

Interactions between iron and vitamin A, riboflavin, copper, and zinc in the etiology of anemia



Michael B. Zimmermann

*Laboratory for Human Nutrition, Swiss Federal Institute of Technology, Zurich, Switzerland
Contact: michael.zimmermann@ilw.agrl.ethz.ch*

MICHAEL ZIMMERMANN

Michael obtained his MD from Vanderbilt University School of Medicine and his MSc in Nutritional Science at the University of California in Berkeley, both in the USA. He is currently Senior Scientist in the Laboratory for Human Nutrition at the Swiss Federal Institute of Technology in Zurich (ETHZ), visiting Professor at Wageningen University in the Netherlands, and holds the Unilever Endowed Chair in International Health and Micronutrients. Michael's research focus is nutrition and metabolism, including the effects of micronutrient deficiencies on thyroid function, and he has won many awards for his work.

INTRODUCTION

The prevalence of anemia is particularly high in developing countries, where 39% of children under five years old, 48% of 5-14 year old children, 42% of all women, and 52% of pregnant women are anemic (1). It is estimated that about half of the anemia is due to iron deficiency (2), and the remainder due to other causes, such as nutritional deficiencies (e.g., deficiencies of vitamin A, riboflavin, folic acid and vitamin B₁₂), infectious disorders (particularly malaria, HIV and tuberculosis), hemoglobinopathies, and ethnic differences in normal Hb distributions (3, 4). In sub-Saharan Africa, anemia affects 50-80% of children (1, 5). In Côte d'Ivoire, using specific indicators of iron status, iron deficiency was detected in approximately 50% of anemic children (6). Although iron deficiency is often assumed to be the cause of most anemia in children in developing regions, a recent Thai study demonstrated that this is not always the case; among 567 6-12 year old children in the rural Northeast, the prevalence of anemia was 31%. Hemoglobinopathies, suboptimal vitamin A status, and age were the major predictors of hemoglobin concentrations (7). Only a small proportion of anemia in the children was associated with iron deficiency. In a study in Sri Lankan children (8), the prevalence of anemia was 50% in males and 58% in females, but among the anemic children, only 30% of males and 48% of females were iron deficient.

Multiple micronutrient deficiencies often coexist in populations in developing countries. Concurrent deficiencies of vitamin A and iron are common in African schoolchildren (9, 10). In a recent survey of Sri Lankan adolescents, iron deficiency was found in 30% of males and 48% of females; folate and zinc deficiencies were found in 54% and 55% of the children, respectively (8). The relative risks of having at least two deficiencies of iron, folate and/or zinc among the anemic children were 1.6 (95% CI; 0.6-4.2) among boys

and 0.8 (95% CI; 0.5-1.5) among girls. A deficiency of one micronutrient may influence the absorption, metabolism and/or excretion of another micronutrient. An example is the aggravation of iodine deficiency goiter by iron deficiency anemia (10), an effect mediated through impairment of the iron dependent thyroid enzyme, thyroperoxidase. This review will focus on interactions between iron deficiency and four other micronutrients – vitamin A, riboflavin, copper, and zinc – in the etiology of anemia (**Table 13.1**).

VITAMIN A

Vitamin A (VA) deficiency (VAD) affects less than 30% of the global population (11). The most vulnerable groups are women of reproductive age, infants and children (12), the same age groups at highest risk for anemia (13, 14). The link between VAD and anemia has been recognized for many years. Surveys in developing countries have generally reported positive correlations between serum retinol (SR) and hemoglobin (Hb) concentrations, with stronger associations in populations with poorer VA status. These include studies in Central American schoolchildren ($r=0.21$, $P<0.05$) (15); Indian children ($r=0.52$, $P<0.001$) (16); Malawian adolescents ($r=0.16$, $P=0.08$) (17) and Austrian adults ($r=0.56$, $P<0.001$) (18). In pooled data from Vietnam, Chile, Brazil, Uruguay, Ecuador, Venezuela, Guatemala and Ethiopia, VA status and Hb were highly correlated ($r=0.77$, $P<0.0001$) (19). In Indonesia, infants and their mothers with SR <0.7 $\mu\text{mol/L}$ had a 2.4 fold increased risk for iron deficiency anemia (IDA) (20). In Tanzania, pregnant women with Hb values <90 g/L were 2.2 fold more likely to have VAD (21).

Hodges, et al. (19) fed adults three different VA deficient diets together with mineral and vitamin supplements for 1-2 years. Despite daily

Table 13.1: Overview of potential interactions of iron and vitamin A, riboflavin, copper and zinc in the etiology of anemia.

Micronutrient interaction	Effect	Mechanism
Vitamin A (VA) – iron	VA repletion in combined iron- and VA-deficient populations improves Hb	May increase resistance to infection, and thereby reduce circulating hepcidin levels triggered by inflammation, increasing dietary iron absorption and mobilization to the marrow
		May improve dietary iron absorption
		May directly stimulate erythropoiesis by increasing renal erythropoietin synthesis
Riboflavin – iron	Compared to iron supplements given alone, riboflavin and iron supplementation produce a greater increase in Hb	May improve mobilization of iron from stores
		May increase dietary iron absorption
Copper – iron	Copper deficiency produces a “functional” iron deficiency anemia, that is responsive to copper but not iron	Reduced hephaestin activity impairs dietary iron absorption by reducing iron efflux from the enterocyte
		Reduced ceruloplasmin activity decreases iron availability to the marrow by reducing iron efflux from the liver and spleen
Zinc – iron	Concurrent zinc and iron supplementation may reduced the efficacy of the iron to increase Hb	May compete at the divalent metal transporter 1 (DMT 1) for transfer from the lumen into the enterocyte

intakes of 18-19 mg iron, mild anemia developed after approximately six months. As SR levels fell from adequate ($>30 \mu\text{g/dL}$), to 20-30 $\mu\text{g/dL}$, to low ($<20 \mu\text{g/dL}$), mean Hb values fell from 156 to 129 to 119 g/L. The anemia was not responsive to iron therapy until the subjects were repleted with VA. Children receiving a soup fortified with iron and vitamin C increased serum iron levels and transferrin saturation to a greater extent when SR levels were $>40 \mu\text{g/dL}$ as compared to $<20 \mu\text{g/dL}$ (22) suggesting VA status affects mobilization of storage iron.

