Micronutrient Interactions on Risk of Infantile Anemia: Going beyond Iron Alone!

Lena Davidsson

Laboratory for Human Nutrition, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, Rüschlikon, Switzerland

Introduction

Iron deficiency in its most severe form results in anemia (iron deficiency anemia, IDA) and since anemia (Hb concentration or Hct below established cutoff levels) is relatively easy to diagnose, the prevalence of anemia has often been used as a proxy for IDA. Although this approach can be useful in settings where iron deficiency is the major cause of anemia, it is less valid in regions where the etiology of anemia is more complex. For example, malaria, HIV and helminth infections as well as other infections causing inflammatory responses are important factors contributing to the high prevalence of anemia in many developing countries. In addition, other nutritional deficiencies (vitamin B₁₂, folate and vitamin A) as well as hemoglobinopathies need to be considered in some areas.

The focus of this review is on the major causes, nutritional and non-nutritional, of anemia. Special attention has been given to recent studies reporting on the etiology of anemia or on the coexistence of nutrient deficiencies in different population groups. In addition, the interaction and overlap between micronutrient deficiencies and infectious/inflammatory disorders in settings where anemia prevalence is high have been highlighted. Due to the limited information available in infants, this review is largely based on relevant human studies in other age groups.
The Etiology of Anemia

Anemia and IDA during Early Life: The Impact of Birth Weight, Fetal Anemia and Maternal Anemia

Full-term breast-fed infants have generally been assumed to have adequate iron status during the first 4–6 months of life, although the iron content of human milk is very low. In addition, iron bioavailability from human milk (measured as erythrocyte incorporation of iron-stable isotopes) has been demonstrated to be low, resulting in very limited amounts of dietary iron available for the infant [1]. However, after the first few months of life, when iron stores have been depleted, additional dietary iron needs to be supplied for the rapidly expanding blood volume and replacement of iron losses [2]. As iron losses are small early in life, iron requirements are largely determined by body stores at birth and growth rate. The impact of birth weight on estimated requirements of absorbed iron during the 1st year of life is clearly illustrated by Fomon [3]: 0.55 mg absorbed iron/day for infants with a birth weight of 3.5 kg (and an estimated body weight of 10.5 kg at 1 year) and 0.75 mg absorbed iron/day for infants with a birth weight of 2.5 kg (estimated body weight 10 kg at 1 year). These estimates highlight the importance of access to appropriate complementary foods with adequate iron content and emphasizes the importance of optimizing iron bioavailability during early life to prevent the development of iron deficiency and IDA. The World Health Organization (WHO) recommends the introduction of complementary foods, in addition to human milk, at 6 months of age [4]. The recent review by Kramer and Kakuma [5] concludes that, although data are scarce, exclusive breast-feeding for 6 months without iron supplementation may compromise the hematological status of infants in developing countries.

There is little information about the prevalence of anemia and IDA in infants during the first few months of life. A recent study of breast-fed infants in Honduras (n = 131) and Sweden (n = 101) reported a less than 3% prevalence of IDA at 4 months of age [6]. All children were born at term (birth weight >2,500 g). However, Honduran children had a lower mean birth weight and larger weight gain during the first 4 months of life and a significantly lower Hb, mean corpuscular volume (MCV) and ferritin compared to Swedish children at 4 months. All children were exclusively breast-fed until 6 months and partially breast-fed until 9 months. Iron supplementation (1 mg/kg/day), starting at 4 or 6 months, significantly reduced IDA in Honduran children at 9 months (9%), as compared to non-supplemented children (29%). At 6 months, 9% of supplemented Honduran children had IDA compared to 19% IDA in non-supplemented children. The prevalence of IDA was 3% in Swedish children receiving placebo at 9 months and similar to the supplemented children. In this study, the criteria for IDA was based on Hb < 110 g/l together with conventional cutoff levels for iron status parameters (ferritin, MCV and zinc protoporphyrin). However, it is important to note that there are no established cutoff values for anemia in young infants (<6 months of age).
WHO [7] defines anemia as Hb < 110 g/l for children 6–59 months of age but does not include any cutoff values for younger children.

Interestingly, iron supplementation increased Hb significantly between 4 and 6 months of age, independent of study site, initial Hb or initial iron status. These results indicate an immature regulation of iron metabolism in early life and that the Hb response to iron supplementation is not a useful criterion of iron deficiency in young infants. The hypothesis that the regulation of iron absorption is immature early in life was tested in a sub-group of the breast-fed Swedish infants. Iron absorption was measured from a small volume of labeled human milk using stable isotope labels of iron at 6 and 9 months of age [8]. The mean iron absorption was 16% at 6 months and not significantly different between supplemented and non-supplemented infants. At 9 months of age, the mean iron absorption was 17% in the supplemented children but significantly higher in non-supplemented children (mean 37%).

The urgent need to define laboratory criteria for anemia in young infants is further indicated by data from Indonesia. The anemia prevalence in 3- to 5-month-old breast-fed infants (n = 990) based on cross-sectional data varied depending on the cutoff level used: 13% (Hb < 90 g/l), 37% (Hb < 100 g/l), and 71% (Hb < 110 g/l) [9]. The proportion of exclusively breast-fed infants was 10–22% in different areas. Maternal anemia and low birth weight (LBW; <2,500 g) were identified as risk factors for infantile anemia. In particular, infants with a combination of LBW and an anemic mother had a high odds ratio (3.68) for anemia (Hb < 100 g/l). No indicators of iron deficiency were included in this study.

