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Iron metabolism in children: Confounding factors

Gary M. Brittenham
College of Physicians and Surgeons, Columbia University, New York.

Abstract

Characterization of iron metabolism in infants and children may be confounded by the diverse effects of developmental, genetic, and acquired influences on iron metabolism and laboratory measurements of iron status, especially in areas with intense perennial transmission of Plasmodium falciparum malaria. In the Pemba iron and folic acid supplementation trial, the coadministration of folic acid with iron is a further confounding factor. Because the design of the Pemba iron and folic acid supplementation study did not include a group that received iron supplementation without folic acid, the observed increase in serious adverse events cannot be ascribed unequivocally to iron alone, to folic acid alone, or to the combination of the two. In interpreting the results from the Pemba iron and folic acid supplementation trial, additional analyses of existing data from the trial and from earlier studies in the area could help clarify the roles of iron and folic acid.

Keywords

iron supplementation; folic acid supplementation; *Plasmodium falciparum* malaria

Introduction

Characterization of iron metabolism in infants and children may be confounded by a variety of developmental, genetic, and acquired influences, especially in areas with intense perennial transmission of *Plasmodium falciparum* malaria.

Iron metabolism in infants and children

The concise account of iron metabolism provided by Lönnerdal and Kelleher [1] emphasizes the distinctive features of the handling of iron by infants and children. Iron absorption in young infants initially seems independent of iron intake or body iron stores and then, in older infants, gradually assumes a more adult-like pattern [2]. The ontogeny of iron homeostasis in infancy involves progressive modifications in the regulation, expression, and localization of the proteins involved in iron metabolism. The amount and form of iron presented to the upper small intestine are also important determinants of absorption. In some studies, iron supplements given to iron-replete infants impaired growth [3, 4]. The mechanisms are unknown but may involve compromise of zinc status, with effects on growth hormone and immune function [1]. Iron given as a food fortificant seems to avoid these adverse effects.

Please address queries to the author: Gary M. Brittenham, Children's Hospital of New York, Room CHN 10-08, 3959 Broadway, New York, NY 10032-3795, USA; gmb31@columbia.edu..

Hepcidin, a small antimicrobial peptide principally produced by the liver, has been identified as the central regulator of iron homeostasis, controlling absorption from the intestine, export from macrophages, and release from body stores [5]. Hepcidin acts to decrease iron exit from cells (enterocytes, macrophages, hepatocytes) by binding to ferroportin, the iron export protein, leading to its internalization and degradation. The synthesis of hepcidin is decreased by anemia and hypoxemia but increased by inflammation and by iron overload.

The extent to which developmental changes in the regulation of hepcidin expression govern the pattern of iron absorption in infancy is still uncertain, but progress has been made in identifying the underlying signal transduction mechanisms. The multifunctional BMP/Smad signaling pathway is involved (bone morphogenetic protein/protein Smad, named from a combination derived from two homologues, the *Caenorhabditis elegans* protein SMA and the *Drosophila* protein MAD [mothers against *decapentaplegic*]) [6–9], providing a link to a variety of developmental processes. BMPs are cytokines that are members of the transforming growth factor-β (TGF-β) superfamily and have a variety of roles in control of cell proliferation, differentiation, and apoptosis. A parallel interleukin-6 (IL-6)/signal transducer and activator of transcription-3 (STAT3) pathway is important for transcriptional regulation of hepcidin expression in response to inflammation and infection [10]. Further examination of the interactions of these, and probably other, transduction pathways affecting hepcidin expression is needed for a more detailed understanding of the regulation of iron absorption in infancy and childhood.

In areas with intense perennial transmission of *P. falciparum* malaria, malarial infection has profound and complex effects on erythropoiesis and iron metabolism [11, 12]. In many of the areas with *P. falciparum* malaria, gene variants that affect the structure or function of erythrocytes are common, apparently because these have provided a survival advantage. These variants include the thalassemias and hemoglobinopathies as well as polymorphisms of genes for red cell enzymes and membrane proteins [13–15]. Haptoglobin genotypes also seem to be involved in iron homeostasis [16] and to influence the risk of anemia in malarious areas [17]. The Q248H mutation in ferroportin is associated with iron accumulation in adults and might provide some protection against iron deficiency in African children [18].

In populations living in areas with perennial transmission of *P. falciparum*, our ability to interpret laboratory measurements of iron status is limited by the confounding effects on iron homeostasis of malaria itself, other infections, developmental changes in infancy and childhood, and the influences of polymorphisms for genes affecting red blood cell structure and function [14, 19–21].