Data from human studies investigating the influence of VA on absorption are equivocal. In Venezuela, iron absorption from fortified bread meals was increased in the presence of VA or β -carotene (23, 24). Iron absorption from a wheat bread based meal increased from 6.9% to 8.3% when the bread was consumed with 300 μg retinol equivalent (RE) added to drinking water (23). In the second study, iron absorption increased from 3.0 to 8.5% when maize based bread contained 40 μg RE as β -carotene; from 3.0 to 8.3% when a wheat bread contained 51 μg RE as β -carotene; and from 2.1 to 8.8% when a rice meal contained 56 μg RE as β -carotene (24). The authors suggested that VA or β -carotene forms a complex with iron in the digestive tract, and that this complex protects iron from reacting with inhibitors of iron absorption such as phytic acid or phenolic compounds. This seems unlikely, however, since the molar ratios of VA to iron used in their studies were <0.04 . In a later report (25), β -carotene, but not retinol, increased iron uptake in a Caco-2 cell system. This suggests that the effect of retinol on iron incorporation into erythrocytes seen in the earlier studies may not be due to increased iron absorption from the gut but rather on improved mobilization of stored iron for erythropoiesis. In contrast, there was no effect of VA on iron absorption when similar meals were fed to Swiss and Swedish subjects (26). The authors suggested that the contradictory results may be due to lower VA status, and possibly lower over-

all nutritional status, in the Venezuelan subjects who were from a lower socioeconomic population compared to the subjects from Switzerland and Sweden. Another study suggested that iron absorption was inhibited when VA was added to a maize meal fed to VA deficient children in the Côte d'Ivoire, and VA supplementation for three weeks did not affect iron absorption (27). These contradictory data suggest that further research is needed to clarify the effect of VA status on iron absorption.

In pre-school and school-age children (28-32) as well as in nonpregnant and pregnant women (33-36), improving VA status usually increases Hb and reduces anemia, although not all studies agree (37, 38). Provision of VA fortified monosodium glutamate not only improved VA status of preschool children in Indonesia but also increased Hb by 10 g/L over a five month period (28). In Indonesia, anemic pregnant women with marginal VA status were given a placebo, VA (2.4 mg RE/d), iron (60 mg/d) or iron plus VA (60 mg iron/d, 2.4 mg RE/d) (33). After eight weeks, the percentage of women no longer anemic was 16%, 35%, 68% and 97%, respectively. In a similar design, anemic school children in Tanzania received daily a placebo, VA (1.5 mg RE), iron (40 mg), or iron plus VA; the Hb increase in the four groups was 3.6 g/L, 13 g/L, 17.5 g/L, and 22.1 g/L, respectively, after three months (32). Overall, these studies suggest that, in areas where VA and iron intakes are poor, dual fortification or supplementation will likely be more effective in controlling anemia than providing VA or iron alone.

The mechanism by which vitamin A exerts its effect on erythropoiesis remains unclear. Several mechanisms may explain the effect of VA status on anemia (39): 1) decreased resistance to infection in VAD, and, hence, an increase in the anemia of infection; 2) effects on iron absorption and/or metabolism; and 3) direct modulation of erythropoiesis. In developing countries with a

high prevalence of infectious diseases, because VA status modulates immune function, VAD may increase risk for infection, and thereby, the anemia of infection (40). There is little data available to directly support this hypothesis (39). Moreover, in a recent controlled study in North African children free of malaria and hookworm, VA repletion clearly improved Hb, suggesting that this mechanism may not be important in some regions (62).

In animals with VA deficiency, iron is retained in the liver and spleen, and is less available for erythropoiesis (41-44). In VA deficient rats, iron uptake by the bone marrow is impaired (45) and erythrocyte incorporation of ^{59}Fe is decreased (46, 47). In deficient animals, repletion with VA increases utilization of iron in bone and spleen (48). In humans, VA deficiency is associated with a low proportion of transferrin saturation and low iron binding capacity (29, 49). In children, consumption of VA fortified sugar increases serum iron, serum ferritin and the proportion of transferrin saturation (50). Roodenburg, et al. (48, 51) suggested that VAD impairs erythropoiesis and speculated that iron accumulation in the reticuloendothelial system may be due to reduced iron transport due to an inhibition of transferrin synthesis. However, Mejia and Arroyave (49) did not find a decrease in circulating transferrin concentrations in rats with VAD.

Retinoids may stimulate erythropoiesis through a direct effect on the later stages of red cell development (52, 53). In vitro, retinoic acid, synergistically with erythropoietin (EPO), stimulates the formation of erythroid burst-forming unit (BFU-E) colonies (54) and d16 (early) erythroid colonies (55). EPO is a 30400 dalton glycoprotein, produced mainly by renal peritubular cells. It acts on the late stages of erythropoiesis, primarily on colony-forming unit erythroid (CFU-E) cells, and stimulates maturation through the normoblast into reticulocytes and mature erythrocytes (56). The enhancer region of the EPO

gene contains a response element that is regulated by retinoic acid (57). In vitro and in animal models, VA treatment stimulates production of EPO (57). In VA deficient rats, treatment with retinoic acid transiently increases circulating EPO concentrations, which return to original levels after 24 hours (58). Retinoids increase EPO gene transcription in an oxygen dependent manner (59).

It is unclear if vitamin A supplementation increases EPO concentrations in humans. Two studies in malnourished populations have examined the effect of VA supplementation on circulating EPO (38, 60). Compared to iron and folate supplementation, VA, iron and folate supplementation did not affect EPO concentrations in Malawian pregnant women (38). In Tanzanian children, a single dose of VA decreased serum ferritin and EPO concentrations measured after 72 hours (60). However, these studies did not have true controls, and were done in regions endemic for malaria, which influences EPO concentrations (61).