The impact of LBW and fetal anemia on anemia during the first months of life was demonstrated in a study in Malawi [10]. The prevalence of fetal anemia (cord Hb < 125 g/l) and LBW was 23 and 15%, respectively, in a study population of 1,400 live-born singleton infants. Hb was monitored in cohorts of LBW infants and infants with fetal anemia at 2, 4, 6, 9 and 12 months of age and compared to a control group with normal birth weight and normal cord Hb. Iron supplementation was prescribed for 30 days when the Hb concentration was <80 g/l (28–38% of infants in the different groups received iron supplements). Ninety-five percent of all Hb measurements (total 1,241) were <110 g/l and 22% were below 80 g/l. At 2 months of age, 36% of LBW infants, 40% of infants with fetal anemia, 60% of infants with LBW and fetal anemia, and 39% of control infants had Hb < 90 g/l. At 9 months, more than 90% of infants had Hb < 105 g/l. In all infants, regardless of birth weight or fetal anemia, a decrease in Hb was observed during the first 6 months of life. LBW was a significant predictor of anemia later in infancy: at 12 months, the mean Hb concentration was 5.5 g/l lower in LBW infants and 7.9 g/l lower in LBW infants with fetal anemia than in the control group. No statistically significant difference in Hb was observed between infants with both LBW and fetal anemia and infants with only LBW. Additional risk factors for infantile anemia were placental malaria and maternal malaria at delivery.
Thus, reducing the prevalence of LBW and malaria transmission during pregnancy are essential to reduce infantile anemia.

Malaria infection during pregnancy is associated with an increased risk of developing severe maternal anemia and giving birth to LBW infants. Prevention of malaria transmission is therefore of great public health importance in regions where malaria is endemic. The highest risk individuals are primigravidae but recent data from Kenya [11] suggest that the impact of preventing malaria in multigravidae may have been underestimated. Based on placental histology in 912 women, malaria prevalence was 64% in primigravidae and 40% in multigravidae. In gravidities 1–4, active malaria infection was associated with severe maternal anemia. The combination of having both chronic or past placental malaria and severe anemia increased the risk of LBW significantly (odds ratio 4.53 in primigravida, and 13.5 in gravidities 2–4). Women of all parities had a substantially increased risk of LBW and severe anemia as a result of malaria infection in pregnancy. In addition, the study by Shulman et al. [11] and another large study in Kenya by van Eijk et al. [12] (4,608 women) confirm previous reports that HIV-infected pregnant women have higher prevalence of malaria infection than non-HIV-infected women and that HIV infection is associated with severe anemia. In women with higher gravidities, HIV seropositivity was the only statistically significant factor associated with all anemias and with severe anemia [12]. Effective interventions to reduce malaria transmission during pregnancy in HIV-infected women are therefore needed [13].

**IDA and Anemia Associated with Malaria and Other Infectious/Inflammatory Disorders**

Recent data from Côte d’Ivoire [14] clearly demonstrated the limited usefulness of anemia as an indicator of IDA in areas where malaria and other infections are prevalent. In this study, the Hb cutoff levels of the WHO were adjusted (−10 g/l) as all individuals (n = 1,573) were black Africans and a multiple criteria model was used to identify iron deficiency. A large proportion of anemic 2- to 5-year-old children (78%) had IDA while only 22% of anemia cases were associated with iron deficiency in adult men. In schoolchildren and adult women, about 50% of anemic individuals had IDA. The complexity of the etiology of anemia was shown by the overlap between anemia, IDA and infection (indicated by elevated C-reactive protein (CRP) concentrations), in particular in young children (fig. 1). The importance of malaria in the etiology of anemia in preschool children was demonstrated as the malaria parasite load was negatively correlated with Hb in the 2- to 5-year olds. However, a large proportion of anemia cases in schoolchildren, adult women and men (32, 54, 56%) could not be attributed to either iron deficiency or infection. Other nutritional causes of anemia were not evaluated. An important finding in this study was that the serum transferrin receptor was the most useful single indicator of iron deficiency as it was unaffected by malaria or infectious/inflammatory disorders.
Iron deficiency was the major cause of anemia in Ivorian preschool children (2–5 years old) and the importance of iron deficiency in the etiology of anemia during early life was further demonstrated in infants and young children (6–18 months old) in Abidjan [15]. A very high proportion of the 51 children were anemic (Hb < 100 g/l, 85% of infants 6–12 months old and 96% of children 12.9–18 months old; Hb < 110 g/l, all children). Eighty percent (6–12 months) and 92% (12.9–18 months) of children had IDA based on a multiple criteria model. Two children had positive malaria smears. No additional indicators of infection were monitored but as the concentrations of ferritin (an acute phase protein that increases with inflammation) were low, the prevalence of infection/inflammation can be assumed to be low. Thus, the etiology of anemia varied with age and sex in Côte d’Ivoire [14, 15], demonstrating that data cannot be extrapolated from one population group to another in settings where there are multiple causes of anemia.