Mechanisms of iron toxicity

Hershko [22] emphasizes the importance of non-transferrin-bound plasma iron (NTBI) [23] in the pathogenesis of a variety of toxic effects of iron. As elaborated by Ratledge [24], the availability of NTBI could favor the growth of a variety of microorganisms. Consequently, if given in a highly bioavailable form that produces plasma iron concentrations that exceed the plasma transferrin iron-binding capacity, administration of supplemental iron might favor infection by microbial pathogens. In contrast, as Hershko describes in detail [23], the growth of *P. falciparum* in infected red blood cells seems to be independent of host iron status. Effects of supplemental iron on the course of malaria are more likely to be the result of other effects of iron, such as altering the balance between pro- and antiinflammatory cytokines during the course of malarial infection.

The natural history of juvenile hemochromatosis provides perspective on the importance of the control of plasma iron in determining susceptibility to infection. Juvenile hemochromatosis is most often caused by a mutation in *HJV*, the gene for hemojuvelin (on chromosome 1q21[25]). The remaining cases have been identified as the result of mutations in *HAMP*, the gene encoding hepcidin (on chromosome 19q13 [26, 27]). Urinary hepcidin concentrations are greatly decreased [25] or absent [28]. Having little or no hepcidin-mediated regulation of iron recycling and absorption, plasma iron is increased, with near or full saturation of plasma transferrin, and severe iron overload develops by the second decade of life [29]. Nonetheless, at least in the environments in the developed countries where these patients have been examined, no increased risk of infection has been reported. Patients with thalassemia major and transfusional iron overload do have an increased susceptibility to infection with certain organisms (including *Yersinia enterocolitica* and *Klebsiella spp*.), but splenectomy, a variety of immune abnormalities related to blood transfusion, treatment with a siderophore (deferoxamine), and other factors [30] complicate interpretation of the role of increased plasma iron.

The Pemba iron and folic acid supplementation trial

In the Pemba iron and folic acid supplementation trial [31], the coadministration of folic acid with iron is a further confounding factor, especially because of the use in the study of an antifolate as antimalarial therapy. Because the design of the Pemba iron and folic acid study did not include a group that received iron supplementation without folic acid, the observed increase in serious adverse events cannot be ascribed unequivocally to iron alone, to folic acid alone, or to the combination of the two. As described by Metz [32], growth of some strains of *P. falciparum* may be enhanced by host folate. Folic acid supplementation may increase the risk of both malarial infection and of treatment failure associated with administration of the antifolate antimalarial sulfadoxine-pyrimethamine [33]. In interpreting the results from the Pemba iron and folic acid trial supplementation trial, further analyses of existing data from the trial and from earlier studies in the Pemba area could help elucidate the roles of iron, folic acid, and antifolate antimalarial treatment.

Reexamination of antimalarial therapy given in the Pemba iron and folic acid supplementation substudy

To ensure that the antimalarial sulfadoxine–pyrimethamine therapy administered to the Pemba substudy participants did not contribute to the observed differences in rates of hospitalization and death, reexamination of the study data would be helpful to determine whether the number and timing of antimalarial treatments were evenly allocated among the three study groups. At least 487 courses of sulfadoxine–pyrimethamine were administered by the study group to the 2,413 substudy children for “confirmed malaria” during the trial [31]. Because of the long elimination half-life of the sulfadoxine–pyrimethamine combination [34], a single treatment has a prolonged effect. Consequently, differences in the number or timing of the antifolate antimalarial treatments could have had a profound influence on the risk of adverse events related to malaria.

Moreover, effects of iron and folic acid supplementation on the behavior of children conceivably could influence the likelihood of receiving antimalarial treatment. In Pemba, an earlier study found that over 80% of children 4 to 71 months of age were infected with *P. falciparum* throughout the year. In children less than 30 months old, about half had a parasite density > 5,000/mm^3 [35] during the 12 months of the study. In other words, the majority of children in Pemba had persistent parasitemia, and approximately half would have had a high (> 5,000/mm^3) parasite count during the course of a year. In the Pemba substudy, any child with a parasite count above 5,000/mm^3 and an axillary temperature

above 37.5°C was give a dose of sulfadoxine–pyrimethamine [31]. It seems possible that behavioral effects of treatment of iron deficiency could influence the likelihood of receiving antimalarial therapy. For example, with placebo treatment, anemic and iron-deficient children could have been more likely to be withdrawn and apathetic. As a consequence, a febrile illness would have been less likely to be noticed by their caregivers, and these children would have been less likely to receive medical attention. By contrast, if treated with iron and folic acid, anemic and iron-deficient children might then have been more alert and active, fussing and complaining with a febrile illness, and more likely to have been taken for treatment. Given the high prevalence of parasitemia, antimalarial therapy might well have been given preferentially to the anemic and iron-deficient children treated with iron and folic acid both for malaria and for other febrile illnesses that coincided with a high parasite count.

