See corresponding editorials on pages 1283 and 1285.

Afebrile *Plasmodium falciparum* parasitemia decreases absorption of fortification iron but does not affect systemic iron utilization: a double stable-isotope study in young Beninese women[1–3]

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ABSTRACT

Background: Iron deficiency anemia (IDA) affects many young women in sub-Saharan Africa. Its etiology is multifactorial, but the major cause is low dietary iron bioavailability exacerbated by parasitic infections such as malaria.

Objective: We investigated whether asymptomatic *Plasmodium falciparum* parasitemia in Beninese women would impair absorption of dietary iron or utilization of circulating iron.

Design: Iron absorption and utilization from an iron-fortified sorghum-based meal were estimated by using oral and intravenous isotope labels in 23 afebrile women with a positive malaria smear (asexual *P. falciparum* parasitemia; >500 parasites/μL blood). The women were studied while infected, treated, and then restudied 10 d after treatment. Iron status, hepcidin, and inflammation indexes were measured before and after treatment.

Results: Treatment reduced low-grade inflammation, as reflected by decreases in serum ferritin, C-reactive protein, interleukin-6, interleukin-8, and interleukin-10 ($P < 0.05$); this was accompanied by a reduction in median serum hepcidin of ≈50%, from 2.7 to 1.4 nmol/L ($P < 0.005$). Treatment decreased serum erythropoietin and growth differentiation factor 15 ($P < 0.05$). Clearance of parasitemia increased geometric mean dietary iron absorption (from 10.2% to 17.6%; $P = 0.008$) but did not affect systemic iron utilization (85.0% compared with 83.1%; NS).

Conclusions: Dietary iron absorption is reduced by ≈40% in asymptomatic *P. falciparum* parasitemia, likely because of low-grade inflammation and its modulation of circulating hepcidin. Because asymptomatic parasitemia has a protracted course and is very common in malarial areas, this effect may contribute to IDA and blunt the efficacy of iron supplementation and fortification programs. This trial was registered at clinicaltrials.gov as NCT01108939. *Am J Clin Nutr* 2010;92:1385–92.

INTRODUCTION

Iron deficiency anemia (IDA) affects many young women in sub-Saharan Africa and increases maternal and perinatal mortality and reduces work capacity (1). The etiology of IDA in Africa is multifactorial, but the major cause is low dietary iron bioavailability from monotonous cereal-based diets (2), which is exacerbated by chronic parasitic infections such as malaria (3). High-dose iron supplements can improve iron status in areas of endemic malaria (4). However, iron fortification of foods may be a more cost-effective, sustainable, and potentially safer strategy to improve iron intakes and reduce anemia (1, 5). Disappointingly, most trials of iron fortification in malarial-endemic areas of Africa have been ineffective (6) or have had only limited effect (7, 8). One reason for the blunted effect of iron fortification could be poor absorption due to chronic inflammation from parasite diseases, such as malaria.

In sub-Saharan Africa, 74% of the population lives in highly malarial endemic areas (9). In acute febrile malaria, the main mechanisms contributing to anemia are hemolysis, increased splenic clearance of erythrocytes, and, possibly, reduced erythropoiesis (10, 11). The intense inflammation of acute malaria increases cytokine concentrations, such as interleukin (IL)-6 (12, 13), which can stimulate hepatic hepcidin production. High circulating hepcidin can reduce iron absorption from the gut and increase iron sequestration in the reticuloendothelial system (RES) (14) by blocking the iron-transporter ferroportin; the resulting hypoferremia limits the iron available for erythropoiesis and contributes to anemia (15, 16). The use of stable isotopes showed that iron absorption was shown to be decreased in young children recovering from acute malaria (17). Growth differentiation factor 15 (GDF-15) produced by the marrow erythroid compartment inhibits hepcidin expression and can increase iron absorption (18); however, the interaction between GDF-15 and hepcidin in malaria is unclear (19).

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However, acute febrile episodes are much less frequent than asymptomatic *Plasmodium falciparum* parasitemia, which, in areas of perennial transmission, can affect much of the population for most of the year (20). Cross-sectional studies have reported high prevalence rates of 74–95% for asymptomatic *P. falciparum* parasitemia among young women in West Africa (21, 22). It is unknown whether iron bioavailability is reduced by low-level, afebrile parasitemia, which has a protracted course and only a limited inflammatory response (19, 23). If afebrile parasitemia impairs iron absorption, its contribution to iron deficiency and anemia in populations may be more important than acute febrile malaria, which usually affects individuals for only a few days each year (24).

Therefore, measuring the effect of asymptomatic parasitemia on host iron absorption and utilization may provide insights into the etiology of anemia in the tropics. Our hypotheses were as follows: *1*) asymptomatic *P. falciparum* parasitemia in young African women would impair both the absorption of dietary iron and utilization of circulating iron and *2*) the mechanism of this effect would be the presence of low-grade inflammation affecting the balance of circulating hepcidin and GDF-15 concentrations.