The importance of age-dependent factors in the etiology of anemia was also reported in a study of 490 infants and young children in Zanzibar (4–71 months old) [16]. The prevalence of anemia was very high: 80% had a Hb

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**Fig. 1.** Percentage of anemic subjects with iron deficiency on the basis of a multiple criteria model (2 of 3 iron status indexes outside the cutoff values: serum ferritin <30 μg/l; serum transferrin receptor >8.5 mg/l and zinc protoporphyrin >40 μmol/mol heme; without elevated C-reactive protein (CRP); a combination of iron deficiency and elevated CRP, or with neither [14]. ■ = Anemic subjects with iron deficiency and normal CRP concentrations (<10 mg/l); □ = anemic subjects with iron deficiency and elevated CRP concentrations (>10 mg/l); □ = anemic subjects with elevated CRP concentrations (>10 mg/l) and no iron deficiency; ■ = anemic subjects with normal CRP concentrations (<10 mg/l) and no iron deficiency. Reproduced with permission from the *American Journal of Clinical Nutrition*. Copyright Am J Clin Nutr, American Society for Clinical Nutrition.
concentration of <100 g/l. Malaria (but not hookworm infection) was associated with anemia in the youngest age group (children <30 months old). In older children, hookworm infection (but not malaria) was associated with anemia. Helminth infections have been demonstrated to be important factors in the etiology of anemia in schoolchildren and the benefits of deworming programs have been shown in several studies [17, 18]. Helminth infections have not been demonstrated to be of major importance in the etiology of anemia during early life and will therefore not be discussed in this review.

A very high prevalence of anemia has been reported in infants and young children living in resource-poor areas during the last few years (table 1). Many of these studies do not include data on the prevalence of iron deficiency or infection, but it is noteworthy that the data from South Africa [19] and Ethiopia [20] indicate a relatively high prevalence of iron deficiency (42–43% based on low ferritin concentrations). Malaria was very rare in the study population in Ethiopia but children with diarrhea or fever had a significantly increased risk of anemia [20]. Asymptomatic malaria was associated with significantly lower Hb and elevated CRP concentrations in Kenyan infants: the prevalence of elevated CRP concentrations was high (38%), and 18% of children had malaria [21]. An elevated CRP concentration was also associated with lower Hb in 6-month-old Ghanian infants [22]. Interestingly, recent infections were also associated with anemia in 12-month-old apparently healthy, term infants in Europe [23]. The prevalence of anemia was 9% of which 40% was not associated with iron deficiency. The prevalence of IDA was very low, 2.3% [23], and similar to IDA in Swedish infants at 9 months [6]. In addition, stunting was identified as a significant risk factor for anemia in Ethiopia [20] and in children living in Palestinian refugee camps [24].

**Table 1.** Anemia prevalence in infants and young children living in developing countries and in refugee camps

<table>
<thead>
<tr>
<th>Age months</th>
<th>Sample size</th>
<th>Anemia prevalence, %</th>
<th>Study site</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–36</td>
<td>318</td>
<td>69</td>
<td>Kenya</td>
<td>21</td>
</tr>
<tr>
<td>4–24 (4–12)</td>
<td>115 (84)</td>
<td>65</td>
<td>South Africa</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>208</td>
<td>30</td>
<td>Ghana</td>
<td>22</td>
</tr>
<tr>
<td>6–35</td>
<td>6,702</td>
<td>65</td>
<td>Palestinian refugee camps</td>
<td>24</td>
</tr>
<tr>
<td>6–69</td>
<td>2,080</td>
<td>42</td>
<td>Ethiopia</td>
<td>20</td>
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Impact of Malaria Control on Anemia Prevalence in Infants and Young Children

Studies on children up to 9 years of age in Gambia and Kenya showed that, in areas with the most intense levels of transmission, the risk of severe malaria
was greatest during the first 2 years of life [25]. In communities with low to moderate rates of *Plasmodium falciparum* transmission, children experienced a much longer period of risk. Very few studies report on the impact of malaria on anemia during the first few months of life but data based on 446 Nigerian infants (1–6 months old) showed that Hct was significantly lower in infants with malaria parasites than in infants without malaria parasites [26]. Among the infants with malaria, infants 2–2.9 months old had the lowest Hct.

The potential positive and negative effects of malaria chemoprophylaxis were evaluated in more than 800 young infants (8–48 weeks) in Tanzania [27]. Weekly prophylaxis (pyrimethamine plus dapsone) resulted in lower rates of clinical malaria and severe anemia: 60% of first or only episodes of clinical malaria and 64% of multiple episodes were prevented. An important finding in this study is that daily iron supplementation (2 mg/kg, given from 8 to 24 weeks) did not influence the frequency of malaria. This study confirmed that malaria is the major contributor to the etiology of severe anemia in infants in highly endemic areas, accounting for 60% of all episodes while iron deficiency accounted for about 30% of all severe anemia episodes. However, after discontinuation of chemoprophylaxis, an increased frequency of clinical malaria and severe anemia was observed.

The possibilities to maximize the protective effects of chemoprophylaxis during early life were explored by using intermittent malaria prophylaxis in Tanzania. Sulfadoxine-pyrimethamine or placebo was given at routine vaccinations at 2, 3 and 9 months (350 infants/group) and daily iron supplementation was provided between 2 and 6 months to all children [28]. Malaria chemoprophylaxis reduced the number of first or only episodes of clinical malaria by 59% and reduced severe anemia by 50%. Rates of clinical malaria and severe anemia did not increase after discontinuation of the drug. Recent economic analysis supports the inclusion of both malaria chemoprophylaxis and iron supplementation delivered through the Expanded Program on Immunization as part of the control strategies for malaria and severe anemia in parts of sub-Saharan Africa [29]. Based on this analysis, preventive interventions would be less costly than the current malaria and anemia control strategies that rely on clinical case management.

