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Cord Blood Hepcidin: Cross-Sectional Correlates and Associations with Anemia, Malaria, and Mortality in a Tanzanian Birth Cohort Study

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Abstract. Hepcidin, the master regulator of bioavailable iron, is a key mediator of anemia and also plays a central role in host defense against infection. We hypothesized that measuring hepcidin levels in cord blood could provide an early indication of interindividual differences in iron regulation with quantifiable implications for anemia, malaria, and mortality-related risk. Hepcidin concentrations were measured in cord plasma from a birth cohort (N = 710), which was followed for up to 4 years in a region of perennial malaria transmission in Muheza, Tanzania (2002–2006). At the time of delivery, cord hepcidin levels were correlated with inflammatory mediators, iron markers, and maternal health conditions. Hepcidin levels were 30% (95% confidence interval [CI]: 12%, 44%) lower in children born to anemic mothers and 48% (95% CI: 11%, 97%) higher in placental malaria–exposed children. Relative to children in the lowest third, children in the highest third of cord hepcidin had on average 2.5 g/L (95% CI: 0.1, 4.8) lower hemoglobin levels over the duration of follow-up, increased risk of anemia and severe anemia (adjusted hazard ratio [HR] [95% CI]: 1.18 [1.03, 1.36] and 1.34 [1.08, 1.66], respectively), and decreased risk of malaria and all-cause mortality (adjusted HR [95% CI]: 0.78 [0.67, 0.91] and 0.34 [0.14, 0.84], respectively). Although longitudinal measurements of hepcidin and iron stores are required to strengthen causal inference, these results suggest that hepcidin may have utility as a biomarker indicating children's susceptibility to anemia and infection in early life.

INTRODUCTION

As the master regulator of bioavailable iron, hepcidin is a critical mediator of child health in malaria-endemic regions. The maintenance of adequate iron stores is essential for the production of hemoglobin and the rapid expansion of red cell mass that occurs in young children, and iron deficiency during the first "1,000 days" of development has been linked to cognitive, motor, and behavioral deficits that can last into adulthood.[1,2] The first 30 months of age also represent a key window of vulnerability to severe malaria infection and death.[3,4] Until children have developed naturally acquired resistance against *Plasmodium* parasites, hepcidin plays a particularly valuable role in host defense. Within hours of pathogenic insult, hepcidin expression is induced by cytokines and causes the increased internalization and degradation of the iron transporter ferroportin.[5–8] The loss of functional ferroportin causes the inhibition of dietary iron absorption via enterocytes and promotes sequestration of iron in reticuloendothelial cells with a net effect of iron restriction from pathogens.[6,9,10]

Iron restriction is important to the host's defensive response across multiple stages of malaria infection. Numerous in vitro and in vivo studies confirm that reducing iron availability through application of iron-chelating agents is an effective method to stunt the proliferation of parasites.[11] During the blood stage, iron deficiency may confer a protective effect by impairing the ability of *Plasmodium falciparum* to invade and propagate between erythrocytes.[12] In addition, recent experiments have also found that hepcidin-induced iron redistribution away from hepatocytes arrests the growth of intrahepatocytic parasites and thereby prevents the occurrence of malaria superinfections.[13] Epidemiological studies lend support to these findings: pregnant women and children who are iron deficient are observed to have lower risks of malarial disease,[14–18] and pediatric iron supplementation has been shown to heighten the risk of both clinical and severe malaria.[19,20] In addition to malaria, elevated iron has been associated with poor prognoses in cases of bacterial and viral infections[21] and may be associated with child mortality in communities with high burdens of communicable disease.

We hypothesized that measuring hepcidin levels in cord blood could provide an early indication of interindividual differences in iron regulation with quantifiable implications for child health during the first years of life. To investigate the theoretical relation between hepcidin and clinical endpoints known to be sensitive to iron homeostasis, we measured cord hepcidin levels in a birth cohort of children followed between 2002 and 2006 in a region of perennial malaria transmission in Muheza, Tanzania. Since little is known about the cross-sectional correlates of cord hepcidin in malaria-endemic regions, we first characterized in detail the associations of hepcidin with host characteristics, maternal health status, and markers of iron and inflammation in cord, maternal peripheral, and placental blood samples collected at the time of delivery. We then measured the associations between cord hepcidin and the time to anemia-, malaria-, and mortality-related outcomes.

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MATERIALS AND METHODS

Ethics. The U.S. National Institutes of Health International Clinical Studies Review Committee of the Division of Microbiology and Infectious Diseases approved the study procedures, and the institutional review boards of the Seattle Biomedical Research Institute and the National Institute for Medical Research in Tanzania provided ethical clearance. Participating mothers provided written informed consent for themselves and their newborn child. Mothers enrolled during labor were reconsented at the first follow-up visit. Prompt care was provided to sick children in accordance with Tanzanian Ministry of Health protocols. Blood smear results and hemoglobin concentrations were evaluated during the study visits, and health workers had full access to the results for clinical decision-making. All subsequent laboratory measurements were performed on deidentified samples.

Study population. The Mother-Offspring Malaria Study, initiated in 2002 as a prospective birth cohort study of malaria, has been described in detail previously.[22] A total of 1,045 pregnant women (1,075 offspring) between the ages of 18 and 45 years who presented for delivery at the Muheza Designated District Hospital in the Tanga region of Tanzania between September 9, 2002, and October 13, 2005, were invited to participate in the investigation; children were followed up until May 18, 2006. To be eligible for this study, children had to be 1) born to human immunodeficiency virus (HIV)–negative mothers, 2) sickle cell disease free, 3) a singleton birth, and 4) followed for a minimum of 28 days (Figure 1). At the time of hepcidin measurement, plasma samples were no longer available for 20% of the originally recruited sample. However, no substantial differences in baseline variables were found between children with and without measured hepcidin; this suggests that, although the missing hepcidin measurements decreased statistical power, their absence was unlikely to bias results (Supplemental Table 1).