A recent intervention trial in malaria-free school-age children with poor VA and iron status investigated the effect of VA repletion on Hb, iron status, and EPO concentrations (62). Using a double blind, randomized design, Moroccan school-children ($n=81$) were given either VA (200,000 IU) or a placebo at baseline and at five months. At baseline, five and 10 months, Hb, indicators of iron and vitamin A status, and EPO were measured. At baseline, 54% of children were anemic and 77% had low VA status. In the VA group at 10 months, serum retinol improved significantly compared to the control. VA treatment increased mean Hb by 7 g/L and reduced the prevalence of anemia from 54% to 38%. VA treatment increased mean corpuscular volume and decreased serum transferrin receptor, indicating improved iron deficient erythropoiesis. VA decreased serum ferritin, suggesting mobilization of hepatic iron stores. Calculated from the TfR to serum ferritin ratio (TfR/SF), overall body iron stores remained

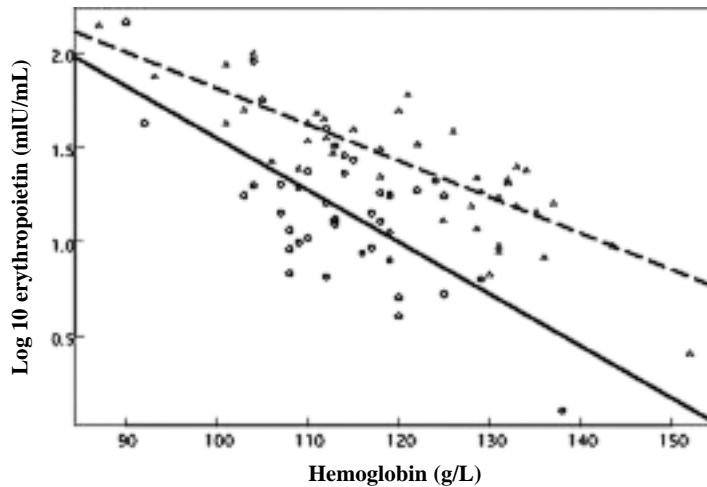


Figure 13.1: Vitamin A and iron deficient schoolchildren ($n=81$) received vitamin A (200,000 IU) or a placebo at baseline and at five months. Compared to placebo (—) vitamin A repletion (- - -) increased circulating erythropoietin (EPO) concentrations and decreased the slope of the regression line of $\log(10)$ EPO on Hb at 10 months. This effect is likely explained by a direct VA-mediated stimulus of erythropoiesis that downregulated the EPO response to lower Hb concentrations.

unchanged. These findings argue against a VA-mediated increase in iron absorption. Rather, they suggest VA repletion causes redistribution of iron from stores to the marrow for erythropoiesis. In the VA group at 10 months, there was an increase in EPO and a decrease in the slope of the regression line of $\log(10)$ EPO on Hb (**Figure 13.1**). Several mechanisms may explain the change in the slope of the regression line. It may represent physiologically appropriate EPO levels for children with Hb at the lower range of the distribution after improvements in iron deficient erythropoiesis. Alternatively, a direct VA-mediated stimulus of erythropoiesis may have downregulated the EPO response to lower Hb concentrations. Enhanced erythropoiesis may lower circulating EPO levels in anemia (63), due to internalization and degradation of the EPO-EPO receptor complex in maturing erythroid cells (64). In summary, in children deficient in VA and iron, VA supplementation mobilizes iron from existing stores to support increased erythropoiesis, an effect likely mediated by increases in circulating EPO (62).

RIBOFLAVIN

Riboflavin (vitamin B₂) is required for many metabolic pathways, usually as a precursor of the flavin coenzyme, NAD, in oxidation reactions (65). Riboflavin deficiency is particularly common in regions where intakes of dairy products and meat are low (66). Deficiency causes impaired growth, cheilosis, angular stomatitis, glossitis and dermatitis, and impaired vision (67). Schoolchildren, in both developing and industrialized countries, are an age group at high risk for riboflavin deficiency (68).

Riboflavin deficiency may also impair erythropoiesis and contribute to anemia (69, 70). Suggested mechanisms for this effect of riboflavin deficiency are decreased mobilization of iron from stores (71, 72), decreased iron absorption, and increased iron losses (73-75). Although these mechanisms have been investigated in animals, there is little data from humans. A stable isotope study in adult Gambian men showed a greater increase in hemoglobin with riboflavin supple-

mentation compared to controls' (both groups received iron supplements) (76). But this study did not demonstrate an increase in iron absorption, suggesting the improvement in hemoglobin may have been due to iron mobilization from stores (76). In three trials in children and pregnant women, compared to iron supplements given alone, riboflavin and iron supplementation produced a greater increase in hemoglobin, although the results may have been confounded by concomitant folic acid supplementation (77-79). A study in pregnant women reported that the combination of iron and riboflavin supplementation, or riboflavin supplementation alone, did not prevent a decrease in hemoglobin, although the decrease was less in the treated groups than the control group (80). Riboflavin supplementation given concurrently with iron improves the response to iron supplementation in adult males and schoolchildren (81, 82). Another trial reported no additional benefit of riboflavin plus iron compared to iron supplementation alone in young adults (83). Similarly, no effect could be found in a riboflavin supplementation trial in Croatian schoolchildren with adequate hemoglobin levels (84). Taken together, these data suggest that the effect of riboflavin status on hemoglobin is variable, and may be confounded by the multifactorial etiology of anemia, particularly in countries in sub-Saharan Africa.

The few published studies investigating riboflavin status in African children suggest that riboflavin deficiency may be widespread. A study in Botswanan children reported that 33-40% of children had an erythrocyte glutathione reductase activation coefficient (EGRAC) ≥ 1.4 (85). In a study in Kenya, approximately one third of children were riboflavin deficient, as measured as red blood cell riboflavin (86). In a survey of 2-5 year old South African children, intakes of riboflavin were less than half of the recommended daily allowance (RDA) (87). A food survey in central Côte d'Ivoire found low consumption of dairy products and meat, the usual major dietary

sources of riboflavin, but riboflavin intakes were not reported (88).

A recent study in 5-15 year old children in south-central Côte d'Ivoire examined the prevalence of riboflavin deficiency in children, estimated the riboflavin content of the local diet and determined if riboflavin deficiency predicts anemia and/or iron deficiency (89). Three-day weighed food records were done to determine riboflavin intakes. Prevalence of anemia in the sample was 52%; 59% were iron deficient, and 36% suffered from iron deficiency anemia. Only 2% of children were VA deficient. Plasmodium parasitemia was found in 49% of the children. Milk and meat intakes were low, and the median intake of riboflavin was 0.42 mg/day, only 47% of the estimated average requirement for this age group. The prevalence of riboflavin deficiency was 65%. Age, elevated C-reactive protein (CRP) and iron deficiency were significant predictors of hemoglobin. Riboflavin deficient children free of malaria were more likely to be iron deficient (odds ratio; 3.07; 1.12-8.41), but riboflavin deficiency did not predict hemoglobin and/or anemia in this age group (89). Thus, these data did not support a detrimental effect of riboflavin deficiency on anemia, as suggested by earlier studies (69, 90).