SUBJECTS AND METHODS

Subjects

The study was carried out in young Beninese women recruited in Natitingou and Toucountouna in the Atacora department in Northern Benin. This is an area of endemic seasonal or perennial malaria transmission. The main transmission season is from May to November (25), during which time the percentage of subjects with a positive blood test result for malaria is from ≈60% to 80% (26). The inclusion criteria were as follows: *1*) female aged 16–40 y, *2*) body weight <65 kg, *3*) not pregnant (confirmed by pregnancy testing) and not breastfeeding, *4*) no chronic medical illnesses, 5) no medicinal iron at the time of entry into the study, *6*) a positive malaria smear (asexual *P. falciparum* parasitemia; >500 parasites/μL blood), *7*) no clinical symptoms (eg, headache, malaise) and lack of fever (<37.5°C) or self-reported fever in past 7 d, *8*) no infection with soil-transmitted helminths (hookworms, bilharziosis, *Ascaris lumbricoides*, *Trichuris trichiura*), and *9*) no severe anemia (hemoglobin <8.0 g/dL).

Sample size calculations indicated that ≥16 women should be included for paired comparisons based on 80% power to detect a 40% significant difference in iron absorption, an SD of 8.2% for log-transformed absorption data (based on previous Swiss Federal Institute of Technology Zurich studies), and a type I error rate of 5%. We anticipated that there could be a substantial dropout rate during the intensive 6-wk study; therefore, we enrolled 23 subjects. The subjects provided informed written consent. Ethical approval for the study was given by the ethical review committee at the Ministry of Health in Benin and the Swiss Federal Institute of Technology Zürich (Zurich, Switzerland).

Iron-absorption and utilization study

The women were studied while infected, then treated, and then restudied (**Figure 1**). Iron absorption and utilization were estimated by using stable-isotope techniques in which the incorporation into erythrocytes of an oral ^{57}Fe dose and an intravenous ^{58}Fe dose was measured 14 d after administration (27, 28). On day 1, a baseline venous blood sample was drawn after an overnight fast for determination of isotopic composition and iron status. The subjects then received a test meal (iron-fortified sorghum porridge; *see* below) labeled with ^{57}Fe as NaFeEDTA, which was fed under standardized conditions and close supervision. Each test meal contained 3 mg labeled ^{57}Fe. One hour later, 2 mL of an aqueous solution containing 100 μg ^{58}Fe as iron citrate was taken into a syringe and, via a 250-mL infusion bag leading into a 0.9% saline drip, slowly infused over 50 min (29). At completion of the infusion, 10 mL normal saline was injected into the 250-mL bag, the bag was rinsed by rotation and inversion, and the saline was infused into the subject. This was done twice; we assumed there was no residual isotope remaining in the bags after this rinsing procedure. The rate of intravenous infusion of iron was based on the estimated 2 μg/min plasma appearance of iron normally absorbed from the gastrointestinal tract (30). No intake of food and fluids was allowed for 4 h after the test meal intake. Fourteen days later (day 15), a second venous blood sample was drawn after an overnight fast, and treatment and prophylaxis were started. To treat malaria, 4 tablets Malarone (250 mg atovaquone + 100 mg proguanil hydrochloride; GlaxoSmithKline, Middlesex, United Kingdom) were administered on 3 consecutive days. After treatment, one tablet Malarone was administered daily as prophylaxis until the study was completed. Before the women were restudied, there was a 10-d waiting period to allow infection-related inflammation to subside (Figure 1). During the 10 d, blood smears were repeated to check whether the women were free of malaria parasites. In addition, a stool smear was done to confirm the absence of soil-transmitted helminths. On day 25, a third venous

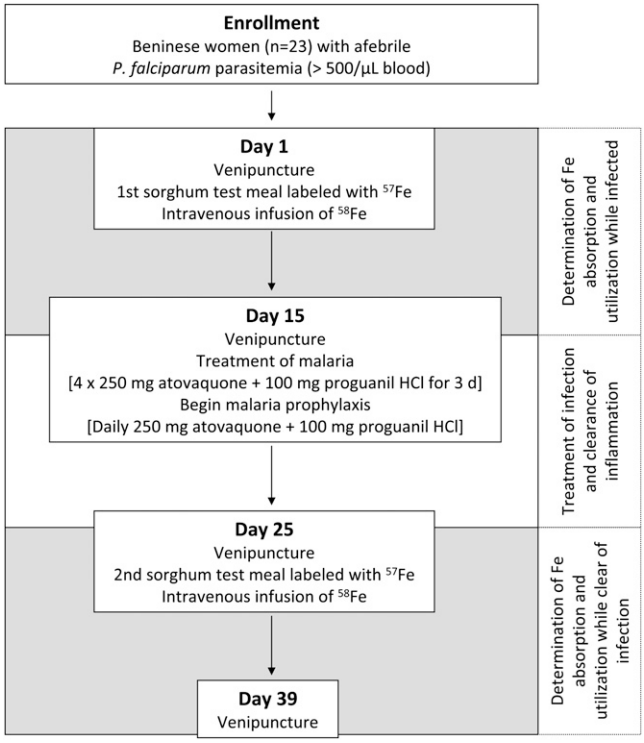

FIGURE 1. Overview of the study design.