Sampling procedures. Trained project nurses and assistant medical officers administered questionnaires to mothers and collected clinical information using standardized forms. Maternal peripheral blood samples and placentas were collected at delivery, and placental blood was extracted from placental tissue by mechanically pressing full-thickness placental tissue. Cord blood samples were collected immediately after parturition from venous umbilical blood vessels using routine procedures for cord clamping and vessel cannulation. Blood samples were collected in ethylenediaminetetraacetic acid for anticoagulation and fractionated by centrifugation at 3,000 *g* for 3 minutes. Plasma samples were frozen at −70°C until the immunoassays were performed. Clinicians monitored children's health statuses during sick visits and at routine visits occurring on a biweekly basis during the first 12 months of life and a monthly basis for any follow-up beyond the first year. Children's hemoglobin was measured at sick visits and during routine visits at approximately 3, 6, 12, 18, 24, 30, 36, 42, and 48 months of age. Parasitemia by *P. falciparum* was determined after counting 200 leukocytes on Giemsa-stained thick blood smear of a sample collected by heel or finger prick during child visits.

Case definitions. In both the children under 4 years of age and pregnant women, all-cause anemia and severe anemia were defined as hemoglobin concentrations < 110 and < 70 g/L, respectively.[23] Malaria was defined as the detection of *P. falciparum* parasitemia in peripheral blood. Severe malaria was identified clinically as a *P. falciparum*-positive blood smear with one or more of the following symptoms: severe anemia (i.e., hemoglobin concentration < 50 g/L), respiratory distress (i.e., respiratory rate > 50 breaths/minute in neonates and > 40 breaths/minute in older children with two of the following: nasal flaring, intercostal indrawing, subcostal recession, and grunting), > 1 convulsion episode in 24 hours, coma (i.e., Blantyre score < 3), prostration (i.e., inability to sit upright in a child normally able to do so or drink in a child too young to sit), hypoglycemia (i.e., blood glucose < 40 mg/dL), renal failure (i.e., urine output < 12 mL/kg/day), hemoglobinuria, jaundice, and shock (i.e., cold extremities, rapid heart rate, and/or systolic blood pressure < 50 mmHg). Placental malaria was defined as the detection of *P. falciparum* parasitemia in placental blood.

Laboratory procedures. The 25-amino acid bioactive isoform of hepcidin was detected in cord blood plasma using the hepcidin-25 (human) EIA kit (Bachem, Bubendorf, Switzerland) as previously described.[24] A dilution series of standard hepcidin-25 was run on each plate with values ranging

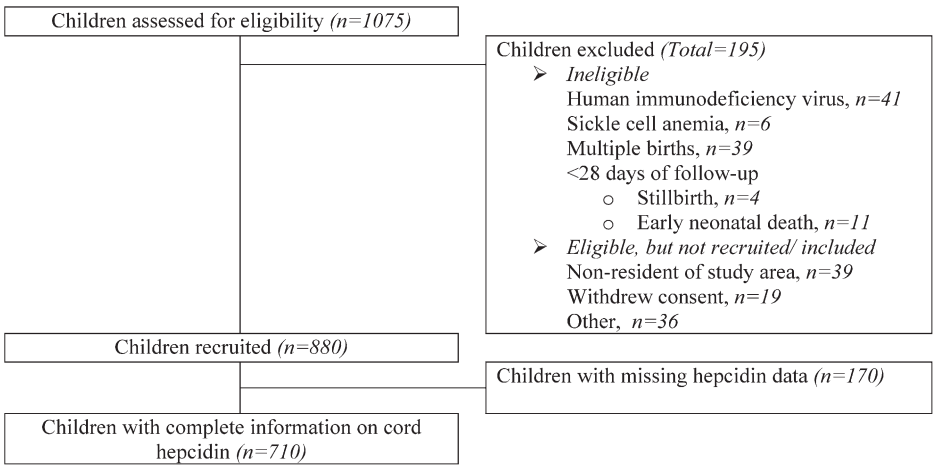

FIGURE 1. Selection of the study sample.

from 25 to 0.05 ng/mL. Sample values were interpolated using logistic 4-parameter nonlinear curve fitting in GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA). If hepcidin readings initially fell outside the linear portion of the standard curve, samples were rerun at appropriate dilution. If samples exhibited a high coefficient of variation (> 15%) between duplicates, they were rerun, and the new value was substituted. Soluble inflammatory and iron markers in cord, maternal peripheral, and placental blood plasma were measured using commercially available multiplex, bead-based platforms (BioPlex®; BioRad, Irvine, CA) and custom-made assay kits as previously described.[25] Samples that did not produce detectable concentrations of a given marker were assigned a value of half the limit of detection of that marker. Sickle cell trait was determined by cellulose acetate paper electrophoresis (Helena Laboratories, Beaumont, TX), and α-thalassemia was determined by polymerase chain reaction.[26]

Statistical analysis. The statistical approach used to describe the cross-sectional correlates of hepcidin was adapted from the methodology used by the Fibrinogen Studies Collaboration.[27] Positively skewed continuous variables were $\log_e$-transformed before analysis. Cross-sectional correlations between hepcidin and continuous traits were first quantified by Pearson's *r*. Mean levels of $\log_e$-transformed cord hepcidin were plotted against the mean for each eighth (i.e., corresponding to approximately one half of a standard deviation) of continuous traits. This approach allowed assessment of the shape of any association with cord hepcidin without assuming linearity a priori. Univariate linear regressions were then used to evaluate mean percent differences in hepcidin per level of categorical variables and by standard deviation of continuous variables; the mean percent differences were estimated with the formula $(e^{\beta} - 1) \times 100\%$, where the β coefficient represents the mean difference in $\log_e$-transformed hepcidin level. The coefficient of determination (r^2) from a linear regression was used to quantify the proportion of variance in cord hepcidin explained by the measured correlates.