**HIV Infection**

Anemia is common in HIV-infected individuals but it is unclear what proportion of anemia can be attributed to iron deficiency, the infection itself and/or associated conditions such as malaria and other infections. For example, 9-month-old HIV-infected Ugandan infants had a very high prevalence of anemia (91%; n = 165). The control group of noninfected infants of similar age was small (n = 39) and also had very high prevalence of anemia, 80% [30]. The prevalence of IDA (based on low ferritin) was 44 and 45% in infected and
noninfected infants but was probably an underestimate as ferritin is an acute phase reactant [30]. A number of recent studies have reported that HIV-infected pregnant women had a significantly higher prevalence of anemia than noninfected pregnant women, for example in Zimbabwe [31], Malawi [32] and in Burkina Faso [33]. The prevalence of anemia in HIV-infected pregnant women in Tanzania was also reported to be very high (83%) and 28% of the women had Hb < 85 g/l [13]. Hb and acute phase proteins were significantly correlated, indicating an association between anemia and the inflammatory response to infectious disease [32]. Furthermore, data from Malawi and Tanzania suggest an association between more advanced HIV disease and lower Hb [13, 32].

Iron deficiency and IDA are difficult to estimate under these conditions but based on low ferritin (<12 µg/l), 28 and 17% of HIV-infected and non-infected Malawian pregnant women had IDA. By using a higher cutoff level (<30 µg/l), 50 and 32% were estimated to have IDA [32]. The higher cutoff level for ferritin has been established based on bone marrow smears in Malawian pregnant women [34]. Low ferritin (<12 µg/l) was found in 63 and 64% of HIV-infected and non-infected women in Zimbabwe [31]. Based on red blood cell morphology, 44% of all women had some evidence of iron deficiency in the study in Tanzania [13], although most (74%) anemia cases were not accompanied by morphological changes in erythrocytes in the study in Burkina Faso [33]. The potential risks and benefits of iron supplementation for HIV-infected infants and pregnant women require further evaluation through carefully controlled clinical trials [35].

Vitamin Deficiencies in the Etiology of Anemia

Vitamin deficiencies often coexist with anemia. However, there is very little information about the role of vitamin deficiencies in the etiology of anemia and the public health impact of vitamin deficiencies on anemia is unclear. Although several studies have evaluated the hematological response to vitamin supplementation, in particular vitamin A supplementation, many studies do not provide conclusive data on the effect of the vitamin per se. In addition, the interpretation of data is often limited as information about the nutritional status of the study population, in particular about well-defined vitamin deficiencies, is not always included. For more information about the role of vitamins in the etiology of anemia, please see the recent reviews by Fishman et al. [36] and Semba and Bloem [37].

Folate

Based on an extensive review of supplementation trials, it was concluded that folate supplementation can prevent megaloblastic anemia in severely deficient individuals. Although a few studies have reported an improved Hb concentration, most studies have been unable to demonstrate any effect in
the absence of severe, overt folate deficiency or megaloblastic anemia [36]. Pregnant women are generally assumed to be at high risk of folate deficiency and routine prenatal iron supplements include folate. In addition, individuals living in malaria endemic regions are assumed to be at higher risk of folate deficiency due to hemolysis of red blood cells [36].

The transfer of methyltetrahydrofolate from mother to fetus is thought to protect the fetus from folate deficiency, unless the mother has severe folate deficiency [for review see, 38]. Preterm infants and LBW infants have lower body stores at birth, higher growth rates and are therefore susceptible to folate deficiency during early life. However, there is very little data on the influence of folate supplementation on the severity of anemia in premature infants.

Vitamin B$_{12}$

Megaloblastic anemia caused by vitamin B$_{12}$ deficiency is morphologically indistinguishable from megaloblastic anemia caused by folate deficiency [38]. Vitamin B$_{12}$ deficiency is assumed to be less common than folate deficiency and has been suggested to be largely due to malabsorption rather than to insufficient intake [36]. However, population groups with a low intake of animal products are considered at risk of developing vitamin B$_{12}$ deficiency. Vitamin B$_{12}$ supplementation has been demonstrated to prevent megaloblastosis but has not been reported to influence the Hb concentration in pregnant women. As for folate, the importance of vitamin B$_{12}$ in the etiology of anemia has not been defined. For example, low serum vitamin B$_{12}$ concentrations were found in about 20% of pregnant anemic women in Tanzania [39] and in about 50% of pregnant women in Nepal [40] but was not significantly associated with anemia in either of these studies.

The ability of the placenta to accumulate vitamin B$_{12}$ results in higher plasma vitamin B$_{12}$ concentration in newborn term infants than in maternal plasma [38]. However, insufficient human milk vitamin B$_{12}$ content can induce vitamin B$_{12}$ deficiency in young infants resulting in failure to thrive during the first few months of life and progressing to megaloblastic anemia [38]. For example, the plasma vitamin B$_{12}$ concentration was deficient or low in 47% lactating Guatemalan women and the holotranscobalamin concentration was low in 32% of the women, indicating vitamin B$_{12}$ malabsorption [41]. The human milk vitamin B$_{12}$ concentration was low in 31% and negatively correlated with infant urinary methylmalonic acid. Urinary methylmalonic acid was elevated in 12% of infants indicating vitamin B$_{12}$ deficiency. The folate concentration in plasma was low or deficient in 9% of women.

