Comparison of iron status 28 d after provision of antimalarial treatment with iron therapy compared with antimalarial treatment alone in Ugandan children with severe malaria[1,2]

Sarah E Cusick,[3] Robert O Opoka,[4] Andrew S Ssemata,[4] Michael K Georgieff,[3] and Chandy C John[3,5]*

[3]Department of Pediatrics, University of Minnesota School of Medicine, Minneapolis, MN; and [4]Department of Paediatrics and Child Health, Makerere University College of Health Sciences, Kampala, Uganda

ABSTRACT

Background: The provision of iron with antimalarial treatment is the standard of care for concurrent iron deficiency and malaria. However, iron that is given during a malaria episode may not be well absorbed or used, particularly in children with severe malaria and profound inflammation.

Objectives: We aimed to *1*) determine baseline values of iron and inflammatory markers in children with severe malarial anemia (SMA), children with cerebral malaria (CM), and community children (CC) and *2*) compare markers in iron-deficient children in each group who received 28 d of iron supplementation during antimalarial treatment with those in children who did not receive iron during treatment..

Design: Seventy-nine children with CM, 77 children with SMA, and 83 CC who presented to Mulago Hospital, Kampala, Uganda, were enrolled in a 28-d iron-therapy study. Children with malaria received antimalarial treatment. All children with CM or SMA, as well as 35 CC, had zinc protoporphyrin (ZPP) concentrations ≥80 μmol/mol heme and were randomly assigned to receive a 28-d course of iron or no iron. We compared iron markers at day 0 among study groups (CM, SMA, and CC groups) and at day 28 between children in each group who were randomly assigned to receive iron or to not receive iron.

Results: At day 0, children with CM and SMA had greater values of C-reactive protein, ferritin, and hepcidin than those of CC. At day 28, interactions between study and treatment group were NS. Children in the no-iron compared with iron groups had similar mean values for hemoglobin (115 compared with 113 g/L, respectively; $P = 0.73$) and ZPP (124 compared with 124 μmol/mol heme, respectively; $P = 0.96$) but had lower median ferritin [101.0 μg/L (95% CI: 84.2, 121.0 μg/L) compared with 152.9 μg/L (128.8, 181.6 μg/L), respectively; $P \leq 0.001$] and hepcidin [45.8 ng/mL (36.8, 56.9 ng/mL) compared with 83.1 ng/mL (67.6, 102.2 ng/mL), respectively; $P < 0.011$].

Conclusions: Severe inflammation is a characterization of children with CM and SMA. The withholding of iron from children with severe malaria is associated with lower ferritin and hepcidin at day 28 but not a lower hemoglobin concentration. This trial was registered at clinicaltrials.gov as NCT01093989. *Am J Clin Nutr* 2016;103:919–25.

Keywords: hepcidin, iron, malaria, timing of iron supplementation, inflammation

INTRODUCTION

Child iron status and malaria risk strike a precarious balance in malaria-endemic regions. Iron is essential for brain development, but iron supplementation, particularly in iron-replete children, may increase risk of malaria and even death. A 2006 randomized controlled trial in malaria-endemic Pemba Island, Tanzania (1), first brought the potential danger of this interaction to the global stage although a troublesome relation between iron and infection had been suggested in the literature for more than a century (2, 3).

In malaria-endemic areas, young children frequently have iron deficiency and malaria concurrently. When to start iron treatment in iron-deficient children who also have malaria is a critical question. The current standard of care is to start iron therapy at the same time as antimalarial treatment in children with malaria who are anemic or determined to be iron-deficient by another indicator (4), but many studies that followed this regimen have reported only modest gains in hemoglobin and persistent anemia (5–7).

A potential explanation for these suboptimal gains in hemoglobin is that the effectiveness of iron therapy given concurrently with antimalarial treatment is diminished by malaria-associated inflammation and the resulting hepcidin-induced impairment of gut iron absorption and release of body iron from functional compartments. Higher blood concentrations of hepcidin and ferritin have both been shown to predict a poorer incorporation of supplemental iron into red blood cells in children who were recovering from postmalarial anemia (8). Values of both of these indicators decline with antimalarial treatment and consequent reduction of inflammation alone (9), but the degree of change in

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[2] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

[5] Present address: Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN.

*To whom correspondence should be addressed. E-mail: scusick@umn.edu.

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these indicators in the absence compared with presence of supplemental iron is not known to our knowledge.