For the prospective analyses, the primary exposure was hepcidin measured from cord plasma, which was evaluated both continuously and in tertiles (histogram provided in Supplemental Figure 1; cutoffs provided in Supplemental Table 2). Associations of cord hepcidin with repeated measurements of hemoglobin (N = 6,121 visits) and parasitemia (N = 5,493 visits with ≥ 1 parasite per 200 white blood cells) were evaluated using linear mixed-effects models with village- and child-specific random effects, quadratic time trends, and adjustment for placental malaria status, cord blood levels of $\log_e$ ferritin and $\log_e$ C-reactive protein, and concurrent malaria infection status. Fractional polynomial models of best fit were used to visualize the nonlinear associations between hemoglobin concentrations and age for children with above and below median cord hepcidin levels. For survival analyses, Cox proportional hazard models were used to calculate the hazard ratios (HRs) for the times to the first episode of anemia, severe anemia, malaria, severe malaria, and child death. Models were adjusted for potential confounders, including village of residence, placental malaria status, and cord blood levels of $\log_e$ ferritin and $\log_e$ C-reactive protein. To allow HRs to be compared informatively across any pair of hepcidin tertiles and without depending on the precision within any arbitrarily selected baseline group, 95% confidence intervals (CIs) were estimated from floated variances.[28] All statistical analyses were performed using Stata, version 12 (StataCorp LP, College Station, TX). *P* values were from two-sided tests.

RESULTS

In 710 children followed up over 1,482.0 person-years (median = 2.2 years per child), 639 experienced anemia, 274 experienced severe anemia, 590 experienced malaria, 98 experienced severe malaria, and 32 died (Supplemental Table 2). Child deaths were primarily caused by infectious diseases, including malaria (N = 11), sepsis, fevers of unknown origin, and diarrheal diseases (N = 9), respiratory infections (N = 4), and meningitis (N = 3). Deaths from noncommunicable diseases included congenital anomalies (N = 2), leukemia (N = 1), intestinal obstruction (N = 1), and aspiration (N = 1). The overall median concentration of cord hepcidin was 28.1 ng/mL (interquartile range: 14.3, 50.1).

Cross-sectional correlates of cord hepcidin. Table 1 describes the prevalence of the categorical traits in the study sample and their cross-sectional associations with cord hepcidin. Although cord hepcidin levels did not vary statistically significantly by sex, hemoglobin S trait, α-thalassemia, maternal gravidity, bed net status at enrollment, or detection of interleukin 4 in blood samples, cord hepcidin levels did appear to reflect maternal anemia and malaria exposure near the time of delivery. In the 594 dyads with known maternal hemoglobin status, hepcidin was 30% (95% CI: 12%, 44%) lower in infants born to mothers with anemia, defined as hemoglobin < 110 g/L, at the time of delivery. Infants born during the high season for malaria transmission had 28% (95% CI: 5%, 57%) higher hepcidin than children born during the low-transmission season. Similarly, placental malaria–exposed infants had 48% (95% CI: 11%, 97) higher cord hepcidin relative to unexposed infants. In addition, hepcidin levels were elevated in children with detectable concentrations of interferon-γ from all three of the blood sources.

Figures 2 and 3, as well as Supplemental Figures 2 and 3, explore the shapes of the associations between the continuous traits and $\log_e$ hepcidin. The shape plots in Figure 2 suggest that cord hepcidin correlates neither with birth weight (Figure 2A) nor with maternal age (Figure 2B). Consistent with the aforementioned relationship between cord hepcidin and maternal anemia, cord hepcidin was positively, but weakly associated with maternal hemoglobin levels as a continuous trait (r = 0.08) (Figure 2C). Post hoc analyses showed that mothers' hemoglobin levels strongly correlated with their peripheral blood ferritin levels: maternal hemoglobin increased by 4.8 g/L (95% CI: 1.6, 7.9) for each standard deviation increase in maternal peripheral blood ferritin, suggesting iron deficiency was likely a key contributor to the observed cases of maternal anemia.

Of the cord blood markers presented in Figure 3, hepcidin was generally positively associated with the measured inflammatory mediators, excluding interleukin 5 (Figure 3E). The strongest correlations were with C-reactive protein (r = 0.31), ferritin (r = 0.28), and tumor necrosis factor (r = 0.26) (Figure 3A, H, and I). Although the shape of association between tumor necrosis factor receptor I and hepcidin reflected that of tumor necrosis factor (Figure 3B), tumor necrosis factor receptor II did not appear to be associated with cord hepcidin levels (Figure 3C). In addition, although cord levels of soluble transferrin receptor had an overall null

TABLE 1
Cross-sectional associations of cord hepcidin with categorical traits measured at the time of delivery

	Variable	Total	*N* (%)	Mean percent difference (95% CI) compared with reference group	*P* value
Clinical markers	Sex	710			0.54
	Female		340 (47.9)	Reference	
	Male		370 (52.1)	−6 (−23, 15)	
	Sickle cell trait	708			0.50
	AA		592 (83.6)	Reference	
	AS		116 (16.4)	10 (−16, 44)	
	α-thalassemia	699			0.53
	α/α		321 (45.9)	Reference	
	α/α-3.7		293 (41.9)	−7 (−25, 15)	
	α-3.7/α-3.7		85 (12.2)	−16 (−39, 16)	
	Gravidity	710			0.47
	Primigravidae		202 (28.5)	Reference	
	Secundigravidae		167 (23.5)	−5 (−28, 26)	
	Multigravidae		341 (48.0)	−13 (−31, 10)	
	Maternal anemia at delivery	594			0.002**
	Anemia−		259 (43.6)	Reference	
	Anemia+		335 (56.4)	−30 (−44, −12)	
	Delivery season	710			0.01*
	Low malaria transmission		369 (52.0)	Reference	
	High malaria transmission		341 (48.0)	28 (5, 57)	
	Placental malaria	710			0.008*
	Placental malaria−		613 (86.3)	Reference	
	Placental malaria+		97 (13.7)	48 (11, 97)	
	Bed net at enrollment	594			0.12
	Bed net+, treated		90 (15.2)	Reference	
	Bed net+, untreated		274 (46.1)	9 (−22, 51)	
	Bed net−		230 (38.7)	−16 (−40, 18)	
Blood markers	Interleukin 4_{CB}	710			0.57
	Below limit of detection		640 (90.1)	Reference	
	Detectable		70 (9.9)	10 (−21, 54)	
	Interleukin 4_{PER}	667			0.08
	Below limit of detection		597 (89.5)	Reference	
	Detectable		70 (10.5)	35 (−3, 88)	
	Interleukin 4_{PLC}	680			0.37
	Below limit of detection		520 (76.5)	Reference	
	Detectable		160 (23.5)	12 (−12, 42)	
	Interferon γ_{CB}	710			0.04*
	Below limit of detection		559 (78.7)	Reference	
	Detectable		151 (21.3)	30 (2, 66)	
	Interferon γ_{PER}	667			0.06
	Below limit of detection		527 (79.0)	Reference	
	Detectable		140 (21.0)	27 (−1, 63)	
	Interferon γ_{PLC}	680			0.002**
	Below limit of detection		320 (47.1)	Reference	
	Detectable		360 (52.9)	39 (13, 71)	