blood sample was drawn after an overnight fast for determination of isotopic composition and confirmation of iron status. The subjects then received the second test meal and the second intravenous dose. Fourteen days later (day 39), a venous blood sample was drawn after an overnight fast. Because prophylaxis against bacterial and intestinal parasite infections is common in this area, the subjects received 500 mg ciprofloxacillin (Bayer, Leverkusen, Germany) every 2 d from day 1 (evening) through day 39 and 400 mg albendazole (GlaxoSmithKline) on days 0 and 21.

Test meal preparation

A reddish sorghum variety typically found in northern Benin was soaked for 12 h and then milled. The resulting sorghum flour was fermented in excess water for 24 h, dried at 37°C in an oven, and then milled again. To prepare test meal servings, 60 g of the fermented sorghum flour was mixed with 200 g mineral water, and the resulting slurry was added to 300 g previously boiled mineral water and then boiled for 6 min. During boiling, 18 g sugar was added with continual stirring. After cooling, portions based on 50 g dry weight of sorghum flour were weighed as test meal servings. All ingredients were purchased in bulk and used for the entire study. The food portions were prepared freshly on each study day, and the 3 mg fortification iron (as $Na^{57}FeEDTA$) was added at the time of feeding after the porridge cooled.

Test meal analysis

Iron was analyzed by graphite-furnace atomic absorption spectrophotometry (GF-AAS, AA240Z; Varian, Palo Alto, CA) after mineralization by microwave digestion (MLS ETHOSplus, MLS GmbH; Leutkirch, Germany) using a mixture of HNO_3 and H_2O_2. The phytic acid (PA) concentration was measured by using a modification of the Makower method (31), in which iron was replaced by cerium in the precipitation step. After the mineralization of food samples, inorganic phosphate was determined according to Van Veldhoven and Mannaerts (32) and converted into PA concentrations. The total polyphenol content was measured by using a modification of the Folin-Ciocalteau method, as suggested by Singleton (33) and was expressed as gallic acid equivalents.

Preparation of isotopically labeled iron

$Na^{57}FeEDTA$ was prepared in solution from ^{57}Fe-enriched elemental iron. The metal was dissolved in 2 mL HCl, diluted with water, and stored in polytetrafluoroethylene containers. The resulting $FeCl_3$ solution was mixed with a freshly prepared aqueous Na_2EDTA solution ($Na_2EDTA \cdot H_2O_2$; Sigma Chemical Co, St Louis, MO) at a molar ratio of 1:1 (Fe:EDTA) 20 min before test meal administration. The resulting NaFeEDTA solution was added to the test meal (34). Iron citrate, enriched with ^{58}Fe, was prepared for intravenous infusion from elemental ^{58}Fe according to the method described previously (35). The solution was divided into ampules containing 100 μg Fe, sterilized, and checked for pyrogens. Enrichment of isotopic labels was 95.5% for ^{57}Fe and 93.1% for ^{58}Fe. The isotopic composition of the stable-isotope labels was measured by using negative thermal ionization–mass spectrometry (28).

Parasite diagnosis

Thick and thin blood smears were stained by using the Giemsa coloration technique and were examined independently by 2 experienced microscopists. During screening, one blood smear from the subjects was examined. After inclusion in the study, the subjects' blood smears were examined in duplicate. Parasite density was quantified against leukocytes; fields containing $\geq$200 leukocytes were counted; if $<$10 parasites were identified, the counting continued to 500 leukocytes. These counts were converted to the number of parasites per μL blood, assuming 8000 leukocytes per 1-μL blood. One hundred high-power fields were examined before a slide was declared negative. Rapid malaria diagnostic tests (Paramax-3; MD Doctors Direct GmbH, Egg b, Zurich, Switzerland) were used to support the results of the blood smears. Two KatoKatz thick smears were prepared from each stool sample. The smears were examined immediately after preparation under a light microscope, and all hookworm eggs were counted. Subsequently, the smears were re-read, and the number of eggs of *A. lumbricoides*, *Schistosoma mansoni*, and *T. trichiura* was determined. Urine samples were collected for a pregnancy test (hCG Urine with OBC; Unipath Ltd, Bedford, United Kingdom), the detection of *Schistosoma haematobium*, and the detection of blood, glucose, and bilirubin. *S. haematobium* infection was assessed by using the syringe filtration technique. Ten milliliters of urine (collected between 1000 and 1400) was filtered with micropore filters, a drop of Lugol (Merck, Darmstadt, Germany) was placed on the filter, and the filter was examined under a microscope; the number of *S. haematobium* eggs was counted. Blood, glucose and bilirubin were detected by using test strips for urinalysis (Uriscan; MD Doctors Direct GmbH).