In the current study, we assessed changes in iron and inflammatory markers over a 28-d period in iron-deficient children with one of 2 forms of severe malaria [cerebral malaria (CM)[6] or severe malarial anemia (SMA)] who started a 28-d course of iron therapy concurrently with antimalarial treatment or who were not given iron. Specifically, we aimed to *1*) determine values of iron and inflammatory markers in children with SMA or CM and also in healthy community children (CC) before antimalarial treatment and *2*) establish whether the day 28 values of iron and inflammatory markers differed between children in each group who received iron with their antimalarial treatment compared with those who received antimalarial treatment alone. Results of this trial may inform the identification of a safer and more effective time to start iron therapy during the postmalaria recovery period in the millions of children worldwide with co-occurring iron deficiency and severe malaria.

METHODS

Study participant enrollment, treatment, and follow-up

Between June 2010 and December 2013, we enrolled Ugandan children aged from 18 mo to 5 y who presented to Mulago Hospital, Kampala, Uganda, with SMA (n = 77) or CM (n = 79) and also healthy CC (n = 83) into a randomized study of the short-term effects on iron and inflammatory markers of giving iron at the time of antimalarial treatment or giving antimalarial treatment alone to children with severe malaria. Children within this cohort who had iron deficiency, which was defined as a zinc protoporphyrin (ZPP) concentration ≥80 μmol/mol heme, were randomly assigned either to receive a 1-mo course of daily iron sulfate treatment that started when oral medication could be tolerated or to not receive iron. The treatment allocation was determined by simple random assignment that was stratified by study group (i.e., CM, SMA, and CC groups). Iron was given as 2 mg liquid oral ferrous sulfate · kg^{-1} · d^{-1} for 4 wk. Children who were assigned to not receive iron were not given a placebo liquid but otherwise received follow-up care that was identical to that in the iron group.

We chose a ZPP concentration ≥80 μmol/mol heme to define iron deficiency because children with a ZPP concentration greater than this cutoff in the Pemba study, in which malaria and iron deficiency were coendemic, benefited from iron supplementation (1) and because ZPP results are available immediately. Only iron-deficient children were eligible to be randomly assigned to receive iron or to not receive iron because of risk of iron supplementation in iron-replete children living in malaria-endemic areas (1). Children with ZPP <80 μmol/mol heme were not eligible for random assignment and were not given iron but were included in the study as a non–iron-deficient reference group.

[6] Abbreviations used: CC, community children; CM, cerebral malaria; CRP, C-reactive protein; SMA, severe malarial anemia; sTfR, soluble transferrin receptor; ZPP, zinc protoporphyrin.

At baseline, caregivers of children who received iron were instructed by a study nurse how to draw up and dispense the iron syrup with a medicine dropper, and they were given an iron-therapy card on which to keep track of the doses administered. These cards were verified by study monitors in subsequent biweekly home visits.

CM was defined as having *1*) a coma (Blantyre coma score ≤2 or Glasgow coma score ≤8), *2*) *Plasmodium falciparum* on a blood smear, and 3) no other known cause of coma. SMA was defined as *P. falciparum* on a blood smear plus a hemoglobin concentration ≤50 g/L. CC were recruited from the same family or neighborhoods as children with CM or SMA and were within the same age range as the children with severe malaria. Eligibility criteria for CC were *1*) being 18 mo to 5 y of age for the iron study, *2*) being currently healthy, and 3) having no illness that required medical care within the previous 4 wk. A known chronic illness was an exclusion criterion for all study groups.

All children with severe malaria were treated for malaria according to the following current guidelines of Mulago Hospital: receiving quinine from the start of the study through December 2012 and receiving artesunate from January 2013 to the end of the study (December 2013). Quinine was given at a dose of 10 mg/kg every 8 h for 24 h until the child could take oral medication. This administration was followed by a 3-d oral dose of artemether and lumefantrine. Artesunate was given at a dose of 2.4 mg/kg at 0, 12, and 24 h and followed by a 3-d dose of artemether and lumefantrine. CC with asymptomatic malaria were treated and not excluded from the study. When children with severe malaria were stabilized and informed consent was obtained from the caregiver, a 5–7-mL venous blood sample was drawn into a heparinized blood collection tube. Whole blood was immediately dispensed for hemoglobin determination with the use of Hemocontrol (HemoCue AB) and for the measurement of ZPP with the use of a hematofluoromter (Aviv Biomedicals). The remaining blood was centrifuged at 3000 rpm for 10 min at room temperature, and plasma was collected and stored onsite at −80°C until shipment to the University of Minnesota for measurements of iron and inflammatory markers.