CB = cord blood; CI = confidence interval; PER = maternal peripheral blood; PLC = placental blood.
* $P < 0.05$,
** $P < 0.005$.

correlation with hepcidin (in part, due to an outlying lowest eighth), these data provide an indication that soluble transferrin receptor concentrations in cord blood may have an inverse association with cord hepcidin levels (Figure 3J).

The correlations between hepcidin and markers from the maternal peripheral blood (Supplemental Figure 2) are consistent in shape with those of the cord samples (Figure 3). Although most inflammatory mediators were positively and approximately linearly associated with cord hepcidin levels, tumor necrosis factor receptor II, interleukin 5, and soluble transferrin receptor measured in maternal peripheral blood were, again, not observed to be associated with hepcidin (Supplemental Figure 2C, E, and J). In the placental blood sample, hepcidin levels were associated with the same inflammatory mediators, but also had additional modest, positive correlations with interleukin 5 ($r = 0.10$) and soluble transferrin receptor ($r = 0.21$) (Supplemental Figure 3E and J).

Table 2 summarizes the continuous traits and their cross-sectional associations with cord hepcidin. Overall, hepcidin levels were positively correlated with markers of inflammation, including interleukins 6 and 10. With the exceptions of interleukin 5 and soluble transferrin receptor, relations between blood markers and cord hepcidin were materially consistent between cord, maternal peripheral, and placental samples. Across all continuous traits, C-reactive protein levels measured in placental blood were the one most highly correlated with cord hepcidin levels (*r*: 0.37, 95% CI: 0.30, 0.43). Of note, placental C-reactive protein levels were, on average, 390% (95% CI: 230%, 630%) higher in placental malaria-positive dyads than those of placental malaria-negative pairs. Moreover, adjustment for placental levels of C-reactive protein caused the correlations of cord hepcidin with high-transmission season at delivery and placental malaria to lose statistical significance ($P = 0.22$ and 0.76, respectively); in

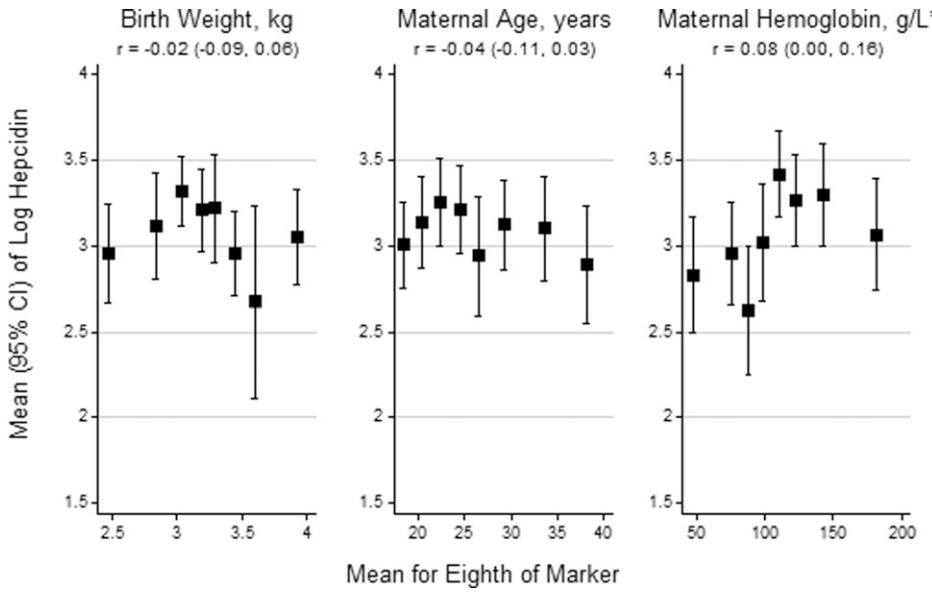

FIGURE 2. Mean $\log_e$ hepcidin levels within eighths of clinical markers measured at the time of delivery plotted against the mean of the clinical marker value in each eighth for assessment of the shape of association with hepcidin. Bars: 95% confidence interval (CI). $^*P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$.

