<td>Afebrile <em>P. falciparum</em> malaria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasitemia, parasites/μL of blood</td>
<td>2160 (1520–3360)</td>
<td>0b</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>4.9 (0.6–28.3)b</td>
<td>0.1 (0.1–0.1)b</td>
</tr>
<tr>
<td>AGP, g/L</td>
<td>0.87 (0.76–1.06)b</td>
<td>0.66 (0.60–0.72)b</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>6.5 (2.3–17.9)b</td>
<td>3.2 (2.0–10.9)b</td>
</tr>
<tr>
<td>Hepcidin, nmol/L</td>
<td>2.7 (1.5–5.5)b</td>
<td>1.3 (&lt;0.5–2.2)b</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>130 ± 11.8a</td>
<td>129 ± 8.8a</td>
</tr>
<tr>
<td>Anemia, % (n)</td>
<td>17.6 (3)</td>
<td>17.6 (3)</td>
</tr>
<tr>
<td>CRP, µg/L</td>
<td>89.8 (70.6–129.4)a</td>
<td>58.8 (39.0–69.2)b</td>
</tr>
<tr>
<td>TIR, mg/L</td>
<td>8.7 (7.1–10.2)a</td>
<td>7.7 (6.3–8.7)a</td>
</tr>
<tr>
<td>Iron deficiency, % (n)</td>
<td>58.8 (10)</td>
<td>35.3 (6)</td>
</tr>
<tr>
<td>Hookworm infection (n = 16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEC, EPG</td>
<td>1896 (1362–4380)a</td>
<td>0b</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.2 (0.2–0.6)a</td>
<td>0.3 (0.2–1.2)a</td>
</tr>
<tr>
<td>AGP, g/L</td>
<td>0.75 (0.61–0.89)a</td>
<td>0.73 (0.58–0.81)a</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>7.9 (2.3–32.8)a</td>
<td>9.6 (5.2–26.2)a</td>
</tr>
<tr>
<td>Hepcidin, nmol/L</td>
<td>1.5 (&lt;0.5–1.9)a</td>
<td>1.0 (&lt;0.5–3.0)a</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>133 ± 13.4a</td>
<td>133 ± 13.9a</td>
</tr>
<tr>
<td>Anemia, % (n)</td>
<td>12.5 (2)</td>
<td>12.5 (2)</td>
</tr>
<tr>
<td>CRP, µg/L</td>
<td>41.2 (32.1–50.7)b</td>
<td>46.9 (30.7–63.6)a</td>
</tr>
<tr>
<td>TIR, mg/L</td>
<td>8.6 (7.2–9.9)a</td>
<td>8 (6.0–9.7)b</td>
</tr>
<tr>
<td>Iron deficiency, % (n)</td>
<td>56.3 (9)</td>
<td>50.0 (8)</td>
</tr>
<tr>
<td>S. haematobium infection (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg output, eggs/10 mL of urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>49.5 (34.5–78.5)a</td>
<td>0b</td>
</tr>
<tr>
<td>AGP, g/L</td>
<td>1 (0.4–1.5)a</td>
<td>1 (0.5–2.1)a</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>0.81 (0.62–0.83)a</td>
<td>0.73 (0.60–0.92)a</td>
</tr>
<tr>
<td>Hepcidin, nmol/L</td>
<td>6.6 (5.7–17.6)a</td>
<td>11.4 (6.2–18.6)a</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>130 ± 10.8a</td>
<td>129 ± 11.0a</td>
</tr>
<tr>
<td>Anemia, % (n)</td>
<td>12.5 (1)</td>
<td>12.5 (2)</td>
</tr>
<tr>
<td>CRP, µg/L</td>
<td>49.8 (40.9–70.8)a</td>
<td>54.8 (44.5–66.4)a</td>
</tr>
<tr>
<td>TIR, mg/L</td>
<td>7.2 (5.4–9.6)a</td>
<td>6.2 (5.4–7.3)a</td>
</tr>
<tr>
<td>Iron deficiency, % (n)</td>
<td>37.5 (3)</td>
<td>12.5 (1)</td>
</tr>
</tbody>
</table>

1Treatment was administered on day 15. Values within the same parameter with different superscript letters were statistically significant (P < 0.05) within groups between the different time points. AGP, α1-acid glycoprotein; CRP, C-reactive protein; EPG, eggs/l g of stool; FEC, fecal egg count; PF, plasma ferritin; TIR, soluble transferrin receptor.