All children returned to the hospital 28 d after study enrollment. A venous blood sample was drawn from each child and processed and stored with the use of the same procedures as used for the baseline sample.

Testing of iron-deficiency markers and iron-deficiency definitions

Plasma concentrations of ferritin (Ramco Laboratories Inc.), soluble transferrin receptor (sTfR) (Ramco Laboratories Inc.), and hepcidin (Bachem Holding AG) were measured with the use of an ELISA assay at the University of Minnesota. Plasma concentrations of C-reactive protein (CRP) were measured with the use of a Luminex immunoassay (Milliplex MAP kit; EMD Millipore).

In addition to the primary definition of iron deficiency that was based on ZPP that determined whether a child would be randomly assigned to received iron therapy, we also examined the prevalence of alternate definitions of iron deficiency including those that were based on a low ferritin concentration (<12 μg/L; or <30 μg/L if the CRP concentration was >10 mg/L) or high

sTfR (>8.3 mg/L) (10, 11). Anemia was defined as a hemoglobin concentration <110.0 g/L (12).

Ethics

Written informed consent was obtained from parents or guardians of all study participants. Ethical approval was granted by the institutional review boards for human studies at Makerere University School of Medicine, the University of Minnesota, The Uganda National Council for Science and Technology, and the Uganda National Drug Authority.

Statistical analysis

A 1-factor ANOVA was used to compare baseline values of each indicator across the 3 study groups (CM, SMA, and CC). Tukey's test was used to make post hoc pairwise comparisons between groups. Outcome measures that were not normally distributed were log transformed before analysis. To avoid negative logarithms, 1 mg/L was added to all CRP values before log transformation. A logistic regression was used to test group differences in categorical variables.

To assess the effect of iron plus antimalarial treatment compared with that of antimalarial treatment alone on the value of each indicator after 28 d, a 2-factor ANOVA was used with the outcome measure of the child's concentration of each indicator on day 28 and the predictor variables study group, treatment group, and their interaction (study group × treatment group). Again, outcome measures that were not normally distributed were log transformed before analysis. Group-specific results were calculated as adjusted means with 95% CIs. For outcomes that were log transformed, means were computed on the log scale and back transformed to the original scale. Treatment and group effects for outcome measures that were analyzed on the log scale were described with the use of ratios rather than with differences. For the analysis of the day 28 values of indicators in children in the iron compared with no-iron groups, CC who had ZPP concentrations <80 μmol/mol heme at baseline (and, thus, were not randomly assigned to either regimen of iron therapy) were not included. All statistical analyses were done with the use of STATA 12 statistical software (StataCorp LP).

RESULTS

A total of 239 children were enrolled in the study (79 children with CM, 77 children with SMA, and 83 community control children). All children in the CM and SMA groups, and 35 of 83 CC (42.2%) had ZPP concentrations ≥80 μmol/mol heme and were randomly assigned to receive iron or to not receive iron. The 48 CC who had ZPP concentrations <80 μmol/mol heme did not receive iron at either time point but otherwise received follow-up care that was identical to that of children who were randomly assigned to receive iron therapy or to not receive iron therapy. Of the 239 children enrolled, 9 children with CM died before the initial discharge from the hospital and before receiving any iron therapy; 2 children (one child with CM and one child with SMA) left the hospital before follow-up directions could be given; consent was withdrawn for 2 children (one CC and one child with SMA); and 4 children moved out of the catchment area during the 4 wk after enrollment (one child with CM and 3 children with SMA), which resulted in 222 children with complete data at baseline and at day 28. In these 222 children, 68 children had CM (37 children in the iron group and 31 children in the no-iron group), 72 children had SMA (36 children in the iron group and 36 children in the no-iron group), and 82 children were CC (19 children in the iron group and 16 children in the no-iron group; there were 47 children with ZPP concentrations <80 μmol/mol heme).

Baseline characteristics of study groups

The mean age and percentage of boys and girls did not differ significantly between groups (**Table 1**). As expected, hemoglobin differed significantly between study groups and was highest in CC and lowest in children with SMA, all of whom had hemoglobin concentrations ≤50 g/L because this was an inclusion criterion for this diagnosis. Twenty-two percent of CC (n = 18 of 83) were anemic at baseline. Only 2 children with CM were not anemic; 42 of 79 children with CM (53%) had hemoglobin concentrations <70 g/L, and 11 of 79 children with CM (13.9%) had hemoglobin concentrations ≤50 g/L, thereby also meeting the definition for SMA. Baseline values of ZPP were significantly higher in children with CM who had a baseline hemoglobin concentration ≤50 g/L than in those with a baseline hemoglobin concentration >50 g/L, but no other iron or inflammatory marker was significantly different between the 2 groups (**Supplementary Table 1**)

Extreme inflammation characterized children in both CM and SMA groups. Both children with CM and those with SMA had CRP, ferritin, and hepcidin concentrations that were significantly higher than those of CC, with median ferritin and CRP values close to 1000 μg/L and 1000 mg/L, respectively, in children with CM. However, of these 3 indicators, only hepcidin differed significantly between children in the CM and SMA groups and was ~3 times greater in the CM group than in the SMA group ($P < 0.001$).