COPPER

Copper deficiency is a rare cause of anemia (91, 92), which is usually microcytic (93), but also normocytic and macrocytic anemia have also been reported (94). It may be associated with neutropenia and thrombocytopenia (95), and is responsive to dietary supplementation with copper but not with iron (96). How copper deficiency causes anemia is uncertain. The divalent metal transporter 1 (DMT1), the duodenal iron transporter, is also a physiologically relevant copper transporter, and these metals could potentially compete with each other for uptake into entero-

cytes (97). Mild copper deficiency alters gene expression of proteins involved in iron metabolism (98). Copper dependent enzymes are necessary for red cell maturation: Ferroxidase II is responsible for the oxidation of iron during erythropoiesis (99), and copper deficient animals with low levels of cytochrome oxidase only poorly synthesize heme from ferric iron and protoporphyrin (100).

Copper deficiency impairs dietary iron absorption in animals (101) and humans (102). Moreover, animals and humans consuming copper deficient diets develop iron deficiency anemia in addition to accumulating iron in the gut, liver, and spleen (103, 104). These effects are likely mediated through the copper-containing ferroxidases, ceruloplasmin (105) and hephaestin (106) that modulate iron efflux from cells. Ceruloplasmin is found primarily in the circulation, and modulates iron homeostasis in the liver and other tissues. Hephaestin is found at the basolateral membrane of enterocytes in the small intestine, and required for efficient dietary iron absorption (107). Iron absorption in sex-linked anemic mice (sla) is impaired due to a defect in the (108). Sla mice take up iron normally from the intestinal lumen into enterocytes, but are unable to transfer it adequately to the circulation. In a study by Chen, et al. (109), mice were fed a copper deficient diet or a control diet for six weeks. In the copper deficient mice, ceruloplasmin ferroxidase activity was circa 50% that of the controls', and enterocyte hephaestin was also significantly reduced. Efflux of iron from the enterocyte to the circulation involves ferroportin 1, the basolateral iron exporter (110) and hephaestin, a process that is regulated systemically by the liver derived peptide hepcidin (111). In the Chen, et al. study (109), in copper deficient mice, hepatic hepcidin expression was 40% of controls' and enterocyte ferroportin 1 levels were increased tenfold, suggesting systemic iron deficiency. However, although copper deficiency impairs dietary iron absorption and results in iron deficiency anemia,

copper deficiency in the general population is rare, so this interaction is not likely to be of public health importance.

ZINC

Iron deficiency anemia is frequently the result of low dietary iron absorption due to low intakes of meat and high intakes of inhibitors, such as phytate and polyphenols. These same dietary factors decrease bioavailability of zinc (112). Although the data do not suggest that zinc deficiency plays a role in anemia, deficiencies of iron and zinc often coexist, and supplements containing both iron and zinc could be of value in vulnerable populations. However, several studies have suggested concurrent zinc supplementation may reduce the efficacy of iron, possibly by impairing iron absorption. However, high intake of nonheme iron inhibits the absorption of zinc (113-115), and conversely, a high ratio of dietary zinc to iron can inhibit iron absorption (116, 117). These interactions have been reported when the micronutrients were given in a water solution in adults (116, 117), but not when given in infant formula or meals (115, 117-120). The mechanism of this interaction is not clear, but may involve competition for absorption in the small intestine. The DMT 1 transports both iron and zinc ions (121). It is possible that high concentrations of zinc reduce absorption of iron into the enterocyte, although this has not been demonstrated in mammalian systems. The effects of zinc supplementation during mid and late infancy on intestinal iron transport mechanisms were recently investigated using a suckling rat model (122). Earlier in infancy, zinc supplementation was associated with increased enterocyte iron retention, decreased hephaestin, and increased ferroportin 1 expression. During late infancy, these effects were absent. The data suggest that zinc supplementation may reduce iron absorption through increased enterocyte iron retention, possibly due to reduced hephaestin levels (122).

In a recent six month randomized controlled supplementation trial in Vietnam, infants received daily either 10 mg iron, 10 mg zinc, 10 mg iron plus 10 mg zinc, or a placebo. The combined iron–zinc supplementation was as effective as iron supplements to control iron deficiency and anemia (123). Similar efficiency of combined iron–zinc and iron supplementation alone on iron status was also reported in Mexican children (124–126). In contrast, in studies in Indonesian infants (127, 128) with the same doses and duration of zinc and iron supplementation used in the Vietnam study (123), the combined iron–zinc supplementation was less effective than iron alone on improvement of iron status. These data suggest that concurrent zinc supplementation

reduced the efficacy of the iron (128). Differences in baseline iron status between the studies may help explain the differing results; in the Vietnam study (123) there was a greater severity of anemia and iron deficiency than in the Indonesian studies (128).

In a recent review, Fischer Walker, et al. (129) concluded that zinc supplementation alone does not appear to have negative effects on iron status that are clinically relevant. However, the review also suggested that when zinc supplements are given with iron supplements, iron status does not improve as much as when iron is given alone. Further research is needed to clarify the effects of joint zinc and iron supplementation.