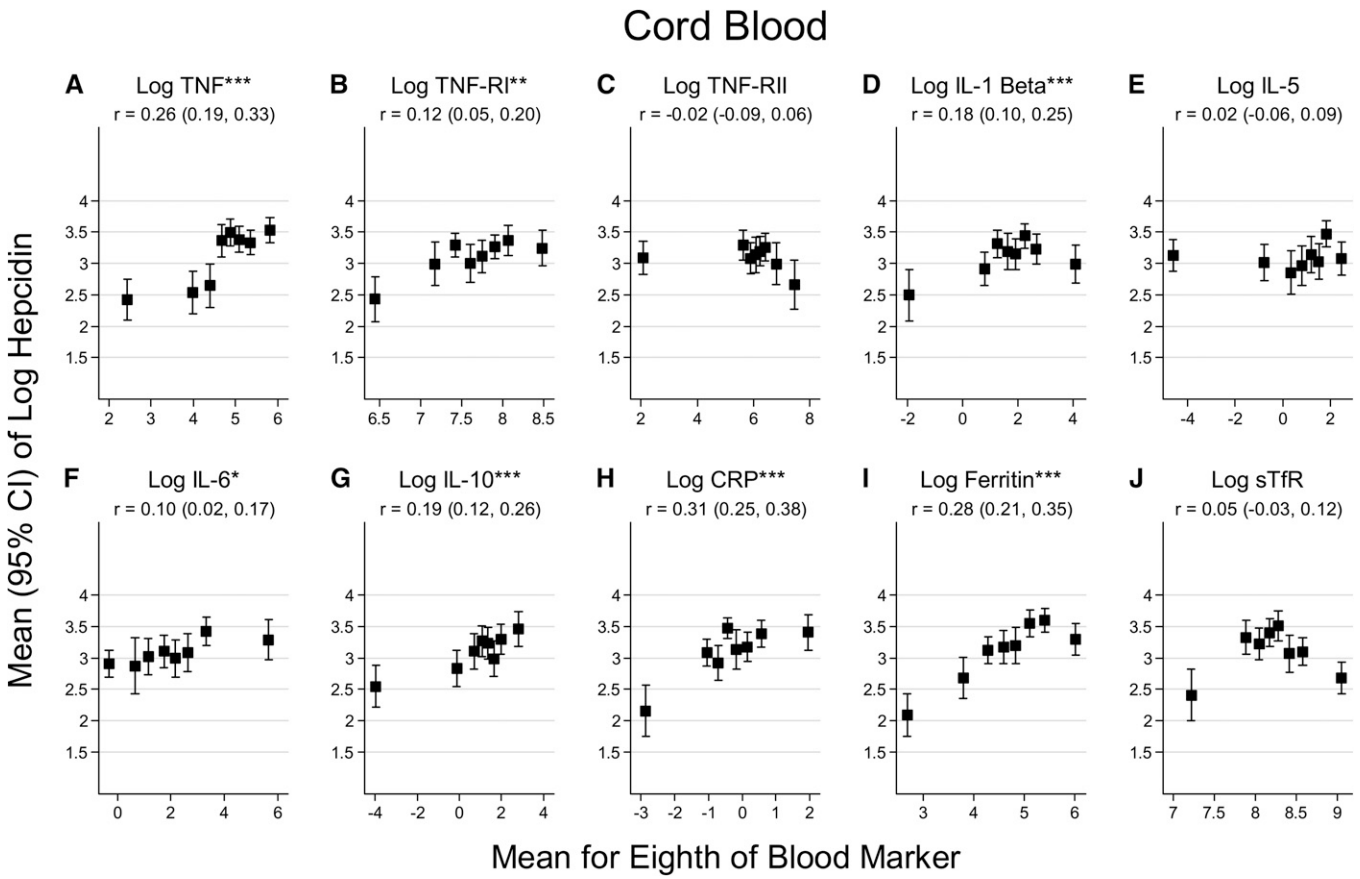

FIGURE 3. Mean $\log_e$ hepcidin levels within eighths of $\log_e$ cord blood markers plotted against the mean of the cord blood marker value in each eighth for assessment of the shape of association with cord hepcidin: (**A**) tumor necrosis factor, (**B**) tumor necrosis factor receptor I, (**C**) tumor necrosis factor receptor II, (**D**) interleukin 1β, (**E**) interleukin 5, (**F**) interleukin 6, (**G**) interleukin 10, (**H**) C-reactive protein, (**I**) ferritin, and (**J**) soluble transferrin receptor. Bars: 95% confidence interval (CI). $^*P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$. CRP = C-reactive protein; IL = interleukin; sTfR = soluble transferrin receptor; TNF = tumor necrosis factor; TNF-RI = tumor necrosis factor receptor I; TNF-RII = tumor necrosis factor receptor II.

TABLE 2
Cross-sectional associations of cord hepcidin with continuous traits measured at the time of delivery