Of the remaining iron markers, both ZPP and sTfR differed significantly between the groups. ZPP was high in all children with severe malaria, but the median ZPP concentration was >100 μmol/mol heme in children with SMA than in children with CM. Children with SMA also had the highest concentrations of sTfR, whereas children with CM had the lowest sTfR concentrations of all 3 groups.

With concurrent severe inflammation, a determination of dietary iron deficiency with the use of standard cutoffs was not meaningful in the CM or SMA groups at baseline. In CC, 17 of 83 children (20.5%) had iron deficiency with the use of the ferritin and CRP cutoffs, 8 of 83 children (9.6%) had elevated sTfR concentrations, and as stated previously, 35 of 83 children (42.2%) had elevated ZPP concentrations. Although 27 of 83 (32.5%) of CC had CRP concentrations ≥10 mg/L, only 7 CC (8.4%) had CRP concentrations >50 mg/L. No community child had a fever, and only 2 CC had smear-confirmed *P. falciparum*.

Twenty-eight-day values of iron and inflammatory indicators in iron and no-iron groups

Summaries of day 0 and 28 values of each indicator disaggregated by study and treatment groups are presented in **Table 2**. No interaction was detected between treatment group and study group for the day 28 value of any iron or inflammatory

TABLE 1
Baseline values of iron and inflammatory markers by study group[1]

	Cerebral malaria	Severe malarial anemia	Community children	Overall *P*
n	79	77	83	
Age, y	3.1 ± 1.0[2]	2.8 ± 1.0	3.1 ± 0.9	0.09
Male, *n* (%)	42 (53.2)	46 (59.7)	35 (42.2)	0.16
Hemoglobin, g/L	69.9 ± 18.9[a]	37.4 ± 9.5[b]	117.2 ± 12.3[3,c]	<0.001
ZPP, μmol/mol heme	266 (184, 344)[4,a]	394 (240, 585)[b]	76 (62, 107)[c]	<0.001
Ferritin, μg/L	1051 (676, 1455)[5,a]	667 (398, 1242)[a]	34.6 (17.0, 69.3)[b]	<0.001
sTfR, mg/L	4.1 (3.0, 4.9)[5,a]	7.0 (5.0, 9.7)[b]	5.0 (4.2, 6.3)[c]	<0.001
Hepcidin, ng/mL	152.8 (89.6, 287.9)[a]	55.5 (21.1, 169.5)[b]	20.0 (11.3, 39.7)[c]	<0.001
CRP, mg/L	822.5 (555.4, 1103.9)[5,a]	581.3 (334.6, 842.7)[a]	4.2 (1.1, 15.2)[b]	<0.001

[1] *P* values were determined with the use of an ANOVA for continuous variables and a logistic regression for sex. All continuous variables other than age and hemoglobin were log transformed before the analysis. Means or medians within a row that do not share a common superscript letter were significantly different ($P > 0.05$) after the use of Tukey's test to account for multiple comparisons. CRP, C-reactive protein; sTfR, soluble transferrin receptor; ZPP, zinc protoporphyrin.
[2] Mean ± SD (all such values).
[3] $n = 82$.
[4] Median; 25th, 75th percentiles in parentheses (all such values).
[5] $n = 78$.

indicator ($P > 0.05$ for all study group × treatment group interaction terms in all models). Therefore, the adjusted mean of each outcome measure at day 28 is presented by treatment group (**Table 3**) and by study group (**Table 4**) as well as the pooled treatment effect and pooled group effect for each outcome measure.

After 28 d, the mean hemoglobin concentration rose to ≥110.0 g/L in all study groups, and the day 28 hemoglobin value did not differ in the iron compared with no-iron treatment groups in any study group (Table 2) or when pooled across study groups (Table 3). Hemoglobin concentrations remained the lowest in children with SMA but no longer differed significantly in the groups after accounting for multiple comparisons (Table 4). The prevalence of anemia also declined to 34.6% in CM children, 35.8% in SMA children, and 23.3% in CC (excluding CC with baseline ZPP concentrations <80). This prevalence did not differ in the 3 groups ($P = 0.37$) and also did not differ between children who received iron and those who did not received iron in any group ($P > 0.05$ for all comparisons; data not shown).