REFERENCES

1. WHO/U CF/UNU. Iron deficiency anemia assessment, prevention, and control. Geneva: World Health Organization, 2001.
2. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. *Lancet* 2006 (in press).
3. Nestel P. Adjusting hemoglobin values in program surveys. Washington, DC: International Nutritional Anemia Consultative Group (INACG), 2002.
4. Lynch SR. The impact of iron fortification on nutritional anaemia. *Best Pract Res Clin Haematol*. 2005;18(2):333–46.
5. United Nations ACC/SCN. Fourth report on the World nutrition situation. Geneva: UN ACC/SCN in collaboration with The International Food Policy Research Institute (IFPRI), 2000:132.
6. Asobayire FS, Adou P, Davidsson L, Cook JD, Hurrell RF. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Cote d'Ivoire. *Am J Clin Nutr*. 2001; 74(6):776–82.
7. Thurlow RA, Winichagoon P, Green T, Wasantwisut E, Pongcharoen T, Bailey KB, Gibson RS. Only a small proportion of anemia in northeast Thai schoolchildren is associated with iron deficiency. *Am J Clin Nutr* 2005;82(2):380–7.
8. Hettiarachchi M, Liyanage C, Wickremasinghe R, Hilmers DC, Abrahams SA. Prevalence and severity of micronutrient deficiency: a cross-sectional study among adolescents in Sri Lanka. *Asia Pac J Clin Nutr*. 2006;15(1):56–63.
9. Zimmermann MB, Wegmüller R, Zeder C, Chaouki N, Biebinger R, Hurrell RF, Windhab E. Triple fortification of salt with microcapsules of iodine, iron and vitamin A. *Am J Clin Nutr* 2004;80:1283–90.
10. Zimmermann MB, Adou P, Zeder C, Torresani T, Hurrell RF. Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in the Côte d'Ivoire. *Am J Clin Nutr* 2000;71:88–93.
11. WHO. Vitamin A deficiency and iodine deficiency disorders: prevalence estimates for the glob-

- al burden of disease. Micronutrient Deficiency Information System (MDIS). Geneva: World Health Organization, 2001.
12. West KP. Extent of vitamin A deficiency among preschool children and women of reproductive age. *J Nutr* 2002;132:2857S–66S.
 13. WHO/UNICEF/UNU (United Nations Children's Fund, United Nations University). IDA: prevention, assessment and control. Report of a joint WHO/UNICEF/UNU consultation. Geneva: World Health Organization, 1998:1–9.
 14. Cook JD. Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol* 2005;18(2):319–32.
 15. Majia LA, Hodges RE, Arroyave G, Viteri F, Torun B. Vitamin A deficiency and anemia in Central American children. *Am J Clin Nutr* 1977;30:1175–84.
 16. Mohanram M, Kulkarni KA, Reddy V. Hematological studies in vitamin A deficient children. *Int J Vitam Nutr Res* 1977;47:389–93.
 17. Fazio-Tirrozzo G, Brabin L, Brabin B, Agbaje O, Harper G, Broadhead R. A community based study of vitamin A and vitamin E status of adolescent girls living in the Shire Valley, southern Malawi. *Eur J Clin Nutr* 1998;52:637–42.
 18. Wenger R, Ziegler B, Kruspl W, Syre B, Brubacher G, Pillat B. [Relationship between vitamin status (A, B1, B2, B6, and C), clinical features and nutritional habits in a population of old people (author's transl)]. *Wien Klin Wochenschr* 1979; 91:557–63.
 19. Hodges RE, Sauberlich HE, Canham JE, et al. Hematopoietic studies in vitamin A deficiency. *Am J Clin Nutr* 1978;31:876–85.
 20. Dijkhuizen MA, Wieringa FT, West CE, Muherdiyantiningsih, Muhilal. Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia. *Am J Clin Nutr* 2001;73:786–91.
 21. Hinderaker SG, Olsen BE, Lie RT, et al. Anemia in pregnancy in rural Tanzania: associations with micronutrients status and infections. *Eur J Clin Nutr* 2002;56:192–9.
 22. van Stuijvenberg ME, Kruger M, Badenhorst CJ, Mansvelt EPG, Laubscher JA. Response to an iron fortification programme in relation to vitamin A status in 6-12-year-old schoolchildren. *Int J Food Sci Nutr* 1997;48:41–9.
 23. Layrisse M, Garcia-Casal MN, Solano L, et al. The role of vitamin A on the inhibitors of nonheme iron absorption: preliminary results. *J Nutr Biochem* 1997;8:61–7.
 24. Garcia-Casal MN, Layrisse M, Solano L, Baron MA, Arguello F, Llovera D, Ramirez J, Leets I, Tropper E. Vitamin A and beta-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. *J Nutr* 1998; 128:646–50.
 25. Garcia-Casal MN, Leets I, Layrisse M. Beta-carotene and inhibitors of iron absorption modify iron uptake by Caco-2 cells. *J Nutr* 2000;130:5–9.
 26. Walczyk T, Davidsson L, Rossander-Hulthen L, Hallberg L, Hurrell RF. No enhancing effect of vitamin A on iron absorption in humans. *Am J Clin Nutr* 2003;77:144–9.
 27. Davidsson L, Adou P, Zeder C, Walczyk T, Hurrell R. The effect of retinyl palmitate added to iron-fortified maize porridge on erythrocyte incorporation of iron in African children with vitamin A deficiency. *Brit J Nutr* 2003;90:337–43.
 28. Muhilal, Permeisih D, Idjradinata YR, Muherdiyantiningsih, Karyadi D. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. *Am J Clin Nutr* 1988;48:1271–6.
 29. Mejia LA, Chew F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. *Am J Clin Nutr* 1988;48:595–600.
 30. Bloem MW, Wede IM, Egger RJ, Speek AJ, Schrijver J, Saowakontha S, Schreurs WHP. Iron metabolism and vitamin A deficiency in children in Northeast Thailand. *Am J Clin Nutr* 1989;50:332–8.
 31. Semba RD, Muhilal, West KP Jr, Winget M, Natadisastra G, Scott A, Sommer A. Impact of vitamin A supplementation on hematological indicators of iron metabolism and protein status in children. *Nutr Res* 1992;12:469–78.
 32. Mwanri L, Worsley A, Ryan P, Masika J. Supplemental vitamin A improves anemia and