2Two children assigned to the *P. falciparum* group had very low *P. falciparum* parasitemia (32 and 160 parasites/μL of blood) at day 43, and one child in the *S. haematobium* group had a very low egg count on day 43 (8 eggs/10 mL of urine). These children were not excluded for the statistical analysis.

3Statistical difference tested with the Friedman test and as post hoc test values were pairwise compared with the Wilcoxon rank-sum test by using Bonferroni-adjusted significance levels.

4Median; 25th–75th percentile in parentheses (all such values).

5Statistical difference tested with 1-factor ANOVA with log-transformed data and Bonferroni post hoc test.

6Mean ± SD (statistical difference tested with 1-factor ANOVA and Bonferroni post hoc test) (all such values).

7Anemia was defined as hemoglobin concentration <120 g/L.

8Iron deficiency was defined as hemoglobin concentration <120 g/L.

Within-group comparisons before and after treatment revealed the following significant differences: in the afebrile malaria group, CRP, AGP, IL-6, PF, TIR, and hepcidin were significantly lower after treatment (all P < 0.01), and in the hookworm group, TIR decreased significantly with treatment (P = 0.004). In the *S. haematobium* group, there were no significant differences (Table 1).

Iron absorption and utilization

In the *P. falciparum* group, geometric mean iron absorption was 12.9%, which increased to 23.6% (P = 0.003) after treatment (Table 2). In the hookworm group, geometric mean iron absorption before treatment was 32.2% and was significantly higher than in both the *P. falciparum* group (P < 0.001) and the *S. haematobium* group (20.2%; P < 0.001). Treatment of hookworm and *S. haematobium* infection did not significantly improve iron absorption. On day 29 posttreatment, iron absorption in the 3 groups did not differ (P = 0.206). Geometric mean iron utilization ranged from 74.2% to 88.0% between the 3 groups (Table 2) with no significant between- or within-groups differences before and after treatment.
Correlations among variables in the malaria group

At baseline before malarial treatment, serum hepcidin significantly correlated with IL-6 but none of the other measures of inflammation (CRP or AGP). Before treatment, iron status (PF and TfR) was correlated with iron absorption and utilization, but serum hepcidin was not. Iron utilization, but not absorption, before treatment negatively correlated with *P. falciparum* parasitemia (Table 3).

DISCUSSION

The main findings of this study are as follows: first, in children aged 11–17 y, afebrile *P. falciparum* reduces iron absorption from a FeSO₄ syrup, whereas hookworm infection does not, second, neither infection reduces iron utilization. Our data provide evidence that afebrile *P. falciparum* produces low-grade systemic inflammation that increases serum hepcidin and thereby reduces iron absorption, whereas hookworm infection does not.

For afebrile *P. falciparum*, our findings confirm those of Cercamondi and colleagues (12), who reported that Beninese women absorbed 10.2% iron from a fortified sorghum porridge during afebrile *P. falciparum* and 17.6% after antimalarial treatment. They also found that afebrile *P. falciparum* did not affect iron utilization, as measured by the erythrocyte incorporation of intravenously administered ⁵⁸Fe. Compared with our study, children had higher parasitemia (2160 compared with 880 parasites/µL of blood), greater intensity of inflammation...
Iron deficiency and anemia are well-known consequences of hookworm infection (42–44). Hookworms attach to the intestinal mucosa, and large amounts of blood can be lost through microcacerations during heavy infections; gastrointestinal losses of >2 mg iron/d has been estimated in children with heavy hookworm infections (42). However, it appears that inflammation at the site of attachment remains localized to the intestinal mucosa and is not apparent systemically (45). Our findings support this claim: in our subjects with light- and moderate-intensity hookworm infection, there was no evidence of systemic inflammation, and clearance of the infection did not significantly affect inflammatory biomarkers or serum hepcidin (Table 1). However, it is possible that ongoing blood loss (46) could have increased iron turnover and contributed to higher iron absorption on day 1 in the hookworm group (Table 2) compared with the other 2 groups. This would be in line with the TIR concentration, which significantly decreased 4 wk after treatment, indicating that the iron need decreased after hookworm infection was cleared.