Blood analysis

Hemoglobin was measured in whole blood on the day of collection by using HemoCue hemoglobin 201+ (HemoCue AG, Wetzikon, Switzerland); anemia was defined as hemoglobin $<$12 g/dL (36). Serum was separated and frozen at -20°C with no freeze-thaw cycles. Serum ferritin (SF) and high-sensitivity C-reactive protein (CRP) were measured with an IMMULITE automatic system (DPC Bühlmann GmbH, Aschwil, Switzerland). Expected high-sensitivity CRP concentrations for healthy individuals were $<$3 mg/L. Serum transferrin receptor (TfR) was determined by using enzyme-linked immunosorbent assays (ELISA) (Ramco Laboratories Inc, Houston, TX); the normal range in healthy blood donors is 2.9–8.3 μg/mL. Iron deficiency was defined by an elevated sTfR ($>$8.3 μg/mL) and/or a low SF ($<$15 μg/L), although these indexes were likely confounded by the low-grade infection (37). IDA was defined as hemoglobin $<$12 g/dL and sTfR $>$8.3 μg/mL and/or SF $<$15 μg/L. Erythropoietin was measured by using ELISA (IBL ELISA; Immunobiological Laboratories, Hamburg, Germany); this assay has a reference range of 4–36 mU/mL in adults. GDF-15 was determined by ELISA for human GDF-15 (DuoSet; R&D Systems, Minneapolis, MN). Serum hepcidin measurements were performed by a combination of weak cation-exchange chromatography and time-of-flight mass spectrometry. An internal standard (synthetic hepcidin-24; Peptide International Inc, Louisville, KY) was used for quantification (38). Peptide spectra were generated

on a Microflex LT matrix-enhanced laser desorption/ionization time-of-flight mass spectrometry platform (Bruker Daltonics, Bremen, Germany). Serum hepcidin-25 concentrations were expressed as nmol/L. The lower limit of detection of this method was 0.5 nmol/L; the average CVs were 2.7% (intrarun) and 6.5% (interrun). The median reference concentration of serum hepcidin-25 in healthy Dutch adults is 4.2 nmol/L (range: 0.5–13.9 nmol/L) (39). Total serum iron and transferrin concentrations were measured on an Aeroset (Abbott Laboratories, Abbott Park, IL) by using Roche and Abbott reagents, respectively; the transferrin concentration was converted to total-iron-binding capacity (TIBC) by using the equation transferrin × 25. Cytokines were analyzed in duplicate by using a Bio-Plex Pro assay (Bio-Rad Laboratories, Hercules, CA), which included: IL-2, IL-4, IL-6, IL-8, IL-10, and granulocyte macrophage colony-stimulating factor (GM-CSF), γ-interferon (IFN-γ), and tumor necrosis factor-α (TNF-α). The limit of detection for each assay was calculated as the concentration of analyte on the standard curve for which the corresponding fluorescence value was 2 SDs greater than the blank. Values below the limit of detection were reported as the limit of detection. Whole blood was mineralized by microwave digestion, and iron was separated by anion-exchange chromatography and a subsequent solvent-solvent extraction step into diethylether. Iron was analyzed by negative thermal ionization–mass spectrometry with a magnetic sector field mass spectrometer (Finnigan MAT 262; Thermo Finnigan, Bremen, Germany) equipped with a multicollector system for simultaneous ion beam detection; isotopic dilution calculations were done as described previously (28).

Data analysis

Data were analyzed by using SPSS 13.0 for Windows (SPSS, Chicago, IL) and Excel (XP 2002; Microsoft, Seattle, WA). The amount of ^{57}Fe and ^{58}Fe label present in the blood was calculated from isotope dilution (28). Circulating iron was calculated from the blood volume based on height and weight (40) and from the hemoglobin concentration (infection period: mean of days 1 and 15; infection-free period: mean of days 25 and 39). The amount of stable isotope administered was used to calculate the fractional ^{57}Fe and ^{58}Fe incorporation into erythrocytes after 14 d. 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 (30, 35). Results of iron and inflammation indexes were presented as means ± SDs if normally distributed. If not normally distributed, the results were presented as medians with 25th–75th percentile ranges. Values were logarithmically transformed before statistical analysis and tested for normality with the Shapiro-Wilk test. If normally distributed after log transformation, paired *t* tests were used for comparisons between the 2 time points before and after treatment. If still not normally distributed after log transformation, Wilcoxon's signed-rank tests were used for comparison. Correlations were run by using log-transformed data. For normally distributed values, Pearson's correlations were performed. Spearman's rho correlations were used for values still not normally distributed after log transformation. Multiple linear regressions were done with log changes (day 25 to day 1) in iron absorption and utilization as the dependant variables and including the changes in log SF, log hepcidin, log TfR, log GDF-15, and log IL-10 as covariates. $P < 0.05$ was considered significant.