- growth in anemic school children in Tanzania. *J Nutr* 2000;130:2691–6.
33. Suharno D, West CE, Muhilal, Karyadi D, Hautvast GAJ. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. *Lancet* 1993;342:1325–8.
34. Chawla PK, Puri R. Impact of nutritional supplements on hematological profile of pregnant women. *Indian Pediatr* 1995;32:876–80.
35. Kolsteren P, Rahman SR, Hilderbrand K, Diniz A. Treatment for iron deficiency anaemia with a combined supplementation of iron, vitamin A and zinc in women of Dinajpur, Bangladesh. *Eur J Clin Nutr* 1999;53:102–6.
36. Muslimatun S, Schmidt MK, Schultink W, West CE, Hautvast JGAJ, Gross R, Muhilal. Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. *J Nutr* 2001;131:85–90.
37. Fawzi WW, Msamanga GI, Spiegelman D, Urassa EJ, McGrath N, Mwakagile D, Antelman G, Mbise R, Herrera G, Kapiga S, Willett W, Hunter DJ. Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. *Lancet* 1998;351:1477–82.
38. Semba RD, Kumwenda N, Taha TE, Mtimavalye L, Broadhead R, Garrett E, Miotti PG, Chipangwi JD. Impact of vitamin A supplementation on anaemia and plasma erythropoietin concentrations in pregnant women: a controlled clinical trial. *Eur J Haematol* 2001;66:389–95.
39. Semba RD, Bloem MW. The anemia of vitamin A deficiency: epidemiology and pathogenesis. *Eur J Clin Nutr* 2002;56:271–81.
40. Means RT, Jr. The anaemia of infection. *Baillieres Best Pract Res Clin Haematol* 2000;13:151–162.
41. Staab DB, Hodges RE, Metcalf WK, Smith JL. Relationship between vitamin A and iron in the liver. *J Nutr* 1984;114:840–4.
42. Sklan D, Halevy O, Donaghue S. The effect of different dietary levels of vitamin A on metabolism of copper, iron and zinc in the chick. *Int J Vit Nutr Res* 1987;57:11–18.
43. Beynen AC, Sijtsma KW, van den Berg GJ, Lemmens AG, West CE. Iron status in rats fed a purified diet without vitamin A. *Biol Trace Elem Res* 1992;35:81–4.
44. Roodenburg AJC, West CE, Yu S, Beynen AC. Comparison between time-dependent changes in iron metabolism of rats as induced by marginal deficiency of either vitamin A or iron. *Br J Nutr* 1994;71:687–99.
45. Sijtsma KW, van den Berg GJ, Lemmens AG, West CE, Beynen A. Iron status in rats fed on diets containing marginal amounts of vitamin A. *Br J Nutr* 1993;70:777–85.
46. Mejia LA, Hodges RE, Rucker RB. Clinical signs of anemia in vitamin A-deficient rats. *Am J Clin Nutr* 1979;32:1439–44.
47. Gardner R, Hodges R, Rucker R. Fate of erythrocyte iron in vitamin A deficient rats. *Fed Proc* 1979;38:762.
48. Roodenburg AJC, West CE, Hovenier R, Beynen AC. Supplemental vitamin A enhances the recovery from iron deficiency in rats with chronic vitamin A deficiency. *Br J Nutr* 1996;75:623–36.
49. Mejia LA, Arroyave G. Lack of direct association between serum transferrin and serum biochemical indicators of vitamin A nutriture. *Acta Vitaminol Enzymol* 1983;5:179–84.
50. Mejia LA, Arroyave G. The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala. *Am J Clin Nutr* 1982;36:87–93.
51. Roodenburg AJ, West CE, Beguin Y, et al. Indicators of erythrocyte formation and degradation in rats with either vitamin A or iron deficiency. *J Nutr* 2000;11:223–30.
52. Rusten LS, Dybedal I, Blomhoff HK, Blomhoff R, Smeland EB, Jacobsen SE. The RAR-RXR as well as the RXR-RXR pathway is involved in signaling growth inhibition of CD34 β erythroid progenitor cells. *Blood* 1996;87:1728–36.
53. Perrin MC, Blanchet JP, Mouchiroud G. Modulation of human and mouse erythropoiesis by thyroid hormone and retinoic acid: evidence for specific effects at different steps of the erythroid pathway. *Hematol Cell Ther* 1997;39:19–26.

54. Douer D, Koeffler HP. Retinoic acid enhances growth of human early erythroid progenitor cells in vitro. *J Clin Invest* 1982;69:1039–41.
55. Correa PN, Axelrad AA. Retinyl acetate and all-trans-retinoic acid enhance erythroid colony formation in vitro by circulating human progenitors in an improved serum-free medium. *Int J Cell Cloning* 1992;10:286–91.
56. Fisher JW. Erythropoetin: physiology and pharmacology update. *Exp Biol Med* 2003;228:1–14.
57. Evans, T. Regulation of hematopoiesis by retinoid signalling. *Exp Hematol* 2005;33:105–61.
58. Okano M, Masuda S, Narita H, Masushige S, Kato S, Imagawa S, Sasaki R. Retinoic acid up-regulates erythropoietin production in hepatoma cells and in vitamin A-depletion rats. *FEBS Lett* 1994;349:229–33.
59. Kambe T, Tada-Kambe J, Kuge Y, Yamaguchi-Iwai Y, Nagao M, Sasaki R. Retinoic acid stimulates erythropoietin gene transcription in embryonal carcinoma cells through the direct repeat of a steroid=thyroid hormone receptor response element half-site in the hypoxia-response enhancer. *Blood* 2000;96:3265–71.
60. Cusick SE, Tielsch JM, Ramsan M, Jape JK, Sazawal S, Black RE, Stoltzfus RJ. Short-term effects of vitamin A and antimalarial treatment on erythropoiesis in severely anemic Zanzibari pre-school children. *Am J Clin Nutr* 2005;82:406–12.
61. Burgmann H, Looareesuwan S, Kapiotis S, Viravan C, Vanijanonta S, Hollenstein U, Wiesinger E, Presterl E, Winkler S, Graninger W. Serum levels of erythropoietin in acute *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 1996;54:280–3.
62. Zimmermann MB, Biebinger R, Rohner F, Dib A, Zeder C, Hurrell RF, Chaouki N. Vitamin A supplementation in children with poor vitamin A and iron status increases erythropoietin and hemoglobin concentrations without changing total body iron. *Am J Clin Nutr* 2006;84:580–6.
63. Bray GL, Taylor B, O'Donnell R. Comparison of the erythropoietin response in children with aplastic anemia, transient erythroblastopenia, and iron deficiency. *J Pediatr* 1992;120:528–32.
64. Gross AW, Lodish HF. Cellular trafficking and degradation of erythropoietin and novel erythropoiesis stimulating protein (NESP). *J Biol Chem* 2006;281:2024–32.
65. McCormick DB. Co-enzymes, biochemistry. In: R Dulbecco, ed. *Encyclopedia of human biology*. San Diego: Academic Press, 1997:847–64.
66. Neumann CG, Bwibo NO, Murphy SP, Sigman M, Whaley S, Allen LH, Guthrie D, Weiss RE, Demment MW. Animal source foods improve dietary quality, micronutrient status, growth and cognitive function in Kenyan school children: background, study design and baseline findings. *J Nutr* 2003;133:3941S-9S.
67. FAO/WHO. Thiamin, riboflavin, niacin, vitamin B6, pantothenic acid and biotin. In: *Human vitamin and mineral requirements*. Rome: FAO, 2001:27–51.
68. Powers HJ. Riboflavin (vitamin B-2) and health. *Am J Clin Nutr* 2003;77:1352–60.
69. Foy H, Kondi A. A case of true red cell aplastic anaemia successfully treated with riboflavin. *J Pathol Bacteriol* 1953;65:559–64.
70. Foy H, Kondi A, Mbaya V. Effect of riboflavin deficiency on bone marrow function and protein metabolism in baboons. Preliminary Report. *Br J Nutr* 1964;18:307–18.
71. Sirivech S, Frieden E, Osaki S. The release of iron from horse spleen ferritin by reduced flavins. *J Biochem* 1974;143:311–5.
72. Powers HJ. Riboflavin-iron interactions with particular emphasis on the gastrointestinal tract. *Proc Nutr Soc* 1995;54:509–17.
73. Adelekan DA, Thurnham DI. The influence of riboflavin deficiency on absorption and liver storage of iron in the growing rat. *Br J Nutr* 1986;56:171–9.
74. Powers HJ, Wright AJ, Fairweather-Tait SJ. The effect of riboflavin deficiency in rats on the absorption and distribution of iron. *Br J Nutr* 1988;59:381–7.
75. Butler BF, Topham RW. Comparison of changes in the uptake and mucosal processing of iron in riboflavin-deficient rats. *Biochem Mol Biol Int* 1993;30:53–61.
76. Fairweather-Tait SJ, Powers HJ, Minski MJ, Whitehead J, Downes R. Riboflavin deficiency and iron absorption in adult Gambian men. *Ann Nutr Metab* 1992;36:34–40.