Folate and vitamin B$_{12}$ deficiencies should be considered whenever the initial evaluation of anemia indicates the presence of macrocytosis [42]. Treatment of megaloblastic anemia with folate can mask concomitant B$_{12}$ deficiency which can lead to severe neurological consequences and megaloblastic anemia should be treated by folate and vitamin B$_{12}$. 
Vitamin A

Epidemiological surveys have shown that the prevalence of anemia is high in populations where vitamin A deficiency is prevalent and several studies have demonstrated increased Hb concentrations after vitamin A supplementation [36, 37]. However, the interpretation of results is complicated as information about the proportion of subjects with concurrent vitamin A deficiency and anemia as well as information about other causes of anemia in the study populations is often missing. In addition, most studies have used plasma or serum retinol concentrations as indicators of vitamin A deficiency. However, retinol concentrations are depressed during infection due to the acute phase response [43] and are therefore often of limited value to identify subclinical vitamin A deficiency in individuals. More specific biochemical indicators of vitamin A status such as the modified relative dose response could be useful to better define vitamin A status in individuals and to assess the response to vitamin A supplementation on Hb concentration [44].

The mechanisms by which vitamin A deficiency cause anemia have not been clearly demonstrated [37]. Several different potential mechanisms have been proposed, including impaired differentiation and proliferation of hematopoietic cells, reduced mobilization of body iron stores and/or effects on iron metabolism through sequestration of iron during the acute phase response to infection [for review see, 36, 37]. As vitamin A plays an important role in immune function, it has been suggested that vitamin A may reduce anemia through its impact on anemia associated with infection. To date, there are little data to support this hypothesis directly [37]. It has also been proposed that vitamin A supplementation may increase the synthesis of erythropoietin, although recent data from a study in pregnant women do not provide evidence that vitamin A supplementation increases hemoglobin and plasma erythropoietin concentrations [45]. In addition, interactions between vitamin A and iron, resulting in enhanced iron absorption in humans, have been suggested [for review see, 36]. However, the enhancing effect of vitamin A on iron absorption observed in adult Venezuelan subjects was recently evaluated but not confirmed in healthy European adults [46] or in African children with subclinical vitamin A deficiency (personal observations).

Multiple Causes of Anemia

Studies in Pregnant Women

A cross-sectional study of 150 anemic (Hb < 110 g/l) pregnant women in Malawi reported on a wide spectrum of factors associated with anemia [34]. Iron deficiency was the most common micronutrient deficiency: 55% of women had serum ferritin <30 μg/l and, based on bone marrow smears in 88 women, 61% were iron deficient. Of the iron-replete women, 74% had elevated CRP concentrations, indicating the importance of infectious/inflammatory disorders
in the etiology of anemia in this population. The study by van den Broek [34] highlights the coexistence of micronutrient deficiencies as 23% were iron deficient (without evidence of folate, vitamin B$_{12}$ or vitamin A deficiencies), 32% were deficient in iron and one or more of the other micronutrients, 26% were not iron deficient but had evidence of one of the other micronutrient deficiencies (most often vitamin A), and 19% were not deficient in any of the micronutrients evaluated. Of these women, more than half (54%) had elevated CRP concentrations. The prevalence of HIV infection was high in the overall study population (48%).

That iron deficiency is a major cause of anemia in pregnant women was also confirmed recently in Tanzania [39] and Nepal [40, 47]. As in the study in Malawi [34], low concentrations of serum or plasma retinol were found in many women [39, 40, 47]. In addition, major risk factors for anemia included hookworm infection and malaria [39, 47] – *Plasmodium vivax* in the study by Dreyfuss et al. [47]. Hookworm infection was very common (74%) and hookworm infection intensity was the strongest predictor of iron status [47]. All these studies indicate that a stronger focus on preventing, diagnosing and treating infections in pregnancy is called for.

*Studies in Infants and Young Children*

The importance of iron deficiency in the etiology of anemia can be determined by the response to iron supplementation in anemic individuals. For example, about 40–45% of anemic infants and young children in Ghana (6–18 months old) were still anemic after 2 months of intervention with iron drops or ‘sprinkles’, indicating that iron deficiency was not the only cause of anemia in these children [48]. At the end of a 12-month (supervised) supplementation study in young Mexican children (18–36 months old), approximately 30% of children were still anemic [49]. The lack of response to iron in this study was attributed to chronic undernutrition and the high prevalence of other micronutrient deficiencies (vitamin B$_{12}$, vitamin A, vitamin E).

That micronutrient deficiencies coexist and overlap was also demonstrated in lactating Indonesian mothers and their infants (2.4–5 months old) [50]. The prevalence of anemia was high (>50%) in both mothers and infants and concurrent micronutrient deficiencies (iron, vitamin A and zinc) were common. Mothers and infants with low plasma retinol (<0.7 µmol/l) had a 3.8- and 2.5-fold higher risk of anemia, respectively, and a 2- to 3-fold higher risk of being iron or zinc deficient than individuals without marginal vitamin A deficiency. Individuals with elevated CRP or ferritin concentrations were excluded from the data analysis.

It is often assumed that nutritional problems cluster in individuals because they share causal factors such as poverty. A recent analysis of data based on the Honduras National Micronutrient Survey 1996 investigated the co-occurrence of anemia (Hb < 110 g/l), risk of vitamin A deficiency
(serum retinol <1.05 \mu mol/l) and stunting in children 12–35.9 months old (n = 633) and 36–59.9 months old (n = 610) [51]. In younger children, 77% had at least one of the nutritional problems evaluated in this study and 51% had more than one problem. Corresponding values for the older children were 64 and 44%, respectively. Although there was statistical evidence for co-occurrence between these nutritional problems, differences between expected and observed prevalences were small. Based on these results, the 3 conditions should be treated as virtually independent when designing intervention strategies in Honduras. The relevance of these findings to other countries needs to be evaluated.