RESULTS

Four hundred thirty-six women were screened. The prevalence of *S. mansoni* and hookworm was 26% and 14%, respectively. The prevalence of asymptomatic parasitemia in women with a negative stool smear was 31%, but most of the positive smears had <500 parasites/μL blood.

The baseline characteristics of the women enrolled in the study (n = 23) are shown in **Tables 1** and **2**. The mean (± SD) age was 20.2 ± 4.8 (range: 16–35) y, and the mean (±SD) body mass index (in kg/m^2) was 21.5 ± 2.4 (range: 17.4–24.8). During the study, no subjects had detectable soil-transmitted helminths, and none developed fever or other clinical signs of infection. The median parasite value at enrollment was 1320 counts/μL. However, at entry into the study on day 1 (1–2 d later), the median parasite count had decreased to 880 parasites/μL and by day 15, 14 of the women no longer had detectable asexual *P. falciparum* parasitemia. After treatment and continuous prophylaxis, no subject had detectable parasites on days 25 and 39.

Iron indexes before and after malarial treatment are shown in Table 1. At baseline (day 1), 22% of women were anemic, the prevalence of iron deficiency was 13%, and 9% had IDA. Clearance of parasitemia was associated with a marked decrease in SF and a smaller but still significant increases in sTfR and decreases in erythropoietin and GDF-15 (Tables 1 and 2). There was no significant change in serum iron or serum transferrin, whereas TIBC showed a small but significant decrease with malarial treatment (Table 1).

Infection/inflammation indexes before and after malarial treatment are shown in Table 2. Clearance of parasitemia was associated with a reduction in low-grade inflammation; there were significant decreases in CRP, IL-6, IL-8, and IL-10 between days 1 and 25. At entry into the study on day 1, 7 women had CRP concentrations >3 mg/L. After treatment, no subjects had CRP concentrations >3 mg/L. The reduction in low-grade inflammation was accompanied by a reduction in median hepcidin of ≈50%. Serum concentrations of IL-2, IL-4, GM-CSF, IFN-γ, and TNF-α (data not shown) for all subjects, except one, were

TABLE 1
Iron indexes in Beninese women (n = 23) before and after treatment of asymptomatic *Plasmodium falciparum* parasitemia

Iron indexes	Day 1 (before treatment)	Day 25 (after treatment)	P
Hemoglobin (g/dL)	13.4 ± 15[1]	12.7 ± 12	0.027[2]
Serum ferritin (μg/L)	71 (29–99)[3]	37 (21–57)	0.001[4]
Serum transferrin receptor (mg/L)	5.2 (4.8–7.3)	5.6 (5.2–7.6)	0.007[2]
Total iron-binding capacity (mmol/L)	68.0 (63.0–73.0)	63.0 (57.5–70.5)	0.006[2]
Serum iron (μmol/L)	13.3 ± 5.6	12.7 ± 6.0	0.473[2]
Transferrin saturation (%)	19.9 ± 9.1	20.7 ± 11.3	0.912[2]
Serum erythropoietin (IU/L)	11.2 (8.0–16.3)	8.9 (7.2–13.6)	0.032[2]

[1] Mean ± SD (all such values).
[2] Paired *t* test (log transformed).
[3] Median; 25th–75th percentile in parentheses (all such values).
[4] Wilcoxon's signed-rank test (log transformed).

TABLE 2
Inflammation indexes in Beninese women (n = 23) before and after treatment of asymptomatic *Plasmodium falciparum* parasitemia[1]

Inflammation indexes	Day 1 (before treatment)	Day 25 (after treatment)	P
Parasites (no./μL)	880 (123–2760)	0 (0)	0.001[2]
C-reactive protein (mg/dL)	0.9 (0.4–5.7)	0.30 (0.3–0.4)	0.001[2]
Hepcidin (nmol/L)	2.7 (1.0–4.6)	1.4 (0.7–2.4)	0.005[2]
GDF-15 (pg/mL)	497 (400–612)	381 (341–432)	0.003[2]
Interleukin-6 (pg/mL)	1.32 (0.96–1.92)	1.27 (0.70–1.32)	0.031[2]
Interleukin-8 (pg/mL)	7.31 (4.58–10.03)	4.18 (2.60–5.67)	0.001[3]
Interleukin-10 (pg/mL)	7.38 (4.44–13.93)	2.94 (1.91–2.94)	0.001[3]

[1] All values are medians; 25th–75th percentiles in parentheses. GDF-15, growth differentiation factor 15.
[2] Wilcoxon's signed-rank test (log transformed).
[3] Paired t test (log transformed).

below the detection limit at days 1 and 25. In the subject with detectable concentrations, concentrations on day 25 were 128.1, 1.5, 21.8, 324.2, and 40.4 pg/mL, respectively.