77. Charoenlarp P, Pholpothi T, Chatpunyaporn P, Schelp FP. The effect of riboflavin on the hematologic changes in iron supplementation of school-children. *Southeast Asian J Trop Med Public Health* 1980;11:97–103.
78. Powers HJ, Bates CJ, Prentice AM, Lamb WH, Jepson M, Bowman H. The relative effectiveness of iron and iron with riboflavin in correcting a microcytic anaemia in men and children in rural Gambia. *Hum Nutr-Clin Nutr* 1983;37:413–25.
79. Suprpto B, Widodo, Suhanantyo. Effect of low-dosage vitamin A and riboflavin on iron-folate supplementation in anaemic pregnant women. *Asia Pac J Clin Nutr* 2002;11:263–7.
80. Powers HJ, Bates CJ, Lamb WH. Haematological response to supplements of iron and riboflavin to pregnant and lactating women in rural Gambia. *Hum Nutr Clin Nutr* 1985;39:117–29.
81. Buzina R, Jusic M, Milanovic N, Sapunar J, Brubacher G. The effects of riboflavin administration on iron metabolism parameters in a school-going population. *Int J Vitam Nutr Res* 1979;49:136–43.
82. Ajayi OA, Okike OC, Yusuf Y. Haematological response to supplements of riboflavin and ascorbic acid in Nigerian young adults. *Eur J Haematol* 1990;44:209–12.
83. Powers HJ, Bates CJ. Micronutrient deficiencies in the aetiology of anaemia in a rural area in the Gambia. *Trans R Soc Trop Med Hyg* 1987;81:421–5.
84. Suboticanec K, Stavljenic A, Schalch W, Buzina R. Effects of pyridoxine and riboflavin supplementation on physical fitness in young adolescents. *Int J Vitam Nutr Res* 1990;60:81–8.
85. Abrams SA, Mushi A, Hilmers DC, Griffin IJ, Davila P, Allen L. A multinutrient-fortified beverage enhances the nutritional status of children in Botswana. *J Nutr* 2003;133:1834–40.
86. Siekmann JH, Allen LH, Bwibo NO, Demment MW, Murphy SP, Neumann CG. Kenyan school children have multiple micronutrient deficiencies, but increased plasma vitamin B-12 is the only detectable micronutrient response to meat or milk supplementation. *J Nutr* 2003;133:3972S–80S.
87. Faber M, Jogessar VB, Benade AJ. Nutritional status and dietary intakes of children aged 2-5 years and their caregivers in a rural South African community. *Int J Food Sci Nutr* 2001;52:401–11.
88. Staubli-Asobayire F. Development of a food fortification strategy to combat iron deficiency in the Ivory Coast. Zurich: Die Eidgenössische Technische Hochschule Zürich (ETH), 2000.
89. Rohner F, Zimmermann MB, Wegmueller R, Tschannen AB, Hurrell RF. Riboflavin deficiency is highly prevalent in school-age children in Côte d'Ivoire but does not increase risk for anaemia. *Br J Nutr* 2006 (in press).
90. Foy H; Kondi A. Anaemias of the Tropics: East Africa; with special reference to proteins and liver damage. *Trans R Soc Trop Med Hyg* 1958;52:46–70.
91. Todd L, Godber I, Gunn I. Iatrogenic copper deficiency causing anaemia and neutropenia. *Ann Clin Biochem* 2004;41:414–6.
92. Harless W, Crowell E, Abraham J. Anemia and neutropenia associated with copper deficiency of unclear etiology. *Am J Hematol* 2006;81(7):546–9.
93. Kahn MJ, Leissinger C. Underproduction anemias. In: Williams ME, Kahn MJ, eds. American Society of Hematology self-assessment program. 2nd ed. Massachusetts: Blackwell Publishing, 2005:71–85.
94. Miyoshi I, Saito T, Iwahara Y. Copper deficiency anaemia. *Br J Haematol* 2004;125:106.
95. Fuhrman MP, Herrmann V, Masidonski P, Eby C. Pancytopenia after removal of copper from total parenteral nutrition. *Jpen-Parenter Nutr* 2000;24:361–6.
96. Reeves PG, Demars LC. Signs of iron deficiency in copper-deficient rats are not affected by iron supplements administered by diet or by injection. *J Nutr Biochem* 2006;17:635–42.
97. Sharp P. The molecular basis of copper and iron interactions. *Proc Nutr Soc* 2004;63(4):563–9.
98. Auclair S, Feillet-Coudray C, Coudray C, Schneider S, Muckenthaler MU, Mazur A. Mild copper deficiency alters gene expression of proteins involved in iron metabolism. *Blood Cells Mol Dis* 2006;36(1):15–20.
99. Linder M, Hazegh-Azam M. Copper biochem-