Interactions between Micronutrients

Iron and Zinc

A potential risk of interactions between micronutrients on absorption, in particular between iron and zinc, has been discussed related to supplementation and food fortification. For example, short-term absorption studies have demonstrated that iron reduced zinc absorption in a dose-dependent way when given in aqueous solutions. However, when iron was added to test meals, zinc absorption was not affected. Also, zinc impaired iron absorption from water solutions but had no effect when added to foods [for review see, 52]. Cases of microcytic hypochromic anemia have been reported after long-term zinc supplementation [52] but the data are not conclusive. For example, the inclusion of zinc to a prenatal iron and folate supplement had no effect on Hb or ferritin concentrations in pregnant Peruvian women [53]. Few studies have evaluated the potential long-term interactions between nutrients and their affects on Hb, in particular in infants.

The proportion of Indonesian infants (4 months old at the start of the study) with anemia (Hb < 110 g/l) after 6 months of intervention was significantly lower in infants supplemented with 10 mg iron/day (28%) as compared to infants receiving zinc (10 mg/day, 62%) or placebo (66%) but was also significantly lower than in infants receiving both iron and zinc (46%) [54]. The proportion of infants with IDA was not significantly different between infants receiving only iron (3%) or iron plus zinc (8%). IDA was found in 30–35% of infants supplemented with only zinc or placebo. These results indicate some differences in the efficacy of combined iron/zinc supplements as compared to iron alone to combat anemia. However, the results are difficult to evaluate as no baseline measurements of iron (or zinc) status were included in the study.

Iron (and Zinc) and Vitamin A

The interactions between vitamin A and iron metabolism, in particular the influence of vitamin A on Hb concentration, were discussed earlier in this

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review. In addition, a positive effect of iron on vitamin A status was recently demonstrated in pregnant Indonesian women [44]. Vitamin A status, measured by the modified relative dose response technique, improved significantly more in women receiving a combination of iron and vitamin A than in women supplemented with iron or vitamin A. The mechanism of the enhancing effect of iron on vitamin A status is not known.

Improved vitamin A status, measured as the plasma retinol concentration, was also observed in young Mexican children (18–36 months old) after 6 months of supplementation with iron or iron and zinc [55]. The effect was more evident in children who were initially deficient in zinc, iron or vitamin A. Interactions between zinc and vitamin A have been reported previously and these results support the observations that zinc supplementation has a positive effect on vitamin A metabolism when zinc status is impaired.

Conclusions

Anemia can be an indicator of poor nutrition and/or of poor health. Interventions to reduce the prevalence of infants born with LBW and to prevent malaria transmission during pregnancy are essential to reduce infantile anemia. Iron deficiency is a major cause of nutritional anemia and strategies to increase iron intake and to improve iron bioavailability from the diet are important to combat anemia. Vitamin deficiencies often coexist with anemia but the public health impact of vitamin deficiencies on anemia is unclear. Furthermore, infections contribute to the high prevalence of anemia in many countries and effective malaria control and deworming programs can reduce the prevalence of childhood anemia significantly in areas where these infections are common. Thus, a holistic approach is needed to combat anemia, including improved sanitation, access to health care and overall better nutrition.

References

Etiology of Anemia

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Discussion

Dr. Young: In relation to the etiology of anemia and at least in reference to the adult and in particular the mother, as you were talking I was reminded of Vitteri's observations a number of years ago indicating that there was a high correlation between the size of the circulating hemoglobin pool and the lean body mass of the individual. Could you comment on the role of the active tissue metabolic demand as a determinant of circulating hemoglobin levels, in addition to the impact of the anemia-related micronutrients which are the focus of this conference and your presentation?

Dr. Davidsson: I don’t think there is very much information about this and this is again related to the difficulties in evaluating, for instance, the circulating mass of hemoglobin. In particular we are very interested in trying to do that during pregnancy as it is again extremely difficult to evaluate status measurements during pregnancy because of plasma expansion, etc. We have been trying to use stable isotopes to evaluate the circulating hemoglobin mass by isotope dilution. I appreciate your comments and I think it is something that is very important, but I am not sure that we really know very much about how to relate the need for iron to other aspects. There is an overall limitation on how we define iron deficiency. There was a discussion yesterday about the circulating transferrin receptor which we found to be very useful, but of course no indicator is perfect, so we are making very little progress on the overall status measurements, but on iron I think we are somewhat better off than for example for zinc status measurements. It is not a good answer to your question, I am afraid.

Dr. Al Frayh: The observation made between iron deficiency anemia and infection is well known to clinicians as well as to scientists, but for me as a clinician the question is whether it is recommended in the presence of chronic infection, for example malaria, HIV, cystic fibrosis, the current infections of pulmonary origin like bronchiectasis. Would you advise iron to be given along with zinc and vitamin A as a prophylaxis or would you wait until certain laboratory indexes are clear in order to start iron supplementation? In other words should clinicians rely on hemoglobin, ferritin, or are there other indexes that you would advise looking at before you start or recommend iron supplementation?