In the Pemba trial, the investigators state that “we did not find any association of recovery from sulfadoxine–pyrimethamine-treated malaria episodes or subsequent recrudescence with iron and folic acid versus placebo (unpublished data)” [31]. Given the evidence elsewhere in Tanzania of the rapid emergence of resistance to sulfadoxine–pyrimethamine [36] and of studies finding that folic acid supplementation may increase the risk of treatment failure with this antifolate antimalarial [37–40], a full presentation of these data would be valuable.

Reanalysis of a previous study in Pemba of iron supplementation without folic acid

Between 1996 and 1997, an earlier study in Pemba [35, 41, 42] examined the effects of 12 months of low-dose (10 mg/day) iron supplementation without folic acid in a community-based sample of 614 children 4 to 71 months old, using a double-blind, placebo-controlled, randomized design. Although this smaller study did not examine clinical malarial outcomes, iron supplementation had no overall effect on either the prevalence of malaria infection or parasite densities. At baseline and at the end of the 12-month trial, both hemoglobin and zinc protoporphyrin were measured in samples of venous blood. Accordingly, the effects of iron supplementation without folic acid on the prevalence of malaria infection and on parasite densities could be examined with respect to iron status and anemia. As an example, if a lower prevalence of malarial infection, lower parasite densities, or both were found only in the “iron-deficient and anemic” group that received iron, then this reanalysis would provide additional evidence supporting a strategy of targeting iron supplementation to children with evidence of iron deficiency.

In addition, in the 2002–2003 Pemba iron and folic acid supplementation substudy, after 12 months of supplementation, venous blood samples were available for 308 children in the iron and folic acid group without zinc and for 327 children in the placebo group. Comparisons with the similarly sized iron (without folic acid) and placebo groups in the 1996–1997 Pemba study would permit an assessment of the contribution of folic acid to the hematological response to supplementation. If no significant beneficial effect were found, this result would provide further evidence for excluding folic acid from universal supplementation of infants and children, as suggested by Metz [32], i.e., in areas with a high prevalence of anemia, intense perennial malarial transmission, and limited medical facilities using antifolate antimalarials as first-line therapy.

These additional examinations of existing data would be invaluable in clarifying the contributions of iron, folic acid and antifolate antimalarial treatment to the pathogenesis of the adverse events reported in the Pemba iron and folic acid supplementation trial.