The native iron content of the sorghum used to prepare the test meals was 3.4 ± 0.2 mg/100 g, but the total iron content in the sorghum flour was 8.9 ± 0.5 mg/100 g due to the introduction of iron, which occurred during local milling. Phytate and polyphenol contents of the sorghum flour were 421 ± 24 mg and 37.5 ± 0.5 mg/100 g, respectively. The meal based on 50 g flour therefore contained 1.7 mg native Fe, 2.8 mg contaminant Fe, 3 mg Fe as NaFeEDTA, 211 mg phytate, and 18.8 mg polyphenols.

Iron absorption and utilization before and after malarial treatment are shown in **Figures 2** and **3**. There was a significant 70% increase in oral iron absorption with treatment: geometric mean absorption (95% CI) on days 1 and 25 were 10.2% (7.4–14.0%) and 17.6% (13.5–22.3%), respectively; 18 of 23 women showed an increase in absorption (Figure 2). In contrast, there was no significant change in systemic utilization of the intravenous iron; 14 of 23 women showed a decrease in utilization after treatment (Figure 3).

Associations between iron absorption and iron and inflammation indexes are shown in **Table 3**. On day 1, during infection, SF, CRP, and GDF-15 showed significant negative correlations with iron absorption, whereas TfR and TIBC showed significant positive correlations. After malarial treatment (day 25), SF, serum iron, serum transferrin, and hepcidin showed significant negative correlations with iron absorption. On day 1, systemic iron utilization negatively correlated with GDF-15 ($r = -0.578$, $P < 0.01$); otherwise, there were no relevant significant associations between the measured indexes and systemic utilization. Change (Δ) in iron absorption (from days 25 to day 1) was negatively correlated with ΔSF ($r = -0.493$, $P < 0.05$), ΔCRP ($r = -0.574$, $P < 0.01$), and ΔIL-8 ($r = -0.426$, $P = 0.05$) and was positively correlated with ΔTIBC ($r = 0.415$, $P < 0.05$). ΔIron utilization (day 25 − day 1) was positively correlated with ΔIL-10 ($r = 0.422$, $P < 0.05$).

In multivariate models with log Δiron absorption and Δutilization as the dependent variables and including log ΔSF, Δhepcidin, ΔTfR, ΔGDF-15, and ΔIL-10 as covariates, ΔSF ($P = 0.008$), ΔGDF-15 ($P = 0.017$), and ΔTfR ($P = 0.025$) were significant predictors of Δiron absorption ($r^2 = 0.598$), and ΔGDF-15 ($P < 0.000$) and ΔIL-10 ($P < 0.001$) were predictors of Δiron utilization ($r^2 = 0.621$).

DISCUSSION

This study showed that afebrile malarial parasitemia, a common condition in endemic areas, decreases dietary iron absorption. This effect appears to be due to low-level inflammation modulation of serum hepcidin. This effect may help explain why iron supplements and iron fortification of staple foods or complementary foods may be less effective in malarial endemic areas (6–8). Fractional iron absorption in the present study was relatively high, which was likely due to the use of NaFeEDTA, an iron chelator, as the iron fortificant (41–45). The test meal in the present study included ≈2.8 mg contaminant Fe introduced by local milling; we assumed that its bioavailability in our test meal was negligible (46, 47).

Our study was the first to directly quantify iron incorporation into erythrocytes from dietary iron and systemic iron during, and after treatment, of asymptomatic malarial parasitemia. The advantage of using both an oral and an intravenous tracer is that it allows separation of dietary iron bioavailability into its 2