Dr. Davidsson: I think we will hear a lot more about HIV infection in one of the later talks today. I don’t think there is any consensus on changing the recommendations when it comes to iron supplementation, for example, of pregnant women, etc. during HIV infection. Malaria has of course been discussed a lot and there is a review issued by the International Nutritional Anemia Consultative Group [1] that is available, and I think the conclusion was that there is really no contraindication. What is also important from the 2 studies that I refer to [2, 3], which present very new data from Tanzania, is that at least one of them showed that there was no negative effect of iron supplementation in these very young children. But of course it is a very specific type of setting with a very high transmission rate. It is very difficult to be
general on these things, and I think malaria in particular had a lot of attention because some of the old studies showed a negative effect on parasite load after iron was given. But that was again in the very extreme conditions, and I think that has been moved away from. There is a recent review on infection and iron in a supplement to the *Journal of Nutrition* [4] based on the meeting in Belmont last year. But yes, I think there are a number of questions that are still not answered on these aspects.

*Dr. Mousa:* Regarding the relation between the iron deficiency anemia and other micronutrients, on admission to hospital we used to screen all the iron deficiency anemia patients for vitamin D deficiency and surprisingly there was a very high incidence, almost up to 60% of those who have an iron deficiency anemia also have a vitamin D deficiency. I don't think these mechanisms are related to each other but I think this is a matter of poor nutrition in the mothers. I don't think there is a mechanism related to the bioavailability or any mechanism related to iron and vitamin D, especially if you go back to the history of the mother and the baby, it is just simply malnutrition, that's it.

*Dr. Davidsson:* There are a number of associations made in epidemiological studies with proxy for malnutrition as you say, looking at stunting, etc. related to anemia. Anemia can be an indicator of poor nutrition or poor health, and it is of course very multifactorial. I think that it is very important to remember that there are a number of reasons why a child or a pregnant woman is anemic. It is very difficult to really tease out what is causing the anemia. As I said, many of the micronutrient deficiencies have an overlap with iron deficiency. If that is really what is causing anemia is not clear because there are very few direct evaluations made on how children, or any individual, respond to micronutrient supplementation. With the difficulties we have in really establishing who is deficient in a specific nutrient, it is also very difficult to evaluate how individuals with different nutritional status respond to the supplementation and if that supplementation has an effect on hemoglobin. Those data are really not there. There have been a number of studies on vitamin A supplementation but again the mechanism is still debated. What is causing the effect of vitamin A on hemoglobin response?

*Dr. Zlotkin:* I continue to be confused by the etiology of the anemia in the first 6 months of life, and I think this problem is particularly important because if you think why the WHO recommended the introduction of complementary foods at 6 months and beyond, one of the reasons they chose 6 months was because of the observation that in the West, infants are protected from developing iron deficiency for around 6 months by the stores they are born with and by the absorption of the small amount of iron in human milk. So the issue of the development of anemia in the first 6 months of life is really very important, important to the point of it being one of the major factors that effect the WHO recommendation. When I think of the infant in the first 6 months of life in general, they are not bleeding so they aren't loosing blood. In general they are being fed breast milk so their intake of iron is quite low, but in the West it is generally true that by 6 months of age they will not be anemic whereas in the developing world by 6 months of age, as you showed, 60–70% of the infants will have documentable anemia. So my conclusion is that this must have something to do with the mother much more than the infant. My understanding of the physiology of the placenta is very rudimentary but it is the placenta that has the highest concentration of transferrin receptor of all organs and the transferrin receptor concentration is what is important for the transfer of iron from the mother to the fetus. Do we know anything about the effect of malaria or HIV or chronic undernutrition on the transfer of iron from the mother to the fetus, and is this something that we should spend more time and perhaps money on to understand?
Dr. Davidsson: I don’t think we know very much about it but I definitely agree with you that this is a very important issue and it has been completely neglected for the reasons you mentioned, i.e. an assumption that the breast-fed child with an adequate normal birth weight will not develop iron deficiency anemia during approximately 6 months. The calculations by Fomon [5] clearly show, as you say, that there is not a lot of iron loss, it is really a matter of growth. The faster you grow, the more need for iron you have, and the smaller you are when you are born, the less iron you have in your liver store. The iron concentration in breast milk is very low and there is probably a reason for that as well that we don’t know. Iron absorption from breast milk is not very high as we have shown and as was also recently demonstrated by another group [6, 7]. So the breast-fed baby is exposed to very little dietary iron. I agree with you that it is the pregnant woman who is of course extremely important here and low birth weight puts that baby at risk. Why do you have a low birth weight baby? Malaria, HIV infection, chronic undernutrition, etc., will all be very important factors for the risk of having a low birth weight baby. When we talk about countries like Bangladesh, for example, where approximately 40% of the babies are born with low birth weight, this is of course a huge problem, and this has been alluded to many times during this conference. To ensure a good pregnancy progression, to try to minimize the risk or reduce the risk of having low birth weight babies is a very important factor. We heard about micronutrient supplementation during pregnancy in Nepal, again in a very poor population, where you start looking at the maternal weight at the start of the pregnancy which, as Dr. Christian stated, was around 40 kg, and weight gain is very small in these women. It is of course a combination of many factors, but yes I totally agree with you.

Dr. Beard: I have a couple of questions and perhaps a comment. One question I have is: what is the appropriate definition of anemia? Our esteemed colleague from Saudi Arabia asked a very clinically oriented question and on one of your slides, I think it was from your Ivory Coast work, 100% of the children were anemic. I want to remind all of us that there are several definitions of anemia. There is a clinical definition that all the clinicians in the room are very conscious of which is, there is insufficient oxygen transport to meet normal metabolic requirements. Then there is the public health definition which is a statistically derived deviation from the norm. They are not necessarily the same thing. So when we want to define anemia in young children what are we talking about? Are we talking about a clinical outcome or are we talking about a public health outcome? So there are two different things. The final comment I would like to make is that anemia is not always a bad thing. We have assumed that a low hemoglobin concentration is a negative result of something happening. I want to remind you of the older literature from Africa in the 1960s, primarily some Dutch investigators showed very clearly that a normal adaptive response to protein energy malnutrition is a decreased hemoglobin. There is decreased erythropoiesis because the metabolic rate goes down so the body is not going to waste the 3 sources making hemoglobin. If one wasn’t conscious of that relationship then all these people would be defined as anemic, and this is a bad thing. It was not bad, it was normal physiology.