References

1. Lönnerdal B, Kelleher SL. Iron metabolism in infants and children. Food Nutr Bull. 2007; 28:S491–9. [PubMed: 18297887]
2. Domellöf M, Lönnerdal B, Abrams SA, Hernell O. Iron absorption in breast-fed infants: Effects of age, iron status, iron supplements, and complementary foods. Am J Clin Nutr. 2002; 76:198–204. [PubMed: 12081835]
3. Dewey KG, Domellöf M, Cohen RJ, Rivera L Landa, Hernell O, Lönnerdal B. Iron supplementation affects growth and morbidity of breast-fed infants: Results of a randomized trial in Sweden and Honduras. J Nutr. 2002; 132:3249–55. [PubMed: 12421836]
4. Idjradinata P, Watkins WE, Pollitt E. Adverse effect of iron supplementation on weight gain of iron-replete young children. Lancet. 1994; 343:1252–4. [PubMed: 7910275]
5. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. Annu Rev Nutr. 2006; 26:323–42. [PubMed: 16848710]
6. Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, Samad TA, Campagna JA, Chung RT, Schneyer AL, Woolf CJ, Andrews NC, Lin HY. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet. 2006; 38:531–9. [PubMed: 16604073]
7. Truksa J, Peng H, Lee P, Beutler E. Bone morphogenetic proteins 2, 4, and 9 stimulate murine hepcidin 1 expression independently of Hfe, transferrin receptor 2 (Tfr2), and IL-6. Proc Natl Acad Sci USA. 2006; 103:10289–93. [PubMed: 16801541]
8. Wang RH, Li C, Xu X, Zheng Y, Xiao C, Zerfas P, Cooperman S, Eckhaus M, Rouault T, Mishra L, Deng CX. A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. Cell Metab. 2005; 2:399–409. [PubMed: 16330325]
9. Niederkofler V, Salie R, Arber S. Hemojuvelin is essential for dietary iron sensing, and its mutation leads to severe iron overload. J Clin Invest. 2005; 115:2180–6. [PubMed: 16075058]
10. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood. 2006; 108:3204–9. [PubMed: 16835372]
11. Menendez C, Fleming AF, Alonso PL. Malaria-related anaemia. Parasitol Today. 2000; 16:469–76. [PubMed: 11063857]
12. Roberts DJ, Casals-Pascual C, Weatherall DJ. The clinical and pathophysiological features of malarial anaemia. Curr Top Microbiol Immunol. 2005; 295:137–67. [PubMed: 16265890]
13. Wambua S, Mwangi TW, Kortok M, Uyoga SM, Macharia AW, Mwacharo JK, Weatherall DJ, Snow RW, Marsh K, Williams TN. The effect of alpha+-thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. PLoS Med. 2006; 3:e158. [PubMed: 16605300]
14. Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casals-Pascual C, Roberts DJ. Malaria and the red cell. Hematology Am Soc Hematol Educ Program. 2002:35–57. [PubMed: 12446418]
15. Williams TN. Red blood cell defects and malaria. Mol Biochem Parasitol. 2006; 149:121–7. [PubMed: 16797741]
16. Van Vlierberghe H, Langlois M, Delanghe J. Haptoglobin polymorphisms and iron homeostasis in health and in disease. Clin Chim Acta. 2004; 345:35–42. [PubMed: 15193975]
17. Atkinson SH, Rockett K, Sirugo G, Bejon PA, Fulford A, O'Connell MA, Bailey R, Kwiatkowski DP, Prentice AM. Seasonal childhood anaemia in West Africa is associated with the haptoglobin 2-2 genotype. PLoS Med. 2006; 3:e172. [PubMed: 16637741]
18. Kasvosve I, Gomo ZA, Nathoo KJ, Matibe P, Mudenge B, Loyevsky M, Gordeuk VR. Effect of ferroportin Q248H polymorphism on iron status in African children. Am J Clin Nutr. 2005; 82:1102–6. [PubMed: 16280445]
19. Domellöf M, Dewey KG, Lönnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be reevaluated. J Nutr. 2002; 132:3680–6. [PubMed: 12468607]
20. Hentze MW, Muckenthaler MU, Andrews NC. Balancing acts: Molecular control of mammalian iron metabolism. Cell. 2004; 117:285–97. [PubMed: 15109490]
21. Mamiro PS, Kolsteren P, Roberfroid D, Tatala S, Opsomer AS, Van Camp JH. Feeding practices and factors contributing to wasting, stunting, and iron-deficiency anaemia among 3–23-month old

children in Kilosa district, rural Tanzania. J Health Popul Nutr. 2005; 23:222–30. [PubMed: 16262018]