	Variable	Total	Mean (SD)	Mean percent difference (95% CI) per one SD higher level	*P* value
Clinical markers	Birth weight, kg	710	3.2 (0.4)	−2 (−12, 8)	0.67
	Maternal age, years	710	25.9 (6.1)	−5 (−14, 5)	0.35
	Maternal hemoglobin, g/L	594	108.3 (40)	13 (1, 26)	0.04*
Blood markers	Log_e tumor necrosis $factor_{CB}$	710	4.6 (1.2)	43 (30, 57)	< 0.001**
	Log_e tumor necrosis $factor_{PER}$	667	3.0 (2.0)	32 (19, 45)	< 0.001**
	Log_e tumor necrosis $factor_{PLC}$	680	5.8 (0.8)	33 (21, 47)	< 0.001**
	Log_e tumor necrosis factor-receptor I_{CB}	710	7.6 (0.7)	18 (7, 31)	0.001**
	Log_e tumor necrosis factor-receptor I_{PER}	667	6.9 (0.7)	28 (16, 42)	< 0.001**
	Log_e tumor necrosis factor-receptor I_{PLC}	680	8.5 (0.9)	26 (14, 39)	< 0.001**
	Log_e tumor necrosis factor-receptor II_{CB}	710	5.8 (2.0)	−2 (−12, 8)	0.63
	Log_e tumor necrosis factor-receptor II_{PER}	667	3.7 (3.6)	−8 (−17, 2)	0.13
	Log_e tumor necrosis factor-receptor II_{PLC}	680	6.7 (1.1)	−1 (−11, 10)	0.85
	Log_e interleukin $1\beta_{CB}$	710	1.6 (1.9)	27 (15, 39)	< 0.001**
	Log_e interleukin $1\beta_{PER}$	667	0.4 (2.4)	29 (16, 43)	< 0.001**
	Log_e interleukin $1\beta_{PLC}$	680	4.2 (1.1)	20 (9, 34)	< 0.001**
	Log_e interleukin 5_{CB}	710	0.3 (2.2)	2 (−8, 13)	0.68
	Log_e interleukin 5_{PER}	667	0.1 (2.4)	0 (−10, 11)	0.98
	Log_e interleukin 5_{PLC}	680	1.8 (1.5)	15 (4, 27)	0.008*
	Log_e interleukin 6_{CB}	710	2.0 (1.9)	14 (3, 26)	0.009*
	Log_e interleukin 6_{PER}	667	2.7 (1.6)	23 (11, 36)	< 0.001**
	Log_e interleukin 6_{PLC}	680	6.4 (1.3)	26 (14, 39)	< 0.001**
	Log_e interleukin 10_{CB}	710	0.7 (2.0)	30 (17, 43)	< 0.001**
	Log_e interleukin 10_{PER}	667	2.0 (1.6)	15 (4, 28)	0.006*
	Log_e interleukin 10_{PLC}	680	2.6 (1.4)	27 (15, 40)	< 0.001**
	Log_e C-reactive $protein_{CB}$	707	−0.3 (1.4)	54 (39, 69)	< 0.001**
	Log_e C-reactive $protein_{PER}$	651	3.1 (1.9)	38 (25, 53)	< 0.001**
	Log_e C-reactive $protein_{PLC}$	676	1.6 (1.9)	64 (49, 80)	< 0.001**
	Log_e $ferritin_{CB}$	710	4.6 (1.0)	46 (32, 60)	< 0.001**
	Log_e $ferritin_{PER}$	667	3.0 (1.4)	27 (15, 40)	< 0.001**
	Log_e $ferritin_{PLC}$	680	6.6 (0.9)	29 (16, 43)	< 0.001**
	Log_e soluble transferrin $receptor_{CB}$	707	8.2 (0.6)	6 (−4, 18)	0.21
	Log_e soluble transferrin $receptor_{PER}$	651	8.4 (0.7)	−3 (−12, 8)	0.59
	Log_e soluble transferrin $receptor_{PLC}$	676	9.7 (1.2)	34 (21, 48)	< 0.001**

CB = cord blood; CI = confidence interval; PER = maternal peripheral blood; PLC = placental blood; SD = standard deviation.
* $P < 0.05$,
** $P < 0.005$.

contrast, the inverse association between cord hepcidin and maternal anemia at delivery remained significant after controlling for C-reactive protein ($P = 0.007$).

In summary, cord hepcidin levels reflect the maternal health conditions of anemia and placental malaria at the time of delivery and are sensitive to inflammatory and iron-related processes at the maternal–child interface. In total, the measured markers explained approximately 37% of the variance in cord hepcidin level. Although the high levels of correlation between blood markers make it difficult to disentangle the individual relations with hepcidin, C-reactive protein, tumor necrosis factor, and ferritin appear to be the strongest correlates in these analyses.

Cord hepcidin and risks of pediatric anemia, malaria, and mortality. In adjusted linear mixed-effects models, children in the top third of the cord hepcidin distribution had, on average over the duration of follow-up, 2.5 g/L (95% CI: 0.1, 4.8) lower levels of hemoglobin ($P = 0.038$) than children in the lowest third. The best-fit fractional polynomials for the time profile of hemoglobin in children with above and below median levels of cord hepcidin provide evidence that the divergence in hemoglobin trajectories precedes the nadir of the physiologic anemia of infancy and can persist through the first 3 years of life (Figure 4). Confirming the clinical relevance of these observations, children in the highest tertile for cord hepcidin had increased risk of all-cause anemia (adjusted HR [95% C]): 1.18 [1.03, 1.36]) (Figure 5) and severe anemia (adjusted HR [95% CI]: 1.34 [1.08, 1.66]) (Supplemental Figure 4) relative to the lowest tertile. In addition, children with relatively higher concentrations of cord hepcidin also had decreased risk of first malaria infection (i.e., based on survival analyses of time to first parasitemia) (adjusted HR [95% CI] for top versus bottom third: 0.78 [0.67, 0.91]) and all-cause mortality (adjusted HR [95% CI] for top versus bottom third: 0.34 [0.14, 0.84]) (Figure 5). Notably, only one of the 11 malaria-related deaths occurred in a child in the highest tertile of cord hepcidin (Figure 6). Despite these relations, cord hepcidin levels did not appear to be significantly associated with a child's risk of severe malaria (adjusted HR [95% CI] for top third versus bottom third: 1.27 [0.89, 1.80]) (Supplemental Figure 4) nor repeated measurements of parasitemia (mean percent difference [95% CI] in parasite density over the duration of follow-up for top versus bottom third cord hepcidin: 10% [−15, 41]).

DISCUSSION

Using data from a birth cohort of 710 children residing in a perennially endemic, high-transmission region of Tanzania, we observed that cord hepcidin levels are sensitive to inflammatory mediators, iron markers, and maternal health conditions at the time of delivery. Although roughly one-third of the variance in cord hepcidin was attributable to the acute conditions measured at the time of birth, the longitudinal

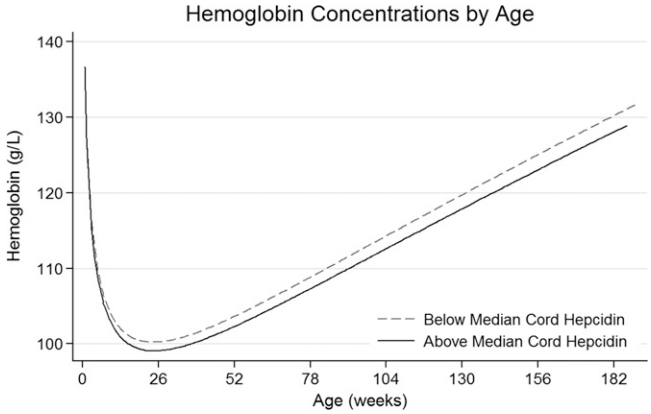

FIGURE 4. Fractional polynomial best-fit model of children's hemoglobin concentrations over follow-up by age in weeks, stratified by cord hepcidin level at birth (N = 683 children, N = 6,121 hemoglobin measurements). The median hepcidin level, 28.1 ng/mL, was derived from the full population with available hepcidin measurements (N = 710). In linear mixed-effects models adjusted for placental malaria, malaria infection status, and cord blood levels of ferritin and C-reactive protein with village- and child-specific random effects and quadratic time trends, children with above median cord hepcidin levels had on average 2.4 g/L (95% confidence interval [CI]: 0.5, 4.3) lower hemoglobin levels (P = 0.012) over the duration of follow-up.