Dr. Davidsson: That is a very important comment and I think this definition of anemia is a crucial part of it, and I think many of us are very familiar with the discussions and perhaps the need to redefine anemia. What I showed you and what is widely used is of course the WHO definitions. The definition of anemia in early life is much more complicated because of these rapid changes. Dr. Zlotkin said that a preterm baby is not a small version of a term baby, but term babies during the first months of life are not very homogenous groups of individuals because so many things happen so quickly. Therefore, we have to be careful about the definition of anemia, and also to be very careful about the definition of the different stages of anemia.
Severe anemia is clearly a high-risk condition that is associated with negative health outcomes, there is no doubt about that. I think we know very little about the consequences of moderate or mild anemia. What does mild or moderate anemia mean and how do these conditions influence an individual with chronic undernutrition, together with other micronutrient deficiencies? That is an open question. I am not aware of any clear data to really set specific cutoff levels or recommendations for that because I think the information is just not really there, which is of course a big limitation on something that should be looked at much more carefully.

Dr. Verhoef: Let me come back to the earlier question posed by our colleague from Saudi Arabia about whether or not to give iron to people who have infections. I think that there is increasing evidence that the anemia of chronic infection is probably not caused by iron deficiency. Administration of iron to resolve that anemia is not efficacious and might even cause adverse effects. For example, the administration of iron does not improve the anemia of HIV but there are some indications that it may actually exacerbate HIV infection. Having said that, in many developing countries, iron deficiency anemia may co-exist and is superimposed on the anemia of infection. In the case of malaria, it has been shown in a study from The Gambia that iron supplements given concurrently with antimalarial chemotherapy assists in hematological recovery from febrile malaria attacks [8]. I am not certain whether we should try to control malaria-associated mild anemia in children free of symptoms. Community-based surveys shows that the prevalence of such children can be very high in endemic areas. There is no doubt that we certainly should try to control malaria associated with severe anemia. The reason why I am uncertain is that on one hand asymptomatic infection predisposes to a subsequent febrile attack. On the other hand, there is little evidence of the functional effects of mild malarial anemia in these children, and they are presumably building up immunity. Thus it might be that if you treat or try to control malaria in these asymptomatic children you might actually put them at high risk of subsequent death. As a last point, I would like to come back to the vitamin A question. I may have wrongly received the impression that you seemed somewhat ambiguous about whether or not vitamin A deficiency causes anemia. I think that this has been shown conclusively in several randomized placebo-controlled trials, although there may be external factors that determine the hematological response to vitamin A supplementation. Would you like to comment on that?

Dr. Davidsson: As I said, the mechanism(s) of how vitamin A is associate with anemia is not clear. Again, just coming back to this issue about infection, anemia of chronic disease is not associated with iron deficiency, it is a specific condition. Of course if we haven't diagnosed iron deficiency there is no reason to believe that that individual will respond to iron. Anemia is a general indicator of poor health or poor nutrition and therefore it is so important to know the etiology of anemia. I think that is one of the reasons why there is so much confusing and conflicting data on the efficacy and effectiveness of various interventions where hemoglobin has been measured as an outcome. If we give iron to an individual who is not iron-deficient, why would he respond to iron?

Dr. Barclay: I have a question following on Dr. Zlotkin's question about the relationship between maternal nutrition and the resulting infant nutrition, and it relates to micronutrient interactions. It is well known that vitamin C has a positive effect on iron absorption. I was just wondering if there is any recent information concerning the possibility of supplementing the mothers with vitamin C or with fruit juice containing vitamin C, and would that have an effect on iron absorption in the infant from breast milk? Could you also say a few words also about the role of lactoferrin in iron absorption?
Dr. Davidsson: I am not aware of any information about the role of vitamin C in breast milk. As Dr. Allen discussed, you can change water-soluble vitamins rather quickly by supplementing and also, as we have shown recently, by dietary interventions, giving orange juice to lactating women. I am not aware of any studies that have demonstrated an effect of vitamin C on iron absorption from breast milk. It is extremely difficult to measure iron absorption from breast milk and it just hasn’t been done as far as I am aware. Lactoferrin and its role in human milk have been discussed for a number of years. We were very interested in that a few years ago and did a study where we measured iron absorption from breast milk in infants, using stable isotope techniques, with and without lactoferrin present. We did not find an enhancing effect at all for lactoferrin, on the contrary iron absorption increased when we removed lactoferrin. These data were published in 1994 in Pediatric Research [6]. This was a small group of infants in Davis, California, in whom we measured iron absorption from expressed breast milk that was labeled with stable isotopes of iron, and we removed lactoferrin in one part of the milk. What you can see here is that human milk had iron absorption, based on erythrocyte incorporation, of about 10% in these normal healthy breast-fed infants, and it went up to a mean of 19% after we had removed lactoferrin. So the presumed positive influence on iron absorption of human lactoferrin has not been demonstrated. Lactoferrin binds iron, we know that very well, and it probably has a lot of important influences on bacterial growth, etc., and that is what I think is most interesting today, not iron absorption per se.

References