- istry and molecular biology. *Am J Clin Nutr* 1996;63:797S–811S.
100. Cizewski Culotta V, Gitlin JD. Disorders of copper metabolism. In: Scriver CR, Beaudett A, et al., eds. *Metabolic and molecular bases of inherited disorders*, 8th ed. New York: McGraw-Hill, 2001:3105–26.
101. Reeves PG, DeMars LC. Copper deficiency reduces iron absorption and biological half-life in male rats. *J Nutr* 2004;134:1953–57.
102. Chirulescu Z, Suciuc A, et al., Possible correlation between the zinc and copper concentrations involved in the pathogenesis of various forms of anemia. *Internal Med* 1990;28:31–5.
103. Lee GR, Nacht S, Lukens JN, Cartwright GE. Iron metabolism in copper-deficient swine. *J Clin Invest* 1968;47:2058–69.
104. Danks DM. Copper deficiency in humans. *Ciba Found Symp* 1980;79:209–25.
105. Harris ZL, Durley AP, Man TK, Gitlin JD. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. *Proc Natl Acad Sci USA* 1999;96:10812–7.
106. Chen H, Attieh ZK, Su T, Syed BA, Gao H, Alaeddine RM, Fox TC, Usta J, Naylor CE, et al. Hephaestin is a ferroxidase that maintains partial activity in sex-linked anemia mice. *Blood* 2004;103:3933–9.
107. Petrak J, Vyoral D. Hephaestin – a ferroxidase of cellular iron export. *Int J Biochem Cell Biol* 2005;37:1173–8.
108. Vulpe CD, Kuo YM, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat Genet* 1999;21:195–9.
109. Chen H, Huang G, Su T, Gao H, Attieh ZK, McKie AT, Anderson GJ, Vulpe CD. Decreased hephaestin activity in the intestine of copper-deficient mice causes systemic iron deficiency. *J Nutr* 2006;136(5):1236–41.
110. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, Miret S, Bomford A, Peters TJ, et al. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol Cell* 2000;5:299–309.
111. Frazer DM, Wilkins SJ, Becker EM, Vulpe CD, McKie AT, Trinder D, Anderson GJ. Hepsidin expression inversely correlates with the expression of duodenal iron transporters and iron absorption in rats. *Gastroenterology* 2002;123:835–44.
112. Lonnerdal B. Dietary factors influencing zinc absorption. *J Nutr* 2000;130(5S):1378S–83S.
113. Solomons NW, Jacob RA. Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. *Am J Clin Nutr* 1981;34:475–82.
114. Valberg LS, Flanagan PR, Chamberlain MJ. Effects of iron, tin, and copper on zinc absorption in humans. *Am J Clin Nutr* 1984;40:536–41.
115. Sandstrom B, Davidsson L, Cederblad A, Lonnerdal B. Oral iron, dietary ligands and zinc absorption. *J Nutr* 1985;115:411–4.
116. Crofton RW, Gvozdanovic D, Gvozdanovic S, et al. Inorganic zinc and the intestinal absorption of ferrous iron. *Am J Clin Nutr* 1989;50:141–4.
117. Rossander-Hulten L, Brune M, Sandstrom B, Lonnerdal B, Hallberg L. Competitive inhibition of iron absorption by manganese and zinc in humans. *Am J Clin Nutr* 1991;54:152–6.
118. Davidsson L, Mackenzie J, Kastenmayer P, et al. Dietary fiber in weaning cereals: a study of the effect on stool characteristics and absorption of energy, nitrogen, and minerals in healthy infants. *J Pediatr Gastroenterol Nutr* 1996;22:167–79.
119. Haschke F, Ziegler EE, Edwards BB, Fomon SJ. Effect of iron fortification of infant formula on trace mineral absorption. *J Pediatr Gastroenterol Nutr* 1986;5:768–73.
120. Fairweather-Tait SJ, Wharf SG, Fox TE. Zinc absorption in infants fed iron-fortified weaning food. *Am J Clin Nutr* 1995;62:785–9.
121. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997;388:482–8.
122. Kelleher SL, Lonnerdal B. Zinc supplementation reduces iron absorption through age-dependent changes in small intestine iron transporter expression in suckling rat pups. *J Nutr* 2006;136(5):1185–91.

123. Berger J, Ninh NX, Khan NC, Nhien NV, Lien DK, Trung NQ, Khoi HH. Efficacy of combined iron and zinc supplementation on micronutrient status and growth in Vietnamese infants. *Eur J Clin Nutr* 2006;60(4):443–54.
124. Rosado JL, Lopez P, Munoz E, Martinez H, Allen LH. Zinc supplementation reduced morbidity, but neither zinc nor iron supplementation affected growth or body composition of Mexican preschoolers. *Am J Clin Nutr* 1997;65:13–9.
125. Allen LH, Rosado JL, Casterline JE, et al. Lack of hemoglobin response to iron supplementation in anemic Mexican preschoolers with multiple micronutrient deficiencies. *Am J Clin Nutr* 2000;71:1485–94.
126. Munoz EC, Rosado JL, Lopez P, Furr HC, Allen LH. Iron and zinc supplementation improves indicators of vitamin A status of Mexican preschoolers. *Am J Clin Nutr* 2000;71:789–94.
127. Dijkhuizen MA, Wieringa FT, West CE, Martuti S, Muhilal. Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth. *J Nutr* 2001;131:2860–5.
128. Lind T, Lonnerdal B, Stenlund H, Ismail D, Seswandhana R, Ekstrom EC, et al. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. *Am J Clin Nutr* 2003;77:883–90.
129. Fischer Walker C, Kordas K, Stoltzfus RJ, Black RE. Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. *Am J Clin Nutr* 2005;82:5–12.