investigation suggests that cord hepcidin may provide an early indication of persistent interindividual differences in iron regulation. Children with relatively higher cord hepcidin levels had heightened susceptibility to anemia and protection from first malaria infection and mortality during the early life course. These results are consistent with the hypothesis that deleterious effects of increased hepcidin on iron availability for erythropoiesis may be counterbalanced by beneficial effects for pathogen control.[29]

Similar to the pediatric studies that have found high concentrations of hepcidin during episodes of malaria parasitemia,[30–35] we observed that cord hepcidin levels were elevated for children born during the high-transmission season and to mothers experiencing placental malaria at delivery. This link between placental malaria infection and hepcidin also aligns with a genome-wide expression analysis that found malaria-infected placentas had a 4-fold upregulation of hepcidin RNA.[36] However, our results differ from the findings of the Van Santen and others' (2011) study, which found no association of placental malaria with cord hepcidin levels in a small sample (N = 69) of primigravid Gabonese mother–child dyads.[37] With the exception of tumor necrosis factor receptor II and interleukin 5, $\log_e$ hepcidin levels had continuous, approximately linear positive associations with the inflammatory mediators, which were highly consistent across the cord, maternal peripheral, and placental blood samples. Of the measured blood markers, the inflammatory marker C-reactive protein was the strongest correlate of hepcidin. The strength of this association may be attributable, in part, to the fact that hepcidin is more tightly temporally coupled with C-reactive protein than cytokines, which have shorter half-lives, in the acute-phase reaction that is activated by normal birth processes.[38]

Markers of iron status were also linked to cord hepcidin levels. In agreement with a recent study of children born to mothers with severe iron deficiency anemia,[39] we found that children born to mothers with anemia at the time of delivery had markedly lower cord hepcidin levels, an association that

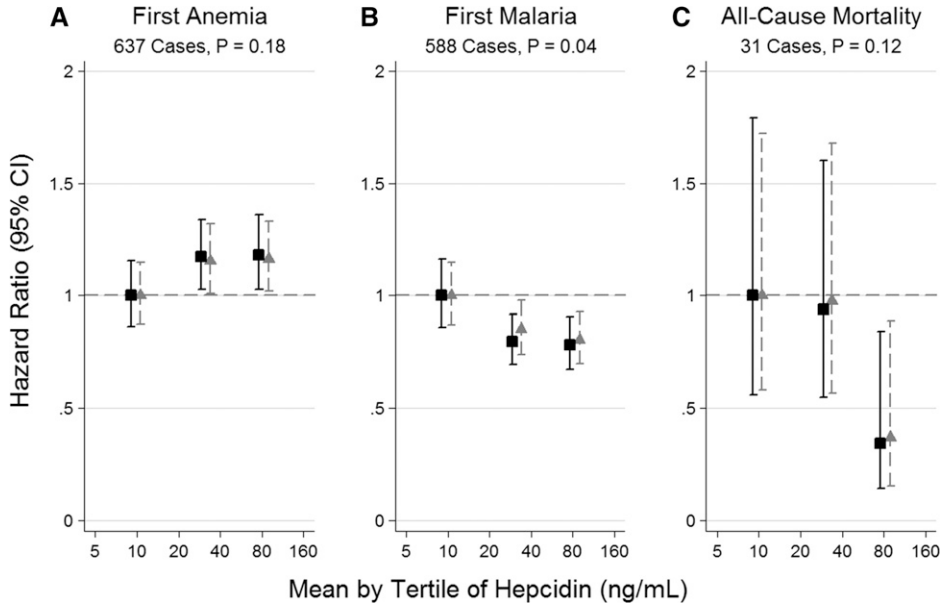

FIGURE 5. Hazard ratios (HRs) for (**A**) anemia, (**B**) malaria, and (**C**) all-cause mortality by tertile of cord hepcidin (N = 707). Gray triangles indicate univariate models; black squares indicate models with further adjustment for village, placental malaria status, and cord levels of ferritin and C-reactive protein. Tertile cutoffs (i.e., 0.195–19.6 ng/mL for tertile 1, 19.7–41.6 ng/mL for tertile 2, and 41.7–319 ng/mL for tertile 3) were derived from the full population with available hepcidin measurements (N = 710); 95% confidence intervals (CIs) were estimated from floated variances. P values were from tests of heterogeneity. Assuming a log-linear relationship, the adjusted HRs (95% CI) for a one standard deviation higher value of cord hepcidin were 1.07 (0.99, 1.17) for anemia, 0.91 (0.83, 0.98) for malaria, and 0.85 (0.61, 1.18) for all-cause mortality.