*S. haematobium* infections are consistently associated with anemia (20, 47) that may be a result of blood loss, autoimmune hemolysis, and inflammation (17). A recent meta-analysis of randomized controlled trials concluded that treatment of individuals with *S. haematobium* significantly decreased anemia (20). In *S. haematobium* infection, adult worms reside in the blood vessels of the bladder wall, and eggs produced by the female worms damage the surrounding tissue, leading to blood loss (48). Hematuria is common in individuals with *S. haematobium* infection (17–19). Daily urinary iron losses in Kenyan children with very high (>200 eggs/10 mL of urine) and low to high (16–177 eggs/10 mL of urine) *S. haematobium* infection intensities were 652 µg and 278 µg, respectively (49). The infection may also cause a local granulomatous inflammatory response (50), but this is less well documented. In a study in Malian children infected with *S. haematobium*, 49% had hematuria but only 8% had an elevated CRP, and there was no correlation between biomarkers of inflammation and infection severity (18). Children infected with *S. haematobium* in the current study showed no evidence of systemic inflammation (Table 1), despite the fact that the median egg count in our sample was 49 eggs/10 mL of urine and infections with >50 eggs/10 mL of urine are considered heavy infections (28). Even the child with the heaviest infection (178 eggs/10 mL of urine) showed no evidence of inflammation (CRP of 0.57 mg/L, AGP of 0.64 g/L, and normal hemoglobin concentration of 130 g/L). However, these results must be interpreted with caution because the sample size was low, and it is possible that longer-term infections with *S. haematobium* in older age groups lead to formation of granulomas that cause tissue damage, resulting in inflammation and thereby influencing iron absorption.

Our study had limitations. First, compared with the desired sample size of 18 children per group, it needed to detect a 40% difference in iron absorption, the sample sizes of the *P. falciparum* group (n = 17), and the hookworm group (n = 16) were slightly below the target. However, we feel a β error is unlikely to explain why iron absorption and utilization did not differ with treatment in this parasitic worm infection, because the pre- and posttreatment absorption values were nearly identical. Second, despite considerable efforts and screening >1000 children, only 8 subjects met our inclusion criteria for the *S. haematobium* group. This group is thus underpowered, and results have to be interpreted with caution. Third, the iron status of our study cohorts was relatively good, and the influence of infection on iron metabolism in children with low iron status needs to be investigated. Fourth, 4 children in the hookworm group and 5 in the *S. haematobium* group initially were co-infected with *P. falciparum*, and hence they were treated 4 wk before the iron absorption measurements. Although there was no indication of systemic inflammation when the absorption study was made, we cannot rule out an influence of the prior malaria infection or of the antimalarial treatment. Fifth, other variables that can influence iron metabolism, such as concurrent deficiencies of vitamin A and riboflavin, or inherited hemoglobinopathies, were not assessed. Sixth, because of the invasiveness of our study, especially the intravenously administered iron dose and the fact that children remained infected for at least 2 wk after diagnosis, we conducted our study with older children, even though younger children (<5 y) are more prone to anemia.

Strengths of our study included direct quantification of iron absorption and utilization by using both an oral and an intravenous tracer before and after treatment of the infections. Moreover, interpretation of many studies of iron and infection are limited by the high rate of coinfection with multiple parasite species, making attribution difficult. We rigorously enrolled only children with single infections and also prophylaxed against potential recurrent coinfection during the study.

In conclusion, our findings provide important new insights into the metabolism of iron and the etiology of anemia in children infected with hookworm. Hookworm infection in our subjects, unlike afebrile *P. falciparum* malaria, did not cause low-grade inflammation or increase serum hepcidin and did not influence iron absorption or iron utilization. Hence, our findings suggest that anemia caused by hookworm infection is predominantly attributable to iron deficiency secondary to blood loss. In contrast, anemia in afebrile *P. falciparum* malaria is attributable to iron deficiency secondary to decreased dietary iron absorption in addition to an increased lysis or removal of infected red blood cells, as well as a decrease in the recycling of red blood cell iron.

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the research; DG and CZ: conducted the laboratory work; HT: measured the hepcidin; DG and CZ: performed the statistical analysis; DG, RHJ, JU, MBZ, and RW: wrote and revised the manuscript and had primary responsibility for the final content. All authors read and approved the final manuscript. The authors declared no conflicts of interest with respect to this study.

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