Values of ZPP at day 28 did not differ by treatment group (considered for separate study groups or pooled across study groups) or by study group with all groups having a median value of slightly >100 μmol/mol heme (Tables 2–4). However, the prevalence of ZPP concentrations ≥80 μmol/mol heme remained high in each study group (CM: 87.9%; SMA 91.6%; CC: 82.9%), and this prevalence did not differ significantly between the groups ($P = 0.61$). Within each study group, the prevalence of a high ZPP at day 28 also did not differ between children in the iron and no-iron treatment groups [percentages in the iron group and in the no-iron group: CM, 86.1% and 90.0% ($P = 0.72$); SMA, 94.3% and 88.9% ($P = 0.67$); and CC, 73.7%, 93.8% ($P = 0.19$)].

sTfR concentrations at day 28 also did not differ between the iron and no-iron groups (Table 3), and values at day 28 in both severe-malaria groups were greater than in the CC group (Table 4).

For all 3 study groups individually and pooled across groups, ferritin and hepcidin values were significantly lower at day 28 in children who did not receive iron than in children who received iron (Table 3). Ferritin was greatest at day 28 in children with SMA and lowest at day 28 in CC (Table 4), whereas hepcidin was greater in children with SMA than in CC, but the difference between children with SMA and children with CM was NS (Table 4).

CRP declined substantially in both severe-malaria groups, but was not significantly different in children who received iron than in children who did not receive iron. No study-group differences persisted in CRP concentrations at day 28 (Table 4).

In all children who were randomly assigned to receive iron or who did not receive iron, 9 children (9.3%) in the iron group and 6 children (7.2%)in the no-iron group had a malaria infection in the first 28 d, but this difference was NS ($P = 0.62$).

DISCUSSION

In the current study, we aimed to characterize the iron and inflammatory profiles of children with CM and those with SMA at the time of acute disease and 28 d after treatment of malaria and to establish the effect of the provision of antimalarial treatment with or without iron on iron and inflammatory markers after 28 d. Our primary findings were as follows: *1*) both children with CM and those with SMA had severe inflammation and functional iron deficiency at the time of acute illness, but hepcidin was more profoundly affected by CM, which suggested a greater degree of inflammation in that condition; and *2*) in both groups, the withholding of iron and provision of antimalarial treatment alone were associated with equivalent hemoglobin and ZPP concentrations but lower concentrations of ferritin and hepcidin on day 28 than resulted from the provision of iron concurrently with antimalarial treatment.

Without biomarkers that could definitively differentiate between dietary iron status and inflammation, the results have 2 potential alternative explanations. The first explanation is that children who received iron had successfully improved iron stores and overall iron status despite the inflammation at the time of enrollment and, for this reason, had higher ferritin and hepcidin concentrations on day 28. The second explanation is that children who did not receive iron had fuller mobilization of storage iron

TABLE 2
Baseline and day 28 values of iron and inflammatory markers by study group and treatment group[1]