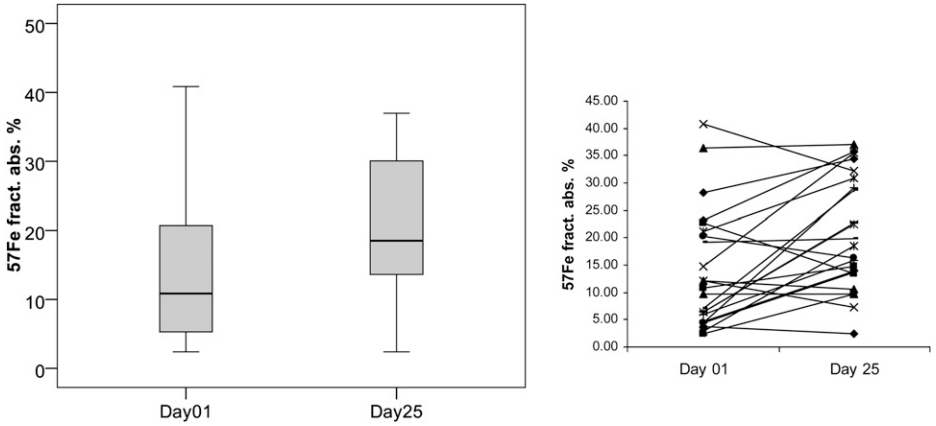

FIGURE 2. Iron absorption in Beninese women (n = 23) who consumed a sorghum porridge labeled with 3 mg ^{57}Fe as NaFeEDTA before (day 1) and after (day 25) malarial treatment. Iron absorption on day 1 was significantly lower than that on day 25 (Wilcoxon's signed-rank test, $P = 0.008$). The box plots show the median and 25th and 75th percentiles. Whiskers in the plots represent the highest and lowest values. The line graph shows individual iron absorption of the 23 women before and after treatment. fract. abs., fractional absorption.

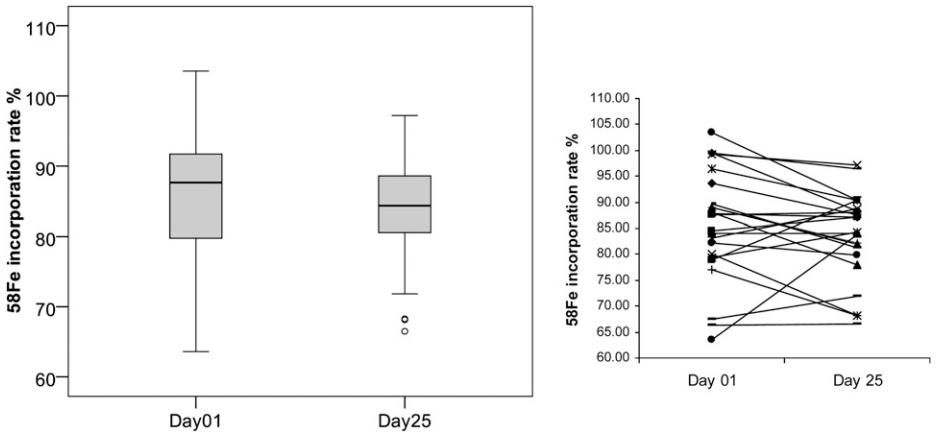

FIGURE 3. Erythrocyte iron utilization in Beninese women (n = 23) who received an intravenous dose of 100 μg ^{58}Fe-labeled iron citrate before (day 1) and after (day 25) malarial treatment. Iron utilization did not differ significantly between the 2 d (Wilcoxon's signed-rank test, $P = 0.107$). The box plots show the median and 25th and 75th percentiles. Whiskers in the plots represent the highest and lowest values that are not outliers. Outliers (values that are between 1.5 and 3 times the interquartile range) are represented by circles beyond the whiskers. The line graph shows individual iron utilization of the 23 women before and after treatment.

components: intestinal absorption and systemic utilization. Acute febrile malaria causes sequestration of RES iron and hypoferremia (48), which often coexists with normal or increased bone marrow iron (49). Release of iron from macrophages, like intestinal cells, is impaired with increased hepcidin and responsible for anemia by restricting iron supply to erythrocytes. In contrast with subjects with acute febrile malaria, our subjects with afebrile parasitemia had no evidence of hypoferremia during infection: the decrease in hepcidin with parasite clearance was not associated with a change in serum iron or in the percentage of serum transferrin. In contrast, asymptomatic parasitemia decreases dietary iron absorption, which suggests that iron export via ferroportin from enterocytes is sensitive to even small changes in hepcidin in the low-normal range. This finding is consistent with a previous cross-sectional study that used stable isotopes in healthy Swiss women, in which mean iron absorption from a test meal increased and serum hepcidin concentrations decreased slightly (50).

TABLE 3
Univariate correlations of iron absorption with iron and inflammation indexes on day 1 (before malarial treatment) and day 25 (after malarial treatment) in Beninese women (n = 23) with asymptomatic *Plasmodium falciparum* parasitemia[1]

	Iron absorption on day 1	Iron absorption on day 25
Serum ferritin[2]	−0.554[3]	−0.784[3]
Serum transferrin receptor[4]	0.576[3]	—
Total-iron-binding capacity[4]	0.472[5]	—
Serum iron[2]	—	−0.473[5]
Transferrin saturation[2]	—	−0.456[5]
C-reactive protein[2]	−0.491[5]	—
Hepcidin[2]	—	−0.679[3]
GDF-15[4]	−0.446[5]	—

[1] GDF-15, growth differentiation factor 15.
[2] Spearman's rho correlations.
[3] $P < 0.01$ (2-tailed).
[4] Pearson's correlation coefficients.
[5] $P < 0.05$ (2-tailed).