22. Hershko C. Mechanism of iron toxicity. Food Nutr Bull. 2007; 28:S500–9. [PubMed: 18297888]
23. Hershko C, Graham G, Bates GW, Rachmilewitz EA. Non-specific serum iron in thalassaemia: An abnormal serum iron fraction of potential toxicity. Br J Haematol. 1978; 40:255–63. [PubMed: 708645]
24. Ratledge C. Iron metabolism and infection. Food Nutr Bull. 2007; 28:S515–23. [PubMed: 18297890]
25. Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dubé MP, Andres L, MacFarlane J, Sakellaropoulos N, Politou M, Nemeth E, Thompson J, Risler JK, Zaborowska C, Babakaiff R, Radomski CC, Pape TD, Davidas O, Christakis J, Brissot P, Lockitch G, Ganz T, Hayden MR, Goldberg YP. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet. 2004; 36:77–82. [PubMed: 14647275]
26. Roetto A, Papanikolaou G, Politou M, Alberti F, Girelli D, Christakis J, Loukopoulos D, Camaschella C. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet. 2003; 33:21–2. [PubMed: 12469120]
27. Lanzara C, Roetto A, Daraio F, Rivard S, Ficarella R, Simard H, Cox TM, Cazzola M, Piperno A, Gimenez-Roqueplo AP, Grammatico P, Volinia S, Gasparini P, Camaschella C. Spectrum of hemojuvelin gene mutations in 1q-linked juvenile hemochromatosis. Blood. 2004; 103:4317–21. [PubMed: 14982873]
28. Papanikolaou G, Tzilianos M, Christakis JI, Bogdanos D, Tsimirika K, MacFarlane J, Goldberg YP, Sakellaropoulos N, Ganz T, Nemeth E. Hepcidin in iron overload disorders. Blood. 2005; 105:4103–5. [PubMed: 15671438]
29. De Gobbi M, Roetto A, Piperno A, Mariani R, Alberti F, Papanikolaou G, Politou M, Lockitch G, Girelli D, Fargion S, Cox TM, Gasparini P, Cazzola M, Camaschella C. Natural history of juvenile haemochromatosis. Br J Haematol. 2002; 117:973–9. [PubMed: 12060140]
30. Vento S, Cainelli F, Cesario F. Infections and thalassaemia. Lancet Infect Dis. 2006; 6:226–33. [PubMed: 16554247]
31. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK, Kabole FM. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: Community-based, randomised, placebo-controlled trial. Lancet. 2006; 367:133–43. [PubMed: 16413877]
32. Metz J. Folic acid metabolism and malaria. Food Nutr Bull. 2007; 28:S540–9. [PubMed: 18297892]
33. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. Pharmacol Rev. 2005; 57:117–45. [PubMed: 15734729]
34. Gatton ML, Martin LB, Cheng Q. Evolution of resistance to sulfadoxine-pyrimethamine in *Plasmodium falciparum*. Antimicrob Agents Chemother. 2004; 48:2116–23. [PubMed: 15155209]
35. Mebrahtu T, Stoltzfus RJ, Chwaya HM, Jape JK, Savioli L, Montresor A, Albonico M, Tielsch JM. Low-dose daily iron supplementation for 12 months does not increase the prevalence of malarial infection or density of parasites in young Zanzibari children. J Nutr. 2004; 134:3037–41. [PubMed: 15514272]
36. Mugittu K, Abdulla S, Falk N, Masanja H, Felger I, Mshinda H, Beck HP, Genton B. Efficacy of sulfadoxine-pyrimethamine in Tanzania after two years as first-line drug for uncomplicated malaria: Assessment protocol and implication for treatment policy strategies. Malar J. 2005; 4:55. [PubMed: 16297234]
37. Carter JY, Loolpapit MP, Lema OE, Tome JL, Nagelkerke NJ, Watkins WM. Reduction of the efficacy of antifolate antimalarial therapy by folic acid supplementation. Am J Trop Med Hyg. 2005; 73:166–70. [PubMed: 16014852]
38. Dzinjalamala FK, Macheso A, Kublin JG, Taylor TE, Barnes KI, Molyneux ME, Plowe CV, Smith PJ. Blood folate concentrations and in vivo sulfadoxine-pyrimethamine failure in Malawian children with uncomplicated *Plasmodium falciparum* malaria. Am J Trop Med Hyg. 2005; 72:267–72. [PubMed: 15772319]

39. Mulenga M, Malunga P, Bennett S, Thuma P, Shulman C, Fielding K, Greenwood B. Folic acid treatment of Zambian children with moderate to severe malaria anemia. Am J Trop Med Hyg. 2006; 74:986–90. [PubMed: 16760508]

40. van Hensbroek MB, Morris-Jones S, Meisner S, Jaffar S, Bayo L, Dackour R, Phillips C, Greenwood BM. Iron, but not folic acid, combined with effective antimalarial therapy promotes haematological recovery in African children after acute falciparum malaria. Trans R Soc Trop Med Hyg. 1995; 89:672–6. [PubMed: 8594693]

41. Stoltzfus RJ, Kvalsvig JD, Chwaya HM, Montresor A, Albonico M, Tielsch JM, Savioli L, Pollitt E. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: Double blind, placebo controlled study. BMJ. 2001; 323:1389–93. [PubMed: 11744561]

42. Stoltzfus RJ, Chway HM, Montresor A, Tielsch JM, Jape JK, Albonico M, Savioli L. Low dose daily iron supplementation improves iron status and appetite but not anemia, whereas quarterly anthelminthic treatment improves growth, appetite and anemia in Zanzibari preschool children. J Nutr. 2004; 134:348–56. [PubMed: 14747671]