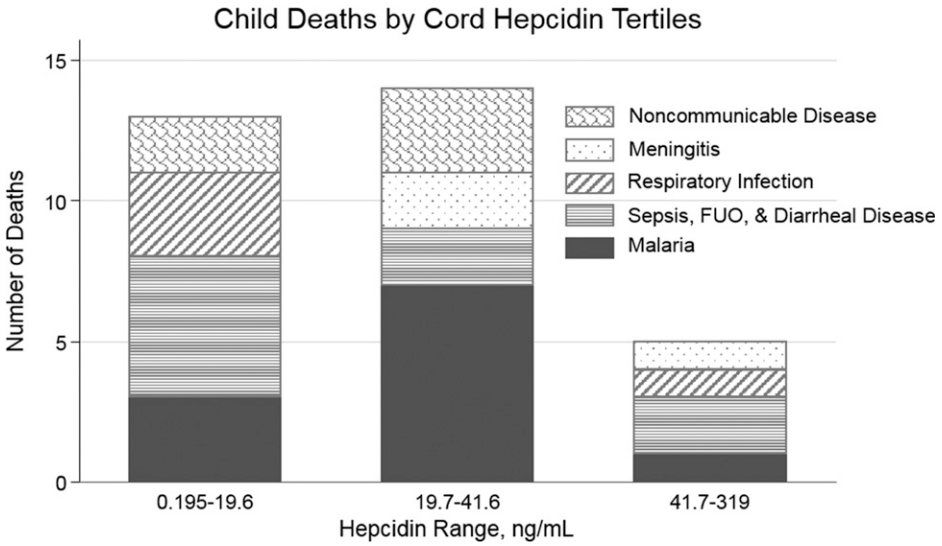

FIGURE 6. The distribution of child deaths by tertile of cord hepcidin. Tertile cutoffs (i.e., 0.195–19.6 ng/mL for tertile 1, 19.7–41.6 ng/mL for tertile 2, and 41.7–319 ng/mL for tertile 3) were derived from the full population with available hepcidin measurements (N = 710). FUO = fever of unknown origin.

was robust to further adjustment for placental inflammation. Consistent with prior investigations, cord hepcidin had a positive, dose–response relationship with cord ferritin.[40,41] The positive association between cord hepcidin and placental levels of soluble transferrin receptor was initially unexpected given earlier findings of an inverse association from the transgenic Thep27 mouse line, in which fetal hepcidin from constitutively hepcidin-expressing offspring was shown to downregulate placental transferrin receptor mRNA.[42] However, further investigation showed that high (i.e., above median) placental levels of soluble transferrin receptor were coincident with lowered maternal ferritin (P = 0.019, Wilcoxon rank-sum test) and normal cord ferritin (P = 0.48, Wilcoxon rank sum test), suggesting that the elevated placental soluble transferrin receptor could be, as previously described, a compensatory mechanism by which the placentas of iron-depleted mothers may enhance transfer of iron to the fetuses despite maternal iron deficiency, thereby mediating conflicting maternal–fetal iron demands.[43]

Building on the hypothetical framework of the Atkinson and others' (2015) study that explored whether healthy child hepcidin concentrations could influence subsequent susceptibility to malaria, we investigated the prospective associations of cord hepcidin with risks of anemia, malaria, and mortality.[35] Unlike the earlier study based on older Kenyan children,[35] we did observe that the cord hepcidin was associated with modestly delayed onset of early malaria infections, findings which resonate with mouse models of hepcidin-mediated malaria protection.[13,44] Nevertheless, higher hepcidin levels were not significantly associated with decreased parasitemia nor severe malaria risk. A possible explanation is that hepcidin may be protecting children from early life infections by inhibiting liver-stage growth, as has been shown in murine models, but not materially altering children's ability to control the severity of infections that reach the blood stage.[13]

The findings that hepcidin could be associated with increased risk of anemia and decreased risk of all-cause mortality could be of greater public health interest. Overall, these results add to the growing concern that elevated hepcidin can impair pediatric iron absorption and promote anemia during critical periods of child development. However, we note that the relationship between hepcidin and anemia risks were nonlinear, and further research will be needed to understand the mechanisms underlying the threshold effects. Although the cross-sectional analyses suggest measuring hepcidin at the time of delivery may be inefficient (i.e., due to the hepcidin's substantial correlations with inflammatory mediators stimulated by the birth process), there may be value in ascertaining children's "usual" levels of hepcidin at later time points (e.g., through monthly measurement during the first 3 months). If the usual hepcidin level in infancy could serve as a prognostic indicator of a child's future iron regulation, then hepcidin testing could facilitate the targeted distribution of iron to those children who are most likely to absorb and use it.[45–47] On the other hand, if the relation between low hepcidin and susceptibility to mortality is a true association and, hypothetically, mediated by iron availability (i.e., if low cord hepcidin → elevated iron absorption → increased mortality), then providing additional iron to children with lower baseline levels of hepcidin without accounting for contemporaneous infectious disease risk could, in theory, make them more vulnerable to early death, such has been observed in the pediatric multinutrient supplementation trials.[19,20] Future investigations should also consider whether interindividual differences in hepcidin could manifest in differential susceptibility to other iron-requiring pathogens, such as *Vibrio* spp.[48] and HIV-1, or macrophage-tropic pathogens, such as *Mycobacterium tuberculosis*.[21]

To date, this is the largest study to measure cord hepcidin in sub-Saharan Africa and the first to prospectively investigate the associated risks of anemia, malaria, and mortality from the time of birth. Nevertheless, this study had some limitations. First, the blood markers examined here are known to fluctuate rapidly and are highly influenced by the acute conditions (e.g., duration of labor, gestational age) at the time of birth. Second, consideration of reverse causation is necessary

for interpreting the cross-sectional correlates of hepcidin described here; for example, it is known that hepcidin levels can both respond to and control circulating iron in the plasma.[49] Third, additional studies with longitudinal measurements of hepcidin and iron stores are required to strengthen causal inference and to further evaluate whether iron absorption and/or availability are part of the mechanistic pathways underlying these prospective associations. Finally, the generalizability of this study is likely limited to regions with high infectious disease burdens. We also recognize that the overall distribution of cord hepcidin levels were low compared with reference standards measured in European neonates, possibly reflecting differences in dietary iron and genetics.[41]

In conclusion, the results of this study highlight the likely involvement of hepcidin in the maintenance of iron homeostasis at the maternal–fetal interface and suggest that measuring hepcidin during infancy may provide an early indication of a child's future susceptibility to anemia and iron-requiring pathogens.

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