	CM		SMA		CC		
					ZPP concentration ≥80 μmol/mol heme		ZPP concentration <80 μmol/mol heme
	Iron (n = 37)	No iron (n = 31)	Iron (n = 36)	No iron (n = 36)	Iron (n = 19)	No iron (n = 16)	Not randomly assigned (n = 47)
Hemoglobin,[2] g/L							
Day 0	70.7 ± 18.4	69.6 ± 17.7	36.1 ± 9.1	39.3 ± 9.6	112.0 ± 14.4	115.0 ± 11.4	120.3 ± 9.3
Day 28	118.6 ± 15.5	114.0 ± 10.0	107.0 ± 20.1	113.8 ± 17.7	118.2 ± 11.5	118.8 ± 10.6	123.5 ± 8.0
ZPP,[3] μmol/mol heme							
Day 0	261 (191, 327)[4]	275 (161, 344)	450 (233, 609)	345 (239, 591)	111 (97, 192)	109 (94, 135)	66 (57, 74)
Day 28	109 (92, 162)	123 (100, 151)	125 (98, 172)	116 (95, 159)	107 (79, 196)	108 (89, 137)	65 (55, 80)
Ferritin,[5] μg/L							
Day 0	879 (615, 1474)	1063 (669, 1446)	724 (443, 1407)	502 (312, 1160)	57.3 (19.8, 78.9)	33.9 (13.3, 76.4)	34.2 (18.8, 61.4)
Day 28	148 (81.7, 349)	105 (37.5, 233)	285 (138, 367)	197 (103, 301)	64.2 (27.6, 92.4)	38.0 (22.8, 56.9)	28.8 (19.2, 43.3)
sTfR,[6] mg/L							
Day 0	3.9 (2.9, 4.7)	4.1 (3.5, 5.3)	6.2 (3.9, 8.6)	7.3 (5.2, 10.6)	6.4 (4.5, 8.4)	5.1 (4.2, 7.4)	4.5 (3.8, 5.2)
Day 28	6.4 (5.3, 9.0)	9.4 (7.1, 11.3)	8.0 (6.5, 10.8)	9.3 (6.9, 11.3)	6.4 (4.2, 7.6)	5.9 (4.3, 7.4)	5.1 (4.5, 6.1)
Hepcidin,[7] ng/mL							
Day 0	147 (89.6, 277)	170 (82.3, 320)	34.7 (16.9, 164)	55.5 (33.5, 213)	26.5 (8.6, 35.3)	20.1 (13.8, 33.0)	19.4 (11.6, 41.2)
Day 28	87.8 (44.2, 143)	39.7 (27.6, 83.7)	106 (50.6, 190)	72.4 (33.7, 130)	52.8 (29.6, 125)	23.1 (13.2, 62.1)	25.8 (17.0, 41.2)
CRP,[8] mg/L							
Day 0	662 (479, 955)	856 (521, 1368)	567 (301, 810)	624 (424, 934)	7.6 (1.0, 31.1)	2.4 (1.1, 11.5)	4.1 (1.4, 14.2)
Day 28	8.2 (1.8, 56.1)	5.5 (1.0, 53.2)	26.3 (3.5, 105)	22.9 (3.2, 86.4)	4.7 (1.7, 204)	32.6 (11.1, 117)	6.3 (0.2, 68.8)

[1]For children with nonmissing data for both days 0 and 28. No group × study group interactions were significant (all $P > 0.05$). CC, community children; CM, cerebral malaria; CRP, C-reactive protein; SMA, severe malarial anemia; sTfR, soluble transferrin receptor; ZPP, zinc protoporphyrin.

[2]All values are means ± SDs. n values were as follows: for CM, 28 in the iron group and 27 in the no-iron group; for SMA, 34 in the iron group and 33 in the no-iron group; and for CC, 17 in the iron group and 13 in the no-iron group.

[3]n values were as follows: for CM, 36 in the iron group and 30 in the no-iron group; for SMA, 35 in the iron group; and for CC with ZPP concentrations <80 μmol/mol heme, 45.

[4]Median; 25th, 75th percentiles in parentheses (all such values).

[5]n values were as follows: for CM, 36 in the iron group; for SMA, 35 in the no-iron group; and for CC with ZPP concentrations <80 μmol/mol heme, 45.

[6]n values were as follows: for CM, 36 in the iron group; for SMA, 35 in the iron group and 35 in the no-iron group; and for CC with ZPP concentrations <80 μmol/mol heme, 45.

[7]n values were as follows: for SMA, 35 in the iron group and 35 in the no-iron group; and for CC with ZPP concentrations <80 μmol/mol heme, 45.

[8]n values were as follows: for CM, 36 in the iron group; for SMA, 35 in the iron group and 34 in the no-iron group; and for CC with ZPP concentrations <80 μmol/mol heme, 45.

and potentially less residual inflammation from the initial severe-malaria episode and, for these reasons, had lower ferritin and hepcidin concentrations at day 28.

It was difficult to distinguish between these explanations in the current study because we only had day 28 measurements of the multiple iron markers, each of which had different half-lives and all of which were still very likely to be in flux as the disease processes resolved. Although the mean hemoglobin concentration was in the normal range and was equivalent between the 2 groups at day 28, ~30% of children in both iron and no-iron groups remained anemic. The body preferentially distributes iron to the red blood cells over storage pools (13), and thus, it might be assumed that hemoglobin would normalize before ferritin would after iron supplementation. However, a recent study in toddlers in Ivory Coast (14) suggested that this assumption may be incorrect because the daily consumption of iron-fortified food significantly increased plasma ferritin but not hemoglobin after 9 mo with >70% of children remaining anemic at the end of the follow-up period. Thus, it is possible that the greater ferritin in the iron group may have reflected greater iron stores and more iron available to the developing brain. Whether brain iron status was differentially affected by the 2 treatment approaches in this study is unknown because of the lack of biomarkers that index brain iron status.