Previous studies of iron metabolism and hepcidin during malaria have focused on changes during treatment of acute febrile infection. Hepcidin regulation is complex during malaria infection: hepcidin production may be upregulated by inflammatory cytokines (15, 51, 52); however, at the same time, hypoxia associated with malarial anemia and possibly with erythropoietic factors such as GDF-15, may down-regulate hepcidin production (53, 54). The inflammatory stimuli appear to predominate, because studies have generally found a positive relation between malaria and circulating hepcidin (55). In anemic Tanzanian children aged <3 y of age with febrile *P. falciparum* malaria, urinary hepcidin strongly increased and was positively correlated with inflammation; antimalarial treatment led to a rapid decrease in urinary hepcidin, TNF-α, IL-6, and IL-10 and in a reversal of hypoferremia (48).

In contrast with previous studies, our Beninese subjects had lower levels of parasitemia, no clinical symptoms, and probable partial immunity from repeated exposure (56). Treatment of parasitemia decreased inflammation (CRP, SF, IL-6, IL-8, and IL-10), which was associated with a significant fall in serum hepcidin. Thus, our findings suggest that the low-grade inflammation in asymptomatic malaria is sufficient to trigger a small increase in serum hepcidin. Similarly, because SF is an acute phase protein, the resolution of low-grade inflammation likely explains the ≈48% decrease in SF associated with treatment of parasitemia (Table 1). Iron absorption was lower in those subjects with more inflammation (as reflected in higher SF and/or higher CRP concentrations) on day 1 or higher hepcidin on day 25 (Table 3). During experimental *P. falciparum* malaria infection in Dutch adults (57), low parasitemia and its treatment was associated with an increase in IL-6, a decline in serum iron and serum transferrin, and an increase in SF; there was an increase in serum hepcidin in 4 of 5 volunteers. Parasitemia and its treatment were associated with a transient decrease in reticulocyte hemoglobin content over 3 d, which was followed by a compensatory reticulocytosis, which suggests impaired iron incorporation into developing erythrocytes. In contrast, we directly measured systemic iron utilization by using labeled intravenous iron and found no effect of treatment of parasitemia. This suggests that parasitemia-associated inflammation may reduce iron release

from macrophages rather than impair iron incorporation during erythropoiesis. Compared with previous studies, geometric mean incorporation of an intravenous iron dose in the present study (83–85%) was lower than in young Thai women (93%) (29), but was somewhat higher than in older British men (79%) (30).

In our study, there were small but significant decreases in both erythropoietin and GDF-15 with clearance of parasitemia. Acute malaria may impair iron homeostasis through a direct and/or indirect suppressive effect on erythropoiesis (11, 58). Increased hepcidin production may contribute by directly inhibiting erythroid progenitor proliferation and survival (59). Decreased erythropoietin production and/or sensitivity during malaria may also play a role (10, 60, 61), but most studies have found an adequate host erythropoietin response to malaria infection (62). In the study by Doherty et al (17), erythropoietin was significantly lower in children recovering from febrile malaria than in anemic children without malaria. In contrast, in our study of mostly nonanemic women, there was a decrease in erythropoietin with treatment, which suggested that erythropoietin was not limiting erythropoiesis during asymptomatic parasitemia. A small but significant fall in serum GDF-15 with clearance of parasitemia was observed. Because circulating GDF-15 fell along with hepcidin, the improvement in iron absorption did not appear to be the result of a "marrow" signal from GDF-15 to increase iron absorption, but rather to a decrease in circulating hepcidin.

In our studies, we did not have an additional control group of women without malaria who received antimalarial treatment and then underwent measurements of iron absorption and/or utilization. Thus, we cannot entirely rule out a potential effect of the antimalarial agents per se on iron metabolism, independent of their effect on *P. falciparum*. We administered prophylactic ciprofloxacillin and albendazole during the 6-wk study period to avoid potential confounding of the results by common local infections and associated inflammation. This was successful in that none of the subjects developed an infection during the study that would have led to their exclusion. Although ciprofloxacillin showed some in vitro activity against the blood stage of *Plasmodium* spp. (63, 64), it was not effective against *Plasmodium* infections (65, 66). Therefore, the negative blood smears in several of the subjects on day 15 (before antimalarial treatment) were likely explained either by self-clearance, which is thought to commonly occur in endemic areas (67, 68), or by the lack of sensitivity of blood smears to detect subpatent (eg, liver stage) parasitemia (69).

Afebrile parasitemia is very common in endemic areas and often has a protracted course (19, 23). Because it reduces iron absorption, it may be an important contributor to IDA in young women with poor diets but high iron requirements because of menstrual blood losses and repeated pregnancies. In this context, the benefits of intermittent treatment of malaria on anemia rates (70, 71) may be due not only to a reduction in acute malarial anemia, but also to the periodic clearance of asymptomatic parasitemia and a resulting improvement in dietary iron absorption.

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