An alternative explanation for our findings of lower ferritin and hepcidin in the no-iron group was that the initial inflammation from malaria was more completely resolved in this group, which led to greater iron absorption in the gut and egress from functional compartments. CRP concentrations at day 28 were not different in children who received iron than in those who did not receive iron. However, because CRP rises and declines acutely with inflammatory insults (15), it is possible that we missed differences in CRP concentrations between the iron and no-iron groups that occurred before our assessment point at 4 wk after treatment of malaria. The fact that both ferritin and hepcidin concentrations remained elevated at day 28 in both the iron and no-iron groups compared with normal values may provide some evidence that these markers were still declining from their high baseline values. Additional time points of assessment would have been necessary to know whether the ferritin concentrations measured at day 28 reflected a decline as the state of inflammation resolved or a rebound with iron supplementation after normalization at a previous time point.

TABLE 3
Adjusted values of iron and inflammatory markers at day 28 by treatment group[1]

	Iron	No iron	Pooled treatment effect[2]	*P*
Hemoglobin,[3] g/L	113.4 (109.9, 116.9)	114.9 (111.3, 118.5)	1.5 (−3.5, 6.5)	0.73
ZPP, μmol/mol heme	124 (114, 135)	124 (114, 135)	1.0 (0.88, 1.12)	0.96
Ferritin, μg/L	152.9 (128.8, 181.6)	101.0 (84.2, 121.0)	0.66 (0.51, 0.85)	<0.01
sTfR, mg/L	7.2 (6.6, 7.9)	8.1 (7.3, 8.9)	1.12 (1.0, 1.3)	0.21
Hepcidin, ng/mL	83.1 (67.6, 102.2)	45.8 (36.8, 56.9)	0.55 (0.41, 0.74)	<0.001
CRP, mg/L	16.6 (11.1, 24.9)	19.2 (12.5, 29.4)	1.2 (0.64, 2.1)	0.25

[1]Except where noted, all values are geometric means; 95% CIs in parentheses. CRP, C-reactive protein; sTfR, soluble transferrin receptor; ZPP, zinc protoporphyrin.

[2]Pooled treatment effect at day 28 was determined with the use of a 2-factor ANOVA with the factors study group, treatment group, and their interaction (study group × treatment group) and was calculated as follows: for hemoglobin, values in the no-iron group minus values in the iron group; and for all other indicators, the ratio of values in the no-iron group to values in the iron group. All variables other than hemoglobin were log transformed before the analysis. The study group × treatment group interaction was NS for any outcome.

[3]All values are arithmetic means; 95% CIs in parentheses.

The lower hepcidin in children in the no-iron group may be particularly important because Prentice et al. (16) reported that lower hepcidin concentrations best predicted the incorporation of supplemental iron into hemoglobin in Gambian toddlers who were recovering from postmalaria anemia. Although values of iron and inflammatory markers at day 28 reflected an improvement in iron status, they also suggested that more recovery and perhaps additional iron supplementation are needed. The high prevalence (~80%) of elevated ZPP that persisted at day 28 in both treatment groups and in all study groups also likely reflected both persistent suboptimal iron status accompanied by the residual inhibition of hemoglobin synthesis. sTfR concentrations were not different between treatment groups but remained elevated in children with severe malaria compared with CC, thus also suggesting suboptimal iron status in the children with severe malaria at the day 28 time point. The lower hepcidin concentrations in the no-iron group on day 28 suggested that supplemental iron may have been better absorbed and incorporated into red blood cells if it was given at this time, but in the current study, we were unable to determine whether this improved absorption would have been as a result of poorer dietary iron status, less residual inflammation with more-complete mobilization of iron from stores, or both factors.

In apparent contrast to our results and those of Doherty et al. (8) are those of Glinz et al. (17) who found that iron absorption was equivalent at days 0 and 14 in a group of anemic Malawian toddlers aged 12–24 mo who were recovering from uncomplicated malaria. However, several differences in study design, including the absence of baseline iron markers in the delayed iron group and the difference in the timing of iron delay, made it difficult to compare their results to our own. In addition, the dose of iron (e.g., 30 mg/d) was large and perhaps masked the differences in absorption that would have occurred with a smaller dose. Although it may be argued that these results support giving a larger iron dose to overcome the hepcidin-induced impairment of iron absorption during malarial inflammation, a recent study by Pasricha et al. (18) suggested that this approach may not be effective or safe. Thus, future studies should define the timing and the dose of iron therapy to be provided to iron-deficient, malaria-infected children.

TABLE 4
Adjusted values of iron and inflammatory markers at day 28 by study group[1]

	CM	SMA	CC	*P*-pooled group effect	CM compared with SMA[2]	CM compared with CC[2]	SMA compared with CC[2]
Hemoglobin,[3] g/L	116.4 (112.2, 120.5)	110.3 (106.5, 114.1)	118.5 (112.9, 124.1)	0.03[4]	−6.0 (−12.6, 0.9)	2.2 (−6.3, 10.7)	8.0 (1.5, 16.3)
ZPP, μmol/mol heme	121 (110, 133)	131 (120, 144)	117 (102, 133)	0.29	1.1 (0.92, 1.3)	0.97 (0.79, 1.2)	0.89 (0.73, 1.9)
Ferritin, μg/L	119.6 (97.9, 146.1)	220.6 (181.7, 268.0)	44.0 (33.4, 58.1)	<0.001	1.9 (1.3, 2.6)	0.37 (0.24, 0.56)	0.20 (0.13, 0.30)
sTfR, mg/L	7.6 (6.8, 8.5)	8.7 (7.8, 9.6)	5.9 (5.1, 6.8)	<0.001	1.1 (0.94, 1.4)	0.77 (0.61, 0.96)	0.68 (0.54, 0.85)
Hepcidin, ng/mL	58.9 (46.4, 74.9)	82.6 (65.3, 104.6)	40.5 (29.1, 56.6)	<0.01	1.4 (0.94, 2.1)	0.69 (0.42, 1.1)	0.49 (0.30, 0.80)
CRP, mg/L	13.0 (8.1, 20.7)	23.7 (14.9, 37.5)	18.5 (9.7, 35.3)	0.20	1.8 (0.83, 4.0)	1.5 (0.57, 3.9)	0.82 (0.31, 2.1)

[1]Except where noted, all values are geometric means; 95% CIs in parentheses. Pooled study group effect at day 28 was determined with the use of a 2-factor ANOVA with the factors study group, treatment group, and their interaction (study group × treatment group). All variables other than hemoglobin were log transformed before the analysis. Tukey's test was used to account for pairwise comparisons between groups. The study group × treatment group interaction was NS for any outcome. CC, community children; CM, cerebral malaria; CRP, C-reactive protein; SMA, severe malarial anemia; sTfR, soluble transferrin receptor; ZPP, zinc protoporphyrin.

[2]Adjusted mean values (95% CIs) of the second group minus those of the first group were calculated for hemoglobin, and ratios of adjusted mean values of the second group to those of the first group (95% CIs) were calculated for all other indicators.

[3]All values are arithmetic means; 95% CIs in parentheses.

[4]Overall *F* test was significant ($P = 0.03$), but Tukey's post hoc procedure showed no pair of groups were different at $P < 0.05$.

Our study also revealed some key differences in the pathophysiology of CM and SMA with regard to body-iron regulation via hepcidin. Although hepcidin, ferritin, and CRP were all highly elevated in both severe-malaria groups, only hepcidin was significantly different between the groups, whereby it was higher in CM children. This difference in hepcidin alone was likely the product of the following 2 opposing factors that govern hepcidin concentrations (19–21): inflammation, which leads to high concentrations in CM children, and profound anemia, which is known to suppress hepcidin concentrations, in SMA children. The group difference at day 28 between CM and SMA children, although NS, was in the opposite direction, whereby SMA children had higher hepcidin concentrations than those of CM children, which perhaps reflected persistent inflammation because children in the SMA group tended to have a higher CRP concentrations at day 28 than those of children with CM, although this difference was NS

In conclusion, both children with CM and those with SMA who received antimalarial treatment without iron therapy had equivalent hemoglobin and ZPP concentrations but significantly lower ferritin and hepcidin concentrations after 28 d as than did children who received both antimalarial treatment and iron therapy. These results suggest that starting iron supplementation during the period of malaria-induced inflammation may, nevertheless, improve iron stores or, alternately, that delaying the start of iron therapy by 4 wk in children with these types of severe malaria may prime them for better absorption of oral iron and a more-complete incorporation of iron into red blood cells when iron is subsequently initiated. Because of the potential danger of iron supplementation in malaria-endemic areas, the timing of the provision of iron supplementation to that when the iron can be most optimally used by the host is critical. Additional studies with long-term outcomes, including iron status, morbidity, and neurobehavioral development, are needed before risks and benefits associated with delaying iron can be definitively established.

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